REGULATION OF TUMOR SUPPRESSOR PROTEIN p53 IN CELLULAR STRESS AND TUMORIGENESIS

SARI TOJKANDER

MOLECULAR AND CANCER BIOLOGY PROGRAM, BIOMEDICUM HELSINKI,
DEPARTMENT OF VIROLOGY, HAARTMAN INSTITUTE
AND
HELSINKI GRADUATE SCHOOL OF BIOTECHNOLOGY
AND MOLECULAR BIOLOGY, UNIVERSITY OF HELSINKI,
FINLAND

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the
Faculty of Medicine of the University of Helsinki,
for public criticism in the Lecture Hall 2, Biomedicum Helsinki
Haartmaninkatu 8, Helsinki
on January 12th, 2007, at 12 noon.

Thesis supervisor
Professor Marikki Laiho
Molecular and Cancer Biology Program
Biomedicum Helsinki and
Haartman Institute
University of Helsinki
Helsinki, Finland

Thesis reviewers
Docent Ismo Ulmanen
Department of Molecular Medicine
National Public Health Institute
Helsinki, Finland

Professor Kirsi Vähäkangas
Department of Pharmacology and Toxicology
University of Kuopio
Kuopio, Finland

Thesis opponent
Associate Professor Mats Ljungman
Department of Radiation Oncology
Division of Cancer Biology
University of Michigan
Michigan, USA

ISBN 978-952-92-1450-1 (paperback)
ISBN 978-952-10-3607-1 (PDF)

Helsinki University Press
Helsinki 2006

ABSTRACT

Functional loss of tumor suppressor protein p53 is a common feature in diverse human cancers. The ability of this protein to sense cellular damage and halt the progression of the cell cycle or direct the cells to apoptosis is essential in preventing tumorigenesis. Tumors having wild-type p53 also respond better to current chemotherapies. The loss of p53 function may arise from *TP53* mutations or dysregulation of factors controlling its levels and activity. Probably the most significant inhibitor of p53 function is Mdm2, a protein mediating its degradation and inactivation. Clearly, the maintenance of a strictly controlled p53-Mdm2 route is of great importance in preventing neoplastic transformation. Moreover, impairing Mdm2 function could be a nongenotoxic way to increase p53 levels and activity. Understanding the precise molecular mechanisms behind p53-Mdm2 relationship is thus essential from a therapeutic point of view.

The aim of this thesis study was to discover factors affecting the negative regulation of p53 by Mdm2, causing activation of p53 in stressed cells. As a model of cellular damage, we used UVC radiation, inducing a complex cellular stress pathway. Exposure to UVC, as well as to several chemotherapeutic drugs, causes robust transcriptional stress in the cells and leads to activation of p53. By using this model of cellular stress, our goal was to understand how and by which proteins p53 is regulated. Furthermore, we wanted to address whether these pathways affecting p53 function could be altered in human cancers.

In the study, two different p53 pathway proteins, nucleophosmin (NPM) and promyelocytic leukemia protein (PML), were found to participate in the p53 stress response following UV stress. Subcellular translocations of these proteins were discovered rapidly after exposure to UV. The alterations in the cellular localizations were connected to transient interactions with p53 and Mdm2, implicating their significance in the regulation of p53 stress response. NPM was shown to control Mdm2-p53 interface and mediate p53 stabilization by blocking the ability of Mdm2 to promote p53 degradation. Furthermore, NPM mediated p53 stabilization upon viral insult. We further detected a connection between cellular pathways of NPM and PML, as PML was found to associate with NPM in UV-radiated cells. The observed temporal UV-induced interactions strongly imply existence of a multiprotein complex participating in the p53 response. In addition, PML controlled the UV response of NPM, its localization and complex formation with chromatin associated factors.

The relevance of the UV-promoted interactions was demonstrated in studies in a human leukemia cell line, being under abnormal transcriptional repression due to expression of oncogenic PML-RARα fusion protein. Reversing the leukemic phenotype with a therapeutically significant drug was associated with similar complex formation between p53 and its partners as following UV. In conclusion, this thesis study identifies novel p53 pathway interactions associated with the recovery from UV-promoted as well as oncogenic transcriptional repression.

TABLE OF CONTENTS

ORIGINAL PUBLICATIONS

The thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Kurki, S., Latonen, L. and Laiho M. Cellular stress and DNA damage invoke temporally distinct Mdm2, p53 and PML complexes and damage-specific nuclear relocalization. *J. Cell Sci.* **116**: 3917-3925 (2003).

II Kurki, S., Peltonen, K., Latonen, L., Kiviharju, M., Ojala, P. M., Meek D. and Laiho, M. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. *Cancer Cell*. **5**: 465-475 (2004).

III Kurki, S., Syrjäkari, H. and Laiho, M. NPM and PML interact and cooperate in the regulation of p53 pathway in cell stress and tumorigenesis. Manuscript, submitted.

ABBREVIATIONS

6-4PP	(6-4)-photoproduct
ALCL	Anaplastic large cell lymphoma
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
ARF	alternative reading frame
ATM	Ataxia telangiectasia mutated
ATO	arsenic trioxide, As_2O_3
ATR	Ataxia telangiectasia-related
Bcl-2	B-cell lymphoma 2
BER	base excision repair
BLM	Bloom syndrome
C	carboxy
CDK	cyclin dependent kinase
CDKI	cyclin dependent kinase inhibitor
CPD	cyclobutane-type pyrimidine dimer
CS	Cockayne syndrome
DBD	DNA binding domain
DFC	dense fibrillar component
DRB	5, 6-dichloro-1-β-D-ribofuranosylbenzimidazole
DSB	double strand break
FC	fibrillar center
FRAP	fluorescence recovery after photobleaching
GC	granular component
GGR	global genomic repair
HAT	histone acetyl-transferase
HDAC	histone deacetylase
Hdm2	human Mdm2
HIPK2	homeodomain-interacting protein kinase-2
HR	homologous recombination
IFN	interferon
IR	ionizing radiation
JNK	c-Jun N-terminal kinase
KSHV	Kaposi´s sarcoma associated herpesvirus
MAP	mitogen-activated protein
Mdm2	murine double minute 2
MDS	Myelodysplastic syndrome
MEF	mouse embryo fibroblast
MMR	mismatch repair
N	amino
NB	nuclear body
NER	nucleotide excision repair

NES	nuclear export signal
NHEJ	non-homologous end-joining
NLS	nuclear localization signal
NoLS	nucleolar localization signal
NPM	nucleophosmin
NPMc+	cytoplasmic NPM mutant
PCNA	proliferating cell nuclear antigen
PI-3-K	phosphoinositide-3-kinase
PML	Promyelocytic leukemia
PRD	proline-rich domain
RA	retinoic acid
RAR	retinoic acid receptor
Rb	retinoblastoma
RPA	replication protein A
RXR	retinoic-X receptor
SUMO	small ubiquitin-related modifier
TAD	transactivation domain
TCR	transcription-coupled repair
TET	tetramerization domain
TSA	trichostatin A
UV	ultraviolet
UVC	ultraviolet C radiation
Wt	wild type
XP	Xeroderma pigmentosum

INTRODUCTION

Cancer, a disease defined as abnormal proliferation and invasion of the cells, is one of the major causes of death in the western societies. The cellular changes leading to this disease may take several years to develop and due to the longer lifespan of the population, the frequency of cancer has increased dramatically.

The multistep process of cancer development requires several genetic changes over a long period of time. Each cell contains the genetic information that has to be replicated and passed to the next progeny in the process of cell cycle. This hereditary code is, however, altered constantly due to external pressure and the DNA in most of the cells experience numerous mutations every day. To support the precise genetic code from one cell generation to the next one, the cells have developed a number of regulatory pathways to monitor the entire process. Despite the high fidelity of this machinery, some occasional mistakes can be passed by this system and be further transferred to the progeny. Errors in the control of the damage response may lead to the accumulation of genetic lesions and multiple phases of clonal selection eventually results in uncontrolled growth and predisposition to cancer.

One of the key proteins in the regulation of the genomic integrity is tumor suppressor protein p53. p53 can prevent accumulation of harmfull mutations, inhibiting tumor-promotion. Upon exposure to various kind of damage, p53 protein is activated and halts the cell cycle to give the repair machinery some time to solve the errors in the hereditary material. In case of excessive damage, however, the DNA may be in an unrepairable condition and the cell may have to choose a cell death pathway instead of the growth arrest to insure maintenance of the genome. The ability of p53 to induce this programmed cell death, apoptosis, is probably its major function in preventing the neoplastic transformation.

The early events leading to cellular p53 response are not totally understood, even though they have been studied extensively over the past two decades. Inactivation of the p53 pathway is very common in cancers and reactivation a potential key factor in killing tumor cells. Although preventing the incidence of cancer by eliminating the risk factors would probably be the most effective way of reducing the number of cancer cases, new therapeutic possibilities are required. Activation of the p53 pathway may have an important role in this process. Thus, knowing the factors that affect the function of p53 are critical to understand in detail. This study has concentrated on exploring the proteins that regulate p53 stability and functional activity in DNA-damaged cells. In addition, the aim was to find how these regulatory steps could be defective in human cancers.

REVIEW OF THE LITERATURE

CONTROL OF CELL PROLIFERATION

Formation of a multi-cellular organism requires proper regulation of the cell division and death in the developmental stage as well as in the renewal and maintenance of the functions of different tissues and organs in the adult body. Cells reproduce themselves by transmitting their genetic information to their daughter cells in a strictly controlled process of cell cycle. Any mistakes in the network of cell cycle-regulatory proteins may lead to aberrations of cellular functions in the next progeny and alterations in these key proteins of the cycle is thus a very common feature for a number of cancers.

The cell cycle

The eukaryotic cell cycle is divided into four phases (Figure 1) (reviewed in Nurse, 2000). The duplication of DNA takes place during the synthesis or S phase of the cycle. This phase of the cycle is the most time consuming, requiring usually about half of the cell cycle time. The segregation of the newly synthesized chromosomes to new daughter cells occurs in the mitosis, M phase of the cycle. Two gap phases, G1 and G2, are inserted between the synthesis and mitosis phases of the cycle. G1 and G2 phases provide the cell more time to grow and produce proteins required for DNA synthesis and cell division. During these periods, the cell can also monitor outside and inside signalling to ascertain that the conditions are appropriate for proceeding further in the cycle. Cells are also able to exit the division cycle to stay in a quiescent state, G0.

The cell cycle control system is conserved in all eukaryotes. The central regulatory proteins responsible for this system are the cyclins and their partners, cyclin dependent kinases, CDKs (Nurse, 2000). Sequential activation and inactivation of these protein complexes allows progression through the cycle (Figure 1). During G1, CDK4 and its homologue CDK6 are activated by complex formation with D-type cyclins. The expression of cyclin D is controlled by the mitogen-activated protein kinase (MAPK) pathway, playing a major role in entry to G1 phase (Lavoie et al., 1996). CDK4/6 phosphorylate and inactivate retinoblastoma protein (pRb), a tumor suppressor protein responsible for normal cell cycle progression (Weinberg, 1995; Classon and Harlow, 2002). In early G1, pRB is in its hypophosphorylated form, blocking the synthesis of DNA through inactivation of transcription factor E2F. When CDK4/6-cyclin D complex becomes active, it allows phosphorylation of pRB in late G1, leading to release of E2F and transcription of genes involved in DNA synthesis (Weinberg, 1995; Classon and Harlow, 2002). At the end of G1, CDK2 complexes with cyclin E and commits the cell to DNA replication phase (Tsai et al., 1993). CDK2 further phosphorylates pRb, resulting in complete inactivation of pRb. In S phase cyclin A replaces cyclin E from the complex and regulates DNA replication (Pagano et

al., 1992). The same cyclin can bind mitotic CDK1 and participate in the G2/M transition, although the key mitotic regulator is cyclin B in complex with CDK1 (Smits and Medema, 2001).

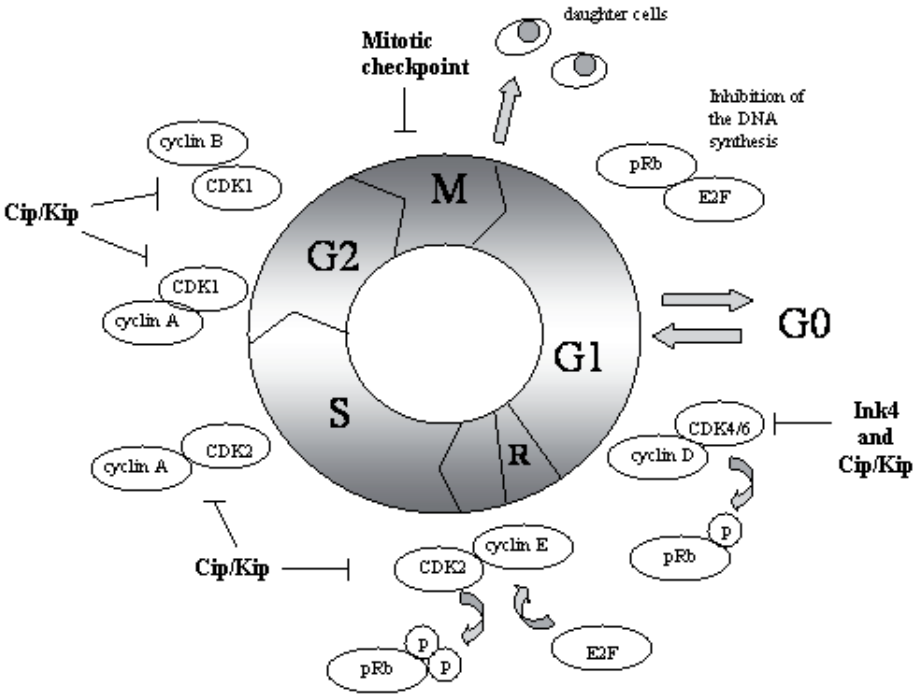

Figure 1. Regulation of the cell cycle. Progression through the cell cycle is precisely controlled by fluctuating activities of the cyclin-CDK complexes and CDK target pRb. See text for further details. CDK, cyclin-dependent kinase; P, phosphorylated; R, restriction point.

The oscillations in the activities of different CDK-cyclin complexes is influenced by several different factors, including rise and fall in the levels of cyclins through proteolytic degradation, inhibitory phosphorylations of the CDKs and binding of CDKs by their specific inhibitory proteins, cyclin dependent kinase inhibitors, CDKIs (Nurse, 2000). The CDKIs are further divided into two families: Ink4- and Cip/Kip-families, of which the Ink4 family members, $p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$ and $p19^{Ink4d}$ prevent the activity of CDK4/6 by utilizing the same binding domain as D-type cyclins and Cip/Kip family proteins, $p21^{Cip1}$, $p27^{Kip2}$ and $p57^{Kip2}$ which bind and inactivate CDK1, CDK2 as well as CDK4/6 (Pines, 1997; Pavletich, 1999) (Figure 1).

Cell cycle checkpoints

Cells are in a continuous pressure on facing endogenous damage and stress due to changes in their environment and alterations in the growth conditions. Preserving genetic stability in the next generation of cells requires that cells are able to sensor and respond to extra- and intracellular signalling and that events in the cell cycle are properly timed and occur in an exact order. In addition, these events can occur only once in a cycle. To secure normal functions, cells have a specific control system that monitors the condition of the cell and is able to delay the progression of the cycle subsequent to damage, contact inhibition, senescence or growth inhibitory signals from other cells. These control points, referred to as checkpoints, screen and holdup the cell cycle at specific phases of the cycle and gain time for the cell to respond to the situation (Hartwell and Weinert 1989; Bartek and Lukas 2001b; Lukas et al., 2004).

The cell cycle checkpoints operate in late G1, S-phase and G2/M transition of the cycle and are controlled by the activation of pathways involving Cip/Kip and Ink4 family members (Figure 1). Transition from G1 phase to S phase requires the presence of growth factors. During mid and late G1, cells sensor outside and inside signalling for favourable conditions and request for a license to continue in the cycle. Loss in mitogenic signalling leads to rapid degradation of cyclin D and inhibits the cells from entering the S phase (Matsushime et al., 1991). In the presence of mitogenic signalling, active CDK4/6-cyclin D complex drives the cells into S phase through a point after which there is no return. If cells are allowed to bypass this restriction point (R) they start to synthesize DNA and the division is completed without extracellular signals, unless the conditions for some reason turn unfavourable (Pardee 1989; Bartek and Lukas 2001a &b) (Figure 1). Loss in the control of this restriction point appears to be a universal feature in the development of tumors, leading to aberrant mitosis. The S phase checkpoint insures proper replication of DNA and that the genetic material has been duplicated only once per cycle before cell division. In addition, this checkpoint monitors correct duplication of the centrosomes. After progression to G2, the cells can still assess the condition of the replicated DNA and halt the cycle if required. Lastly, the mitosis checkpoint, also referred to as spindle point checkpoint, monitors the attachment of chromosomes to the mitotic spindle. Any negative signal from an unattached kinetochore blocks progression to anaphase and delays the cycle.

DNA damage checkpoints

Genotoxic stress and errors in the DNA replication processes challenge the damage sensoring system continuously. Depending on the damaging source, genetic material is subjected to different kind of mutations. Ionizing and ultraviolet radiation, mutagenic compounds and reactive oxygen species from metabolic pathways of the cell each cause particular type of lesions, triggering specific signalling cascades. The DNA damage checkpoints are evolution-

ary conserved and they largely overlap with the cell cycle checkpoints (Zhou and Elledge, 2000) (Figure 2). G1/S checkpoint delays entry into S phase if damaged DNA is discovered. In S phase, the replication-independent intra-S checkpoint slows down the DNA replication in damaged cells. G2/M checkpoint inhibits cells from going into mitosis if cells are exposed to DNA damage during G2 or they have unrepairable damage left from the preceeding phases of the cycle.

The DNA damage checkpoint system composes of multiple factors forming a complex signalling pathway. Although the checkpoints are in the different phases of the cycle, many of their signalling components are common and share the same upstream events (Bartek and Lukas 2001b; Lukas et al., 2004). Proteins of the checkpoint pathways can be divided into three groups: sensoring factors that initially recognize the damaged site, signal transducers that mediate the signal further in the cascade and effector proteins, which induce the final response in the pathway (Iliakis et al., 2003). One of the most prominent sensors and activators of the checkpoint responses are the phosphoinositide-3-kinases (PI-3-Ks), Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia-related (ATR) kinases (Abraham, 2001; Shiloh, 2003). Activation of these primary sensors of lesions is followed by DNA damage in G1/S, S and G2/M phases of the cycle. Despite partly overlapping functions of these kinases, ATM seems to be more important in double strand breaks (DSBs) induced by ionizing radiation, while ATR responds to broader range of damage types, including UV radiation. ATM and ATR work through a signalling cascade involving checkpoint kinases, Chk1 and Chk2. In the activation of G1/S checkpoint, Chk1/Chk2 kinases mediate the degradation of Cdc25A phosphatase, holding cyclin E/CDK2 complexes inactive through an inhibitory phosphorylation (Mailand et al., 2000; Bartek and Lukas, 2001a). This response is acute, leading to a transient cell cycle arrest (Lukas et al., 2004). A slower and more sustained response is mediated by the p53 protein through its key downstream effector p21, resulting in delayed inhibition of the cyclin E/CDK2 complex (Sherr and Roberts, 1999; Bartek and Lucas, 2001b; Wahl and Carr, 2001). The intra-S-phase checkpoint is able to delay the progression of the cycle in a p53-independent manner, operating mainly through Cdc25A degradation pathway and cyclin E/CDK2, cyclin A/CDK2 or alternatively through Nbs1/SMC1 pathway (Falck et al., 2002; Yazdi et al., 2002). Activation of both of these pathways cause a transient delay of the cycle, rather than a proper cell cycle arrest. In G2/M phase of the cycle the ATM/ATR and Chk1/Chk2 signalling cascades lead to inactivation of cyclin B/CDK1 complexes through inhibition of the Cdc25c (Abraham, 2001; Nyberg et al., 2002) (Figure 2). Additionally, p53 operates through p21, GADD45 and 14-3-3σ proteins for long term silencing of cyclin B/CDK1 (Taylor and Stark, 2001), although this p53-induced pathway is probably not essential for the sustained G2 arrest. Even though the upstream signalling is overlapping in most of the checkpoint pathways, the downstream signalling in response to various kinds of damage follow separate routes and may have distinct outcomes (reviewed in Lukas et al., 2004). Despite the final response of each route, the common task of

these pathways is to ultimately eliminate the DNA lesions and secure the genetic stability of the cells.

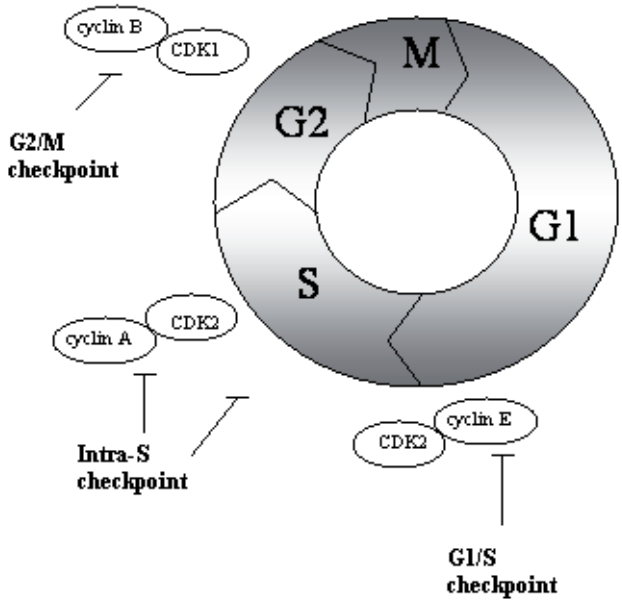

Figure 2. DNA damage checkpoints. The cells respond to DNA lesions by halting progression of the cycle through several independent pathways leading to inhibition of CDK-cyclin complexes. See text for details.

Repair of the damaged DNA

During the cell cycle arrest the cells have time to deal with the damage they have experienced. Human cells have several, partly overlapping, mechanisms for repairing different kind of damage lesions. Plenty over 100 genes are involved in the different repair pathways (Wood et al., 2001), summarized in table 1.

Direct reversal is probably the simplest repair pathway. This single enzyme reaction is responsible for the removal of DNA adducts, like miscoding methylated bases, caused by DNA alkylating agents or endogenous catabolites. Several DNA methyltransferases are involved in this process (Mishina et al., 2006). The most common repair pathways are base excision repair (BER) and nucleotide excision repair (NER). BER pathway utilizes a group of specific DNA glycosylases for excision of the altered bases and is mainly induced by cellular metabolites (Lindahl and Wood, 1999). NER is a versatile repair pathway, managing several types of lesions and will be later discussed in more detail in the context of its relevance in the UV-induced damage repair. Mismatch repair (MMR) corrects noncomplementary base pairs and other DNA structure-distorting loops during replication as well as in damaged cells (Jiricny, 2000). MMR is a multistep process, consisting of recognition and excision of the incorrect site, resynthesis and ligation of the newly synthesized strand and involves numerous different proteins, including human mutS homolog (MSH) family proteins and mutL homolog 1 (MLH1) forming mismatch recognition complexes, proliferating cell nuclear antigen (PCNA) and replication protein A (RPA) (Jiricny, 2000). The removal of double-strand breaks, induced by ionizing radiation, chemical agents or cellular dysfunctions, occurs through two major pathways. Non-homologous end-joining (NHEJ) is the main DSB-repair pathway in humans, while the second pathway, homologous recombination (HR) is preferred in S and G2 phases of the cell cycle (Haber, 2000). The initiating signal in NHEJ pathway is by the DNA-PK kinase, while the ATM kinase is the major coordinator of the damage response in the HR pathway. Both of these pathways eventually lead to activation of the Mre11-Rad50-Nbs1 complex, involved in sensing and repairing the damaged site (Petrini & Stracker, 2003). In addition to activation of several other repair proteins, these kinase pathways also activate many downstream substrates, like Chk2 and p53, involved in halting the cell cycle. A variety of human diseases are associated with defects in the DNA repair capacity of the cells. Many of these diseases are inherited conditions, leading to a higher mutation rate and predisposition to cancer in the carriers of these defective DNA repair genes, as discussed later.

Repair mechanism	Activating event and special features
Nucleotide excision repair, NER (Hanawalt et al., 2003; Peterson and Côté, 2004)	Several types of bulky lesions induced by UV radiation, chemicals and DNA crosslinking agents
Base excision repair, BER (Lindahl and Wood, 1999)	Altered bases and damage induced mainly by cellular metabolites
Mismatch repair, MMR (Jiricny, 2000)	Noncomplementary base pairs, causing distortion in the DNA helix during replication or DNA damage
Non-homologous end-joining, NHEJ (Haber, 2000; Petrini & Stracker, 2003)	DSBs induced by ionizing radiation and other DNA damaging agents; Main pathway for DSB repair in humans.
Homologous recombination, HR (Haber, 2000; Petrini & Stracker, 2003)	DSBs induced by ionizing radiation and other DNA damaging agents; Activated mainly in S/G2 phases.
Direct reversal (Mishina et al., 2006)	Methylated base-adducts in DNA, caused by DNA methylating agents or cellular catabolites

Table 1. Summary of the main repair pathways in human cells. Several DNA repair systems have evolved for the repair of the damaged DNA caused by cellular processes and metabolic byproducts. These same repair pathways are utilized for the correction of lesions, induced by extracellular agents, which could contribute to accumulation of genetic instability, carcinogenesis as well as lethality due to malfunction in essential cellular pathways.

Apoptosis

Besides the strict control of cell division, equally important for the interests of functional organisms is to control the number of cells by cell death. By inducing an intracellular death program, programmed cell death or apoptosis, cells that are superfluous or cells that could be of threat to the organism, are destroyed. Triggering this suicide pathway may be the only alternative in cells exposed to excessive DNA damage.

The evolutionary conserved program of apoptosis is mainly affected by a family of proteases, caspases, which mediate the cleavage of their specific target proteins. Caspase pathway is activated by intracellular or extracellular stimuli. External activation can occur through the death receptors by ligand binding, which induce a signalling pathway leading to activation of the caspase pathway (Muppidi et al., 2004). Internal signalling requires release of mitochondrial cytochrome c. B-cell lymphoma 2 (Bcl-2) family members are the key regulators of caspases and apoptosis, directly impacting the permeabilization of the outer-membrane of mitochondria and cytochrome c release (Spierings et al., 2005).

This family contains both apoptotic and anti-apoptotic factors and their significance is underlined by the knowledge that lack of the apoptosis inducing factors of this group makes the cells extremely resistant to programmed cell death. On the other hand, the anti-apoptotic factors of this family, like Bcl-2, are overexpressed in several cancer types (Willis and Dyer, 2000).

During the apoptotic response, the release of cytochrome c activates Apaf-1, apoptotic protease-activating factor, triggering caspase pathway and simultaneously blocking other anti-apoptotic factors, like IAPs, inhibitor of apoptosis proteins (Li P. et al., 1997; Zou et al., 1997). The final outcome of this process is associated with specific cellular features including shrinkage of the cell size and disruption of the cytoskeletal structure, breakage of the nuclear envelope as well as fragmentation of the DNA. The remains of the apoptotic cell are rapidly phagocytosed, causing no damage to the neighbouring cells.

UV DAMAGE RESPONSES

UV radiation-induced DNA damage

UV radiation of the sun is associated with skin cancers, including basal cell carcinoma, squamous cell carcinoma and malignant melanoma (de Gruijl, 1999). It is invisible electromagnetic radiation that can be divided into three wavelenght areas: UVA 315-380 nm, UVB 280-315 nm and UVC 190-280 nm, the shorter wavelength radiation being the most harmful (Tyrrell, 1994). Most of the radiation reaching the ground of earth is UVA, but the proportion of the shorter wavelength light is unfortunately increasing due to a decline in thickness of the ozone layer.

UV radiation induces bulky DNA-lesions, cyclobutane-type pyrimidine dimers (CPDs) and structurally more distorting (6-4)-photoproducts (6-4PPs), which cross-link DNA bases, inhibiting transcription (Ravanat et al., 2001; Thoma et al., 1999; Tornaletti et al., 1999; Mitchell et al., 2003). UVA and UVB radiation are somewhat more environmentally relevant as most of the shorter wavelength radiation is still absorbed by the ozone layer. However, the UV-induced transcriptional stress is more efficiently triggered by UVC due to higher energy absorbance by DNA from this type of light (Ravanat et al., 2001) and most of the studies have for this reason used UVC as a model. As the wavelength increases, UV radiation-induced oxidative stress becomes more likely (Kielbassa et al., 1997). UVB causes different proportion of 6-4PPs than does UVC and additionally it causes the oxidative lesions and interacts also with other molecules than DNA. In addition, some DNA strand-breaks and protein-DNA cross-links can be detected after exposure to longer wavelengths of UV radiation.

Cellular responses induced by UV radiation

UV radiation-induced DNA damage evokes a set of cellular responses, including transcriptional inhibition, damage recognition and activation of several signalling pathways (de Gruijl et al., 2001). The early response in UV-damaged cells is provoked by ATR-Chk1 kinase pathway, leading to phosphorylation of several downstream targets. In addition to this pathway, the MAP kinases, Erk, JNK and p38 are essential and activated upon UV damage in a dose dependent manner (Davis, 2000; Bode and Dong, 2003). The changes in the cell surface receptors trigger these intracellular signalling pathways and their activation plays a role in the control of cell growth, changes in the chromatin structure and apoptotic responses (Bode and Dong, 2003). Depending on the damage-induced cascade, different target genes are activate through activation of UV-induced transcription factors, including p53, antiapoptotic factor nuclear factor κB (NFκB) and activating protein 1 (AP-1) (Ryan et al., 2000; Shaulian and Karin, 2002; Chen and Greene, 2004). Activation of a certain cascade is dependent on the amount of UV dose and affects eventually the final transcriptional response, leading to either cell cycle arrest and DNA repair or to apoptosis, if cells are subjected to

excessive amounts of lesions (Gentile et al., 2003). Transcriptional responses differ also depending on the cell type (Valery et al., 2001; Sesto et al., 2002; Gentile et al., 2003). In addition to growth arrest and apoptotic response, exposure to UV light provokes immunosuppression, possibly contributing to neoplastic transformation (Clydesdale et al., 2001).

Nucleotide excision repair

Nucleotide excision repair, NER, is probably the most comprehensive repair pathway, facilitating the repair of a variety of dissimilar lesions in DNA, including UV-induced CPDs and 6-4PPs (Peterson and Côté, 2004). The complex NER pathway is well conserved in bacteria, yeast and mammals (Eisen and Hanawalt, 1999; Petit and Sancar, 1999) and the mammalian NER function requires nearly 30 different factors for full activity (Lindahl and Wood, 1999; Volker et al., 2001). NER is divided into two different subpathways, transcription-coupled repair (TCR) and global genomic repair (GGR) (reviewed in Hanawalt et al., 2003; Peterson and Côté, 2004). TCR is less well understood and repairs damage sites found in transcribed DNA strands of genes, while GGR is a more general repair machinery, correcting lesions throughout the whole genome.

The action of NER involves the following steps: lesion detection in DNA and chromatin remodelling, removal of the lesion and resynthesis of the nucleotide sequence and ligation of the newly-synthesized strand to the pre-existing one (Figure 3). The initial lesion detection in TCR and GGR is done by separate proteins, although many of the other enzymatic processes in GGR and TCR have overlapping factors. The GGR proteins are, however, usually kept at low levels until the cells are exposed to DNA damage. Several of the proteins in Xeroderma pigmentosum (XP) complementation group play a role in both subpathways (Friedberg et al., 2004). For these repair pathways to work efficiently the chromatin structure has to be altered (Smerdon and Lieberman, 1978). In TCR the chromatin accessibility appears to be ensured by the presence of the transcription machinery itself (Friedberg, 2001), while in GGR the chromatin accessibility has to be achieved by other factors (Tijsterman et al., 1999; Friedman, 2001). Initial recognition of lesions by GGR include the mammalian XPC-HR23B-centrin2 complex and XPA protein (Sugasawa et al., 1998; Volker et al., 2001). Additionally, UV-DDB, UV DNA damage binding protein is required in some damage types like CPDs (Tang and Chu, 2002; Wakasugi et al., 2002). These factors can also recruit histone acetylatransferase (HAT) activities to further increase the access to these sites. In TCR, the Cockayne syndrome proteins, CSA and CSB as well as XAB2 protein, initially target NER to stalled RNA pol II on DNA strands (Tornaletti and Hanawalt, 1999; Mitchell et al., 2003). CSB/Rad26 remodelling complex may also facilitate increased accessibility to the damage sites during TCR. In addition, the UVSS, ultraviolet-sensitive syndrome protein has been shown to be essential for properly functioning TCR (Spivak et al., 2002). Further verification and direction of unwinding in the damaged area is performed in both subpathways by a multisubunit transcription factor/repair

protein TFIIH and its partners XPB and XPD (Giglia-Mari et al., 2004). This is followed by incision of the lesion area by XPF and XPG proteins with the help of XPA and RPA proteins, stabilizing the formation of the repair complex. The formed gap is filled when synthesis of a new DNA strand by DNA pol δ/ε and PCNA takes place (de Laat et al., 1999; Peterson and Côté, 2004) (Figure 3).

Failure in NER function leads to increased cancer incidence, as observed in the hereditary Xeroderma pigmentosum in humans (Friedberg, 2004). XP is defined as a group of recessive disorders caused by defects in the nucleotide excision repair genes. The GGR deficient cells of these patients have weaker apoptotic signalling resulting in higher mutation rates and transformation of the surviving cells. Due to this XP patients are very prone to sunlight-induced diseases, like skin cancers, developing both benign and malignant neoplasms (Bootsma, 1993). Cockayne´s syndrome and UV-sensitive syndrome of humans are both diseases associated with defective TCR DNA repair of the cells (Spivak, 2005). However, these syndromes are not associated with increased cancer risk as cells deficient in TCR are even more prone to UV-induced apoptosis (Ljungman and Zhang, 1996). Cell survival as well as cancer incidence so appears to be more dependent on functional GGR than TCR and cellular damage left after deficient TCR function can be still rescued by GGR machinery.

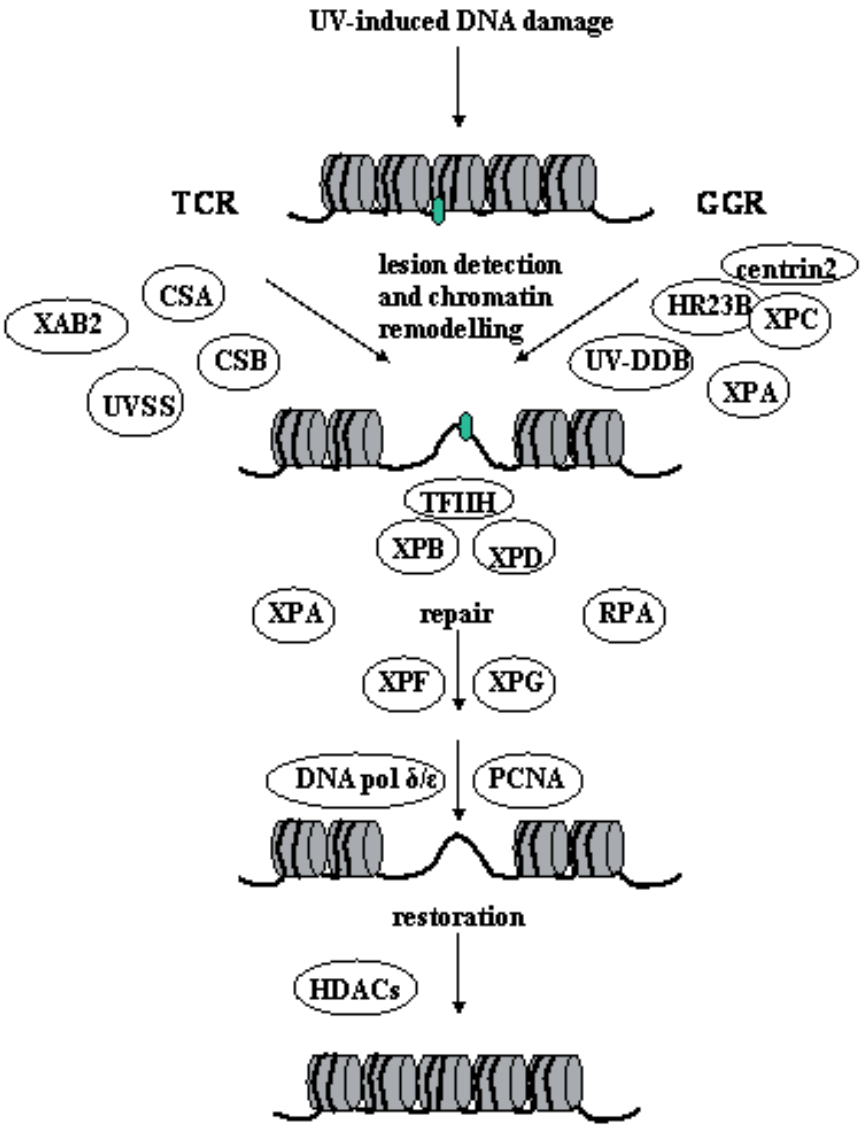

Figure 3. Nucleotide excision repair pathway. NER is composed of two subpathways, TCR and GGR, specified by their differencies in the initial lesion detection factors. The repair of the damage site is overlapping for both pathways, including 1) unwinding of the lesion surroundings by TFIIH and its partners XPB and XBD, 2) incision of the damaged area by XPF and XPG and 3) synthesis of a new DNA strand by DNA pol δ/ε and PCNA. Finally the newly synthesized strand is ligated to the pre-existing one and the structure is restored by chromatin modifiers.

Nucleolar stress response

Nucleoli are specific subcompartments of the nucleus, clearly visible dense structures under the microscope. The main function of these dynamic compartments is to act as ribosome factory. Nucleoli orchestrate the synthesis and processing of ribosomal RNAs (rRNAs) and their assembly to pre-ribosomal particles in specific compartments of the nucleolus (Carmo-Fonseca et al., 2000; Olson & Dundr, 2005). The nucleolus is formed of small fibrillar centers (FCs), which are responsible for the initiation of the rRNA transcription. These structures are surrounded by dense fibrillar component (DFC), processing the nascent rRNA transcripts. Finally the further processing occurs in the granular component (GC), surrounding FC and DFC structures. The rate and efficiency of this process reflects the transcriptional activity of the cell, being high in rapidly proliferating cells. Cancer cells often have very prominent nucleoli (Derenzinin et al., 2000). In addition to its traditional role in ribosome biogenesis, nucleolus has lately been connected to several other functions due to its protein composition (Andersen et al., 2005; Pendle et al., 2005; Leung et al., 2006). Many of the nucleolus-associated proteins have roles in the cell cycle control, aging, viral replication, nuclear export and telomerase activity, reflecting the versatility of the functions of nucleolar compartment (Carmo-Fonseca et al., 2000; Olson et al., 2002). Furthermore, recent results show the importance of nucleolus as a stress sensor, responding to various kind of cellular damage and mediating p53 stabilization (Rubbi and Milner, 2003b; Olson et al., 2004; Mayer et al., 2005).

Exposure of the cells to external and internal stress, including UV radiation, hypoxia, heat shock and nucleotide depletion, causes so called “nucleolar stress” impairing the function of these sub-nuclear compartments (Rubbi and Milner 2003b; Olson et al., 2004; Mayer and Grummt, 2005). All of these stress inducers are basically inhibitors of the transcription and disruption of this key function of the nucleolus leads to reorganization of its structure and several nucleolar proteins are released to nucleoplasm (Olson et al., 2004; Shav-Tal et al., 2005) (Figure 4). For instance, Ki-67, nucleolin, fibrillarin, p120 and Hrad17 have been shown to relocalize from the nucleoli upon UV-induced stress (Chang et al., 1999; Daniely et al., 2002; Rubbi and Milner, 2003; Al-Baker et al., 2004). The structure of the nucleoli reorganizes rapidly upon transcriptional inhibition and the FC compartments move to the perinucleolar area (Panse et al., 1999). These kind of dotted compartments called “nucleolar necklaces” were already described in the 1970´s (Granick & Granick, 1971; Granick, 1975). The function of these necklaces is still not clear, even though several proteins have been reported to colocalize with them upon cellular stress (Fuchsová et al., 2002; Hoogstraten et al., 2002).

The mechanism leading to reorganization in the nucleolar structure and translocations of different proteins to the perinucleolar area is not totally understood, although it is possible that the initiating signal comes from the RNA pol II inhibition by UV radiation. One of the key factors regulating the nucleolar structure could be transcription factor TIFIA, which regulates the activity

of RNA polI (Schnapp et al., 1990). In stressed cells TIFIA is phosphorylated by c-Jun N-terminal kinases (JNK2), disrupting the TIFIA-RNA polI connections and leading to nucleoplasmic TIFIA upon reorganization of the nucleolus (Mayer et al., 2005). The importance of this pathway is highlighted by the fact that inactivation of TIFIA phosphorylation by JNK results in stress-resistance of PolI transcription and rRNA synthesis. The recent reports show the versatility of the nucleolar functions and underline its importance as a stress sensor in damaged cells.

Figure 4. Reorganization of the nucleoli upon transcriptional inhibition. The nucleoli are damage sensors, undergoing rapid morphological changes in stressed cells. The fibrillar centers (FC) relocalize to the perinucleolar area and several nucleolar proteins are released to the nucleoplasm (Olson et al., 2004; Shav-Tal et al., 2005), and may thus affect the stress response through specific targets in the nucleoplasmic compartment.

UNCONTROLLED GROWTH AND CANCER

Every day one single human cell has to deal with thousands of errors in its genome due to endogenous and exogenous damaging agents (Friedberg, 2001). Even though the cells have efficient and overlapping machineries for repairing the altered sites, sometimes the systems and their backups fail and cells with mutated genomes continue multiplying. In the worst situation, the mutations may give the cell selective advantage, allowing it divide more efficiently than the neighbouring ones. Over time the cells may also acquire more genetic alterations that lead to tumorigenesis, driving the cells from normal human cells into cancerous derivatives. As normal cells act in the benefit of the whole organism, by either resting, dividing, differentiating or dying, the cancerous cells have forgotten about these normal rules of cell behaviour leading to uncontrolled growth of the cells at the expense of the whole cell community.

The development of malignant tumors in a long period of time requires sequental steps of mutations contributing to loss of tumor suppressor gene functions and gain of function with oncogenes, as well as epigenetic changes. Bypassing the phenomenon of replicative senescence leads to immortalization of the cells and is prerequisite for the malignant transformation. Basically, six different alterations in the normal cell functions have been suggested to lead to tumorigenesis in most of the cancer types. These features include: Self-sufficiency in maintaining growth signals, unresponsiveness to growth-inhibiting signals, inhibition of the apoptotic pathways, unlimited replication potential, angiogenic signalling and potential to metastasize (Hanahan and Weinberg, 2000). Transforming cells are capable of generating their own growth-inducing signals by producing growth-factors of their own, by inducing their neighbours to release these signals or by switching on the downstream signalling of the growth factors inside the cell. Many of the oncogenes can as well mimick the players in the signalling pathway and promote transfer from the quiescent state to a proliferative one. For example, about a quarter of the human cancers have upregulation of the Ras-signalling pathway, leading to mitogenic signals inside the cell (Medema and Bos, 1993). The insensitivity to growth-inhibiting signals results often from deregulation of the TGF-β pathway and its intracellular targets (Levy and Hill, 2006). TGF-β inhibits cyclin D/CDK4/6 complex, prerequisite for pRb phosphorylation and progression into S phase (Hannon and Beach, 1994; Weinberg, 1995). pRB and its regulatory pathway is one of the main targets in tumorigenesis, in addition to the p53 pathway. Furthermore, unresponsiveness to TGF-β leads to upregulation of growth promoting c-Myc (Adhikary and Eilers, 2005). Features of the transformed cells also include amplification of the centrosomes, contributing to chromosome instability, further giving the tumor cells a more malignant potential (Brinkley and Goepfert, 1998; D`Assoro et al., 2002).

Cancer incidence is increasing, mostly due to longer life time expectancy and living habits. In addition to spontaneous mutations affecting cancer development, some germline mutations, linked to inherited susceptibility to certain cancers, have been found. These mutated genes, usually associated with DNA

damage checkpoints, repair functions and apoptosis, can be either recessively or dominantly inherited and can cause a specific cancer phenotype or just general increased risk of cancer incidence. In addition, phenotypically similar cancers can result from a single gene or a group of genes acting in the same cellular pathway. Usually the cancer–linked genes have several different mutations affecting the activity of its respective protein product, leading to either low-or high-risk predisposition for certain cancers, the high-penetrance mutations affecting its carriers with relatively early age. The most well-studied inherited forms of cancer involve breast and ovarian cancer, which have been linked to mutations in checkpoint proteins BRCA1 and BRCA2 genes (Easton et al., 1993; Miki et al., 1994; Wooster et al., 1995). Other well-known dominantly inherited forms of cancer involve mutations of the Rb gene, causing retinoblastoma of the eye (Ward et al., 1984), APC tumor suppressor gene, mutations of which cause familial adenomatous polyposis (FAP) and high susceptibility to colorectal cancers (Bodmer et al., 1987) and some forms of melanoma, which have been linked to mutations in the CDKN2A gene, encoding p14ARF and p16 proteins (Cannon-Albright et al., 1994). Li-Fraumeni syndrome, causing susceptibility to several kinds of cancers, including breast cancers, soft tissue sarcomas, brain tumors, leukemia, osteosarcoma and adenocortical carcinoma, has been linked to both tumor suppressor protein p53 as well as DNA damage kinase Chk2 (Li and Fraumeni, 1969; Malkin et al., 1990; Bell et al., 1999). Most studied recessively inherited forms of cancers are probably Xeroderma pigmentosum (XP), described earlier in the nucleotide excision repair chapter as well as Bloom syndrome and Ataxia-Telangiectasia, caused by mutations in the BLM and ATM genes respectively (Ellis et al., 1995; Savitsky et al., 1995). These genes are involved in DNA checkpoint functions and repair, and mutations in both cases cause abnormalities in the development as well as increased cancer risk, especially for leukemias and lymphomas (German et al., 1997). Despite these cancer associated genetic disorders, the inherited genetic susceptibility for cancer is still quite rare and most of the cancers are sporadic and occur due to risks caused by the individual itself or the living environment.

TUMOR SUPPRESSOR PROTEIN p53

p53 tumor suppressor protein, also known as "the guardian of the genome" was initially identified as an oncogenic protein, in complex with viral proteins (DeLeo et al., 1979; Kress et al., 1979; Lane and Crawford, 1979; Linzer and Levine, 1979; Melero et al., 1979). Later, this transcription factor was found to be essential for the prevention of tumor formation, dependent on its ability to induce apoptosis. Despite its essential role in inhibition of neoplastic transformation, p53 expression is not essential during the development of mice in utero (Donehower, 1996).

Somatic mutations in p53 are found in approximately 50% of the cancers, positioning p53 as the most frequently mutated gene in human malignancies (Hollstein et al., 1991; Levine et al., 1996). Even one mutant p53 allele can result in a gain-of-function phenotype with acquired new oncogenic properties and inactivation of the wt p53 allele (Lang et al., 2004; Olive et al., 2004; Chan et al., 2004). As mentioned in the previous chapter, p53 is also mutated in Li-Fraumeni syndrome, a rare inherited syndrome predisposing the carriers of p53 germline mutations to early-onset tumors (Varley, 2003). Further support for the essential role of p53 in prevention of cancer formation comes from the mouse models, which show highly increased predisposition to malignancies in p53 null mice and mice with mutated p53 (Donehower et al., 1992; Jacks et al., 1994). The tumor spectrum of p53 null mice include lymphomas, soft tissue sarcomas, brain and lung tumors, while the heterozygous mice have a more variable tumor spectrum corresponding better to tumor incidence in Li-Fraumeni syndrome (Donehower, 1996).

Structure

Human p53 protein, encoded by the *TP53 gene* in chromosome 17, is constructed of 393 amino acids and contains several different functional domains (figure 5). The amino-terminus of p53 forms its transactivation domain (TAD) and is heavily modified in response to cellular stress (Appella and Anderson, 2001). TAD participates in the transcriptional regulation and binds several factors required for p53-mediated response (Lin et al., 1994; Zhu et al., 1998). In addition, regulation of p53 occurs through TAD via binding to Mdm2 protein, which blocks the transactivation activity of p53. The crystal structure of p53-Mdm2 interface has been solved (Kussie et al., 1996). Mdm2 contains a hydrophobic pocket in which the hydrophobic site of the amphipathic p53 α-helix, amino acids 19-26 respectively, are inserted (Chen et al., 1993; Kussie et al., 1996; Bottger et al., 1997). This tight configuration of the binding cleft probably hinders p53 interactions with the transcriptional machinery. The proline-rich domain (PRD) follows the TAD and is presumably involved in the apoptotic response of p53 (Zhu et al., 2000; Baptiste et al., 2002; Edwards et al., 2003). It also mediates the co-factor binding through interaction with acetyltransferase p300 (Dornan et al., 2003).

The central domain of p53 contains its highly conserved DNA binding domain (DBD) required for its transcriptional properties and for identifying the p53 DNA consensus recognition elements on its target promoters (Kern et al., 1991). DBD, composed of a β-sandwich and three loop-based structures, binds different p53 target sequences with variable affinities, resulting in great variations in the transactivation potential (Inga et al., 2002). Most of the tumor-associated p53 mutations occur in this DNA-binding domain of the protein, leading to inactivation of p53 functions through disruption of its sequence-specific binding or through destabilization of its tertiary structure (Cho et al., 1994; Bhullock et al., 1997; Royds and Iacopetta, 2006). The structure is stabilized by a zinc atom, connecting the residues C176 and H179 of the second loop and C238 and C242 of the third loop of DBD (Cho et al., 1994). Interference of this structure disturbes DNA-binding of p53 as well as its tumor suppressive properties.

Carboxy-terminus of p53 is composed of a linker region, oligomerization/tetramerization (TET) site and a basic C-terminal DNA-binding domain (CTD). The oligomerization site is composed of a β-sheet-turn-α-helix structure (Clore et al., 1995), essential for p53 ability to form tetramers. The tetramers are also the most active forms of this protein (Jeffrey et al., 1995; Arrowsmith and Morin, 1996; McLure and Lee, 1998). CTD contains a number of phosphorylation, acetylation, sumoylation and ubiquitination sites associated with the regulation of p53 functions (Appella and Anderson, 2001). While the DBD recognizes specific target sequences, CTD binds DNA without any sequence specificity (Kim and Deppert, 2006; Liu and Kulesz-Martin, 2006). As CTD is also capable of binding various lesions, it has been associated with the DNA damage recognition (Bakalkin, 1995; Lee et al., 1995; Reed et al., 1995).

p53 function can also be influenced via its cellular localization, regulated by the nuclear localization signal (NLS) and nuclear export signal (NES) of the protein (Shaulsky et al., 1991) as well as interactions with some of its partner proteins. p53 has a major NLS in its linker region and two other NLS sequences in its very C-terminal end (Dang and Lee, 1989; Shaulsky et al., 1990). The nuclear export signal (NES) of p53 lies within the tetramerization domain and is possibly masked by the formation of oligomeric forms (Stommel et al., 1999). Another NES has been found in p53 N-terminus, in the Mdm2 binding domain (Zhang and Xiong 2001).

p53 protein is very well conserved (Soussi et al., 1990) and it belongs to a family consisting of two other proteins, p63 and p73 (Yang et al., 2002). These proteins are structurally very similar and have several overlapping duties with p53 in cellular stress response (Yang et al., 2002) besides their specific roles in the development (Irwin and Kaelin, 2001). p63 and p73 isoforms lacking their TAD domain may inhibit p53 function through oligomer formation, while some p53 mutants are able to attenuate the function of p63 and p73 and contribute to oncogenesis in this way (Yang et al., 2002; Olive et al., 2004). In addition to the p53 family members, multiple splice variants from an alternative promoter of p53 are expressed in a tissue-dependent manner and may influence the activity of the full length protein (Bourdon, 2005). These forms can regulate p53

transcriptional activity by enhancing gene expression from specific promoters or by blocking the activity of the full length p53. Furthermore, distinct expression patterns of these isoforms have been discovered in human tumors, possibly affecting the response to therapeutic drugs and general biological features of different cancer types.

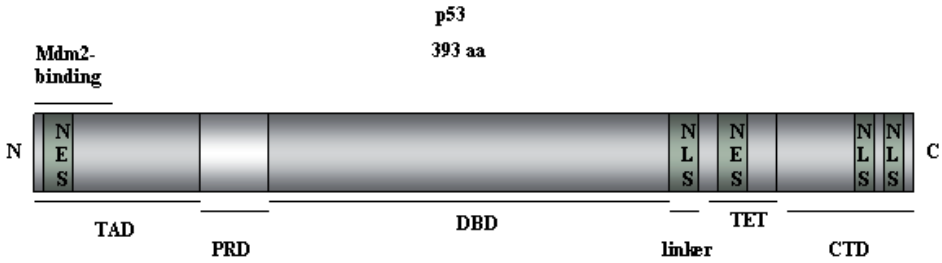

Figure 5. Organization of p53 functional domains. TAD, transactivation domain (aa 1-42); PRD, proline-rich domain (aa 63-97); DBD, DNA binding domain (aa 102-292); linker region (aa 300-318); TET, tetramerization domain (aa 323-356); CTD, C-terminal DNA binding domain (aa 363-393); NES, nuclear export signal; NLS, nuclear localization signal.

Regulation of p53 stability and activity

p53 stability and activity are tightly controlled and the protein levels are kept low and in latent form in unstressed cells. However, in reponse to various kind of cellular damage, p53 is stabilized and its transcriptional activity is rapidly enhanced through several existing mechanisms. The stabilization of p53 is not required for its transactivation activity, suggesting that these events are at least partly independent of each other (Hupp, 1999).

p53 protein is constantly synthesized and its accumulation in response to stress has been thought to be mainly based on inhibition of its degradation, not on de novo gene transcription and translation. A recent paper has, however, shown that increased translation of p53 mRNA is also an important step in the induction of p53 protein in DNA-damaged cells (Takagi et al., 2005). Despite extensive studies on the stabilization mechanisms of this protein, these stress-induced pathways leading to stable p53 are not yet fully understood. Yet, both the activation and stabilization events of p53 have been proposed to include a number of site-and time-specific post-translational modifications, like phosphorylations, acetylations and sumoylation, as well as interactions with other activators. Alterations in the localization pattern of p53 may as well play a role in its function, as nuclear localization of p53 has been shown to be critical for its full activity (Shaulsky et al., 1991).

The major regulation of p53 occurs via its degradation. p53 is degraded through the ubiquitin-proteasome pathway (Maki et al., 1996), which was for the first time discovered from papilloma virus-infected cells (Scheffner et al.,

1990 &1993). The papilloma virus protein E6 was shown to mediate the degradation of p53 and play a role in this way in the oncogenesis of the infected cells (Scheffner et al., 1990 &1993). Later, Mdm2 (murine double minute 2) protein was found to be the major mediator of the ubiquitination and proteasomal degradation of p53 (Haupt et al., 1997; Honda et al., 1997; Kubbutat et al., 1997). The ubiquitination of p53 C-terminal residues competes for its acetylation on the very same sites (Ito et al., 2002; Li et al., 2002). Acetylation by p300/CBP blocks p53 degradation (Ito et al., 2001) and may contribute to p53 activation upon various cellular stress situations (Gu and Roeder, 1997; Lill et al., 1997; Sakaguchi et al.,1998; Liu et al., 1999). In addition to acetylation, several phosphorylations of p53 N-terminus and C-terminus have been proposed to play a key role in controlling p53 stability and activity as well as target gene selection (Siliciano et al., 1997; Banin et al., 1998; Canman et al., 1998; Khanna et al., 1998; reviewed in Xu, 2003). Phosphorylations on p53 N-terminus, especially residues serine 15 and serine 20 have been thought to block the interaction between p53 and Mdm2 in DNA damaged cells and in this way lead to elevation in p53 levels (Shieh et al., 1997; Craig et al., 1999; Prives and Hall, 1999; Unger et al., 1999; Kapoor et al., 2000; Zhang and Xiong, 2001). Later, phosphorylation of threonine 18 was shown to be the only critical residue, affecting Mdm2-p53 interface (Lai et al., 2000; Schon et al., 2002).

Although these phosphorylations in many models contribute to p53-Mdm2 interactions, contrasting reports also exist (Ashcroft et al., 1999) and the in vivo data has showed no evidence of any p53 phosphorylations being critical for its stabilization or activation (Blattner et al., 1999; Xu et al., 2003). Similarly, the in vivo results about the effect of p53 acetylation on the protein stability are conflicting with the previous studies (Feng et al., 2005; Krummel et al., 2005). According to Feng et al., acetylation plays a role in p53 transactivation activity, while the other study proposed that this modification only appears to have a slight effect in fine-tuning the p53 response (Krummel et al., 2005). p53 has also been shown to be modified by SUMO, a small ubiquitin like protein, on its C-terminal Lys386. This modification was proposed to enhance its transcriptional activity (Gostissa et al., 1999; Rodriguez et al., 1999; Melchior and Hengst, 2002), although contrasting results again exist (Kwek et al., 2001). Additional modifications, including neddylation and methylation of p53 have also been discovered under certain stress situations, but their influence on p53 regulation still remains rather unknown.

The C-terminal domain of p53 has been proposed to influence its activity, by mediating the conversion from the latent form to an active DNA-binding protein (Hupp et al., 1992). The latent form of p53 can also be in tetrameric form (Hupp and Lane, 1994), but the regulation of the conversion to active p53 has been proposed to involve allosteric transition through the C-terminal site of p53 in DNA-damaged cells (Hupp and Lane, 1994; Waterman et al., 1995). According to this model, the stress-induced p53 modifications in its CTD would affect positively the ability of DBD to bind its target sequences (Gu and Roeder, 1997; Sakaguchi et al., 1998; Luo et al., 2004). Later, this model has been questioned

by other studies (Ayed et al., 2001; Krummel et al., 2005) and several different hypothesis about the role of CTD in the activation of p53 has emerged. Some studies propose that CTD acts as a negative regulator for DBD by binding DNA non-specifically and that the post-translational modifications of this domain upon stress blocks this function of CTD, allowing sequence-specific binding of DBD (Anderson et al., 1997; Friedler et al., 2005). C-terminus has also been suggested to play a role in enhancing the recognition of specific p53-response elements through the central domain (Ahn and Prives, 2001; McKinney et al., 2004). The study of McKinney et al. (2004) showed the ability of CTD to diffuse linearly on DNA, acting as a positive regulator for the sequence-specific binding, independently of the modification status of CTD. Moreover two studies have shown the requirement for intact C-terminus for the efficient promoter activation of p53 in vivo (Liu et al., 2004; McKinney et a., 2004). In all, the network regulating p53 stability and activity seems to be very complex, showing no simple on-off features.

Mdm2

Mdm2 (murine double minute 2) was first identified from transformed murine 3T3 fibroblasts (BALB/c), amplified in small extrachromosomal nuclear bodies (Cahilly-Snyder et al., 1987; Fakharzadeh et al., 1991). It was later shown to decrease p53 activity, suggesting that this function of Mdm2 was responsible for its oncogenic potential (Momand et al., 1992, Oliner et al., 1992). Mdm2 can inhibit p53 transactivation and act as its E3-ligase, mediating the degradation of p53 through the proteasome pathway (Haupt et al., 1997; Honda et al., 1997; Kubbutat et al., 1997). The degradation through the proteasome pathway requires the polyubiquitination of the target protein (Thrower et al., 2000) and is controlled by three enzymes: ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2 and ubiquitin ligase E3. Mdm2 acts as the E3-ligase enzyme towards p53 and determines its fate to be degraded through this pathway. The importance of Mdm2 as a p53 regulator was proven by mice knock-out studies, where loss of p53 rescued the embryonic lethality of Mdm2 null mice (Jones et al., 1995; Montes De Oca Luna et al., 1995). Mdm2 itself is a p53 target gene, induced by cellular stress (Perry et al., 1993; Saucedo et al., 1999), creating a negative feedback loop between these proteins (Wu et al., 1993).

Mdm2 gene is composed of 12 exons, which locate under two different promoters, the other one being p53 responsive. The two promoters result in two different Mdm2 forms, p90 and p76, of which p76 does not bind p53 and acts as a dominant negative inhibitor of the full length form (Perry et al., 2000). In addition, a number of different *Mdm2* splice variants exists and some of them also control the activity of the full length form (Bartel et al., 2002). *Mdm2* gene is amplified in one third of human sarcomas and approximately 7% of all human cancers (Oliner et al., 1992; Bond et al, 2004). Polymorphisms in its promoter region in a subgroup of human population may also lead to enhanced Mdm2 expression and downregulation of p53 (Bond et al, 2004).

The N-terminal site of the full length 491-amino acid Mdm2 is required for binding the p53 transactivation domain and repression of its activity (Chen et al., 1993, Oliner et al., 1993) (Figure 6). The amino acids 25-109 of Mdm2 form a hydrophobic pocket, which bind the N-terminus of p53 and hide it from the transcriptional machinery (Chen et al., 1993; Kussie et al., 1996). Recently, another binding site between the DBD of p53 and acidic-domain of Mdm2 has been reported (Shimizu et al., 2002; Yu GW et al., 2005). This interaction could possibly stabilize the Mdm2-p53 complex and modulate p53 degradation. Besides mediating p53 degradation and transactivation, Mdm2 may use other means in regulating p53 functions. Mdm2 controls the location of p53 protein, targeting it either to cytoplasm or nucleus (Roth et al., 1998; Freedman et al., 1998), possibly sequestering it from its target genes. The shuttling of Mdm2 requires its nuclear localization (NLS) and nuclear export signals (NES) (Figure 6.). The C-terminus of Mdm2 contains its RING-domain, coordinating the E3-ligase activity and ubiquitination of p53 C-terminal lysines (Nakamura et al., 2000; Rodriguez et al., 2000). Additionally, Mdm2 is able to mediate its own degradation through the same domain (Fang et al., 2000; Honda et al., 2000).

For a long time it was thought that Mdm2 alone promotes p53 polyubiquitination. More recent data suggests that it actually mediates monoubiquitination of p53 on several lysine residues and participates in polyubiquitination in co-operation with other factors (Lai et al., 2001). Because the degradation through the proteasome pathway requires polyubiquitination, other proteins besides Mdm2 must be required for efficient degradation of p53. One of these proteins is p300/CBP (Grossman et al., 1998; Zhu et al., 2001; Grossman et al., 2003). In unstressed cells Mdm2-p300-p53 form complexes, in which p53 is not modified by p300 and stays transcriptionally inactive (Kobet et al., 2000; Ito et al., 2001). The degradation of p53 can take place in both cytoplasm and nucleus (Xirodimas et al., 2001; Joseph et al., 2003), although the cytoplasmic translocation was at first suggested to be prerequisite for p53 degradation (Freedman and Levine, 1998; Tao and Levine, 1999a). The nuclear degradation function appears to be critical for shutting off the p53 activity in later stages of the damage response (Shirangi et al., 2002), while low levels of Mdm2 in unstressed cells induce p53 monoubiquitination and subsequent translocation to the cytoplasm (Geyer et al., 2000; Li et al., 2003). Other factors besides Mdm2 could be responsible for degrading p53 in unstressed cells possesing low Mdm2 levels. Whether the monoubiquitination of p53 by Mdm2 triggers some other unknown p53 functions, still remains to be solved.

In addition to p53 modifications in the Mdm2-p53 interface, Mdm2 protein is also heavily modified in response to cellular stress, possibly affecting their interactions (Meek and Knippschild, 2003; Moll and Petrenko, 2003). Mdm2 is acetylated in its RING domain, inactivating it and leading to p53 transactivation (Wang et al., 2004). Several phosphorylations/dephosphorylations on Mdm2 have also been shown to modify its effect on p53 degradation and inhibition of its transactivation activity in stressed cells (Maya et al., 2001; Okamoto et al., 2002; Blattner et al., 2002). ATM, for instance, phosphorylates Mdm2 on Ser

395 and inhibits p53 degradation (Khosravi et al., 1999; de Toledo et al., 2000; Maya et al., 2001). Mdm2 is also a target of the AKT-kinase pathway, which phosphorylates Mdm2 and targets it to nucleus where it is able to ubiquitinate p53 (Mayo and Donner, 2000). Tumor suppressor protein PTEN on the other hand is able to reverse this action of AKT and protect p53 from the Mdm2-mediated degradation (Mayo et al., 2002; Freeman et al., 2003). DNA damage also promotes new interactions with Mdm2 and its partner proteins, possibly contributing to p53 activation and stabilization. In addition to the control of p53-Mdm2 interaction, the levels of Mdm2 could be critical in regulating p53 stability and activity and Mdm2 protein and mRNA levels have been shown to decrease upon various treatments that lead to elevated levels of p53 (Wu and Levine, 1997; Arriola et al., 1999; Ashcroft et al., 2000; Inoue et al., 2001; Wang et al., 2002).

Besides its function as a regulator of p53 activity, Mdm2 is capable of affecting the cell cycle, DNA repair, basal transcription, differentiation and cell fate determination independently of p53 (reviewed in Ganguli and Wasylyk, 2003). Splice variants of Mdm2 having no p53 binding domain clearly operate in p53-independent functions. Mdm2 binds DNA pol ε (Vlatkovic et al., 2000) and stimulates its activity (Asahara et al., 2003). DNA pol ε has roles in DNA repair, recombination, replication, damage sensing and chromatin remodelling, linking Mdm2 to regulation of these functions. Possible role in ribosome biosynthesis and in translational regulation comes from Mdm2 interaction with L5 (Marcchal et al., 1994). Mdm2 could also affect transcription as it interacts with general transcription factors (Ganguli and Wasylyk, 2003). The cell cycle regulatory role of Mdm2 derives from its ability to bind Rb and perturb Rb-mediated G1-arrest (Xiao et al., 1995) and cooperate with E2F, stimulating E2F-dependent activation of some promoters involved in DNA synthesis (Martin et al., 1995). Interestingly, Mdm2 has also two cell cycle arrest-inducing domains (ID1 and ID2), which do not overlap with p53 interaction domain (Brown et al., 1998). These domains could be lost during the tumorigenesis, as Mdm2 is mostly associated with transformation of the cells. Overexpression of the entire Mdm2 gene predisposis to spontaneous tumor formation in a cell type-dependent manner (Jones et al., 1998). Mdm2 also contributes to the transformed phenotype in the absence of p53 and confers to a growth advantage in cells that lack p53 and Rb and can overcome the cell cycle arrest induced by p107 (Dubs-Poterszman et al., 1995). Tumors with both p53 mutation and Mdm2 amplification are rare but lead to poorer prognosis, further underlining the p53-independent role of Mdm2 in cellular transformation (Cordon-Cardo et al., 1994). Mdm2 promoter is also a target of Ras/MAPK pathway and activation of this pathway can increase Mdm2 levels during neoplastic transformation, giving a growth advantage for the cells (Ries et al., 2000).

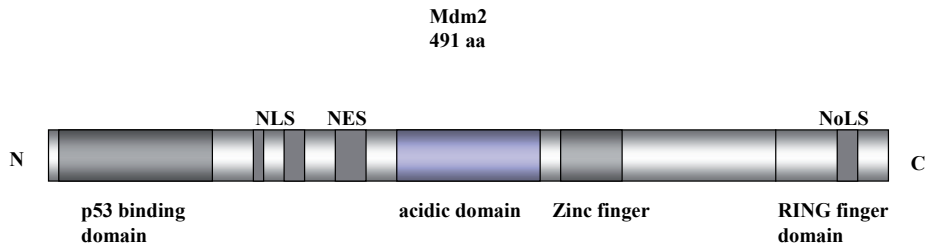

Figure 6. Structure of Mdm2 protein. N, N-terminal domain; C, C-terminal domain; NLS; nuclear localization signal; NES, nuclear export signal; NoLS, nucleolar localization signal.

MdmX

MdmX (Mdm4), a Mdm2 homologue, is also an important negative regulator of p53 activity, as the embryonic lethal phenotype of MdmX null mice is rescued by p53 knock-out (Parant et al., 2001; Finch et al., 2002; Migliorini et al., 2002) and amplification of MdmX directly affects to tumor formation by inhibiting the tumor suppressor activity of p53 (Danovi et al., 2004). Several studies on this Mdm2 homologue has been published, but the relevance of MdmX in the regulation of p53 is not fully understood and many contradictory results exist (reviewed in Marine and Jochemsen, 2005).

MdmX is able to bind p53 and structurally resembles Mdm2, but does not contain the C-terminal domain responsible for the E3-ligase activity. It seems to play a dual role in the regulation of p53 stability and activity, as it can inhibit Mdm2 and stabilize p53 when overexpressed, still keeping p53 in an inactive form (Jackson and Berberich, 2000). However, when the MdmX protein remains at low physiological levels, it co-operates with Mdm2 in p53 downregulation (Gu et al., 2002). One of the mechanisms in regulation of p53 activity could be the inhibitory effect of MdmX on the acetylation of p53 C-terminus (Sabbatini and McCormick, 2002). A recent paper by Toledo et al. (2006) showed a mouse model expressing p53 mutant lacking the proline-rich domain (p53DeltaP) and with reduced p53 apoptotic response. Expression of this mutant rescued the lethal phenotype of MdmX deficiency, but not Mdm2 deficiency. Furthermore, decreasing Mdm2 levels increased p53DeltaP levels without altering its transactivation, suggesting that MdmX mainly regulates p53 activity while Mdm2 controls p53 stability. Interestingly, MdmX is a target for Mdm2-mediated ubiquitination and proteasomal degradation in DNA-damaged cells, indicating that Mdm2 can also insure proper p53 transactivation activity (de Graaf et al., 2003; Kawai et al., 2003). The ubiquitination of MdmX by Mdm2 is also enhanced by tumor suppressor protein ARF, correlating with the ability of ARF to bind Mdm2 (Pan and Chen, 2003). Regardless of many unknown aspects in p53-MdmX-Mdm2 relationship, MdmX probably has as important biological impact on p53 function as its homologue, Mdm2

Other regulators of p53 stability

Mdm2 was for long thought to be the single E3-ligase for p53. Nowadays a few other proteins accomplishing this same duty have been discovered. Two of these factors are Pirh2 and Cop1, also E3-ligases for p53 and able to trigger p53 degradation in a Mdm2-independent manner (Leng et al., 2003; Dornan et al., 2004). Similarly to Mdm2, both of these genes are p53 targets, upregulated in cellular stress and forming an autoregulatory negative feedback loop with p53. Recently, another new E3-ligase, ARF-BP1, was found to be a mediator of p53 degradation (Chen et al., 2005). This protein is a major binding-partner of ARF tumor suppressor protein and its negative effect on p53 levels is prevented by this complex formation. As ARF-BP1 is not a p53 target gene in cellular stress, it could be responsible for maintaining the basal levels of p53, while Mdm2, together with Pirh2 and Cop1, could be the key regulators of p53 in stressed cells as at least Mdm2 levels are regulated by p53 only in response to cellular stress (Figure 7).

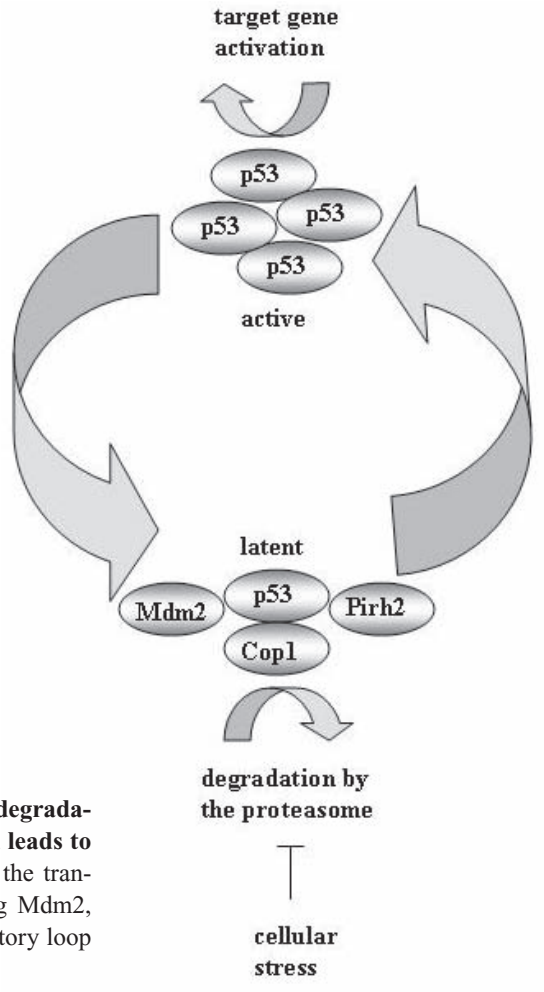

Figure 7. Cellular stress blocks p53 degradation by the proteasome pathway and leads to stable and active p53. p53 regulates the transcription of its target genes, including Mdm2, Pirh2 and Cop1 creating an autoregulatory loop in the control of p53 levels.

p53-mediated stress responses

p53 is a versatile, stress-induced protein, responding to a variety of cellular stress (Duthu et al., 1985; Fritsche et al., 1993; Hall et al., 1993; Lu and Lane; 1993; Graeber et al., 1994; Yamaizumi et al., 1994) (Figure 8). Most of the p53 functions coordinating cellular responses to stress are exerted by transcriptional activation of p53 target genes involved in cell cycle arrest, apoptosis or DNA repair (Ko & Prives, 1996). p53 acts as a transcription factor, recruiting a number of other transcriptional regulators and chromatin modifying proteins, like TFIIH and p300/CBP to promote the transcription of its target genes through DNA-binding upon stress (Xiao et al., 1994; Espinosa and Emerson, 2001). The functional activation of p53 leads to either up-regulation (Harms et al., 2004; Yu and Zhang et al., 2005) or down-regulation (Mirza et al., 2003) of numerous different p53 targets. Depending on the severity of the damage, p53 is able to either promote cell cycle arrest and DNA repair or in the case of excessive damage, lead the cells to apoptosis (Vousden and Lu, 2002). How p53 determines the destiny of the cell is not totally clear. It may, however, be dependent on the levels of p53 (Chen et al., 1996), p53 modifications and interactions or interplay with other regulatory pathways.

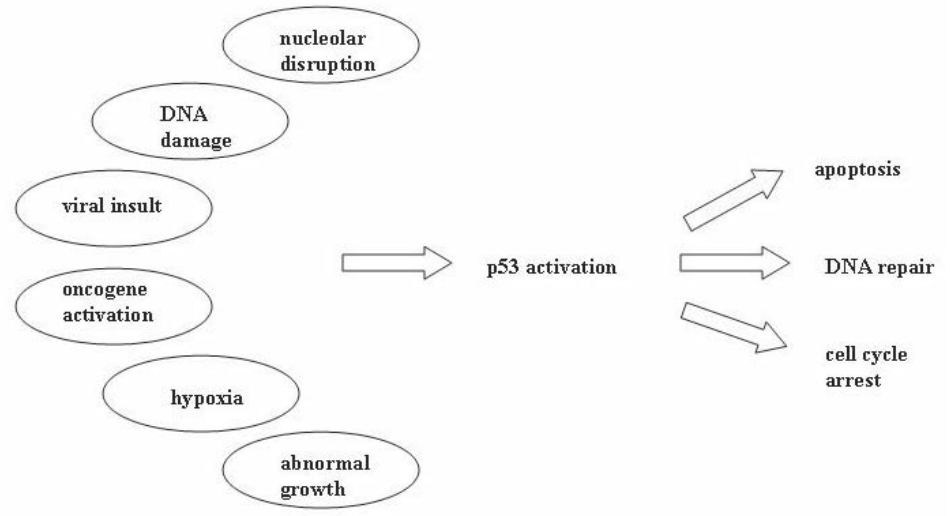

Figure 8. p53 responds to a variety of cellular stress and regulates apoptosis, cell cycle arrest and DNA repair by transcription-dependent and -independent means.

Cell cycle arrest

Following DNA damage, activation of p53 leads to halting the cell cycle progression. The p53-dependent arrest in G1 phase of the cycle mainly relies on its target gene $p21^{WAF1/CIP1}$ (El-Deiry et al., 1993; Dulick et al., 1994; El-Deiry et al., 1994), which inhibits CDKs and concomitantly entry to S-phase (Harper et al., 1993; Xiong et al., 1993). Although p53 is not required for delaying the cell cycle progression in S phase upon DNA damage, it is directly involved in the control of centrosome duplication (Tarapore et al., 2001). In undamaged cells cyclin E/CDK2 complex triggers DNA synthesis as well as centrosome duplication. However, in the presence of impaired DNA synthesis p21-mediated inhibition of the cyclin E/CDK2 complex stops the centrosome cycle and protects the cells from centrosome amplification (Hinchcliffe et al., 1999; Lacey et al., 1999). This control of the centrosome duplication cycle is maintaned in a p53-dependent manner (Tarapore et al., 2001). In G2 phase, the p53-dependent response is controlled by its targets 14-3-3σ and GADD45, in addition to its major transactivation target p21 (Hermeking et al., 1997; Taylor and Stark, 2001). However, p53 seems not to be essential for this checkpoint as many cell types deficient for p53 still accumulate in G2 upon DNA damage (Lukas et al., 2004). In addition, p53 participates in the spindle checkpoint, ensuring proper chromatin segragation and maintenance of ploidy in the daughter cells (Cross et al., 1995).

DNA repair

p53 is a major player in the DNA damage-induced pathways and has an essential role in the maintenance of intact genome (Lane, 1992; Levine, 1997). Its role in several repair networks either directly or indirectly was discovered quite early and evidence for p53 involvement in NER, BER, MMR and the repair of DSBs has come from numerous studies (Reviewed in Sengupta and Harris, 2005; Gatz and Wiesmuller, 2006). p53 regulates the damage repair by either inducing the transcription of repair proteins or through interactions with the repair machinery (Gatz and Wiesmuller, 2006 and references therein) or by recognizing and associating with the damage sites themselves (Bakalkin et al., 1995; Lee et al., 1995; Reed et al., 1995). p53 is also capable of catalyzing the reannealing of the DNA strands (Oberosler et al., 1993; Brain and Jenkins, 1994) and has 3´-5´ exonuclease activity (Mummenbrauer et al., 1996; Huang, 1998; Janus et al., 1999; Skalski et al., 2000).

The evidence for the involvement of p53 in mismatch repair comes mainly from its ability to transactivate some MMR genes, such as MSH2, MLH1 and PMS2 (Scherer et al., 2000; Warmick et al., 2001; Chen and Sadowski, 2005). The role of p53 in BER can be either direct or indirect, and a few BER genes are under the transcriptional regulation of p53 (Offer et al., 1999; Offer et al, 2001; Seo et al., 2002; Zurer et al., 2004; Lu et al., 2004). The regulation of the DSB repair by p53 seems not to require p53 transcriptional activation. Several studies have suggested a direct role for p53 in the repair of DSBs: p53 can bind many

central DSB repair proteins, like Rad51, RPA, BRCA1 and BRCA2, Bloom´s syndrome protein and Werner´s syndrome protein. It also represses HR upon DSBs and stalling of the replication fork, independently of its transactivation activity (Gatz and Wiesmuller, 2006 and references therein). p53 function may thus be required for inhibiting error-prone DSB repair and for halting replication until the damage has been repaired. Similarly p53 can probably contribute to the nonhomologous end-joining of DSBs to secure error-free NHEJ (Bill et al., 1997; Dahm-Daphi et al., 2005).

p53 in nucleotide excision repair

UV radiation and some DNA-damaging agents induce DNA lesions that block the transcription by RNA pol II and trigger nucleotide excision repair (Mello et al., 1995; Selby et al., 1997; Culliane et al., 1999). The formation of DNA damage-induced lesions and transcriptional inhibition acts as a signal for p53 induction (Yamaizumi et al., 1994; Ljungman and Zhang, 1996; Dumaz et al., 1997; Ljungman et al., 1999; Ljungman et al., 2001). p53 seems to have a transcription-dependent and -independent role in NER network . Its function in NER is independent of its stabilization, induced already by smaller amounts of damage. Cells expressing very low levels of wt p53 have been reported to have defective NER (Ford and Hanawalt, 1995; Smith et al., 1995; Wang et al., 1995) and the cells derived from Li-Fraumeni patients or cells infected with HPV-E6 to have defective GGR (Ford and Hanawalt, 1995; Zhu Q et al., 2000). p53´s involvement in GGR has been widely accepted, while its effect on TCR is somewhat contradictory, some papers supporting the association of p53 in TCR (Wang et al., 1995; Mirzayans et al., 1996; McKay et al., 1999; Therrien et al., 1999; Mathonnet et al., 2003) whereas others do not (Ford and Hanawalt, 1995 & 1997; Ford et al., 1998; Wani et al, 2000). The differencies in these results may, however, partly result from variable experimental setups as well as different wavelengths used in the UV studies (Therrien et al., 1999; Mathonnet et al., 2003).

p53 participates in the repair of both UV-induced 6-4PPs and CPDs and its activity is essential for CPD repair (Ford and Hanawalt, 1995; Ford and Hanawalt, 1997; Ford et al., 1998; Bowman et al., 2000). The presence of p53 is required for the repair of other types of DNA adducts as well, induced by exposure to environmental carcinogens, benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE) or benzo(g)chrysene (B(g)CDE) (Lloyd and Hanawalt, 2000; Lloyd and Hanawalt, 2002). p53´s ability to participate in NER comes from its direct interactions with the repair factors of this pathway, like TFIIH (Wang et al., 1995; Gatz and Wiesmuller, 2006 and references therein) and it also controls the expression of XPC, XPE, a GGR repair protein p48 and Gadd45, a factor involved in growth arrest as well as NER (Hwang et al., 1999; Smith et al., 2000; Amundson et al., 2002; Adimoolam et al., 2002; Adimoolam and Ford, 2002; Tan and Chu, 2002). Additionally, p53 may have a role in mediating histone modifications and act as a chromatin accessibility factor to assist in the GGR pathway of the nucleotide excision repair (Rubbi and Milner, 2003a).

p53-induced apoptosis

The apoptosis-inducing activity of p53 is probably its major function in preventing tumor formation (Symonds et al., 1994; Schmitt et al., 2002). p53 coordinates apoptosis by either inducing several pro-apoptotic genes or by repressing anti-apoptotic factors. The most studied and relevant apoptotic p53 targets are Bax, Noxa, PUMA, Apaf-1 and p53AIP1 (Miyshita and Reed, 1995; Oda E. et al., 2000; Oda K. et al., 2000; Moroni et al., 2001; Nakano and Vousden, 2001). These targets play a role in the mitochondrial apoptotic pathway (Chipuk and Green, 2006) and deletion of any of them results in resistance to p53-mediated apoptosis, depending on the stress stimulus and cell type (Jeffers et al., 2003; Shibue et al., 2003; Villunger et al., 2003). p53-mediated gene repression also takes place under some stress conditions. For example, in hypoxic cells p53 supresses the anti-apoptotic survivin to promote activation of the caspase pathway (Hoffman et al., 2002; Hammond and Giaccia, 2005). Additionally, p53 contributes to the external signalling pathway of apoptosis by inducing several different death receptors, like Fas/APO1, PERP and KILLER/DR5 (Muller et al., 1998; Benchimol, 2001) and by participating in their intracellular transport (Bennett et a., 1998).

In addition to the transcriptional regulation of apoptosis, p53 also contributes to the death pathway by directly associating with the Bcl-2 family members in the cytoplasm. p53 is rapidly translocated to the mitochondria in response to multiple death stimuli and binds anti-apoptotic factors Bcl-2 and Bcl-xL as well as apoptotic Bak and Bax to release cytochrome c from the mitochondria (Marchenko et al., 2000; Mihara et al., 2003; Chipuk et al., 2004; Leu et al., 2004). This rapid mitochondrial response inducing apoptosis may represent the immediate death signal, the transcriptional activation being the second-wave response to death stimuli (Erster et al., 2004). The p53 DNA-binding domain probably plays an important role in mediating this first-wave reponse through its interactions with Bcl-2 family proteins (Petros et al., 2004), again underlining the importance of this domain in tumor-suppression.

The apoptotic function of p53 is controlled by several p53-binding and modulating proteins, like the ASPP family proteins (Samuels-Lev et al., 2001). The ASSP family consists of ASSP1, ASPP2 and iASPP proteins, regulating p53-induced apoptosis. iASSP is likely to be an oncoprotein and acts as inhibitor of p53-mediated apoptosis by binding to p53 C-terminus (Bergamaschi et al., 2003). ASPP1 and ASPP2 are activated by DNA damage or oncogenic stress and bind to p53 and other p53 family members and stimulate their ability to induce apoptosis through proapoptotic genes, like Bax, PUMA and PIG3 (Samuels-Lev et al., 2001). The importance of the ASPP proteins is underscored by the finding that the ASPP contacting residues in p53 are mutated with high frequency in human cancers and that either overexpression of iASSP or downregulation of the ASPP1 and 2 are common in human tumors (Gorina and Pavletich, 1996; Samuels-Lev et al., 2001; Bergamaschi et al., 2003). Expression of E2F proteins also regulates p53-mediated apoptosis and E2Fs are induced by several types of

DNA-damaging agents that activate p53 (O´Connor and Lu, 2000). Increased E2F-levels can enhance p53 activity through induction of ASPPs (Fogal et al., 2005b) or through complex formation between p53 and E2F that enhance the apoptotic activity of p53 in damaged cells (Hsieh et al., 2002). In addition to ASPPs and E2Fs, other factors not reviewed in here, may modulate p53 response in the decision to choose the cell death pathway.

Senescence

The phenomenon of senescence prevents normal human fibroblasts from dividing indefinitely in cell cultures (Hayflick, 1965). Senescence is associated with shortening of telomeres in the ends of chromosomes during each cell cycle (Harley et al., 1990) and this shortening can be reversed by the action of telomerase enzyme, promoting the replicative potential of the cells (Bodnar et al., 1998). In addition to tumor suppressor protein Rb, p53 is the major controller of cellular senescence (Ithana et al., 2001; Schmitt et al., 2002b; Beausejour, 2003) and can also induce premature senescence upon oncogenic signalling (Serrano et al., 1997). Cellular senescence is associated with changes in p53 modifications and enhanced p53 transcriptional activity (Atadja et al., 1995; Bond et al., 1996; Vaziri et al., 1997; Webley et al., 2000). ATM and Chk-kinases are recruited to the shortened telomeres and may promote a DNA-damage signalling cascade, leading to phosphorylation of p53 (d´Adda di Fagagna et al., 2003; Gire et al., 2004, Herbig et al., 2004). Subsequently, p53 can be regulated by p300-mediated acetylation, which can be controlled for example by the ING-family protein ING2 (Pedeux et al., 2005). Upon activation, p53 helps to maintain a nonproliferative state in the late passage cells mainly by upregulating p21 expression (Beausejour, 2003). Moreover, it can participate in the cellular senescence by decreasing the expression of the catalytic subunit of telomerase, hTERT (Xu et al., 2000). As cells need to bypass the senescence to become transformed (Shay and Roninson, 2004), this may be an essential function for p53´s tumor suppressive properties.

p53 response to UV radiation

p53 is stabilized and activated by UV radiation and has an essential role in the protective UV response (Maltzman and Czyzyk, 1984; Ziegler et al., 1994). It is the key player in inducing UV radiation-promoted cell cycle arrest or apoptosis upon higher exposure to UV (Decraene et al., 2001). Mutations disturbing these functions are also frequently associated with skin cancers (Ziegler et al., 1994; Hartmann et al., 1996; Haapajärvi et al., 1999). The extent of p53-mediated cellular responses in the skin keratinocytes depend on the age and differentiation state of the cells, being more prominent in differentiated keratinocytes and

impaired in the aged ones (Latonen and Laiho, 2005 and references therein). Additionally, p53 has a protective role in the skin through participitation to the melanin production and tanning process (Nylander et al., 2000).

Most of the studies on p53 responses to DNA damage have been performed with ionizing radiation or cytotoxic damage. Despite some overlapping features of the p53 response to DSBs, many of the UV-induced events in p53 activation are distinct (Reviewed in Latonen and Laiho, 2005). p53 modifications and stabilization for example occur with slower kinetics than with IR (Saito et al., 2003). p53 can be phosphorylated on its N-terminus by the ATM/ATR-pathway (Canman et al., 1998; Khanna et al., 1998; Khosravi et al., 1999; Chebab et al., 2000). Upon UV radiation, p53 is phosphorylated primarly by ATR and its downstream kinase Chk1. UV damage-induced kinases, DNA-PK, p38, HIPK2, JNK, TAFII250 and CK2 also phosphorylate p53 on its N-or C-terminal sites (Xu, 2003 and references therein). In addition, p53 is modified by acetylations and sumoylation upon UV radiation of the cells (Sakaguchi et al., 1998; Ljungman et al., 2001; Melchior and Hengst, 2002). As previously dicussed, these modifications can possibly affect p53 stress response by finetuning its transcriptional activity or by enhancing its stability and interactions with other proteins, although contrasting results exist. Some p53 modifications, like Ser 392 phosphorylation, are associated only with UV damage (Latonen and Laiho., 2005 and references therein) and many of the modifications occur in a dose-dependent manner (Reinke and Lozano, 1997; Latonen et al., 2001). For example, p53 phoshorylation on Ser 46 after higher doses of UV by homeodomain-interacting protein kinase-2 (HIPK2), has been linked to the apoptotic p53 response (D´Orazi et al., 2002).

Low doses of UVC radiation induce a transient p53 activation and cell cycle arrest, while higher doses, leading to persistent transcription blockage, induce slower and more prominent induction of p53. The apoptotic response is associated with the activation of apoptotic p53 target genes and downregulation of anti-apoptotic p53 targets (Cotton and Spandau, 1997; Reinke and Lozano, 1997; Wu and Levine, 1997; Latonen et al., 2001). The transient activation of p53, leading to transactivation of cell cycle-regulatory genes, also leads to induction of Mdm2 levels and is associated with the feedback loop that is lacking from the apoptotic cells (Perry et al., 1993; Latonen et al., 2001).

In addition to the posttranslational modifications, p53 function is modulated by several protein-protein interactions upon UV damage. Prolyl isomerase (Pin1) controls p53 stability upon various kind of stress and regulates p53 activation by modulating its interactions with DNA and some cofactors (Wulf et al., 2002; Zacchi et al., 2002; Mantovani et al., 2004). In UV-damaged cells Pin1 is required for the Chk2 phosphorylation of p53 on Ser 20, leading to dissociation from Mdm2 and p53 stabilization (Zacchi et al., 2002; Berger et al., 2005). Several of the known stress-induced protein-protein associations and effects occur in a UV dose-dependent manner. For example p33ING1b and ASPP1 and ASPP2 proteins contribute to p53-induced apoptosis upon higher doses of UV radiation (Samuels-Lev et al., 2001; Cheung and Li, 2002).

As discussed above, p53 also participates in NER pathway in UV-damaged cells. While p53 participates in the repair of DNA lesions, it is also capable of protecting the cells against UV- or cisplatin-induced apoptosis in a TCR- and transcriptional recovery-dependent manner (McKay and Ljungman, 1999; McKay et al., 1999, 2000 and 2001). TCR-deficient cells undergo massive apoptosis upon UV radiation. Even though the induction of apoptosis correlates with p53 stability, it is not p53-dependent and p53 actually contributes to inhibition of apoptosis in TCR-proficient fibroblasts (Ljungman and Zhang, 1996; McKay et al., 1998; Ljungman et al., 1999; McKay et al., 2001).

p53-PATHWAY PROTEINS

This chapter introduces the most relevant p53-pathway proteins with respect of this study.

ARF

Alternative reading frame (ARF) protein is encoded by the Ink4a/ARF locus, producing both $p16^{INK4a}$ and $p19^{ARF}$ proteins (Quelle et al., 1995). $p19^{ARF}$ (mouse ARF) and $p14^{ARF}$ (human ARF) are nucleolar proteins involved in the p53 pathway (Quelle et al., 1995; Pomerantz et al., 1998; Weber et al., 1999; Zhang and Xiong, 1999). The proteins of the Ink4a/ARF locus control the progression of the cell cycle mainly by regulating the activites of Rb and p53 (Sharpless and DePinho, 1999). The gene products of Ink4a/ARF locus are also commonly inactivated in human cancers.

ARF is activated upon oncogene expression, like Ras, c-Myc, v-Abl and adenovirus E1A, leading to downstream activation of p53 (De Stanchina et al., 1998; Palmero et al., 1998; Radfar et al., 1998; Zindy et al., 1998; Sherr, 2001). ARF may also perform its growth suppressive activities in p53-independent manner (Sugimoto et al., 2003). The activation of p53 downstream of ARF is independent of p53 modifications (de Stantchina et al., 1998) as ARF exerts its p53-inducing functions by binding to Mdm2 RING domain and inhibiting the Mdm2-mediated degradation of p53 (Kamijo et a., 1998; Pomerantz et al., 1998; Stott et al, 1998; Zhang et al., 1998; Honda and Yasuda, 1999). Several mechanisms have been proposed for the ARF-mediated activation of p53, including degradation of Mdm2 by ARF (Zhang et al., 1998), and ARF-mediated relocalization of Mdm2 to nucleoli (Tao and Levine, 1999; Weber et al., 1999), which requires nucleolar localization signal (NoLS) of both proteins (Honda and Yasuda, 1999; Lohrum et al., 2000). This could possibly enable p53 accumulation in the nucleoplasm, although contrasting reports on the importance of this nucleolar Mdm2 relocalization exist (Llanos et al., 2001). Another model has proposed that Mdm2-p53 complex could exit from the nucleus to cytoplasm via nucleoli and that ARF-Mdm2 interaction could interfere with this function (Tao and Levine, 1999). ARF, Mdm2 and p53 have also been detected in the nuclear bodies in the nucleoplasm and this has been linked to ARF´s capability of stabilizing p53 (Xhang and Xiong, 1999). Recently, a new p53 E3-ligase, ARF-BP1, was discovered (Chen et al., 2005). Blockage of the function of ARF-BP1 by ARF may be one way to stabilize p53.

Nucleophosmin

Nucleophosmin, NPM, (also referred to as B23, numatrin or NO38) is an abundant nucleolar phosphoprotein, shuttling constantly between the nucleoli and cytoplasm (Schmidt-Zachmann et al., 1987; Schmidt-Zachmann and Franke, 1988; Borer et al., 1989). Npm is essential for the development and its loss in germ-line

leads to embryonic lethality (Colombo et al., 2005; Grisendi et al., 2005). The first identified functions for this protein involved ribosome biogenesis and transport of the pre-ribosomal particles (Prestayko et al., 1974; Spector et al., 1984; Olson et al., 1986; Herrera et al., 1995; Savkur and Olson, 1998). Depletion of NPM modifies pre-RNA processing and alters maturation of the ribosomes (Ithana et al., 2003; Grisendi et al., 2005). The survival of Npm-/- mouse embryos to mid-gestation does not suggest that NPM is an essential factor in the biogenesis of ribosomes (Colombo et al., 2005; Grisendi et al., 2005). NPM has also been linked to various other cellular processes, including stress response, DNA-repair and maintenance of the genomic integrity (Grisendi et al., 2006).

NPM belongs to a nuclear chaperone family of nucleoplasmins and can act as a chaperone for nucleic acids and proteins (Szebeni and Olson, 1999; Okuwaki et al., 2001). The chaperone activity of NPM has been mapped to its N-terminal domain (Hingorani et al., 2000) (Figure 9). NPM can also act as a histone chaperone and this may reflect its importance in the regulation of chromatin structure and transcription (Okuwaki et al., 2001; Swaminathan et al., 2005). In addition, NPM seems to be involved in the centrosome cycle (Okuda et al., 2002). It associates with unduplicated centrosomes during G1 and is dissociated from the centrosomes by phosphorylation on Thr 199 by cyclin E/CDK2 complex (Okuda et al., 2000; Tokuyama et al., 2001; Okuda et al., 2002; Tarapore et al., 2002). This allows the duplication of centrosomes during S phase of the cycle. Recent results also show that Ran-CRM1 complex, involved in the nucleo-cytoplasmic transport of NPM, is involved in controlling the NPM localization at the centrosomes (Wang et al., 2005). During mitosis, NPM re-associates with centrosomes and colocalizes with NUMA and BRCA1-BARD1 proteins (Zatsepina et al., 1999; Sato et al., 2004). The importance of controlled regulation of NPM during the centrosome cycle is underlined by the finding that centrosome amplification has been detected in the presence of excessively phosphorylated NPM (Saavedra et al., 2003; Zhang et al., 2004) and that its functional loss also leads to centrosome amplification and aneuploidy (Grisendi et al., 2005).

NPM protein exists in two different isoforms, B23.1 and B23.2, due to alternative splicing (Chang and Olson, 1989; Chang and Olson, 1990; Wang et al., 1993). Of these forms B23.1 is the major form and localizes predominantly in the nucleoli and functions in ribosome biogenesis. B23.2, lacking the DNA and RNA binding domain of NPM C-terminus localizes to nucleoplasm and also nucleoli through its interactions with the full length form (Wang et al., 1994; Okuwaki et al., 2002; Wang et al., 1993). In native conditions, NPM exists in an oligomeric form, as a hexamer (Herrera et al., 1996) and the splice variants are able to form multimers as well (Chang and Olson, 1989; Umekawa et al., 1993).

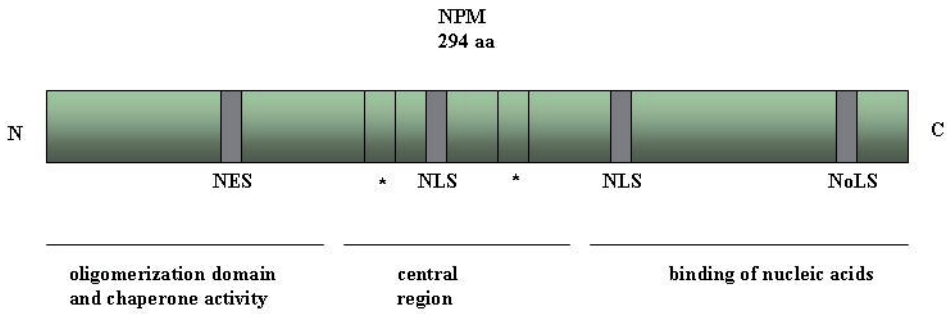

Fig 9. Functional domains of NPM (B23.1). The hydrophobic N-terminus of NPM contains its chaperone activity as well as the domain required for oligomer formation. The central region contains two acidic domains (*), responsible for histone binding. Binding of DNA and RNA occurs through the C-terminal domain, of which 35 last residues are lacking in the spliced form, B23.2. Ribonuclease activity requires both the C-terminal domain and the region between the two acidic domains. Additionally, NPM contains nuclear export signal (NES), two nuclear localization signals (NLS) and a nucleolar localization signal (NoLS).

NPM and cancer

In addition to its role in various cellular functions, NPM has been linked to neoplastic transformation (see Grisendi et al., 2006 for review). NPM is a transcriptional target of Myc-oncogene and may support cell proliferation in transformed cells through enhanced ribosome biosynthesis (Boon et al., 2001; Zeller et al., 2001). Its expression is enhanced in response to mitogenic signals and its protein level is often high in rapidly proliferating and malignant cells (Feuerstein et al., 1988; Chan et al., 1989; Gubin et al., 1999; Dergunova et al., 2002). Overexpression of NPM is detected in several cancer types, including melanoma, prostate, gastric, colon and ovarian carcinomas, possibly reflecting the high translational activity of these cells (Tanaka et al., 1992; Nozawa et al., 1996; Shields et al., 1997; Subong et al., Skaar et al., 1998; Bernard et al., 2003; 1999; Tsui et al., 2004). Opposite to highly proliferating cells, NPM levels in apoptotic or quiescent cells are decreased (Jiang and Yung, 1999; Wu et al., 1999; You et al., 1999). In addition to a supportive role in ribosome biogenesis, high levels of NPM may enhance proliferation by inhibiting apoptotic pathways (Ye, 2005). NPM may for instance inhibit apoptosis by blocking the activity of transcription factor IRF-1 (Kondo et al., 1997) and eukaryotic initiation factor 2 kinase PKR (Pang et al., 2003) or by mediating the anti-apoptotic activity of nerve growth factor, NGF (Ahn et al., 2005).

Interestingly, recent knock-out studies have shown that NPM has also growth-suppressive properties and an important role in the cellular stress response. NPM protein has for long been known to react to various kind of stress and several DNA-damaging or cytotoxic drugs. These cause NPM translocation from the nucleoli to the nucleoplasm (Yung et al., 1985; Chan et al., 1987; Yung et al.,

1990; Bor et al., 1992; Chan, 1992; Wu and Yung, 2002). In DNA-damaged cells NPM can promote repair of lesions by upregulating PCNA protein (Wu et al., 2002) and through regulating the localization of GADD45, a protein involved in DNA repair and chromatin remodelling (Gao et al., 2005). Moreover, Npm-/- cells show increased γ-H2AX-ATM DNA damage foci formation (Colombo et al., 2005) and Npm-/- or hypomorphic MEFs show genomic instability (Grisendi et al., 2005), indicating an essential role for NPM in the maintenance of the integrity of the genome.

NPM binds ARF in the nucleoli in a quantitative manner (Bertwistle et al., 2004). The complex relationship between these proteins has been studied extensively over the last few years and is still not fully understood. ARF has been found to inhibit the growth-promoting effect of NPM by blocking its function in rRNA processing and the transport of pre-ribosomal particles (Savkur et al, 1998; Ithana et al., 2003; Sugimoto et al., 2003; Brady et al., 2004) and by mediating NPM degradation (Itahana et al., 2003). NPM, on the other hand, stabilizes ARF, and ARF mutants lacking NPM binding domain have been shown to be more unstable (Kuo et al., 2004). Moreover, in MEFs lacking both p53 and NPM, ARF is relocalized to nucleoplasm and is found in lower protein levels (Colombo et al., 2005). Such cells are also more prone to transformation. Thus NPM´s potential tumor-suppressive functions could occur through ARF pathway upon oncogenic stress. An opposite study has, however, suggested that ARF function could be inhibited through its nucleolar sequesteration by NPM (Korgaonkar et al., 2005).

NPM may also affect p53 pathway by directly associating with p53 or through controlling p53 pathway proteins. The indirect control of p53 pathway can occur for example through GADD45 nuclear localization. In this way NPM could participate in the p53-mediated growth arrest (Wang et al., 1999; Gao et al., 2005). Several studies suggest that NPM acts as a negative regulator of p53 (Li et al., 2004; Chan et al., 2005; Li et al., 2005). NPM has been proposed to inhibit p53-induced apoptosis in hypoxic cells through reduced p53 Ser 15 phosphorylation by competing for the same kinase (Li et al., 2004; Li et al., 2005). NPM has also been found to affect p53 stability and activity in a positive way upon DNA damage (Colombo et al., 2002). Deletion of *NPM* gene, however, results in p53 activation. This occurs probably through an indirect mechanism due to DNA damage (Colombo et al., 2005) and aneuploidy of these cells (Grisendi et al., 2005).

NPM is associated with several hematopoietic malignancies, including acute myeloid leukemia (AML), anaplastic large cell lymphoma (ALCL) and acute promyelocytic leukemia (APL), through chromosomal translocations forming oncogenic fusion proteins (Raimondi et al., 1989; Morris et al., 1994; Yoneda-Kato et al., 1996; Redner et al., 2002; Chiarle et al., 2003) (Table 2). In addition, NPM is often deleted in myelodysplastic syndrome (MDS), and mice heterozygous for Npm develop hematological abnormalities, resembeling this human syndrome (Olney and Le Beau, 2002; Grisendi et al., 2005, Berger et al., 2006). Mutations causing cytoplasmic NPM (NPMc+) have been detected in AML (Al-

calay et al., 2005; Falini et al., 2005; Falini et al., 2006). The cytoplasmic localization of NPM probably alters its normal nucleolar functions, but it also affects other NPM-binding proteins, including ARF which is relocalized to cytoplasm in NPMc+ -expressing cells (den Besten et al., 2005; Colombo et al., 2006).

The complex nature of NPM has led to several contrasting reports on its role as either an oncogene or tumor suppressor. Most likely NPM has both tumor-suppressive and -promoting functions through its role in ribosome biogenesis and interactions with important tumor suppressor proteins, these functions being dependent on its levels and localization as well as the genetic background of the cell. The aberrant overexpression of NPM could then lead to neoplastic transformation and too low levels again to genomic instability, pointing out the importance of the strict regulation of NPM levels and localization.

hematological malignancy	associated genetic alterations
acute promyelocytic leukemia, APL (Redner et al., 2002)	NPM-RARα fusion due to t(5;17)(q35;q12) translocation
acute myeloid leukemia, AML (Raimondi et al., 1989; Yoneda-Kato et al., 1996; Alcalay et al., 2005; Falini et al., 2005)	NPMc+ mutations; NPM-MLF1 fusion due to t(3;5)(q25;q35) translocation; deletion (-5q35, -5)
anaplastic large cell lymphoma, ALCL (Morris et al., 1994)	NPM-ALK1 fusion due to t(2;5)(p23;q35)
myelodysplastic syndrome, MDS (Yoneda-Kato et al., 1996; Olney and Le Beau, 2002; Berger et al., 2006)	NPM-MLF1 fusion due to t(3;5)(q25;q35) translocation; deletion (-5q35, -5)

Table 2. Genetic alterations of NPM in human hematological malignancies.

Promyelocytic leukemia protein

Promyelocytic leukemia protein, PML, was initially found as a fusion protein with retinoic acid receptor α in patients with specific types of leukemia (de The et al., 1990 & 1991). PML protein is responsible for the formation of so-called PML or nuclear bodies, NBs (Also previously referred to as ND10 or promyelocytic oncogenic domains, PODs). These nuclear matrix-associated structures are usually about 0.2- 0.5 μm in diameter and their number in the nucleus varies between 5-20 (Ascoli and Maul, 1991; Stuurman et al., 1992). The PML bodies were described already in 1984 by Bernstein et al. and unlike the cytoplasmic organelles, these subnuclear compartments are not surrounded by a lipid bilayer. Several regulatory factors, including Sp100, Daxx, SUMO-1, CBP, p53, HIPK2 and some DNA damage repair proteins are localized to these sites in a PML-

dependent manner (Szostecki et al., 1990; Boddy et al., 1996; Kamitani et al., 1998a; Ishov et al., 1999; Kim et al., 1999; Fogal et al., 2000; Li et al., 2000a; Lombard and Guarente, 2000; Zhong et al., 2000a; Boisvert et al., 2001). PML in NB structures is sumoylated (Sternsdorf et al., 1997; Kamitani et al., 1998a & 1998b; Muller et al., 1998). Several studies have suggested that sumoylation of PML is prerequisite for the recruitment of other proteins to these sites (Ishov et al.,1999; Lallemand-Breitenbach et al., 2001; Li & Chen, 2000; Maul et al., 2000; Zhong et al., 2000a), whereas sumoylation of p53 was found to be dispensable for its relocalization to these structures (Fogal et al., 2000). PML itself has three main sumoylation sites, lysines 65, 160 and 490 (Figure 10), of which lysine 160 conjugation by SUMO-1 appears to be the most critical one for PML body formation. Removal of main sumoylation sites from PML protein prevents formation of NBs (Muller et al., 1998; Kamitani et al., 1998, Zhong et al., 2000a), although contrasting reports exist as well (Ishov et al., 1999; Lallemand-Breitenbach et al., 2001). Only the nuclear PML seems to be sumoylated, as colocalization with SUMO was not detected with cytoplasmic PML body aggregates (Ishov et al., 1999).

PML belongs to a family of nuclear proteins, containing one RBCC motif (Borden et al., 1995; Jensen et al., 2001) (Figure 10). PML protein exists as seven different, equally expressed isoforms due to alternative splicing, ranging from 48-97 kDs (de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991; Fagioli et al., 1992; Kastner et al., 1992; Jensen et al., 2001). The N-terminal site is identical in all isoforms, differing only in their C-terminus or the length of the central region (Fagioli et al., 1992; Jensen et al., 2001). The exact functions of all the different isoforms is still not fully understood, even though some isoforms with specified functions exist. For instance, one isoform with cytoplasmic localization and association with TGF-β pathway has been characterized lately (Lin et al., 2004).

The number of PML bodies is known to vary depending on the cell type and condition, stress responses and cell cycle, being highest in G2 phase and then dispersing in the M phase (Koken et al.,1995; Maul et al., 1995; Terris et al., 1995; Everett et al., 1999). The number of NBs starts to increase already in G1 and peaks in G2 due to several fission and fusion events of these structures in S phase (Dellaire et al., 2006b). During mitosis, PML is partly found in so called "Mitotic accumulations of PML protein" (MAPPs) some of which are in physical contact with mitotic chromosomes (Dellaire et al., 2006a). These particles, which no longer contain SUMO, Sp100 or Daxx, may explain the partitioning of PML in dividing cells and contribute to the reformation of PML NBs in new daughter cells. The regulation of PML NB integrity during cell cycle is most likely controlled through its contacts with chromatin (Eskiw et al., 2003; Eskiw et al., 2004).

Multiple functions of PML

Several functions have been suggested for PML and PML NBs. The NBs may act as storage compartments for several proteins, which can be released upon need to other cellular compartments (Everett et al., 1999; Negorev et al., 2001; Borden, 2002). In addition, a role in cell cycle and growth control, apoptosis, viral infections, DNA repair and transcriptional regulation have been proposed for PML and NBs containing several different regulatory factors.

The levels of PML and the number of NBs are induced by interferon, reflecting their role in response to viral infection (Lavau et al. 1995; Stadler et al., 1995; Grotzinger et al., 1996; Gaboli et al., 1998). PML NBs attract several viral proteins, including herpes simplex virus type 1, cytomegalovirus (CMV), adenovirus 5, Ebstein-Barr virus (EBV) and Simian virus 40 (SV40) proteins (Doucas et al., 1996; Ishov & Maul 1996; Maul et al., 1996; Szekely et al., 1996; Ishov et al., 1997; Everett 2001). Some of these proteins are able to disperse the structural integrity of PML NBs, and start the degradation of specific PML body associated proteins (Maul et al., 1993; Everett & Maul, 1994; Maul & Everett 1994; Ahn and Hayward, 1997; Everett et al., 1998; Chelbi-Alix & de The, 1999). The viruses may also utilize PML body associated proteins in their lifecycle by recruiting them for viral replication (Doucas et al., 1996). Alternatively, the PML NB itself could act as the site for viral DNA replication and transcription.

PML protein has growth suppressive properties (Mu et al., 1994; Ahn et al., 1995; Koken et al., 1995; Le et al., 1996; Quignon et al., 1998) and has been accepted as tumor suppressor (Salomoni and Pandolfi, 2002). The expression of PML is lost in several human cancer types of multiple histological origins and its expression status often correlates with the grade and progression of these cancers (Gurrieri et al., 2004a). It also plays a major role in the pathogenesis of acute promyelocytic leukemia, which will be described in more detail in the following chapter. PML overexpression leads to either growth arrest or apoptosis (Le et al., 1998; Pearson and Pelicci, 2001), and together with its partner Daxx, PML participates in nuclear apoptotic pathways (Torii et al., 1999; Zhong et al., 2000c). PML can inhibit the transformation induced by neu (c-erbB2, ERBB2), Ha-Ras and c-Myc as well as mutant p53 (Mu et al., 1994; Liu et al., 1995; Mu et al., 1996). It is also able to modulate the cell cycle progression by affecting several key proteins involved in the G1/S transition. Stable overexpression of PML alters the progression of the cycle and induces growth arrest by lengthening G1 (Mu et al., 1997). The apoptotic function of PML is probably one of its main growth suppressive properties, as PML is required for the induction of several apoptotic pathways, including Fas, tumor necrosis factor (TNF), ceramide, IR and interferons (Quignon et al., 1998; Wang et al., 1998). Pml null mice are protected against several of these pathways. These mouse cells have increased proportion of cells in S phase (Wang et al., 1998a) and they are less sensitive to lethal doses of γ radiation or Fas antibody treatment, supporting the pro-apoptotic role for PML protein (Wang et al., 1998b). Pml-/- mice do not

develop spontaneous tumors, but they are subjected to a greater number of skin papillomas and B- and T- cell lymphomas when exposed to DMBA (dimethylbenzanthracene) and TPA (12-O-tetradecanoylphorbol-13-acetate) (Wang et al., 1998a and 1998b).

In addition, PML is able to recruit several other apoptotic proteins into PML NBs (Quignon et al., 1998). PML IV isoform for example regulates p53 localization to PML NBs through binding p53 central domain and affecting its transcriptional activity (Fogal et al., 2000). Several studies have suggested that PML plays a role in potentiating p53-mediated apoptosis (Fogal et al., 2000; Guo et al., 2000; D´Orazi et al., 2002). PML may for example enhance p53 transcriptional activity towards PIG3 promoter, a gene induced in apoptosis (Fogal et al., 2000), and mediate HIPK2 phosphorylation of p53 upon UV radiation (D´Orazi et al., 2002). In addition to apoptotic control, PML is induced by oncogenic Ras and regulates p53-dependent senescence in response to oncogenic signalling (Ferbeyre et al., 2000; Pearson et al., 2000; Pearson and Pelicci, 2001). Ras has been shown to induce PML-p53-CBP complex formation and p53 acetylation in a PML-dependent manner in vivo (Pearson et al., 2000).

PML has been suggested to participate in RNA synthesis and processing as well as replication and modification of the chromatin structure (de Jong et al., 1996). The control of chromatin structure by PML is likely as PML bodies include transcription coactivator and histone acetyltransferase CBP as well as chromatin modifying proteins, histone deacetylases (HDACs) (LaMorte et al., 1998; Doucas et al., 1999; Von Mikecz et al., 2000; Bandobashi et al., 2001; Boisvert et al., 2001; Wu et al., 2001). PML NBs associate with sites of active transcription (Wang, J. et al., 2004). Nascent transcripts have been shown to accumulate on the surface of PML bodies, although the bodies themselves do not contain RNA or DNA (Boisvert et al., 2000). PML and its most studied partner Sp100 have been linked to transcriptional control, mainly to repression. PML IV interacts with the nonphosphorylated form of Rb and has been shown to be required for the transcriptional repression by Rb and Mad (Mu et al., 1994; Seeler et al., 1998; Vallian et al., 1998; Li et al., 2000b Alcalay et al., 1998; Khan et al., 2001a; Khan et al., 2001b). PML can also control Daxx by sequestering it and inhibiting Daxx-mediated transcriptional repression (Li et al., 2000a). In addition, PML may regulate the expression of particular genes or gene families at specific gene loci, like MHC class I gene family and TP53 gene locus, found in the vicinity of PML bodies (Shiels et al., 2001; Sun et al, 2003).

PML may participate in the recognition and repair of DNA lesions. Several repair factors, including Nbs1, Rad50, Mre11 and Chk2 are localized to these sites and controlled in a damage-dependent manner (reviewed in Dellaire and Bazett-Jones, 2004). PML may also affect genomic stability through Bloom syndrome protein (BLM). *BLM* gene encodes a RecQ DNA helicase, whose loss in Bloom syndrome patients leads to genomic instability and predisposition to cancer due to higher levels of sister-chromatid exchange (SCE) (Ellis et al., 1995). BLM protein colocalizes with PML NBs and cells lacking PML or expressing PML-RARα fusion protein, have abnormal BLM localization. Interest-

ingly, these cells have also higher frequency of SCE, mimicking the phenotype of Bloom syndrome cells (Zhong et al., 1999). The control of genomic stability by PML may also come from its centrosome association as PML IV isoform has been linked to the control of proper centrosome cycle (Xu et al., 2005).

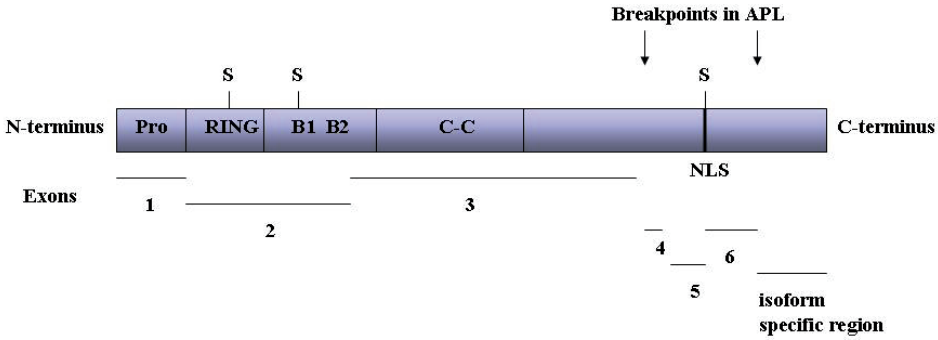

Figure 10. Structure of PML protein. PML exists in seven different cellular isoforms due to alternative splicing. All of the isoforms share common N-terminal region including a proline rich region, followed by a RING finger (C_3HC_4 zinc finger), two cys-rich B-boxes and a -helical coiled-coil domain, together forming the RBCC-domain. The coiled-coil region can mediate the formation of PML homodimers (Perez et al., 1993). Three major sumoylation site at positions 65, 160 and 490 are indicated with S and breakpoints in APL at positions 394 and 552 at the C-terminal site.

Acute promyelocytic leukemia

PML was indicated as a tumor suppressor protein already a decade ago (Mu et al., 1994). As mentioned above, the protein was initially discovered in acute promyelocytic leukemia, APL patients, where PML was found to be fused to retinoic acid receptor α due to a reciprocal translocation event (de The et al., 1990 &1991; Goddard et al., 1991; Kakizuka et al., 1991; Pandolfi et al., 1991; Kalantry et al., 1997). APL is a specific subtype of acute myeloid leukemia, AML, accounting for about 10 % of all AML cases. In APL leukemic cells, blocked at the promyelocytic stage, start to accumulate in the bone marrow. Cytogenetigally, a translocation between chromosomes 15 and 17, PML and RARα genes (t(15;17)(q22;q21)) respectively, is found in most of the APL cases (de The et al., 1990; Goddard et al., 1991; Kakizuka et al., 1991; Pandolfi e al., 1991). Two major breakpoints of PML have been described in APLs (de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991; Pandolfi et al., 1991; Kastner et al., 1992). Depending on the breakpoint either between exons 3 and 4 or downstream of exon 6, the generated PML-RARα form can be *bcr3* (short) or *bcr1* (long) (Huang et al., 1993; Vahdat et al., 1994) (Figure 10). The shorter form localizes to cytoplasmic bodies, CBs, and the patients carrying this *bcr3*

form have generally poorer prognosis than the ones with *bcr1* (Vahdat et al., 1994; Bellodi et al., 2006). While the other allele of PML is lost due to this chromosomal translocation, the remainig one may be wt or mutated. Total loss of functional PML in APL is associates with poor prognosis due to resistance to therapeutic agents (Gurrieri et al., 2004b).

Expression of PML-RARα increases the survival of hematopoietic cell lines and causes resistance to apoptosis (Grignani et al., 1993; Wang et al., 1998). The fusion protein acts as an oncogenic transcription factor, promoting relocalization of PML from NBs through heterodimer formation between PML and PML-RARα coiled-coil domains (Perez et al., 1993). The loss of normal functions of these structures inhibits the tumor suppressive properties of PML and other NB proteins, giving the cells growth advantage (Melnick and Licht, 1999) (Figure 11). The PML NB structure can be reinduced by treatments providing cinical remission (Daniel et al., 1993; Dyck et al., 1994; Weis et al., 1994; Koken et al., 1994). The development of APL in transgenic mice expressing PML-RARα fusion protein has also been described extensively and was shown to occur with incomplete penetrance (Brown et al., 1997; Grisolano et al., 1997; He et al., 1997; He et al., 1998; Kogan et al., 2000; Kogan et al., 2001, Pandolfi et al., 2001). In addition, reduction in the levels of the normal PML form, by crossing Pml-/- mice with PML-RARα transgenic mice, led to an increase and earlier onset in the incidence of leukemia (Rego et al., 2001).

Expression of PML-RARα also leads to dysregulation of the retinoic acid pathway (Kigan et al., 2000). The fusion protein competes with RARα for binding to RA-response elements of RARα target genes and abnormal binding to co-repressor complexes leads changes in transcriptional regulation under physiological concentrations of RA. PML-RARα is also a more potent transcriptional repressor and has abnormal associations with corepressor-histone deacetylase complex, remodelling the general chromatin stucture to a more condensed configuration. Additionally, it is capable of repressing transcription through a pathway indepent of HDACs and corepressors (Segalla et al., 2003). Combination of PML-RARα homodimerization, enhanced corepressor binding and inhibition of the RA-pathway have been proposed to contribute to the hematopoietic differentiation block, accumulation of promyelocytic blasts and the development of APL (Grignani et al., 1993; Grignani et al., 1998; He et al., 1998; Lin & Evans, 2000; Lin et al., 1998; Minucci et al., 2000). PML-RARα can in this way act as a double dominant-negative fusion protein, affecting both the normal PML and RARα functions (Kastner et al., 1992; Perez et al., 1993).

The oncogenic activity of PML-RARα has been associated with its N-terminal C-C domain, which is required for its sumoylation, microspeckle formation and for the inhibitory effect on RA-signalling pathway (Kim et al., 2005). A recent report showed that a sumoylation site, K160, in the N-terminus of PML is essential for the ability of PML-RARα to block differentiation and immmortalize primary hematopoietic precursor cells (Zhu et al., 2005). This transcriptional repression was due to Daxx binding, which was abolished by mutating the K160 residue.

Pharmacological concentrations of RA can release the repressor complexes from PML-RARα and recruit activator complexes, normalizing the function of the cells through differentiation of the leukemic blasts, degradation of the fusion protein and restoration of normal PML NBs (Daniel et al., 1993; Dyck et al., 1994; Koken et al., 1994; Weis et al., 1994; He et al., 1999). The transcriptional response by RA is largely PML-RARα-dependent (Meani et al., 2005). cDNA microarrays of an APL cell line, NB4, has revealed that over 1100 transcripts may be regulated in APL cells in response to RA. Genes involved in the regulation of hematopoietic differentiation cofactors and chromatin modifiers are early targets of RA treatment (Meani et al., 2005). Other targets include factors associated with calcium signalling and IFN-signalling pathways (Zheng et al., 2005).

In addition to RA, arsenic trioxide (ATO, As_2O_3) has proven to be an effective inducer of remission in APL patients (Chen et al., 1997; Zhu et al., 1997). Over 90 % of the patients benefit from a high-dose all-*trans*-retinoic acid (ATRA) or arsenic trioxide (ATO) therapy, which induce a complete remission in most of the cases (Warrell et al., 1991; Chen et al., 1997; Shen et al., 1997; Shao et al., 1998; Shen et al., 2004). Patients treated with RA as a first choice usually relapse at some point, after which they may be switched to arsenic trioxide therapy. ATO has several ways of affecting the differentiation and apoptosis in APL cells. Lower concentrations of ATO (0.5 uM) induce differentiation of the hematopoietic cells, while the higher concentration (2 uM) is apoptotic. Like RA, ATO treatment also leads to the degradation of the fusion protein (Zhu et al., 1999; Lallemand-Breitenbach et al., 2001). In addition, PML protein is degraded upon this treatment (Lallemand-Breitenbach et al., 2001). Compared to RA, ATO also induces changes in less number of regulated genes and they do not involve the RA-pathway. The array study of ATO-treated NB4 cells showed the downregulation of β1 integrins, upregulation of genes involved in the ubiquitin-proteasome pathway and downregulation of genes involved in the RNA processing and protein synthesis (Wang et al., 2003). In general, ATO may exert its effects more at the proteome level, affecting posttranslational and translational modifications. ATO and RA may also have additive effects together, as some genes involved in differentiation, cell cycle and growth control as well as apoptosis regulators have been found to be synergistically modified (Zhen et al., 2005). Moreover, in clinical studies targeting of PML-RARα oncoprotein by combined RA and ATO treatment has led to high-quality disease-free survival (Shen et al., 2004). Basically, the more PML-RARα is degraded, the better the recovery is in APL patients (Shen et al., 2004).

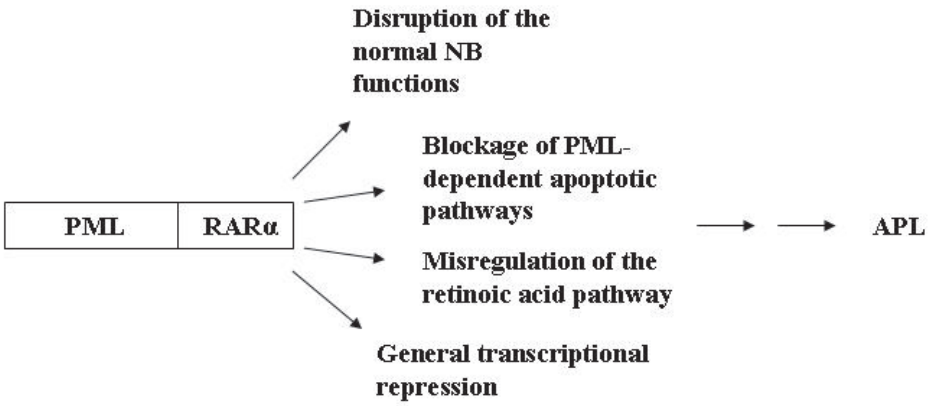

Figure 11. Cellular events leading to development of acute promyelocytic leukemia. Expression of PML-RARα fusion protein disturbs several cellular pathways, including disruption of the normal functions of NB-associated proteins and PML-dependent apoptotic pathways. Blockage of normal RARα and PML functions also inhibits the RA-response and prevents differentiation of the hematopoietic cells. PML-RARα fusion protein may also have a general impact on chromatin structure and transcriptional regulation through HDACs and corepressor recruitment.

AIMS OF THE STUDY

Functional loss of p53 is a common feature for most of the cancers. In addition to p53 mutations, overexpression of its negative regulator Mdm2 may be responsible for the inactivation of this essential pathway in significant proportion of human cancers. Clearly, the maintenance of a strictly controlled p53-Mdm2 circuit is of great importance in controlling p53 functions and preventing tumorigenesis. Understanding the exact molecular mechanisms in p53 pathway is thus essential from therapeutic point of view.

The early events leading to p53 stabilization and activation have been studied extensively over the last decade. Most of the studies have concentrated on the relevance of p53 modifications, occuring in response to various kind of DNA damage. In addition to the posttranslational modifications of p53, its cellular localization and complex formation with other proteins may be critical in the alteration of its function.

In our studies we have used UV radiation as a model of DNA damage. UV damage activates a complex cellular stress response in the cells, leading to transcriptional inhibition and activation of p53. The main aims of this research were:

1) to study the early events in damaged cells leading to release of p53 from the negative pressure of Mdm2
2) compare the cellular localizations of p53 pathway proteins in stressed and unstressed cells
3) find out which proteins could regulate p53 activity and stability in UV-damaged cells and unravel the molecular mechanisms behind them
4) address whether these particular p53 pathways are altered in human cancers

MATERIALS AND METHODS

Cells

The following cell lines were used in the study:

Cell line	cell type and description	source
A375	human malignant melanoma	ATCC, CRL 1619
HL-60	myelocytic leukemia	A.Vaheri (*)
NB4	acute promyelocytic leukemia, APL (Lanotte et al., 1991)	A. Vaheri
p53-/-mdm2-/- MEF	mouse embryonic fibroblasts (Montes de Oca Luna et al., 1995)	G. Lozano (**)
Pml-/- MEF	mouse embryonic fibroblast (Wang et al., 1998)	P.P. Pandolfi (***)
SaOS-2	p53-null human osteosarcoma	ATCC, HTB 85
U2OS	human osteosarcoma	ATCC, HTB 96
U937	promonocytic leukemia cell line	A. Vaheri
WS1	human skin fibroblast	ATCC, CRL 1502

(*) Haartman Institute, University of Helsinki, Helsinki, Finland
(**) University of Texas, M.D. Anderson Cancer Center, Houston, Texas, USA
(***) Memorial Sloan-Kettering Cancer Center, New York, New York, USA

Cell culture

Cells were cultured in a humidified atmosphere at 5% CO_2 at 37°C in Dulbecco´s modified Eagle medium, DMEM supplemented with 10% fetal calf serum (FCS) (GIBCO or PromoCell) (A375 and mouse embryonic fibroblasts), 10% FCS + non-essential amino acids (NAA) (WS1 human fibroblasts) or 15% FCS (SaOS-2, U2OS cells). Suspension cells (HL-60, NB4, U937) were maintained in RPMI, supplemented with 10% FCS. Mononuclear cells were isolated by purification through Ficoll gradients from fresh peripheral blood samples, using established protocols and the lysates were used for studying p53 and NPM levels (III, Fig. 5A).

Treatment of the cell cultures

UV treatments of the cells were carried out with Stratalinker 2400 (Stratagene, La Jolla, CA) and the cells were exposed to either 10 or 35 J/m^2 of UVC (254 nm) (I, II, III). For the inhibition of the proteasome activity, the cells were treated with 10 μM MG132 (Affinity Research Products) (I, II). Histone deacetylase inhibitor experiments were carried out by treating the cells with 100 ng/ml Trichostatin A, TSA (Sigma) (III). Arsenic trioxide (ATO, As_2O_3) (Sigma) was used at 1μM (I) or 2 μM (III) concentrations. retinoic acid (RA) (Sigma) was used at 2 μM concentration (III).

Mutagenesis

Mdm2 deletion mutants Δ89-222 and ΔNoLS (Δ464-471, nucleolar localization-defective mutant Mdm2) were constructed by site-directed mutagenesis (QuickChange Site-directed Mutagenesis Kit, Stratagene) and the products were verified by DNA sequencing.

Transfections

Mouse embryonic fibroblasts were transfected by electroporation (Gene Pulser II, Bio-Rad) with 280 V and 975 μF in Optimem (GIBCO) (I,II). U2OS cells were transfected by lipofection (Lipofectamine 2000, Invitrogen) (II,III) and NB4-suspension cells by Amaxa nucleofector, Kit T, program X-001 (III). The following plasmids were used in transfections: PML III (PML-L) in pSG5, PML IV (PML-3) and PML IV-3K (sumoylation deficient triple mutant PML IV) in pCDNA3 and PML-RARα in pSG5 (obtained from G. del Sal) (I, III); wt Mdm2 and Mdm2 ΔNoLS (Δ464-471, nucleolar localization-defective mutant Mdm2) (I); B231.1-pCHA and B231.2-pCHA (obtained from Dr. Kyosuke Nagata, Okuwaki et al., 2001) (II), SUMO-1 expression vector (obtained from Dr. Jorma Palvimo) (II); Myc-tagged K-cyclin expression vector (originally obtained from Dr. Sibylle Mittnacht, Ellis et al., 1999) (II); Myc-tagged Xenopus NPM (NO38) (obtained from Dr. Marion Schmidt-Zachmann, Zirwes et al., 1997); NPM-ECGFP (described in Kurki et al., 2006) (III); p53-pCDNA3 (III); PG13xRE-luciferase reporter vector (obtained from Dr. Bert Vogelstein) and pRLSV40 Renilla Luciferase control vector (III).

siRNA

siRNA was used to deplete NPM and Mdm2 from U2OS cells (III). The duplex sequences for NPM siRNA were as described in Colombo et al., 2002 (purchased from Dharmacon Research, Inc.). Mdm2 RNAi duplexes containing the sequence 5′UGGUUGCA UUGUCCAUGGC3′ targeting Mdm2 mRNA and SMARTpool Mdm2 siRNA mix were purchased from Dharmacon Research, Inc. The duplexes were transfected into cells by lipofection (Oligofectamine,

Invitrogen). The cells were incubated for 1 (Mdm2 siRNA) or 3 days (NPM siRNA) posttransfection.

Luciferase reporter activity assays

For p53 activity assays, NB4 cells were treated with either ATO or RA 24 hours prior the transfection (III). p53-pDNA3, PG13xRE luciferase reporter vector and Renilla luciferase control vector, pRLSV40, were transfected by using Nucleofector Kit T (Amaxa). Luciferase activities were measured by Dual-Luciferase Reporter Assay System (Promega) and luminometer (DCR-1, Digene Diagnostics) five hours post-transfection. Renilla activity was used to normalize the transfection efficiencies. Fold induction of p53 activity was calculated as a mean value of at least two separate experiments.

Preparation of cellular extracts

Monolayer cells were washed with Tris-buffered saline (TBS). EBC lysis buffer containing 25 mM Tris-HCl pH 8.0, 120 mM NaCl, 0.5% NP-40, 4 mM NaF, 100 μM Na_3VO_4, 1mM phenylmethylsulfonyl fluoride, 100 KIU/ml aprotinin and 10 μg/ml leupeptin was added on the plates and cells were scraped into eppendorf tubes and incubated on ice for 20 minutes. The insoluble fraction was separated from the soluble one by centrifuging the cells with 14 000 rpm for 15 min. The pellet of the insoluble fraction was boiled in Laemmli sample buffer (LSB), containing dithiothreitol (DTT) (100mM) (I, II, III). Suspension cells were pelleted prior to washing with TBS. Cells were suspended into lysis buffer and treated as above to separate the soluble and insoluble fraction (III).

The obtain total cellular lysates, cells were resuspended into urea-Tris buffer containing 9 M urea, 75 mM Tris-HCl (pH 7.0) and 0.15 M 2-mercaptoethanol (III). The suspension was sonicated briefly and protein concentrations were determined by Bio-Rad D_c protein assay kit (Bio-Rad, Hercules, CA). The samples were boiled in LSB-DTT for 5 min. Alternatively, total cell lysates were extracted in LSB-DTT and sonicated briefly before boiling the samples (I).

Immunoprecipitation

After normalization of protein concentrations, cellular lysates were immunoprecipitated with specific antibodies and the samples were collected on GammaBind-G Sepharose beads (Pharmacia Biotech). The beads were washed four times with TBS. Immunocomplexes were boiled in LSB-DTT prior to analysis. The following antibodies were used in immunoprecipitations: anti-acetyl-Histone H3 (06-599, Upstate) (III), anti-Mdm2 mix (SMP14, Santa Cruz Biotechnology; 2A10; IF2, Oncogene Sciences) (I, II), anti-c-Myc 9E10 (Biosite) (II), anti-NPM (Zymed) (II, III), anti-p300 (N-15, Santa Cruz) (III), anti-p53 mix (DO-1, PAb1801, PAb421) (I, II), anti-p53 (FL393, Santa Cruz Biotechnology) (III), anti-PML (PG-M3, Santa Cruz Biotechnology) (I, III), anti-PML (H-238,

Santa Cruz Biotechnology) (III). To exclude unspecific binding, mouse or rabbit IgG (Dako Cytomation, Denmark) were used as negative controls (II, III).

Immunoblotting

Lysates and immunoprecipitates were separated by 7.5%, 9%,10% or 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The samples were transferred to nitrocellulose membrane (Trans-Blot, Transfer Medium, Bio-Rad) and the membranes were blocked with either 5% milk in TBS or 3% bovine serum albumin (BSA). Immunoblotting was carried out by using specific antibody dilutions in 1% BSA. The following antibodies were used in this study: anti-acetyl-Histone H3 (06-599 and 06-942, Upstate) (III), anti-GAPDH (Europa Bioproducts, Cambridge, UK) (II, III), anti-GST (clone BC8E8) (II, III), anti-Histone H3 (FL-136, Santa Cruz Biotechnology) (III), anti-Mdm2 mix (SMP14, Santa Cruz Biotechnology; 2A10; IF2, Oncogene Sciences) (I, II), anti-NPM (Zymed) (II, III), anti-p53 (FL393, Santa Cruz Biotechnology) (I, II, III), anti-p53 (DO-1) (III), anti-PML (PG-M3, Santa Cruz Biotechnology) (I,III), anti-PML (H-238, Santa Cruz Biotechnology) (I,III), anti-SUMO-1 (GMP-1, Zymed) (II) and anti-PCNA (Santa Cruz Biotechnology) (III). The primary antibodies were followed by secondary antibodies coupled to horseradish peroxidase, HRP (Dako Cytomation, Denmark). The washes of the membranes between primary and secondary antibodies were done in TBS containing 0.05% Tween 20 (Amersham Biosciences). The proteins were detected with enhanced chemiluminescence, ECL (Amersham Life Sciences or Millipore).

Immunofluorescence analysis

Monolayer cells were fixed for 20 minutes with 3.5% parafolmaldehyde, PFA, followed by permeabilization with 0.5% NP-40 lysis buffer and blocking with 3% BSA (I, II, III). Suspension cells were centrifuged for 3 minutes 600 rpm on glass slides prior to fixation with PFA (Shandon cytospin II cytocentrifuge, Thermo Electron Corporation) (III). The following primary antibodies were used in the study: anti-Mdm2 mix (SMP14, Santa Cruz Biotechnology; 2A10; IF2, Oncogene Sciences) (I, II), anti-c-Myc 9E10 (Biosite) (II, III), anti-NPM (Zymed) (II, III), anti-NPM (C-19, Santa Cruz Biotechnology) (II), anti-p53 (FL393, Santa Cruz Biotechnology) (I, II, III), anti-p53 (DO-1, PAb421, Pab1801) (I), anti-PML (PG-M3, Santa Cruz Biotechnology) (I,III), anti-PML (H-238, Santa Cruz Biotechnology) (I,III) or anti-PML antibody mix (A-20 and N-19, Santa Cruz Biotechnology). Specific antibodies were detected by secondary antibodies conjugated to fluorochromes. The following secondary antibodies were used: swine anti-rabbit or rabbit anti-goat FITC (I,II), rabbit anti-mouse TRITC (DAKO) (II), goat anti-mouse, goat anti-rabbit or donkey anti-goat antibody conjugated Alexa fluorochromes 488 and 594 (Molecular Probes) (I, II, III). The absence of crossreactivity was verified in separate experiments. DNA was stained with 4´,6-diamidino-2-phenylindole (DAPI) (Molecular Probes) (I, II, III) and RNA

with Syto 12 green fluorescent nucleic acid stain (Molecular Probes) (II). The fluorochromes were visualized with the Zeiss Axioplan 2 Imaging MOT (Jena, Germany) equipped with appropriate filters (Chroma). Images were captured with Zeiss Axiocam CCD-videocamera and image processing and analysis was performed with AxioVision programs, versions 3.0 (I,II) or 4.4 (III). Confocal images in the study I were made with Bio-Rad MRC1024. Staining intensities in the study II were quantified by KS Run 3.0 analysis program (KS 400, Zeiss) from 100 nuclei per each time point.

Fluorescence Recovery After Photobleaching (FRAP) and image analysis

U2OS cells were cultured on LabTek II chambered coverglass (Nalge Nunc International), and transfected with NPM-ECGFP (III). The cells were treated with 35 J/m^2 UVC 24 hours post-transfection. For imaging, the medium was changed to DMEM without phenol red, supplemented with 25 mM Hepes (PromoCell). Zeiss 510 META confocal laser scanning microscope (LSM, Zeiss) with heating stage and Plan-Neofluar 40x oil objective with 1.3 NA was used for photobleaching and imaging of the samples. For imaging, the Argon laser line (458 nm) was set at 2% and for bleaching at 100% with 85% output. The size and shape of each nucleolus was defined with region of interest (ROI) and the ROI was bleached after three scans with 30 iterations. 97 post-bleach images were collected every second.

The image analysis and quantification of the fluorescent intensities were calculated from at least two separate experiments and 8-10 cells. LSM 510 Physiology Software was used for measuring the fluorescent intensities. The method of Rabut and Ellenberg (2005) was used for the analysis of mobile fractions and recovery halftimes. Statistical significance of the results were evaluated as p-values by using Student´s t-test.

In vitro translation

In vitro translation of Mdm2 (I, II), p53 (I, II), NPM (II, III) and different PML isoforms (I, III) were performed with TNT Coupled Reticulosyte Lysate System (Promega) from T7 promoter containing expression vectors of each gene, either in the presence of 20 μCi of ^{35}S-methionine (specific activity 1000 Ci/mmmol, Amersham) (II, III) or unlabeled methionine (I). Translation products were immunoprecipitated as described above. The samples were separated by SDS-PAGE and the proteins were analyzed by autoradiography (II, III) or immunoblotting (I).

GST pulldown assays

Mdm2 GST-fusion proteins (II) (obtained from Dr. David Meek), PML IV-GST (III), GST-NPM (III) and GST-protein control (GST-CRP1) (II) were produced in BL-21 Escherichia coli cells following induction with IPTG. The fusion proteins were captured on glutathione-Sepharose 4B beads (Amersham) for the pull-down experiments. Binding of the specific ^{35}S-methionine labeled partner protein, ^{35}S-NPM (II, III) or ^{35}S-PML (III), was performed in 140 mM NaCl, 0.5% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA and 1 mM PMSF (TNE-buffer) overnight at +4 ˚C under rotation, after which the beads were washed ten times with TNE-buffer and finally with PBS. Washed beads were boiled in LSB containing dithiothreitol (10mM) for 5 minutes and the supernatant was loaded to SDS-PAGE gels. The proteins were analyzed by autoradiography.

Chromatin isolation

Chromatin preparations and nucleoplasmic fractions from U2OS cells and MEFs were performed as described by Mendez and Stillman (2000) with slight modifications (III). The samples were separated by SDS-PAGE and analyzed by immublotting as described above.

RESULTS AND DISCUSSION

UV radiation induces stabilization of the tumor suppressor protein p53 (Maltzman and Czyzyk, 1984). The stabilization is associated with posttranslational modifications of both p53 and its negative regulator Mdm2 (Meek and Knippschild, 2003; Xu, 2003) as well as decreased interaction between them (Latonen et al., 2001). Release of p53 from the negative pressure of Mdm2 promotes transactivation of specific p53 target genes in a dose-dependent manner, leading to either growth arrest and p53-assisted NER pathway or to apoptosis upon excessive damage (Latonen et al., 2001; Gentile et al., 2003; Gatz and Wiesmuller, 2006). In addition to the posttranslational modifications, the complex network resulting in functional p53 requires multiple stress-induced protein-protein interactions, each contributing to p53 activation (Lavin and Gueven, 2006).

Regulation of the Mdm2-p53 interface has a great potential in the development of new therapeutics targeting p53 pathway in cancer cells. Particularly potential factors reinducing the p53 pathway in cancer cells could be proteins interacting with either Mdm2 or p53, uncoupling the degradation pathway and leading to active p53 upon cellular stress. A couple of promising pharmacological inhibitors of this interaction have recently been described (Issaeva et al., 2004; Vassilev et al., 2004; Yang et al., 2005). During our studies of the impact of cellular stress on p53 and Mdm2 interaction, we observed associations of Mdm2 with promyelocytic leukemia protein as well as with nucleolar protein nucleophosmin. The similar localization patterns detected upon certain stress situations as well as the damage-induced relocalizations of these particular proteins led us to study the possible association of PML and NPM in the regulation of p53 pathway through its negative inhibitor Mdm2.

Cellular stress and DNA damage evoke subnuclear translocations of p53 pathway proteins (I, II, III)

PML exists in the nucleus mostly attached to PML NBs, in a detergent- insoluble form (Muller et al., 1998; Lallemand-Breitenbach et al., 2001). The size and number of these bodies is affected by the phase of the cell cycle as well as exposure to cellular stress (Koken et al.,1995; Maul et al., 1995; Terris et al., 1995; Everett et al., 1999). γ radiation for example is known to increase the number and size of NBs and attract p53 to these suborganelles (Pearson and Pelicci, 2001). In contrast to the γ radiation-induced effect on PML NB structure, we found that PML bodies lost their nuclear architecture upon UV radiation of the cells (I, Figure 1A and 6). The NBs were dispersed to smaller microstructures and nucleoplasmic PML staining increased rapidly, starting from one hour after UV exposure. Similar effect on PML NB structures has been earlier described to occur upon heat shock and exposure to heavy metals, like Cd^{2+} (Maul et al., 1995; Ishov et al., 1999; Eskiw et al., 2003; Nefkens et al., 2003). As the level

of PML was not upregulated during the early timepoints (I, Figure 5C) and we detected an increase in the soluble form and decrease in the insoluble form of PML (I, Figure 4A), the evident enhanced nucleoplasmic PML staining was due to release of PML from the NBs to a more soluble form. Similar observations about the disruption of PML body structures were published by Seker et al., 2003 and later by Salomoni et al., 2005. The mechanism for this UV-induced PML NB dispersion is not known, although DNA damage-induced kinase pathways may be involved. UV radiation activates the p38 MAPK and ERK1/2 kinase pathways, which are regulating PML release from the NBs upon Cd^{2+}-exposure (Nefkens et al., 2003). Other suggested mechanisms for PML release include changes in its sumoylation status, which has been proposed to control the formation of PML NBs. Exposure to heat shock, heavy metals or adenovirus E1A expression leads to dispersal of PML bodies through a desumoylation event (Eskiw et al., 2003). Also transcriptional acitivity of the cells may play a role as inhibition of either RNA pol I (Kiesslich et al., 2002) or RNA pol II by DRB (Eskiw et al., 2004; our unpublished results) leads to scattering of PML and its associated proteins to the nucleoplasm, suggesting that any stress leading to inhibition of the transcription may interfere the integrity of these structures. The reason for PML body dispersal upon transcriptional inhibition may result from the alterations in the chromatin structure as only a slight change in the conformation of the surrounding DNA is enough to obstruct the maintenance of intact PML NBs in a SUMO-independent manner (Eskiw et al., 2004). PML NBs themselves do not contain nucleic acids in their core (Boisvert et al., 2000), but they are surrounded by and in extensive contact with chromatin, which maintains the structural integrity of NBs in interphase cells (Eskiw et al., 2003; Eskiw et al., 2004). This could also mean that DNA damage, causing reorganization of the chromatin, may be the only required signal for the instability of PML NBs.

PML translocation also involved the perinucleolar area, which started to show positivity of PML staining in the nucleolar necklace structures, also referred to as nucleolar caps, in UV-radiated cells (Figure 12 & I, Figure 1B). Interestingly, Mdm2 was also detected in these insoluble structures, partly colocalizing with PML (I, Figure 1B, C & D), while p53 remained in the nucleoplasmic fraction (I, data not shown). The significance of this localization pattern of PML and Mdm2 is presently not clear, although it could be indicative of their role in rRNA transcription. PML bodies are know to contain several factors associated with transcription (reviewed by Zhong et al., 2000b). They contain both CBP/p300 and RNA pol II (Von Mikecz et al., 2000) and associate with RNA pol II at the sites of active transcription (Kiesslich et al., 2002). DNA helicase II also associates with intact PML NBs and inhibition of transcription leads to translocation of this protein to the perinucleolar area (Fuchsová et al., 2002). Furthermore, Mdm2 could play a role in transcription through its interaction with RNA and ribosomal protein L5 (Marechal et al., 1994; Elenbaas et al., 1996). Another study, with similar observations on PML and Mdm2 translocation to the perinucleolar area upon DNA-damage caused by cytotoxic drugs, suggested that

the nucleolar localization of these proteins affects p53 stabilization (Bernardi et al., 2004). According to this model PML would potentiate p53 stabilization in stressed cells by sequestering Mdm2 to nucleolus. This hypothesis, however, is unlikely due to the slow kinetics of these events and because only a small fraction of the total Mdm2 is being translocated to nucleoli in stressed cells.

Similarly to UV-induced translocation of PML, we also detected that UV exposure induced translocation of the nucleolar protein NPM to the nucleoplasmic compartment (II, 1A & B, 3A) as well as to the perinucleolar area around to nucleoli (II, data not shown). This kind of translocation has been shown before to occur upon some DNA damaging and cytotoxic drugs (Yung et al., 1985; Chan et al., 1987; Yung et al., 1990; Bor et al., 1992; Chan, 1992; Wu and Yung, 2002), and the relocalization upon UV was recently also reported by Rubbi and Milner (2003b). The translocation was detected in p53 and ARF null cell lines, indicating that it did not require a functional p53 pathway (II, Figure 1A and data not shown). The relocalization was also evident in analysis of the insoluble and soluble fractions of the cells, which showed a decrease of NPM in the nucleoli containing insoluble compartment, associated with an increase in the free, soluble nucleoplasmic fraction (II, Figure ID). A slight increase in the total levels was also detected, as has been earlier shown also by Wu and Yung (2002) (II, 1D). Translocation of nucleophosmin from nucleoli to nucleoplasm is known to require ATP (Wu et al., 1995; Finch and Chan, 1996). The activating signal for this UV-induced effect on NPM distribution is not yet understood. Although the nucleolar structures undergo morphological changes upon transcriptional inhibition, the nucleoli themselves are not completely disrupted. Therefore, nucleolar disruption may not fully explain the release, as observed by staining of the ribosomal RNA in the nucleoli (II, Figure 1C). As NPM is a heavily modified phosphoprotein and its cellular localization is known to be affected at least during the cell cycle by several phosphorylation events, the mechanism could include UV-induced modifications, like phosphorylations (Grisendi et al. 2006, and references therein). Although many of the kinase pathways known to phosphorylate NPM are activated upon UV, we could not find any evidence that they would play a role in UV-induced translocation of NPM (unpublished results). Other posttranslational modifications can be involved as well. An obvious inducer of NPM relocalization could be a direct signal from stalled RNA pol II by UV-induced lesions, causing ribosomal stress and release of NPM upon disturbance of its main functions in the nucleoli.

As both NPM and PML were found in the perinucleolar area of the UV-treated cells, we also studied the colocalization of these proteins. We observed a fraction of NPM and PML in necklace structures, the majority still colocalizing in the nucleoplasmic fraction (III, Figure 1B). Additionally, we found that NPM and PML colocalized in mature PML NBs (III, Figure 1C) as well as at centrosomes of the metaphase cells (unpublished data). The detection of these colocalizations with endogenous proteins and in different compartments, depending on the cell phase and stress, suggests that the association of NPM and PML is physiologically relevant and function in both stressed and unstressed cells.

In addition to the observed colocalizations in UV-treated cells, we detected similar cellular localization patterns of the p53 pathway proteins Mdm2, PML and NPM upon exposure of the cells to other kind of cellular stress as well. Proteasome inhibitor MG132 and arsenic trioxide both induced the number of PML NBs (I, Figure 1A & C). MG132 induces accumulation of several proteins, including many PML NB-associated factors, into the nucleoli (Klibanov et al., 2001; Mattsson et al., 2001; Latonen et al., 2003). The colocalization of PML and Mdm2 was also detected in this compartment as well as in the PML NBs of the proteasome-inhibited cells (I, Figure 1A). Mdm2 was also found to colocalize with NPM upon same conditions in the nucleoli (II, Figure 2A). ATO, on the other hand, induced recruitment of Mdm2 to larger PML NB aggregates (I, Figure 1C), while NPM was not detected in these structures (unpublished data). The relocalization of Mdm2 to these larger NBs upon ATO treatment occured with slower kinetics than seen with other cellular stress inducers, emphasizing the differencies in the timing and spatial distribution of these associations upon exposure to various kind of cellular stress.

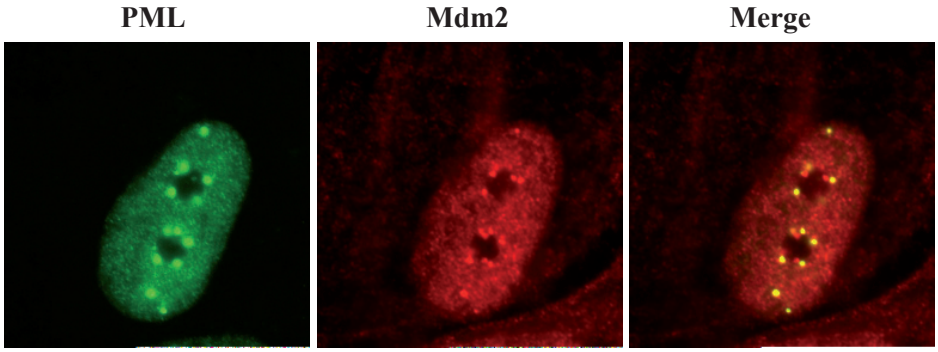

Figure 12. UV-induced nucleolar structures in WS1 human fibroblasts.

UV radiation induces rapid and transient complex formation between p53, Mdm2, PML and NPM (I, II, III)

The ability of PML to affect cellular stress responses of p53 has been amply demonstrated by many studies (Fogal et al., 2000; Guo et al., 2000; D´Orazi et al., 2002; Ferbeyre et al., 2000; Pearson et al., 2000; Pearson and Pelicci, 2001). In addition, the positive effect of NPM on p53 function upon DNA damage of the cells was shown by Colombo et al. (2002). As we had detected colocalizations between these p53 pathway proteins and Mdm2, we started to study their possible effect on p53 function through Mdm2. Moreover, the impact of PML on p53 function upon exposure to UV radiation was largely unknown at the time this study was started.

Studies were performed using several cell lines, WS1, SaOS-2 and U2OS, to detail their dependency on p53 or ARF. The cells were exposed to 35 J/m^2 of UVC radiation to induce UV stress and p53 stabilization and activation. As expected, p53 protein in normal WS1 human fibroblasts accumulated clearly upon this treatment, starting from three hours postradiation (I, Figure 5A & C). As a sign of a transient p53 induction, the feedback loop with Mdm2 was also activated and the levels of Mdm2 protein were increased at later time points leading to enhanced p53-Mdm2 complex formation (I, Figure 5A & C). Interestingly, we also observed complex formation between PML and p53 as well as PML and Mdm2 (I, Figure 5A). These interactions took place early after radiation, prior to p53 stabilization, suggesting a role in the regulation of p53 function in UV-treated cells. To study whether the novel interaction between Mdm2 and PML could be mediated through p53, we performed similar assays in a p53-negative cell line, SaOS-2. As Mdm2-PML interaction was also observed in this cell, in a dose-dependent manner, the respective complex formation was clearly not p53-dependent (I, Figure 4B). In addition, we observed the interaction between these proteins upon MG132-treatment, blocking the proteasomal degradation of both Mdm2 and PML (I, Figure 4B).

To address whether the interactions of the proteins are direct we performed in vitro interaction assays. These studies showed that PML binding to Mdm2 was significantly weakened by using Mdm2-deletion constructs lacking the C-terminal domain of Mdm2 (I, Figure 3B). This domain also contains the Mdm2 RING domain responsible for its E3-ligase activity. As none of the used Mdm2-deletion mutants were fully devoid of interaction between these proteins, Mdm2 may have more than one PML binding site. Mdm2, on the other hand, preferred binding to PML isoform IV, independently of its sumoylation status (I, Figure 3A). Interestingly, p53 has earlier been shown to interact with only this PML isoform, and require PML C-terminus and p53 DBD (Fogal et al., 2000). Considering these results, we performed in vitro competition assays to study whether a shared binding site for p53 and Mdm2 is localized in the PML C-terminus (I, Figure 7). Higher amounts of PML in the in vitro assay increased the association of p53 with Mdm2 and vice versa, the elevation of Mdm2 levels increased p53-PML complex formation. The results suggested that rather than competing for the same binding site on PML, p53, Mdm2 and PML can form trimeric complexes and that this is promoted by the PML-Mdm2 interaction. As both p53 and Mdm2 were found to transiently interact with PML rapidly after UV stress, it is possible that a trimeric complex is formed, playing a role in the UV response of the tumor suppressor protein p53.

Similarly to PML, we found that NPM forms kinetically rapid and transient complexes with Mdm2 early after UV exposure (II, Figure 3B). Moreover, this interaction was independent of the p53 status of the cells (II, Figure 3C). A recent paper on UV-induced responses of NPM and ARF, showed similar association between NPM and Mdm2 following UV-exposure, although with a little bit delayed kinetics (Lee C et al., 2005). In vitro interaction analyses using GST-pull down experiments showed that interaction is dependent on the N- and

C-terminal domains of Mdm2, containing its p53 binding site and the RING domain (II, Figure 2C). Although p53 requires the same binding domain for its interaction with Mdm2, the in vitro competition assays suggested that p53 is able to promote the interaction between NPM and Mdm2. Higher amounts of p53, however, competed for the interaction and decreased NPM-Mdm2 complex formation. As p53-NPM interaction has recently been shown to require the N-terminal site of p53 (Maiguel et al., 2004), also essential for its Mdm2 binding, the higher nucleoplasmic NPM levels upon cellular stress might be able to disrupt the interaction between p53 and Mdm2, leading to inhibition of the p53 degradation by the proteasome and its transcriptional activation.

As we had detected transient interactions between PML, Mdm2 and p53 as well as NPM, Mdm2 and p53 following UV stress, we further studied the association of PML and NPM proteins in these early complexes. These proteins were found to interact within similar kinetics like the other UV-induced transient complexes (III, Figure 1A). Furthermore, the interaction between PML and NPM was independent of the presence of either p53 or ARF (data not shown). In vitro interaction analyses of the interacting domains between NPM and PML showed that several PML isoforms associate with NPM, which was dependent on the intact N-terminus of NPM and its oligomerization domain (III, Figure 2A-C). Interestingly, Mdm2 also required this oligomerization domain for binding to NPM (unpublished results). As all of these transient interactions took place with similar kinetics (I, II, III), the results strongly implicate the existence of a UV-induced multiprotein complex (Figure 13). The presence of NPM and PML in p53-Mdm2 complex may thus potentiate the early events in p53 functional activation following UV damage of the cells.

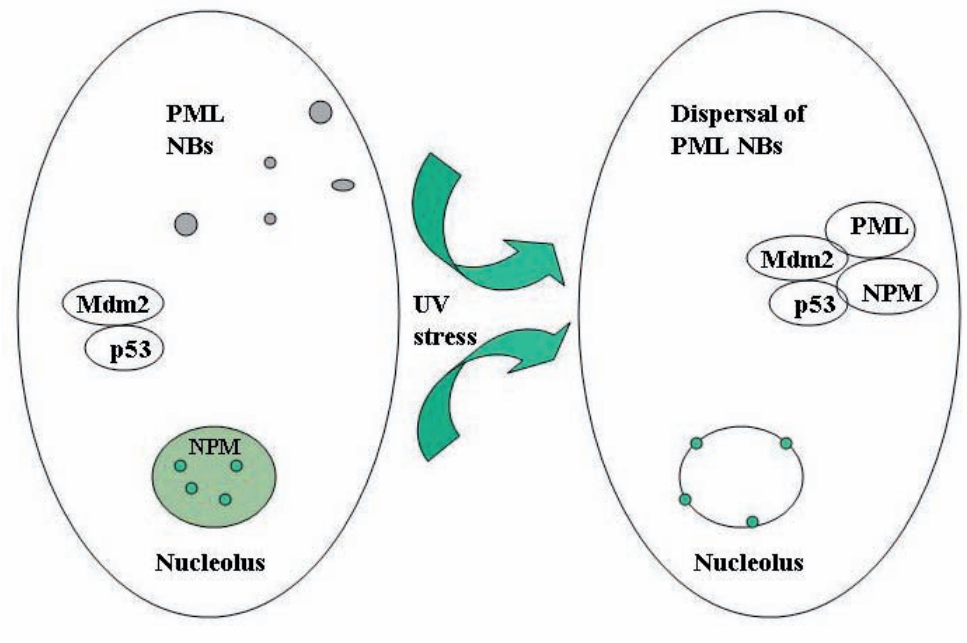

Figure 13. Model for the early events in p53 activation following UV stress. Exposure of cells to UV induces site-specific translocations of the p53 pathway proteins, associated with transient interactions, possibly causing formation of a multiprotein complex involved in p53 stress response.

The impact of PML and NPM on p53 stability and activity (I, II)

p53 stabilization has been shown to take place upon numerous cellular stress situations, like exposure to UV radiation and transcriptional inhibition (Maltzman and Czyzyk, 1984; Ljungman and Zhang, 1996; Ljungman et al., 1999). The stabilization of p53 could involve disruption of the interactions with its negative regulators or direct inhibition of their E3-ligase activities. Our results showed that Mdm2 and PML interact rapidly and kinetically in a transient manner in UV-stressed cells prior to p53 stabilization (I, Figure 5A). This could reflect a role for PML in p53 activation or stabilization. In addition, the in vitro data showed binding of PML to Mdm2 RING finger, possibly affecting its E3-ligase activity towards p53 (I, Figure 3B). An increase in the levels of endogenous p53, associated with enhanced PML-Mdm2 complex, was also detected following ectopic PML expression in U2OS cells (unpublished observations). Moreover, p53 in Pml-/- MEFs is present in a multimodified form compatible with ubiquitinated p53, suggesting that PML is necessary for the inhibition of p53 degradation (Louria-Hayon et al. 2003). Recent data from several laboratories has suggested that PML could influence p53 stability by inhibiting the abil-

ity of Mdm2 to degrade p53, either by directly blocking the E3-ligase activity (Louria-Hayon et al., 2003), through direct interactions (Zhu et al., 2003) or by PML-mediated Mdm2 translocation to nucleoli in stressed cells (Bernardi et al., 2004). One study in a breast carcinoma cell line, MCF-7, also showed that stable suppression of PML expression results in enhanced p53-Mdm2 complex formation and a decrease in p53 levels due to enhanced degradation (Bao-Lei et al., 2006). Several reports have thus shown the importance of PML in the control of basal and stress-induced p53 levels.

PML was shown to be required for p53 stabilization upon γ radiation and certain cytotoxic drugs (Louria-Hayon et al., 2003; Bernardi et al., 2004). To test whether it could also be essential for UV radiation-induced p53 stabilization, we performed both immunofluorescence and western analysis from UV-treated wt and Pml-/- MEFs. Although p53 was present in the PML null cells in more ubiquitinated forms, it was stabilized in a similar manner as in the wt MEFs upon UV exposure (unpublished results, Figure 14). Similar findings were presented in a study of Salomoni et al. (2005), showing equal increase in p53 levels in both wt and Pml-/- MEFs in response to UV. Thus, PML seems essential for p53 stability in unstressed cells and in some, but not all stress-induced pathways. Even though PML does not seem to play a role in UV-promoted stabilization of p53, it could still influence the p53 posttranslational modifications or protein-protein interactions, finetuning p53 target gene activation or repair functions. This is supported by several studies suggesting a role for PML in modifying p53 activity. Furthermore, PML is itself a p53 target, forming in this way a positive-feedback loop in the p53 activation (de Stanchina et al., 2004).

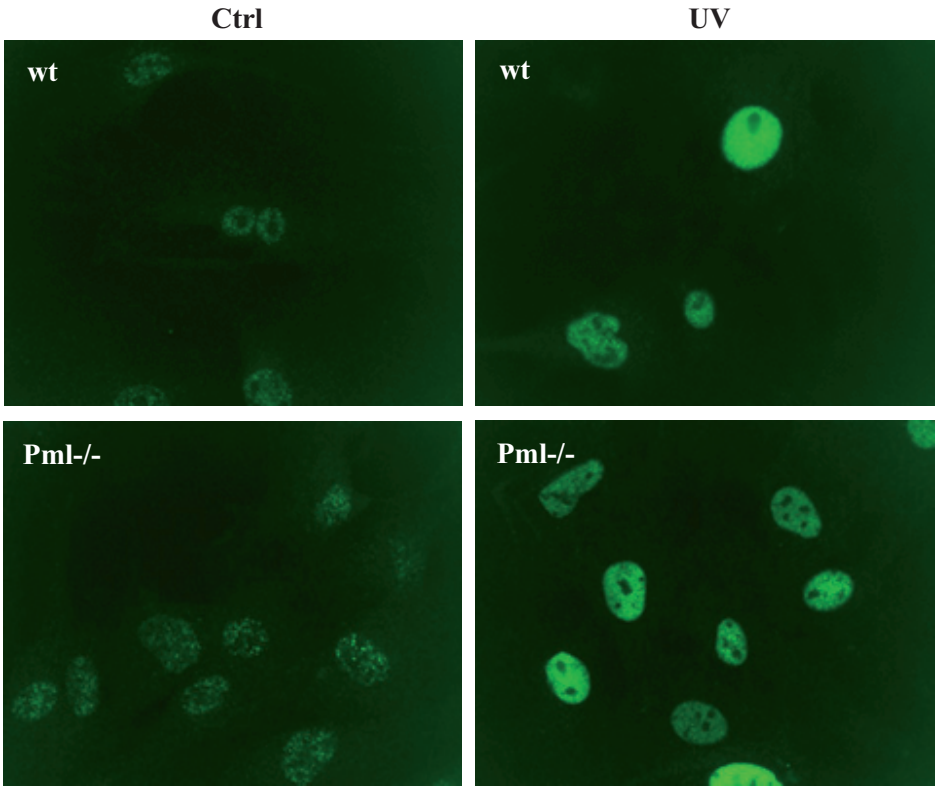

Figure 14. PML is dispensable for p53 stabilization following UV radiation. Immunofluorescence staining of p53 in control and UV-treated (35 J/m^2, 6 h) wt and Pml-/- MEFs.

NPM has been implicated in the regulation of p53 activity in several studies with contrasting results, some suggesting that NPM acts as p53 activator (Colombo et al., 2002; Zou et al., 2005) and others as repressor (Maiguel et al., 2004; Li et al., 2004; Li et al., 2005). We studied the effect of NPM overexpression on p53 levels in U2OS cells and found that ectopic expression of NPM stabilized both p53 and Mdm2 (II, Figure 4A & B). Similar effect of NPM on p53 stability has been shown by Colombo et al. (2002). Additionally, we found that the increase in Mdm2 levels was clearly independent on the p53 transactivation, as the same phenomenon was evident in SaOS-2 cells (II, Figure 4C). The mechanism of stabilization of p53 by NPM could involve inhibition of the Mdm2 E3-ligase activity, as NPM binds Mdm2 RING finger domain (II, Figure 2C). Alternatively, NPM could disrupt the interaction between p53 and Mdm2 due to competing binding domains (II, Figure 2D and Maiguel et al., 2004). To verify that NPM increases p53 stabilization by inhibiting Mdm2, we silenced NPM using siRNA in U2OS cells (II, Figure 5A & B). Depletion of NPM from these cells reduced the basal levels of p53 as well as the UV-induced stabilization of p53 (II, Figure 5C). Moreover, the decrease in the levels of p53 was associated with enhanced

p53 complex-formation with Mdm2, suggesting that NPM can control p53 levels through blocking its interaction with Mdm2 (II, Figure 5C). Further, depletion of Mdm2 together with NPM was able to rescue the negative regulation of p53 by NPM siRNA (II, Figure 5D), confirming the ability of NPM to block Mdm2-mediated degradation of p53 through controlling their interaction.

Several nucleolar proteins have lately been associated with p53 stabilization in stressed cells. Ribosomal proteins L5, L11 and L23 have been shown to inhibit the Mdm2-mediated degradation of p53 (Lohrum et al., 2003; Dai and Lu, 2004; Jin et al., 2004; Zhang et al., 2004). The general mechanism controlling these proteins and their downstream effects on p53 could be the reorganization and disruption of the nucleolar structure upon cellular stress. Cells can tolerate relatively high amounts of DNA damage without stabilizing p53. On the other hand, nucleolar disruption alone, even in the absence of DNA damage, is able to stabilize p53 (Rubbi and Milner 2003b). The structural and functional integrity of the nucleoli was though proposed to be the main signal for p53 stress response (Rubbi and Milner, 2003b). Interference of rRNA processing has also been shown to lead to p53-dependent cell cycle arrest (Pestov et al., 2001). The possibility that nucleoli function in maintaining low p53 levels also fits to the regulation of p53 stability and nucleolar disassembly during the cell cycle (David-Pfeuty et al., 1996). Inhibition of the CDKs also leads to disruption of the nucleolar structure and accumulation of p53 (David-Pfeuty, 1999; David-Pfeuty et al., 2001). In accordance, we also found that NPM is translocated to nucleoplasm upon CDK2 inhibition by roscovitine treatment, with concomitant p53 stabilization (unpublished results). p53 can also be localized to nucleoli in unstressed cells. It is colocalized with the sites of rRNA transcription, suggesting that it can sense inhibition of transcription immediately even before disruption of the nucleolar structure (Rubbi and Milner, 2000). Alternatively, the nucleolar compartment could play a role in p53 degradation, as the ubiquitinated p53-Mdm2 complex has been proposed to travel through the nucleoli on its way to the proteasome machinery. The inhibition of this pathway upon various stress situations could thus affect the nucleolar structure. In stressed cells, NPM may be one of the main factors affecting p53 stability upon nucleolar reorganization. However, p53 is stabilized and activated in Npm-/- cells, suggesting that NPM may not be essential in the maintenance of p53 levels (Colombo et al., 2005; Grisendi et al., 2005). The deletion of *NPM* from mice resulted in p53 activation probably indirectly due to checkpoint activation in cells with mitotic abberations and DNA damage (Colombo et al., 2005). Some of the opposite results on the effect of NPM on p53 activity may result from different experimental settings and cell lines.

Modification of p53 by SUMO has been proposed to affect p53 transcriptional activity (Gostissa et al., 1999; Rodriguez et al., 1999). Conjugation of several proteins by SUMO has been linked to regulation of the cellular localizations, interactions with other proteins and stability of the proteins. We detected a slower migrating form of p53 in the insoluble fraction of the cells early after UV exposure, correlating kinetically with p53 protein complexes (I, Figure 5B).

The slower migrating p53 form corresponded to a SUMO-modified p53, migrating around 65 kDa. Although PML NBs have been linked to sumoylation of certain proteins and the 65 kDa form was detected at the same time frame with p53-PML interaction, we did not find any evidence for p53 translocation to NBs (I, data not shown). Alternatively the 65 kDa p53 form could be bound to the chromatin fraction. Due to the kinetics of the early interactions, NPM was tested for its ability to induce p53 sumoylation. Ectopic expression of NPM led to an increase in a slower migrating, sumoylated p53 form (II, Figure 4F). Furthermore, NPM preferred binding to the sumoylated p53 (II, Figure 4F) and associated with this form in UV-treated cells (II, Figure 4D & E). Although we could not find any evidence for p53 sumoylation taking place in NBs, PML could still potentiate the sumoylation event of p53 by acting as a platform for protein interactions, in other cellular compartment than PML NB.

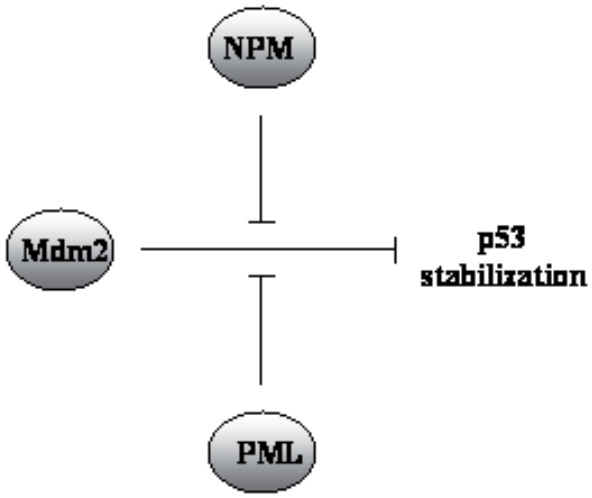

Figure 15. NPM and PML inhibit Mdm2-mediated degradation of p53. Both NPM and PML are regulators of p53 stability and may block the Mdm2-mediated degradation of p53 (Kurki et al., 2003; Louria-Hayon et al., 2003; Zhu et al., 2003; Bernardi et al., 2004; Kurki et al., 2004). NPM seems to be essential for p53 stabilization upon UV radiation (Colombo et al., 2002; Kurki et al., 2004), γ radiation (Colombo et al., 2002), polyamine depletion (Zou et al., 2005) as well as upon viral stress (Kurki et al., 2004). PML plays a role in p53 stabilization upon γ radiation (Louria-Hayon et al., 2003) and after exposure to cytotoxic drugs (Bernardi et al., 2004).

NPM is associated with p53 stabilization in viral insult (II)

NPM interacts with several viral proteins like Rev, HIV and Tat (Fankhauser et al., 1991; Miyazaki et al., 1995; Li, 1997). p53 function is also altered by several viral proteins, including viral cyclin (K-cyclin) (Verschuren et al., 2002). This viral protein is a cyclin-D homologue, encoded by the Kaposi´s sarcoma-associated herpesvirus (KSHV) and is known to induce p53 stabilization, concomitant

with its activation leading to either growth arrest or apoptosis (Verschuren et al., 2002). As NPM was required for p53 stabilization upon DNA damage, we wanted to assess its possible role in p53 stress response.

We transiently expressed K-cyclin in U2OS cells. Immunofluorescence analysis showed a major translocation of NPM to the nucleoplasmic fraction following expression of this protein (II, Figure 6A). This was also evident from the soluble fraction of the cells in western analysis (II, Figure 6B), while the total levels remained unaltered. NPM may be attracted to the nucleoplasmic fraction through its interaction with this viral protein (II, Figure 6C). Alternatively, the expression of this protein could affect the function of the nucleoli as nucleolus is targeted by several viral proteins (Hiscox, 2002). Thus, viral stress by K-cyclin may lead to release of the nucleolar proteins and promote NPM-K-cyclin interaction in the nucleoplasmic compartment.

The effect of K-cyclin expression on p53 levels was similar in U2OS cells as described before in MEFs (Verschuren et al., 2002), leading to stabilization of the protein (II, Figure 6D). Mdm2 protein was stabilized as well (II, Figure 6E). These inductions in the levels of p53 and Mdm2 were associated with increased interactions with NPM as well as decreased interaction between p53 and Mdm2, suggesting that also following this kind of cellular stress NPM is able to affect the negative pressure of Mdm2 on p53 (II, Figure 6D & E). As several viruses are able to target nucleoli (Hiscox, 2002) and adenovirus infection for instance blocks the rRNA synthesis (Castiglia and Flint, 1983), causing nucleoplasmic distribution of NPM (Matthews, 2001), the general pathway affecting p53 in the viral infections could take place through the interference of nucleolar functions.

PML controls the localization of p53 pathway proteins (I, III)

The plurifunctional PML NBs can be divided into subgroups according to their protein composition, size and movement. PML is able target several proteins with variable functions to PML NBs in a cell cycle phase and stress-dependent manner (Dellaire and Bazett-Jones, 2004). Nowadays over forty proteins are found in the database for PML NB proteins, many of these being RING finger proteins (Dellaire et al., 2003). As we had detected colocalization of Mdm2 with PML following DNA damage, inhibition of the proteasome and treatment with ATO, we addressed whether Mdm2 localization was altered by PML itself. Ectopically expressed PML III or IV was able to relocalize Mdm2 in a dose-dependent manner to large PML NB structures in a p53-null background (I, Figure 2). As we did not detect a strong interaction between Mdm2 and PML III in vitro, this relocalization could involve the endogenous PML IV isoform or other associated proteins. Interestingly, in studies of the capacity of PML to relocalize different Mdm2 deletion mutants, we found that Mdm2 lacking its nucleolar localization signal was found in the nucleoli in cells treated with a proteasome inhibitor only when PML was colocalizing with it, pointing towards a role of PML in Mdm2 nucleolar entry (I, data not shown). The nucleolar localization of Mdm2 is usually affected by its nucleolar localization signal (NoLS) in its C-terminal site (Lohrum et al., 2000), but

the results suggest that either PML or some PML associated protein is able to direct Mdm2 to this subnuclear compartment independently of its NoLS sequence. This observations was corroborated by a study of Bernardi et al. (2004), in which they showed the absence of Mdm2 nucleolar localization in stressed Pml-/- cells.

Additionally, PML was able to control the localization of NPM, relocalizing it from the nucleoli to either perinucleolar area, mature PML bodies or to nucleoplasm in a dose dependent manner (III, Figure 2D). NPM translocation was detected with PML III, PML IV, PML IV-3 (sumoylation defective mutant) and PML-RARα fusion protein (III, Figure 2D and data not shown). The immunofluorescence data further confirmed the in vitro data on association of NPM with several PML isoforms and suggested that NPM binding occurs through a common domain of different PML isoforms through its N-terminus. To further test whether PML could have a role in the UV-promoted translocation of NPM, we performed immunofluorescence stainings of the wt and Pml-/- MEFs. The data showed a striking difference already in unstressed Pml null and wt MEFs, NPM being prominently nucleoplasmic in the absence of PML (III, Figure 4A). Further, translocation following UV stress to the nucleoplasmic fraction was also not as evident as in wt MEFs, proposing a defect in the UV response of NPM. Additionally, NPM perinucleolar staining pattern, usually detected at the border of nucleoli within one hour in UV-damaged cells, was delayd in the Pml null cells. Whether this reflects a defect in the reorganization of the nucleolar structure upon transcriptional inhibition remains to be studied.

NPM has an essential role in the maintenance of genomic integrity and Npm-/- cells show more increased staining for γ-H2AX repair foci (Colombo et al., 2005). NPM is also linked to DNA repair and binds chromatin following IR-induced DSBs (Wu et al., 2002; Lee et al., 2005). To verify that the different subcellular localization of NPM in Pml-/- cells was not due to increased DNA- damage of the PML null cells, we performed immunofluorescence stainings with γ-H2AX (III, results not shown). The staining was comparable in wt and Pml null MEFs, suggesting that NPM translocation probably does not occur through a mechanism involving damaged DNA. However, PML may affect NPM localization directly, or alternatively, it could act as a platform protein, mediating some essential modifications or protein-protein interactions involved in the control of NPM localization and its stress response.

PML dictates NPM-chromatin association and NPM-p300 complex formation in DNA-damaged cells (III)

NPM has been shown to associate with histones (Okuwaki et al., 2001) and bind chromatin in γ radiated cells (Lee SY et al., 2005) A recent paper also suggested a role for NPM as a general transcriptional regulator through control of histone acetylation and nucleosomal disassembly (Swaminathan et al., 2005). We studied the possible association of NPM with chromatin in UV-treated U2OS cells (III, Figure 3A). The results showed that NPM is associated with chromatin, without a major change in this property after UV treatment of the cells. As

DNase treatment of the cells only partially released NPM from chromatin, this finding suggested that NPM may actually be more tightly bound to chromatin associated proteins than to DNA itself. In contrast, p53 was increasingly associated with chromatin upon UV radiation, and this association was clearly diminished in DNase-treated cells, in similar manner than the association of acetylated histones (AcH3, lysine9, respectively) with DNA (III, Figure 3A). Furthermore, AcH3 was found to be released from chromatin fractions even without DNase-treatment, in response to UV radiation, indicating a conversion of acetylated histones to a more soluble form upon DNA relaxation.

To address whether NPM association with histones was regulated upon UV treatment, we studied their interaction using coimmunoprecipitation analyses of UV-treated MEFs (III, Figure 3B). The results showed transient complex formation between AcH3 and NPM shortly after radiation. As NPM acetylation by p300 has been linked to its association with histones (Swaminathan et al., 2005) and p300 is also known to regulate chromatin structure through histone modifications in damaged cells (Chan and La Thangue, 2001), we tested a possible involvement of NPM in p300-complexes following UV treatment. p300-NPM interaction was also transiently increased in UV-treated cells, although it was clearly detectable already in control cells (III, Figure 3C). p53, known to bind p300 (Grossman, 2001), was also tested for its p300 interaction upon UV. Their complex formation was also enhanced early after radiation (unpublished results). The association of NPM and p300 was further increased by the presence of trichostatin A (TSA), a known histone deacetylase inhibitor or by overexpression of PML, suggesting that histone acetylation and PML could play a role in regulating this interaction (III, Figure D).

To verify whether PML could control the association of NPM with p300 and chromatin, we isolated chromatin from wt and Pml null MEFs. Regulation of NPM in UV-treated wt MEFs was similar to U2OS, and its nucleoplasmic levels increased upon UV radiation and there was no change in its association with the chromatin (III, Figure 4B). The increase in the nucleoplasmic NPM levels could be due to its translocation from the nucleoli upon rearrangement of the nucleolar structure (II, Figure 1A). Interestingly, this increase was not as evident in the samples of Pml-/- MEFs, suggesting a role for PML in the proper UV response of NPM. The results correlated well with the immunofluorescence data, showing that in Pml-/- MEFs NPM exists in a more soluble fraction, without any major changes in its localization pattern upon UV radiation (III; Figure 4A).

We further asked whether PML influences p300-NPM interaction (III, Figure 3D). Wt and Pml-/- MEFs behaved completely differently with respect of p300 binding upon radiation, as the NPM in wt MEFs transiently interacted with p300 within one hour timepoint and this same interaction was negligible in cells lacking PML (III, Figure 4C). It is therefore possible that PML controls the acetylation of NPM through its association with p300 and influences the histone and chromatin binding properties of NPM.

The dynamic movement of NPM is affected by UV radiation (III)

NPM is translocated to nucleoplasm in response to UV (II) and this translocation is associated with transient complex formation with p53, Mdm2, PML, p300 and AcH3 early after radiation, prior to p53 stabilization (II, III). To address whether these events affect the mobility and movement of NPM, we utilized optical manipulation of the cells using fluorescence recovery after photobleaching (FRAP). In previous studies NPM mobility in unstressed cells has been shown to be high (Phair and Misteli, 2000; Chen and Huang, 2001). We observed that the high mobility of this protein was transiently retained one hour after radiation (III, Figure 3E). The observed kinetics correlate well with the transient NPM protein complexes, suggesting that NPM interactions with its nucleoplasmic partners may affect its dynamic movements in response to UV radiation. Alternatively, the mobility of NPM could be retained by its association with chromatin (III, Figure 3B, C & D). The mobility of NPM was increased with one hour and further six hours after UV damage NPM was almost completely mobile in the nucleoplasm (III, Figure 3E and data not shown). This mobility correlated with the staining pattern, observed in immunofluorescence analysis that could be lost by pretreatment of the cells with NP-40 lysis buffer prior to staining with NPM antibodies (III, Figure 4A).

Hypothetical models for the function of early multiprotein complexes following UV (I, II, III)

The detected stress-induced interactions between p53, Mdm2, PML, NPM, p300 and AcH3 took place at similar kinetics after radiation, and could possibly indicate the formation of a multiprotein complex required for p53 stress response. Several possibilities for the function of these complexes exist. As the interactions occurred prior to p53 stabilization, they could be required for the stability of p53 by inhibiting Mdm2 E3 ligase activity or its interaction with p53. NPM is essential for the elevation in p53 levels in UV-stressed cells (II, Figure 5C). Interestingly, NPM localization is not affected by γ radiation (Syrjäkari et al., unpublished results) and the ubiquitination of p53 is also not affected following this treatment (Maki and Howley, 1997), indicating separate mechanisms leading to p53 stabilization following different kinds of DNA damage. We could not find any evidence for the role of PML in the UV-induced stabilization of p53. Still, PML could provide a platform for the protein-protein interactions and specific modifications, required for p53 stability or transcriptional activity. These interactions could also play a role in the regulation of p53 target gene selectivity. As p300 and AcH3 were also involved in the complexes, they could as well participate in the promoter-specific histone acetylation, required for the activation of specific p53 targets. p53-dependent histone acetylation of certain promoters, including p21 and PUMA, has been shown to occur upon p53 activation (Kaeser and Iggo 2004). In addition, it has been suggested that PML and PML NBs are involved in chromatin remodel-

ling and could also mediate the access to certain promoter regions (Seeler et al., 1998).

To effectively repair the UV-induced DNA damage, the lesions have to be recognized and repaired in the highly condensed chromatin fibers. Histone modifications like acetylation affect structure of the nucleosomes in a modification-dependent manner. This may lead to relaxation between the tight histone-DNA interface and facilitate binding of NER machinery to these sites. Histones are known to be acetylated in response to UV radiation (Ramanathan and Smerdon, 1986), and the repair of UV-induced lesions by NER is also associated with increased histone acetylation (Brand et al., 2001). Specific chromatin accessibility factors are required for this task. p53 was shown to act in this manner and to mediate histone acetylation in a p300-dependent way upon UV damage (Rubbi and Milner, 2003a). Tumor cells lacking functional p53 also have lower levels of acetylated histone H3. The basal acetylation of K9 residue of H3 and increase in acetylation of K9 and K14 after UV damage have been shown to be affected by p53 (Rubbi and Milner, 2003; Allison and Milner, 2003). A recent paper also showed the p53-dependent increase in K9 H3 acetylation upon UV damage in *Drosophila* (Rebollar et al., 2006), suggesting that this function of p53 is conserved. Further, p53 can tether Mdm2 to chromatin, where it is able to bind histones and promote monoubiquitination H2A and H2B through its RING-domain (Minsky and Oren, 2004). PML and NPM have also been proposed to play a role in chromatin modifications due to their protein associations. NPM binds histones and controls their acetylation (Okuwaki et al., 2001; Swaminathan et al., 2005). PML on the other hand interacts with HDACs and p300 and has been linked to both condensation and decondensation of the chromatin structure (LaMorte et al., 1998; Doucas et al., 1999; Von Mikecz et al., 2000; Bandobashi et al., 2001; Boisvert et al., 2001; Wu et al., 2001). PML could as well control chromatin structure and act as a chromatin accessibility factor in UV-radiated cells. Given that the association of NPM with p300 is PML dependent (III, Figure 4C) and that PML controls NPM localization in UV-treated cells (III, Figure 4A) as well as forms transient complexes with it and p53 (I, Figure 5A; III, Figure IA), the apparent multiprotein complex early after radiation could possibly have a role in modifications of the chromatin structure.

p53 is involved in the repair of UV-induced DNA lesions throug affecting NER functions by several ways. p53 also controls the localization of PML to the sites of DNA damage and nucleotide excision repair in UV-treated cells (Seker et al., 2003). PML itself and PML NBs have been proposed to act as damage sensors and control the release and localizations of many different repair proteins (Dellaire and Bazett-Jones, 2004), in this way linking it either directly or indirectly to the repair processes. NPM also has the ability to promote repair of lesions by upregulating PCNA protein (Wu et al., 2002) and through regulating the localization of GADD45 (Gao et al., 2005), although evidence about a direct association to the lesion sites is missing. The possibility that the detected early NPM protein complexes (I, II, III) could affect repair functions, exists, although a direct involvement in the repair process seems quite unlikely.

PML-RARα fusion protein in acute promyelocytic leukemia cells affects the normal function and localization of NPM (III)

Acute promyelocytic leukemia is most often caused by a translocation between *PML* and *RAR*α genes (de The et al., 1990; Goddard et al., 1991; Kakizuka et al., 1991; Pandolfi et al., 1991). In some cases, the partner with RARα in the fusion protein can also be PLZF, NPM, NuMA or Stat5b (Redner, 2002). However, these different subtypes of leukemia display different cytomorphological features as well as penetrance of the disease in transgenic mouse models (Rego et al., 2006). The NPM-RARα fusion protein in APL does not interact with PML and localizes to nucleoli, probably affecting the normal NPM functions and the development of APL (Rego et al., 2006; Rush et al., 2006). During this study we observed the association of PML-RARα with NPM (III) and became interested in their relationship in the development of APL. Even though both NPM and PML have been linked to hematological malignancies and are associated with APL, this connection has not been studied before.

We used a APL cell line, NB4, which has a wt PML allele in addition to the fusion protein PML-RARα (Lanotte et al., 1991). Even though p53 mutations are extremely rare in this type of cancer, p53 status in NB4 cells is mutant (Fleckenstein et al., 2002). Correlating with this status, the levels of p53 were also very high in this cell line, as observed by Western blot experiments (III, Figure 5A). Interestingly, the NPM levels were comparable to other tumor cell lines, A375 and U2OS, but it was prominently localized to the nucleoplasm (III, Figure 5B). This could be due to its sequestration by nucleoplasmic PML-RARα (III, Figure 5B), which clearly formed complexes with NPM (III, Figure 5C). The findings are concordant with the in vitro interaction data and immunofluorescence data showing that the effect of PML-RARα expression on NPM localization pattern (III, Figure 2A, B & D). Nucleolar NPM localization may be crucial for its proper function, as mutant NPMc+ expression disrupts the ARF pathway (Falini et al., 2005), suggesting that the capacity of PML-RARα to sequester NPM may alter cellular functions and subject the neoplastic transformation.

The APL phenotype can be reversed by ATO and RA treatments, which induce degradation of the fusion protein and clinical remission in most APL patients (Zhu J et al., 2001). To verify whether the abnormal localization of NPM in the NB4 APL-cells is due to PML-RARα expression, we treated the cells with these drugs and analyzed NPM localization. The nucleolar staining of NPM became more intense in the ATO- and RA-treated cells without any change in its protein levels, suggesting that a relocalization event occurs in response to PML-RARα degradation (III, Figure 6 A& B). ATO treatment induced an almost complete degradation of the cellular PML and PML-RARα, while RA treatment promoted the formation of normal PML NB structures (III, Figure 6A&B). Immunoprecipitation experiments from cells with similar treatments revealed that RA promoted complex formation between p53, NPM, AcH3 and p300 (III, Figure 6C) and that this was associated with p53 activation as determined by luciferase reporter assays in the presence of exogenous p53 (III, Figure 6D) (Figure 14.). Based on

these results we propose a role for this multiprotein complex in the activation of p53 pathway following RA treatment of APL. Whether PML acts as a crucial factor in the activation of p53 upon this treatment, remains to be studied.

APL cells, expressing PML-RARα, are blocked at the promyelocyte phase. Enhanced co-repressor binding of PML-RARα and inhibition of the RA-pathway have been proposed to result in this differentiation block and APL development (Grignani et al., 1993; Grignani et al., 1998; He et al., 1998; Lin & Evans, 2000; Lin et al., 1998; Minucci et al., 2000). PML–RARα maintains a more condensed chromatin structure due to its association with co-repressor complexes together with HDACs and inactivates target genes by these means (Wu et al., 2001; Segalla et al., 2003). Additionally, the fusion protein maintains the silenced chromatin state by recruiting DNA methyltransferase activities (Villa et al., 2006). Expression of PML-RARα also promotes relocalization of PML from PML NBs, affecting the normal functions of these structures (Dyck et al., 1994; Koken et al., 1994; Weis et al., 1994) and the apoptotic pathways that PML and PML NB proteins are involved in (Takahashi et al., 2004). Furthermore, tumor suppressor protein p53 has been suggested to be inactivated in APL by the complex of PML-RARα and HDACs, blocking the proper p53 response in these cells (Insinga eta al., 2004). Given that HDACs would be the only player in p53 inactivation in these types of cancer, one could expect to see p53-mediated apoptosis in response to histone deacetylase treatment. However, TSA promotes p53-independent apopotosis and we could not detect any p53 activation upon this treatment (III, results not shown). On the other hand, another histone deacetylase inhibitor, RA, was able to induce formation of a multiprotein complex, associated with the activation of p53 pathway (III, Figure 6C & D). Although NPM overexpression has been shown to decrease the sensitivity of human leukemia cells (HL-60) to retinoic-acid-induced differentiation and apoptosis (Hsu and Yung, 1998; Hsu and Yung, 2000; Yung, 2004), we find here that it could possibly act as an activating component of the p53 pathway. Histone modifications and alterations in the chromatin structure seem to be one of the outcomes in p53 binding to its target sequences upon stress. Our findings indicate that PML-RARα, in addition to its various effects on chromatin structure, also disrupts the normal localization and function of nucleolar protein NPM, possibly having an effect in the association of p53 with histone modifying factors and blocking the activation of p53 pathway in APL.

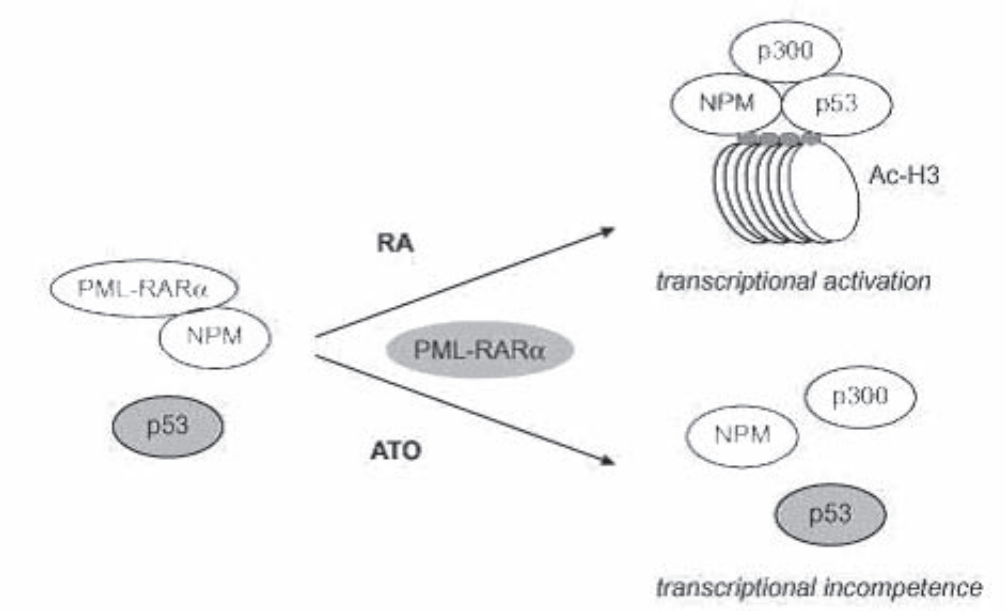

Figure 16. Model for p53 activation in APL cells. NPM associates constitutively with PML-RARα, and is dissociated from this inhibitory interaction by ATO and RA induced degradation of the fusion protein. RA treatment leads to formation of normal PML NBs, and binding and activation of p53 through its interactions with NPM, AcH3 and p300. ATO does not support PML NB formation or interactions between p53, NPM and p300.

CONCLUSIONS

Cancerous cells can be potentially destroyed by activation of the p53 pathway. This thesis work has aimed in finding new contributors to p53 stabilization and activation as well as unravelling the molecular mechanisms behind them. Although many p53 inducing agents have been shown to decrease the levels of p53 negative regulator Mdm2 and in this way lead to p53 accumulation (Wu and Levine, 1997; Arriola et al., 1999; Ashcroft et al., 2000; Inoue et al., 2001; Wang et al., 2002), the stabilization of p53 following transcriptional inhibition does not occur due to diminished Mdm2 protein levels (Ashcroft et al., 2000; O´Hagan and Ljungman, 2004). The blockage of the p53-Mdm2 interface by modifications and newly formed interactions, followed by UV-induced transcriptional inhibition, plays a major role in the regulation of p53 response in this type of damage .

Transcriptional inhibition by UV exposure of the cells promoted subcellular translocations of the p53 pathway proteins Mdm2, NPM and PML, but not p53 itself. The subsequent rapid and transient interactions of NPM and PML with each other as well as with p53 and Mdm2 could be prerequisite for the induction of a proper p53 cellular response. Although, the transcriptional inhibition leading to nucleolar stress response and concomitant release of NPM was found to be essential for p53 stabilization, the exact function of the potential multiprotein complex between p53, Mdm2, NPM and PML is currently not clear and needs to be verified in future studies. The fact that Mdm2 and PML are associated with the nucleolar compartment upon cellular stress, underlines the importance of this subnuclear organelle in the regulation of p53 pathway and suggests that cellular compartmentalization is important in this type of damage response.

NPM and PML are often altered in hematological malignancies and could thus contribute to the oncogenesis through alterations in the p53 pathway. Moreover, PML exerts control over the cellular localization of NPM, its UV response and association with chromatin binding factor p300, events which are disrupted in cells lacking functional PML. The pathogenesis of APL could so be affected through NPM inactivation. The relevance of the UV-induced interactions between these particular p53 pathway proteins is underscored by the finding that therapeutically relevant RA, reversing the APL phenotype, induced similar complexes with p53 and its partners leading to transcriptional activation of p53. The transcriptional changes by UV radiation or oncogenic PML-RARα could thus be overcome by p53 association with NPM and PML, emphasizing their importance in the regulation of the p53 pathway. To address whether NPM and PML dysregulation influences p53 function in other types of cancers and whether they could have potential as therapeutic targets, will have to be determined by future work.

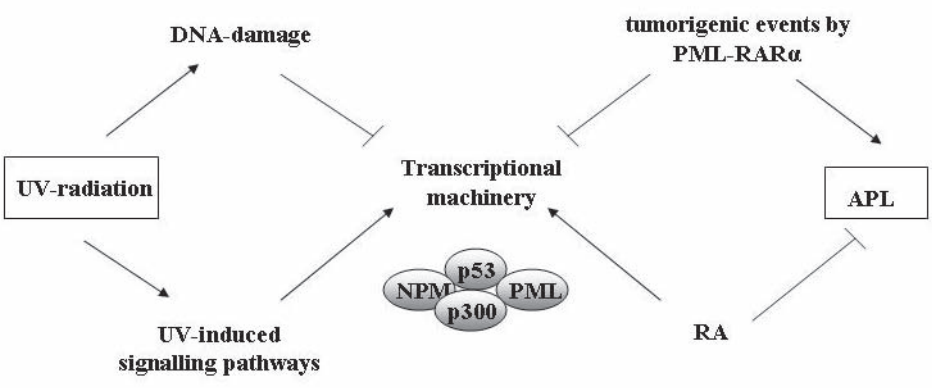

Figure 17. Hypothetical model of the RA- and UV-induced p53 complex. Transcriptional inhibition by UV-induced damage as well as PML-RARα promoted transcriptional repression in APL is overcome by the formation of a hypothetical p53-NPM-PML-p300 multiprotein complex, associated with the recovery of transcriptional competence. APL, acute promyelocytic leukemia; RA, retinoic acid.

ACKNOWLEDGEMENTS

This work was carried out in the Molecular and Cancer Biology Research Program, Biomedicum Helsinki and Department of Virology, Haartman Institute, University of Helsinki. I wish to thank Prof. Kari Alitalo, the head of the Program, for the great working facilities, atmosphere and scientific events during these past years. This has been an excellent chance for me to carry out graduate research.

I am most grateful to my supervisor Prof. Marikki Laiho for the opportunity to work in her group. I have really enjoyed working in this exciting and sometimes maybe too competitive field. I want to thank for this excellent learning experience and for the freedom to work with my research interests. It has been a privilege to carry out research under your guidance.

I also wish to thank my thesis committee members Prof. Jorma Palvimo and Doc. Ismo Ulmanen for their valuable comments and suggestions concerning this study. Ismo Ulmanen is also thanked for thoughtfully reviewing the thesis manuscript. I also wish to express my thanks to Prof. Kirsi Vähäkangas for being the second reviewer of this thesis and for the constructive comments on the manuscript.

It has been a great pleasure to collaborate with our neighbours, Doc. Päivi Ojala and her group member Grzegorz Sarek. Thank you for your ideas and participation to the NPM-K cyclin studies! I am also most grateful to Dr. David Meek for his contribution to the NPM work.

I want to thank all the people in the Laiho lab! The old members of the lab, Annari, Hanna, Virpi, Stina, Jiri, Fan, Max, Marilotta, Anni-Helena, Annika, Riikka, Runar and the present members, Arja, Henna, Karita, Leena, Maija, Mia, Päivi, Sari J and Taija. Thank you for making the atmosphere in the lab so warm and cheerful (especially on Friday afternoons). Thank you for all your help, ideas and company during these years. It has been a pleasure working with you all and I wish you all the best in the future – in and outside the lab.

A special thanks to Maija, Annari and Virpi for helping me with numerous experiments. I could not have done this work without your help.

Leena, thank you for your company and all the science and not-so-science conversations during this time. I have learned a lot from you and wish you the best in your future work! Also, thank you for the hilarious travel company ☺. Mia, thank you for being a great help and always knowing the answer to every question in the lab. Thank you for also sharing the same interests in our furry friends. I wish you all the best with your work - you really deserve it. Karita, thank you

for your contribution to the NPM study, without your help I would probably still be running those Westerns…Henna – thank you for participating in the project during the last two years! And thank you for doing those "final experiments" over and over again…You are such a hard-working person, I know you will get far with your enthusiasm.

I wish to thank all my friends and relatives for their interest in my work! I thank my parents, my late mother Anita and my father Jorma, my brother Sami and his family. A special thanks to my husband Toni who has been forced to learn about PML and the life of p53 during our long Sauna-conversations… thank you for putting up with me even during the tougher times! And I cannot forget about my doggy friends Nero, Susi, Ronja, Mio, Mosse and Mini. Thank you for forcing me to get some fresh air and exercise once in a while! Last but not least I want to thank my little son Samu for making a deadline for this book ☺.

I am grateful to the Graduate School of Biotechnology and Molecular Biology for providing me salary and excellent courses during four years of my study. This work is also supported by the Finnish Cancer Foundation, the Finnish Cultural Foundation, Paulo Foundation, Ida Montin Foundation, K. Albin Johansson Foundation, Oskar Öflund Foundation, Magnus Ehrnrooth Foundation and Orion Pharmos Research Foundation.

Helsinki, January 2007

REFERENCES

Abraham RT. (2001) Cell cycle checkpoint sigmaling through the ATM and ATR kinases. *Gened Dev.* **15**:2177-2196.

Adhikary S, Eilers M. (2005) Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol*. **6**:635-645.

Adimoolam S, Ford JM. (2002) p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. *Proc Natl Acad Sci U S A*. **99**:12985-12990.

Ahn MJ, Nason-Burchenal K, Moasser MM, Dmitrovsky E. (1995) Growth suppression of acute promyelocytic leukemia cells having increased expression of the non-rearranged alleles: RAR alpha or PML. *Oncogene.***10**:2307-2314.

Ahn JH, Hayward GS. (1997) The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. *J Virol.* **71**:4599-4613.

Ahn J, Prives C. (2001) The C-terminus of p53: the more you learn the less you know. *Nat Struct Biol.* **8**:730-732.

Ahn JY, Liu X, Cheng D, Peng J, Chan PK, Wade PA, Ye K. (2005) Nucleophosmin/B23, a nuclear PI(3,4,5)P(3) receptor, mediates the antiapoptotic actions of NGF by inhibiting CAD. *Mol Cell*.**18**:435-445.

Al-Baker EA, Boyle J, Harry R, Kill IR. (2004) A p53-independent pathway regulates nucleolar segregation and antigen translocation in response to DNA damage induced by UV irradiation. *Exp Cell Res***. 292**:179-186.

Alcalay, M., Tomassoni, L., Colombo, E., Stoldt, S., Grignani, F., Fagioli, M., Szekely, L., Helin, K., Pelicci, P.G. (1998) The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. *Mol Cell Biol.* **18**:1084-1093.

Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, Meani N, Diverio D, Bernard L, Tizzoni L, Volorio S, Luzi L, Colombo E, Lo Coco F, Mecucci C, Falini B, Pelicci PG. (2005) Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. *Blood*.**106**:899-902.

Amundson SA, Patterson A, Do KT, Fornace AJ Jr. (2002) A nucleotide excision repair master-switch: p53 regulated coordinate induction of global genomic repair genes. *Cancer Biol Ther.* **1**:145-149.

Andersen JS, Lam YW, Leung AK, Ong SE, Lyon CE, Lamond AI, Mann M. (2995) Nucleolar proteome dynamics. *Nature*. **433**:77-83.

Anderson ME, Woelker B, Reed M, Wang P, Tegtmeyer P. (1997) Reciprocal interference between the sequence-specific core and nonspecific C-terminal DNA binding domains of p53: implications for regulation. *Mol Cell Biol.* **17**:6255-6264.

Appella E, Anderson CW. (2001) Post-translational modifications and activation of p53 by genotoxic stresses. *Eur J Biochem.* **268**:2764-2772.

Arriola EL, Lopez AR, Chresta CM. (1999) Differential regulation of p21waf-1/cip-1 and Mdm2 by etoposide: etoposide inhibits the p53-Mdm2 autoregulatory feedback loop. *Oncogene.* **18**:1081-1091.

Arrowsmith CH, Morin P. (1996) New insights into p53 function from structural studies. *Oncogene.* **12**:1379-1385.

Asahara H, Li Y, Fuss J, Haines DS, Vlatkovic N, Boyd MT, Linn S. (2003) Stimulation of human DNA polymerase ε by Mdm2. *Nucleic Acids Res.* **31**:2451-2459.

Ascoli CA, Maul GG. (1991) Identification of a novel nuclear domain. *J Cell Biol.* **112**:785-795.

Ashcroft M, Kubbutat MH, Vousden KH. (1999) Regulation of p53 function and stability by phosphorylation. *Mol Cell Biol.* **19**:1751-1758.

Ashcroft M, Taya Y, Vousden KH. (2000) Stress signals utilize multiple pathways to stabilize p53. *Mol Cell Biol*. **20**:3224-3233.

Atadja P, Wong H, Garkavtsev I, Veillette C, Riabowol K. (1995) Increased activity of p53 in senescing fibroblasts. *Proc Natl Acad Sci USA*. **92**:8348-8352.

Ayed A, Mulder FA, Yi GS, Lu Y, Kay LE, Arrowsmith CH. (2001) Latent and active p53 are identical in conformation. *Nat Struct Biol.* **8**:756-760.

Bakalkin G, Selivanova G, Yakovleva T, Kiseleva E, Kashuba E, Magnusson KP, Szekely L, Klein G, Terenius L, Wiman KG. (1995) p53 binds single-stranded DNA ends through the C-terminal domain and internal DNA segments via the middle domain. *Nucleic Acids Res.* **23**:362-369.

Bandobashi K, Maeda A, Teramoto N, Nagy N, Szekely L, Taguchi H, Miyoshi I, Klein G, Klein E. (2001) Intranuclear localization of the transcription coadaptor CBP/p300 and the transcription factor RBP-Jk in relation to EBNA-2 and -5 in B lymphocytes. *Virology*. **288**:275-282.

Bao-Lei T, Zhu-Zhong M, Yi S, Jun-Jie Q, Yan D, Hua L, Bin L, Guo-Wei Z, Zhi-Xian S. (2006) Knocking down PML impairs p53 signaling transduction pathway and suppresses irradiation induced apoptosis in breast carcinoma cell MCF-7. *J Cell Biochem.* **97**:561-571.

Baptiste N, Friedlander P, Chen X, Prives C. (2002) The proline-rich domain of p53 is required for cooperation with anti-neoplastic agents to promote apoptosis of tumor cells. *Oncogene.* **21**:9-21.

Bartek J, Lukas J. (2001a) Pathways governing G1/S transition and their response to DNA damage. *FEBS Lett*. **490**:117-122.

Bartek J, Lukas J. (2001b) Mammalian G1- and S-phase checkpoints in response to DNA damage. *Curr Opin Cell Biol*. **13**:738-747.

Bartel F, Taubert H, Harris LC. (2002) Alternative and aberrant splicing of MDM2 mRNA in human cancer. *Cancer Cell.* **2**:9-15.

Bates S, Phillips AC, Clark PA, Stott F, Peters G, Ludwig RL, Vousden KH. p14ARF links the tumour suppressors RB and p53. *Nature*. **395**:124-125.

Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. (2003) Reversal of human cellular senescence: roles of the p16 and p53 pathways. *EMBO J.* **22**:4212-4222.

Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA. (1999) Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science*. **286**:2528-2531.

Bellodi C, Kindle K, Bernassola F, Dinsdale D, Cossarizza A, Melino G, Heery D, Salomoni P. (2006) Cytoplasmic Function of Mutant Promyelocytic Leukemia (PML) and PML-Retinoic Acid Receptor-{alpha}*J Biol Chem.* **281**:14465-14473.

Benchimol S. (2001) p53-dependent pathways of apoptosis. *Cell Death Differ.* **8**:1049-1051.

Bennett M, Macdonald K, Chan SW, Luzio JP, Simari R, Weissberg P. (1998) Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. *Science.* **282**:290-293.

Bergamaschi D, Samuels-Lev Y, O´Neil N, Trigiante G, Crook T, Hsieh JK, O´Connor DJ, Zhong S, Compargue I, Tomlinson M, Kuwabara P, Lin X. (2003) iASSP oncoprotein is key inhibitor of p53 conserved from worm to human. *Nat Genet.* **33**:162-167.

Berger M, Stahl N, Del Sal G, Haupt Y. (2005) Mutations in proline 82 of p53 impair its activation by Pin1 and Chk2 in response to DNA damage. *Mol Cell Biol.* **25**:5380-5388.

Berger R, Busson M, Baranger L, Helias C, Lessard M, Dastugue N, Speleman F. (2006) Loss of the NPM1 gene in myeloid disorders with chromosome 5 rearrangements. *Leukemia*. **20**:319-321.

Bernard K, Litman E, Fitzpatrick JL, Shellman YG, Argast G, Polvinen K, Everett AD, Fukasawa K, Norris DA, Ahn NG, Resing KA. (2003) Functional proteomic analysis of melanoma progression. *Cancer Res.* **63**:6716-6725.

Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KH, Pandolfi PP. (2004) PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat Cell Biol*. **6**:665-672.

Bernstein RM, Neuberger JM, Bunn CC, Callender ME, Hughes GR, Williams R. (1984) Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. *Clin Exp Immunol*. **55**:553-650.

Bertrand P, Saintigny Y, Lopez BS. (2004) p53´s double life: transactivation-independent repression of homolous recombination. *Trends Genet.* **20**:235-243.

Bertwistle D, Sugimoto M, Sherr CJ. (2004) Physical and functional interactions of the ARF tumor suppressor protein with nucleophosmin/B23. *Mol Cell Biol.* **24**:985-996.

Bill CA, Yu Y, Miselis NR, Little JB, Nickoloff JA. (1997) A role for p53 in DNA end rejoining by human cellular extracts. *Mutat Res.* **385**:21-29.

Blander G, Kipnis J, Leal JFM, Yu CE, Schellenberg GD, Oren M. (1999) Physical and functional interaction between p53 and Werner´s syndrome protein. *J Biol Chem.* **274**:29463-29469.

Blattner C, Tobiasch E, Litfen M, Rahmsdorf HJ, Herrlich P. (1999) DNA damage induced p53 stabilization: no indication for an involvement of p53 phosphorylation. *Oncogene.* **18**:1723-1732.

Blattner C, Hay T, Meek DW, Lane DP. (2002) Hypophosphorylation of Mdm2 augments p53 stability. *Mol Cell Biol.* **22**:6170-6182.

Bode AM, Dong Z. (2003) Mitogen-activated protein kinase activation in UV-induced signal transduction. *Sci STKE*. **2003**:RE2.

Boddy MN, Howe K, Etkin LD, Solomon E, Freemont PS. (1996) PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. *Oncogene.* **13**:971-982.

Bodmer WF, Bailey CJ, Bodmer J, Bussey HJ, Ellis A, Gorman P, Lucibello FC, Murday VA, Rider SH, Scambler P, et al. (1987) Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature.* **328**:614-616.

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. (1998) Extension of life-span by introduction of telomerase into normal human cells. *Science.* **279**:349-352.

Boehden GS, Akyuz N, Roemer K, Wiesmuller L. (2003) p53 mutated in the transactivation domain retains regulatory functions in homology-directed double-strand break repair. *Oncogene*. **22**:4111-4117.

Boisvert FM, Hendzel MJ, Bazett-Jones DP. (2000) Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. *J Cell Biol.* **148**:283-292.

Boisvert FM, Kruhlak MJ, Box AK, Hendzel MJ, Bazett-Jones DP. (2001) The transcription coactivator CBP is a dynamic component of the promyelocytic leukemia nuclear body. *J Cell Biol.* **152**:1099-1106.

Bond J, Haughton M, Blaydes J, Gire V, Wynford-Thomas D, Wyllie F. (1996) Evidence that transcriptional activation by p53 plays a direct role in the induction of cellular senescence. *Oncogene.* **13**:2097-2104.

Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell*. **119**:591-602.

Boon K, Caron HN, van Asperen R, Valentijn L, Hermus MC, van Sluis P, Roobeek I, Weis I, Voute PA, Schwab M, Versteeg R. (2001) N-myc enhances the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis. *EMBO J.* **20**:1383-1393.

Bootsma D. (1993) The genetic defect in DNA repair deficiency syndromes. *Eur J Cancer.* **29A**:1482-1488.

Bor AM, Chang FJ, Yung BY. (1992) Phosphoprotein B23 translocation and modulation of actinomycin D and doxorubicin cytotoxity by dipyridamole in HeLa cells. *Int J Cancer.* **52**:658-663.

Borden KL, Boddy MN, Lally J, O'Reilly NJ, Martin S, Howe K, Solomon E, Freemont PS. (1995) The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. *EMBO J.* **14**:1532-1541.

Borden KL. (2002) Pondering the promyelocytic leukemia protein (PML) puzzle: possible functions for PML nuclear bodies. *Mol Cell Biol*. **22**:5259-5269.

Borer RA, Lehner CF, Eppenberger HM, Nigg EA. (1989) Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell.* **56**: 379-390.

Bottger A, Bottger V, Garcia-Echeverria C, Chene P, Hochkeppel HK, Sampson W, Ang K, Howard SF, Picksley SM, Lane DP. (1997) Molecular characterization of the hdm2-p53 interaction. *J Mol Biol.* **269**: 744-756.

Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP, Saville MK, Lane DP. (2005) p53 isoforms can regulate p53 transcriptional activity. *Genes Dev.* **19**:2122-2137.

Bowman KK, Sicard DM, Ford JM, Hanawalt PC. (2000) Reduced global genomic repair of ultraviolet light-induced cyclobutane pyrimidine dimers in simian virus 40-transformed human cells. *Mol Carcinog*. **29**:17-24.

Brady SN, Yu Y, Maggi LB Jr, Weber JD. (2004) ARF imbedes NPM/B23 shuttling in an Mdm2-sensitive tumor suppressor pathway. *Mol Cell Biol.* **24**:9327-9338.

Brain R, Jenkins JR. (1994) Human p53 directs DNA strand reassociation and is photolabelled by 8-azido ATP. *Oncogene*. **9**:1775-1780.

Brand M, Moggs JG, Oulad-Abdelghani M, Lejeune F, Dilworth FJ, Stevenin J, Almouzni G, Tora L. (2001) UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. *EMBO J*. **20**:3187-3196.

Brinkley BR, Goepfert TM. (1998) Supernumerary centrosomes and cancer: Boveri's hypothesis resurrected. *Cell Motil Cytoskeleton.* **41**:281-288.

Brodsky MH, Nordstrom W, Tsang G, Kwan E, Rubin GM, Abrams JM. (2000) Drosophila p53 binds a damage response element at the reaper locus. Cell. **101**:103-113.

Brown D, Kogan S, Lagasse E, Weissman I, Alcalay M, Pelicci PG, Atwater S, Bishop JM. (1997) PMLRARalpha transgene initiates murine acute promyelocytic leukemia. *Proc Natl Acad Sci U S A*. **94**:2551-2556.

Brown DR, Thomas CA, Deb SP. (1998) The human oncoprotein MDM2 arrests the cell cycle: elimination of its cell-cycle-inhibitory function induces tumorigenesis. *EMBO J.* **17**:2513-2525.

Bullock AN, Henckel J, DeDecker BS, Johnson CM, Nikolova PV, Proctor MR, Lane DP, Fersht AR. (1997) Thermodynamic stability of wild-type and mutant p53 core domain. *Proc Natl Acad Sci U S A.* **94**:14338-14342.

Cahilly-Snyder L, Yang-Feng T, Francke U, George DL. (1987) Molecular analysis and chromosomal mapping of amplified genes isolated from a transformed mouse 3T3 cell line. *Somat Cell Mol Genet*.**13**:235-244.

Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, Appella E, Kastan MB, Siliciano JD. (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science*. **281**:1677-1679.

Cannon-Albright L, Goldgar D, Neuhausen S, Gruis N, Anderson D, Lewis CM, Jost M, Tran D, Nyguen K, Kamb A. et al., (1994) Localization of the 9p melanoma susceptibility locus (MLM) to a 2-cM region between D9S736 and D9S171. *Genomics*. **23**:265-268.

Carmo-Fonseca M, Mendes-Soares L, Campos I. (2000) To be or not to be in the nucleolus. *Nat Cell Biol.* **2**:E107-E112.

Castiglia CL, Flint SJ. (1983) Effects of adenovirus infection on rRNA synthesis and maturation in HeLa cells. *Mol Cell Biol.* **3**:662-671.

Chan WY, Liu QR, Borjigin J, Busch H, Rennert OM, Tease LA, Chan PK. (1989) Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. *Biochemistry*. **28**:1033-1039.

Chan PK, Aldrich MB, Yung B. (1987) Nucleolar protein B23 translocation after doxorubicin treatment in murine tumor cells. *Cancer Res.* **47**:3798-3801.

Chan PK. (1992) Characterization and cellular localization of nucleophosmin/B23 in HeLa cells treated with selected cytotoxic agents (Studies of B23-translocation mechanism). *Exp Cell Res.* **203**:174-181.

Chan PK, Qi Y, Amley J, Koller CA. (1996) Quantitation of the nucleophosmin/B23-translocation using imaging analysis. *Cancer Lett.* **100**:191-197.

Chan HM, La Thangue NB. (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *J Cell Sci.***114**:2363-2373.

Chan WM, Siu WY, Lau A, Poon RY. (2004) How many mutant p53 molecules are needed to inactivate a tetramer? *Mol Cell Biol.* **24**:3536-3551.

Chan HJ, Weng JJ, Yung BY. (2005) Nucleophosmin/B23-binding peptide inhibits tumor growth and up-regulates transcriptional activity of p53. *Biochem Biophys Res Commun.* **333**:396-403.

Chang JH, Olson MO. (1989) A single gene codes for two forms of rat nucleolar protein B23 mRNA. *J Biol Chem.* **264**:11732-11737.

Chang JH, Olson MO. (1990) Structure of the gene for rat nucleolar protein B23. *J Biol Chem*. **265**:18227-18233.

Chang MS, Sasaki H, Campbell MS, Kraeft SK, Sutherland R, Yang CY, Liu Y, Auclair D, Hao L, Sonoda H, Ferland LH, Chen LB. (1999) HRad17 colocalizes with NHP2L1 in the nucleolus and redistributes after UV irradiation. *J Biol Chem.* **274**:36544-36549.

Chehab NH, Malikzay A, Appel M, Halazonetis TD. (2000) Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53.*Genes Dev*. **14**:278-288.

Chelbi-Alix MK, de The H. (1999) Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins. *Oncogene*. **18**:935-941.

Chen J, Marechal V, Levine AJ. (1993) Mapping of the p53 and mdm-2 interaction domains. *Mol Cell Biol.* **13**:4107-4114.

Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z. (1996) In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia: As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. *Blood.* **88**:1052-1061.

Chen X, Ko LJ, Jayaraman L, Prives C. (1996) p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.* **10**:2438-2451.

Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, Han ZG, Ni JH, Shi GY, Jia PM, Liu MM, He KL, Niu C, Ma J, Zhang P, Zhang TD, Paul P, Naoe T, Kitamura K, Miller W, Waxman S, Wang ZY, de The H, Chen SJ, Chen Z. (1997) Use of ar-

senic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): I. As2O3 exerts dose-dependent dual effects on APL cells.*Blood*. **89**:3345-3353.

Chen D, Huang S. (2001) Nucleolar components involved in ribosome biogenesis cycle between the nucleolus and nucleoplasm in interphase cells. *J Cell Biol.* **153**:169-176.

Chen L, Chen J. (2003) Mdm2-ARF complex regulates p53 sumoylation. *Oncogene*. **22**:5348-5357.

Chen D, Li M, Luo J, Gu W. (2003) Direct interactions between HIF-1alpha and Mdm2 modulate p53 function. *J Biol Chem.* **278**:13595-13598.

Chen LF, Greene WC. (2004) Shaping the nuclear action of NF-kappaB. *Nat Rev Mol Cell Biol.* **5**:392-401.

Chen J and Sadowski I. (2005) Identification of the mismatch repair genes PMS2 and MLH1 as p53 target genes by using serial analysis of binding elements. *Proc Natl Acad Sci USA.* **102**:4813-4818.

Chen D, Kon N, Li M, Zhang W, Qin J, Gu W. (2005) ARF-BP1/Mule is a critical mediator of the ARF tumor suppressor. *Cell.* **121**:1071-1083.

Cheung KJ Jr, Mitchell D, Lin P, Li G. (2001) The tumor suppressor candidate p33(ING1) mediates repair of UV-damaged DNA. *Cancer Res.* **61**:4974-4977.

Cheung KJ Jr, Li G. (2002) p33(ING1) enhances UVB-induced apoptosis in melanoma cells. *Exp Cell Res.* **279**:291-298.

Chiarle R, Gong JZ, Guasparri I, Pesci A, Cai J, Liu J, Simmons WJ, Dhall G, Howes J, Piva R, Inghirami G. (2003) NPM-ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. *Blood*. **101**:1919-1927.

Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR. (2004) Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science*. **303**:1010-1014.

Chipuk JE, Green DR. (2006) Dissecting p53-dependent apoptosis. *Cell Death Differ*. 13:994-1002.

Cho Y, Gorina S, Jeffrey PD, Pavletich NP. (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science*. **265**:346-355.

Classon M, Harlow E. (2002) The retinoblastoma tumour suppressor in development and cancer. *Nat Rev Cancer.* **2**:910-917.

Clore GM, Ernst J, Clubb R, Omichinski JG, Kennedy WM, Sakaguchi K, Appella E, Gronenborn AM (1995) Refined solution structure of the oligomerization domain of the tumor suppressor p53. *Nat Struc Biol*. **2**:253-254.

Clydesdale GJ, Dandie GW, Muller HK. (2001) Ultraviolet light induced injury: immunological and inflammatory effects. *Immunol Cell Biol*. **79**:547-568.

Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. (2002) Nucleophosmin regulates the stability and transcriptional activity of p53. *Nat Cell Biol.* **4**:529-533.

Colombo E, Bonetti P, Lazzerini Denchi E, Martinelli P, Zamponi R, Marine JC, Helin K, Falini B, Pelicci PG. (2005) Nucleophosmin is required for DNA integrity and p19Arf protein stability. *Mol Cell Biol.* **25**:8874-8886.

Colombo E, Martinelli P, Zamponi R, Shing DC, Bonetti P, Luzi L, Volorio S, Bernard L, Pruneri G, Alcalay M, Pelicci PG. (2006) Delocalization and destabilization of the Arf tumor suppressor by the leukemia-associated NPM mutant. *Cancer Res*. **66**:3044-3050.

Cotton J, Spandau DF. (1997) Ultraviolet B-radiation dose influences the induction of apoptosis and p53 in human keratinocytes. *Radiat Res.* **147**:148-155.

Cordon-Cardo C, Latres E, Drobnjak M, Oliva MR, Pollack D, Woodruff JM, Marcchal V, Chen J, Brennan MF, Levine AJ. (1994) Molecular abnormalitis of *mdm2* and *p53* genes in adult soft tissue sarcomas. *Cancer Res*. **54**:794-799.

Craig AL, Blaydes JP, Burch LR, Thompson AM, Hupp TR. (1999) Dephosphorylation of p53 at Ser20 after cellular exposure to low levels of non-ionizing radiation. *Oncogene.***18**:6305-6312.

Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH, Reid BJ. (1995) A p53-dependent mouse spindle checkpoint. *Science.* **267**:1353-1356.

Cullinane C, Mazur SJ, Essigmann JM, Phillips DR, Bohr VA. (1999) Inhibition of RNA polymerase II transcription in human cell extracts by cisplatin DNA damage. *Biochemistry.* **38**:6204-6212.

D`Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, von Zglinicki TG, Saretzki G, Carter NP, Jackson SP. (2003) A DNA damage checkpoint response in telomere-initiated senescence. *Nature*. **426**:194-198.

Dahm-Daphi J, Hubbe P, Horvath F, El-Awady RA, Bouffard KE, Powell SN, Willers H. (2005) Nonhomologous end-joining of site-specific but not of radiation-induced DNA double-strand breaks is reduced in the presence of wildtype p53. *Oncogene.* **24**:1663-1672.

Dai MS, Lu H. (2004) Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J Biol Chem*. **279**:44475-44482.

Dang CV, Lee WM. (1989) Nuclear and nucleolar targeting sequences of c-erb-A, c-myb, N-myc, p53, HSP70, and HIV tar proteins. *J Biol Chem.* **264**:18019-21803.

Daniel MT, Koken M, Romagne O, Barbey S, Bazarbachi A, Stadler M, Guillemin MC, Degos L, Chomienne C, de The H. (1993) PML protein expression in hematopoietic and acute promyelocytic leukemia cells. *Blood.* **82**:1858-1867.

Daniely Y, Dimitrova DD, Borowiec JA. (2002) Stress-dependent nucleolin mobilization mediated by p53-nucleolin complex formation. *Mol Cell Biol.* **22**:6014-6022.

Danovi D, Meulmeester E, Pasini D, Migliorini D, Capra M, Frenk R, de Graaf P, Francoz S, Gasparini P, Gobbi A, Helin K, Pelicci PG, Jochemsen AG, Marine JC. (2004) Amplification of Mdmx (or Mdm4) directly contributes to tumor formation by inhibiting p53 tumor suppressor activity. *Mol Cell Biol.* **24**:5835-5843.

D'Assoro AB, Lingle WL, Salisbury JL. (2002) Centrosome amplification and the development of cancer. *Oncogene*. **21**:6146-6153.

David-Pfeuty T, Chakrani F, Ory K, Nouvian-Dooghe Y. (1996) Cell cycle-dependent regulation of nuclear p53 traffic occurs in one subclass of human tumor cells and in untransformed cells. *Cell Growth Differ*. **7**:1211-1225.

David-Pfeuty T. (1999) Potent inhibitors of cyclin-dependent kinase 2 induce nuclear accumulation of wild-type p53 and nucleolar fragmentation in human untransformed and tumor-derived cells. *Oncogene.* **18**:7409-7422.

David-Pfeuty T, Nouvian-Dooghe Y, Sirri V, Roussel P, Hernandez-Verdun D. (2001) Common and reversible regulation of wild-type p53 function and of ribosomal biogenesis by protein kinases in human cells. *Oncogene.* **20**:5951-5963.

Davis RJ. (2000) Signal transduction by the JNK group of MAP kinases. *Cell.* **103**:239-252.

Decraene D, Agostinis P, Pupe A, de Haes P, Garmyn M. (2001) Acute response of human skin to solar radiation: regulation and function of the p53 protein. *J Photochem Photobiol.* **B6**:78-83.

de Graaf P, Little NA, Ramos YF, Meulmeester E, Letteboer SJ, Jochemsen AG. (2003) Hdmx protein stability is regulated by the ubiquitin ligase activity of Mdm2. *J Biol Chem.* **278**:38315-38324.

de Gruijl FR. (1999) Skin cancer and solar UV radiation. *Eur J Cancer*. **35**:2003-2009.

de Gruijl FR, van Kranen HJ, Mullenders LH. (2001) UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. *J Photochem Photobiol.* **B63**:19-27.

de Jong L, Grande MA, Mattern KA, Schul W, van Driel R. (1996) Nuclear domains involved in RNA synthesis, RNA processing, and replication. *Crit Rev Eukaryot Gene Expr*. **6**:215-246.

De Laat WL, Jaspers NG, Hoeijmakers JH (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev*. **13**:768-785.

DeLeo AB, Jay G, Appella E, Dubois GC, Law LW, Old LJ. (1979) Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci U S A*. **76**:2420-2424.

Dellaire G, Farral R, Bickmore WA. (2003) The Nuclear Protein Database (NPD): subnuclear localisation and functional annotation of the nuclear proteome. *Nucl Acids Res*. **31**:328-330.

Dellaire G, Bazett-Jones DP. (2004) PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. *Bioessays.* **26**:963-977.

Dellaire G, Eskiw CH, Dehghani H, Ching RW, Bazett-Jones DP. (2006a) Mitotic accumulations of PML protein contribute to the re-establishment of PML nuclear bodies in G1. *J Cell Sci.* **119**:1034-1042.

Dellaire G, Ching RW, Dehghani H, Ren Y, Bazett-Jones DP. (2006b) The number of PML nuclear bodies increases in early S phase by a fission mechanism. *J Cell Sci.* **119**:1026-1033.

den Besten W, Kuo ML, Williams RT, Sherr CJ. (2005) Myeloid leukemia-associated nucleophosmin mutants perturb p53-dependent and independent activities of the Arf tumor suppressor protein. *Cell Cycle.* **4**:1593-1598.

Dergunova NN, Bulycheva TI, Artemenko EG, Shpakova AP, Pegova AN, Gemjian EG, Dudnik OA, Zatsepina OV, Malashenko OS. (2002) A major nucleolar protein B23 as a marker of proliferation activity of human peripheral lymphocytes. *Immunol Lett*. **83**:67-72.

de Stanchina E, McCurrach ME, Zindy F, Shieh SY, Ferbeyre G, Samuelson AV, Prives C, Roussel MF, Sherr CJ, Lowe SW. (1998) E1A signaling to p53 involves the p19(ARF) tumor suppressor. *Genes Dev.* **12**:2434-2442.

de The H, Chomienne C, Lanotte M, Degos L, Dejean A. (1990) The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. *Nature*. **347**:558-5561.

de The H, Lavau C, Marchio A, Chomienne C, Degos L, Dejean A. (1991) The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell.* **66**:675-684.

de Toledo SM, Azzam EI, Dahlberg WK, Gooding TB, Little JB. (2000) ATM complexes with HDM2 and promotes its rapid phosphorylation in a p53-independent manner in normal and tumor human cells exposed to ionizing radiation. *Oncogene.* **19**:6185-6193.

Derenzini M, Trere D, Pession A, Govoni M, Sirri V, Chieco P. (2000) Nucleolar size indicates the rapidity of cell proliferation in cancer tissues. *J Pathol.* **191**:181-186.

Derry WB, Putzke AP, Rothman JH. (2001) Caenorhabditis elegans p53: role in apoptosis, meiosis, and stress resistance. *Science.* **294**:591-595.

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature.* **356**:215-221.

Donehower IA. (1996) The p53-deficient mouse: a model for basic and applied cancer studies. *Semin Cancer Biol.* **7**:269-278.

D´Orazi G, Cecchinelli B, Bruno T, Manni I, Higashimoto Y, Saito S, Gostissa M, Coen A, Marchetti A, Del Sal G, Piaggio G, Fanciulli M, Appella E, Soddu S. (2002) Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. *Nat Cell Biol.* **4**:11-19.

Dornan D, Shimizu H, Burch L, Smith AJ, Hupp TR. (2003) The proline repeat domain of p53 binds directly to the transcriptional coactivator p300 and allosterically controls DNA-dependent acetylation of p53. *Mol Cell Biol.* **23**:8846-8861.

Dornan D, Wertz I, Shimizu H, Arnott D, Frantz GD, Dowd P, O'Rourke K, Koeppen H, Dixit VM. (2004) The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature*. **429**:86-92

Doucas V, Ishov AM, Romo A, Juguilon H, Weitzman MD, Evans RM, Maul GG. (1996) Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. *Genes Dev*. **10**:196-207.

Doucas V, Tini M, Egan DA, Evans RM. (1999) Modulation of CREB binding protein function by the promyelocytic (PML) oncoprotein suggests a role for nuclear bodies in hormone signaling. *Proc Natl Acad Sci U S A*. **96**:2627-2632.

Dubs-Poterszman MC, Tocque B, Wasylyk B. (1995) MDM2 transformation in the absence of p53 and abrogation of the p107 G1 cell-cycle arrest. *Oncogene.* **11**:2445-2449.

Duckett DR, Bronstein SM, Taya Y, Modrich P. (1999) hMutSalpha- and hMutLalpha-dependent phosphorylation of p53 in response to DNA methylator damage. *Proc Natl Acad Sci U S A*. **96**:12384-12388.

Dudenhöffer C, Kurth M, Janus F, Deppert W, Wiesmuller L. (1998) Dissociation of the recombination control and the sequence-specific transactivation function of p53. *Mol Cell Biol.* **18**:5332-5342.

Dulic V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper JW, Elledge SJ, Reed SI. (1994) p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell.* **76**:1013-1023.

Dumaz N, Duthu A, Ehrhart JC, Drougard C, Appella E, Anderson CW, May P, Sarasin A, Daya-Grosjean L. (1997) Prolonged p53 protein accumulation in trichothiodystrophy fibroblasts dependent on unrepaired pyrimidine dimers on the transcribed strands of cellular genes. *Mol Carcinog.* **20**:340-347.

Duthu A, Ehrhart JC, Benchimol S, Chandrasekaran K, May P. (1985) P53-transformation-related protein: kinetics of synthesis and accumulation in SV40-infected primary mouse kidney cell cultures. *Virology.* **147**:275-286.

Dyck JA, Maul GG, Miller WH Jr, Chen JD, Kakizuka A, Evans RM. (1994) A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. *Cell*. **76**:333-343.

Easton DF, Bishop DT, Ford D, Crockford GP. (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am. J Hum. Genet*. **52**:678-701.

Edwards SJ, Hananeia L, Eccles MR, Zhang YF, Braithwaite AW. (2003) The proline-rich region of mouse p53 influences transactivation and apoptosis but is largely dispensable for these functions. *Oncogene.* **22**:4517-4523.

Eisen JA, Hanawalt PC. (1999) A phylogenomic study of DNA repair genes, proteins, and processes. *Mutat Res.* **435**:171-213.

El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*.**75**:817-825.

El-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, et al. (1994) WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res*. **54**:1169-1174.

Elenbaas B, Dobbelstein M, Roth J, Shenk T, Levine AJ. (1996) The MDM2 oncoprotein binds specifically to RNA through its RING finger domain. *Mol Med.* **2**:439-451.

Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J. (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell.* **83**:655-666.

Ellis M, Chew YP, Fallis L, Freddersdorf S, Boshoff C, Weiss RA, Lu X, Mittnacht S. (1999) Degradation of p27(Kip) cdk inhibitor triggered by Kaposi's sarcoma virus cyclin-cdk6 complex. *EMBO J.* **18**:644-653.

Erster S, Mihara M, Kim RH, Petrenko O, Moll UM. (2004) In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. *Mol Cell Biol.* **24**:6728-6741.

Eskiw CH, Dellaire G, Mymryk JS, Bazett-Jones DP. (2003) Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. *J Cell Sci.* **116**:4455-4466.

Eskiw CH, Dellaire G, Bazett-Jones DP. (2004) Chromatin contributes to structural integrity of promyelocytic leukemia bodies through a SUMO-1-independent mechanism. *J Biol Chem.* **279**:9577-9585.

Espinosa JM, Emerson BM. (2001) Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Mol Cell*. **8**:57-69.

Everett RD, Maul GG. (1994) HSV-1 IE protein Vmw110 causes redistribution of PML. *EMBO J*. **13**:5062-5069.

Everett RD, Freemont P, Saitoh H, Dasso M, Orr A, Kathoria M, Parkinson J. (1998) The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. *J Virol.* **72**:6581-6591.

Everett RD, Lomonte P, Sternsdorf T, van Driel R, Orr A. (1999) Cell cycle regulation of PML modification and ND10 composition. *J Cell Sci.* **112**:4581-4588.

Everett RD. (2001) DNA viruses and viral proteins that interact with PML nuclear bodies. *Oncogene.* **20**:7266-7273.

Fagioli M, Alcalay M, Pandolfi PP, Venturini L, Mencarelli A, Simeone A, Acampora D, Grignani F, Pelicci PG. (1992) Alternative splicing of PML transcripts predicts coexpression of several carboxy-terminally different protein isoforms. *Oncogene*. **7**:1083-1091.

Fakharzadeh SS, Trusko SP, George DL. (1991) Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line.*EMBO J.* **10**:1565-1569.

Falck J, Petrini JH, Williams BR, Lukas J, Bartek J. (2002) The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. *Nat Genet.* **30**:290-294.

Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF; GIMEMA Acute Leukemia Working Party. (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med.* **352**:254-266.

Falini B, Bolli N, Shan J, Martelli MP, Liso A, Pucciarini A, Bigerna B, Pasqualucci L, Mannucci R, Rosati R, Gorello P, Diverio D, Roti G, Tiacci E, Cazzaniga G, Biondi A, Schnittger S, Haferlach T, Hiddemann W, Martelli MF, Gu W, Mecucci C, Nicoletti I. (2006) Both carboxy-terminus NES motif and mutated tryptophan(s) are crucial for aberrant nuclear export of nucleophosmin leukemic mutants in NPMc+ AML. *Blood.* **107**:4514-4523.

Fang S, Jensen JP, Ludwig RL, Vousden KH, Weissman AM. (2000) Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J Biol Chem.* **275**:8945-8951.

Fankhauser C, Izaurralde E, Adachi Y, Wingfield P, Laemmli UK. (1991) Specific complex of human immunodeficiency virus type Rev 1 and nucleolar B23 proteins: dissociation by the Rev response element. *Mol Cell Biol.* **11**:2567-2575.

Feng L, Lin T, Uranishi H, Gu W, Xu Y. (2005) Functional analysis of the roles of post-translational modifications at the p53 C terminus in regulating p53 stability and activity. *Mol Cell Biol.* **25**:5389-5395.

Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C, Lowe SW. (2000) PML is induced by oncogenic ras and promotes premature senescence. *Genes Dev.* **14**:2015-2027.

Feuerstein N, Chan PK, Mond JJ. (1988) Identification of numatrin, the nuclear matrix protein associated with induction of mitogenesis, as the nucleolar protein B23. Implication for the role of the nucleolus in early transduction of mitogenic signals. *J Biol Chem.* **263**:10608-10612.

Finch RA, Chan PK. (1996) ATP depletion affects NPM translocation and exportation of rRNA from nuclei. Biochem *Biophys Res Commun.* **222**:553-558.

Finch RA, Donoviel DB, Potter D, Shi M, Fan A, Freed DD, Wang CY, Zambrowicz BP, Ramirez-Solis R, Sands AT, Zhang N. (2002) mdmx is a negative regulator of p53 activity in vivo. *Cancer Res*. **62**:3221-3225.

Fleckenstein DS, Uphoff CC, Drexler HG, Quentmeier H. (2002) Detection of p53 gene mutations by single strand conformational polymorphism (SSCP) in human acute myeloid leukemia-derived cell lines. *Leuk Res*. **26**:207-214.

Fogal V, Gostissa M, Sandy P, Zacchi P, Sternsdorf T, Jensen K, Pandolfi PP, Will H, Schneider C, Del Sal G. (2000) Regulation of p53 activity in nuclear bodies by a specific PML isoform. *EMBO J.* **19**:6185-6195.

Fogal V, Hsieh JK, Royer C, Zhong S, Lu X. (2005) Cell-cycle dependent nuclear retention of p53 by E2F1 requires phosphorylation of p53 at Ser315. *EMBO J.* **24**:2768-2782.

Fogal V, Kartasheva NN, Trigiante G, Llanos S, Yap D, Vousden KH, Lu X. (2005b) ASSP1 and ASSP2 are new transcriptional targets of E2F. *Cell Death Differ*. **12**:369-376.

Ford JM, Hanawalt PC. (1995) Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc Natl Acad Sci U S A.* **92**:8876-8880.

Ford JM, Hanawalt PC. (1997) Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. *J Biol Chem*. **272**:28073-28080.

Ford JM, Baron EL, Hanawalt PC. (1998) Human fibroblasts expressing the human papillomavirus E6 gene are deficient in global genomic nucleotide excision repair and sensitive to ultraviolet irradiation. *Cancer Res.* **58**:599-603.

Freeman DJ, Li AG, Wei G, Li HH, Kertesz N, Lesche R, Whale AD, Martinez-Diaz H, Rozengurt N, Cardiff RD, Liu X, Wu H. (2003) PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. *Cancer Cell*. **3**:117-130.

Friedberg EC. (2001) How nucleotide excision repair protects against cancer. *Nat Rev Cancer*.**1**:22-33.

Friedberg EC. (2004) The discovery that xeroderma pigmentosum (XP) results from defective nucleotide excision repair. *DNA Repair (Amst)*. **3**:183-195.

Friedler A, Veprintsev DB, Freund SM, von Glos KI, Fersht AR. (2005) Modulation of binding of DNA to the C-terminal domain of p53 by acetylation.*Structure.* **13**:629-636.

Fritsche M, Haessler C, Brandner G. (1993) Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene.* **8**:307-318.

Fuchsova B, Novak P, Kafkova J, Hozak P. (2002) Nuclear DNA helicase II is recruited to IFN-alpha-activated transcription sites at PML nuclear bodies. *J Cell Biol*.**158**:463-473.

Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF. (1996) Abnormal centrosome amplification in the absence of p53. *Science*. **271**:1744-1747.

Gaboli M, Gandini D, Delva L, Wang ZG, Pandolfi PP.(1998) Acute promyelocytic leukemia as a model for cross-talk between interferon and retinoic acid pathways: from molecular biology to clinical applications. *Leuk Lymphoma.* **30**:11-22.

Ganguli G, Wasylyk B. (2003) p53-independent functions of Mdm2. *Mol Cancer Res*. **1**:1027-1035.

Gao H, Jin S, Song Y, Fu M, Wang M, Liu Z, Wu M, Zhan Q. (2005) B23 regulates GADD45a nuclear translocation and contributes to GADD45a-induced cell cycle G2-M arrest. *J Biol Chem.* **280**:10988-10996.

Garkavtsev I, Grigoarian IA, Ossovskaya VS, Chernov MV, Chumakov PM, Gudkov AV. (1998) The candidate tumor suppressor p33ING1 cooperates with p53 in cell growth control. *Nature*. **391**:295-298.

Gatz SA, Wiesmuller L. (2006) p53 in recombination and repair. *Cell Death Differ.* **13**:1003-1016.

Gentile M, Latonen L, Laiho M. (2003) Cell cycle arrest and apoptosis provoked by UV radiation-induced DNA damage are transciptionally highly divergent responses. *Nucleic Acids Res.* **31**:4779-4790.

German J. (1997) Bloom´s syndrome. XX. The first 100 cancers. *Cancer Genet Cytogenet*. **93**:100-106.

Geyer RK, Yu ZK, Maki CG. (2000) The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat Cell Biol*. **2**:569-573.

Giglia-Mari G, Coin F, Ranish JA, Hoogstraten D, Theil A, Wijgers N, Jaspers NG, Raams A, Argentini M, van der Spek PJ, Botta E, Stefanini M, Egly JM, Aebersold R, Hoeijmakers JH, Vermeulen W. (2004) A new, tenth subunit of TFIIH is responsible for the DNA repair syndrome trichothiodystrophy group A. *Nat Genet.* **36**:714-9.

Gire V, Roux P, Wynford-Thomas D, Brondello JM, Dulic V. (2004) DNA damage checkpoint kinase Chk2 triggers replicative senescence. *EMBO J.* **23**:2554-2563.

Goddard AD, Borrow J, Freemont PS, Solomon E. (1991) Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. *Science*. **254**:1371-1374.

Gorina S, Pavletich NP. (1996) Structure of the p53 tumor suppressor bound to the ankyrin and SH3 domains of 53BP2. *Science*. **274**:1001-1005.

Gostissa M, Hengstermann A, Fogal V, Sandy P, Schwarz SE, Scheffner M, Del Sal G. (1999) Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. *EMBO J.* **18**:6462-6471.

Graeber TG, Peterson JF, Tsai M, Monica K, Fornace AJ Jr, Giaccia AJ. (1994) Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol.* **14**:6264-6277.

Granick S, Granick D. (1971) Nucleolar necklaces in chick embryo myoblasts formed by lack of arginine. *J Cell Biol.* **51**:636-642.

Granick D. (1975) Nucleolar necklaces in chick embryo fibroblast cells. I. Formation of necklaces by dichlororibobenzimidazole and other adenosine analogues that decrease RNA synthesis and degrade preribosomes. *J Cell Biol.* **65**:398-417.

Grignani F, Ferrucci PF, Testa U, Talamo G, Fagioli M, Alcalay M, Mencarelli A, Grignani F, Peschle C, Nicoletti I, et al. (1993) The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. *Cell*. **74**:423-431.

Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Grignani F, Lazar MA, Minucci S, Pelicci PG. (1998) Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. *Nature.* **391**:815-818.

Grisendi S, Bernardi R, Rossi M, Cheng K, Khandker L, Manova K, Pandolfi PP. (2005) Role of nucleophosmin in embryonic development and tumorigenesis. *Nature.* **437**:147-153.

Grisendi S, Mecucci C, Falini B, Pandolfi PP. (2006) Nucleophosmin and cancer. *Nat Rev Cancer*. **6**:493-505.

Grisolano JL, Wesselschmidt RL, Pelicci PG, Ley TJ. (1997) Altered myeloid development and acute leukemia in transgenic mice expressing PML-RAR alpha under control of cathepsin G regulatory sequences. *Blood.* **89**:376-387.

Grossman SR, Perez M, Kung AL, Joseph M, Mansur C, Xiao ZX, Kumar S, Howley PM, Livingston DM. (1998) p300/MDM2 complexes participate in MDM2-mediated p53 degradation. *Mol Cell.* **2**:405-415.

Grossman SR. (2001) p300/CBP/p53 interaction and regulation of the p53 response .*Eur J Biochem.* **268**:2773-2778.

Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM. (2003) Polyubiquitination of p53 by a ubiquitin ligase activity of p300. *Science*. **300**:342-344.

Grotzinger T, Sternsdorf T, Jensen K, Will H. (1996) Interferon-modulated expression of genes encoding the nuclear-dot-associated proteins Sp100 and promyelocytic leukemia protein (PML). *Eur J Biochem*. **238**:554-560.

Gu Y, Turck CW, Morgan DO. (1993) Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. *Nature.* **366**:707-710.

Gu W, Roeder RG. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell.* **90**:595-606.

Gu J, Kawai H, Nie L, Kitao H, Wiederschain D, Jochemsen AG, Parant J, Lozano G, Yuan ZM. (2002) Mutual dependence of MDM2 and MDMX in their functional inactivation of p53. *Biol Chem.* **27**7:19251-19254.

Gubin AN, Njoroge JM, Bouffard GG, Miller JL (1999) Gene expression in proliferating human erythroid cell. *Genomics.* **59**:168-177.

Guo A, Salomoni P, Luo J, Shih A, Zhong S, Gu W, Pandolfi PP. (2000) The function of PML in p53-dependent apoptosis. *Nat Cell Biol.* **2**:730-736.

Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, Rush LJ, Verbel DA, Cordon-Cardo C, Pandolfi PP. (2004a) Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst.* **96**:269-279.

Gurrieri C, Nafa K, Merghoub T, Bernardi R, Capodieci P, Biondi A, Nimer S, Douer D, Cordon-Cardo C, Gallagher R, Pandolfi PP (2004b) Mutations of the PML tumor suppressor gene in acute promyelocytic leukemia. *Blood.***103**:2358-2362.

Haapajärvi T, Pitkänen K, Laiho M. (1999) Human melanoma cell line UV responses show independency of p53 function. *Cell Growth Differ.* **10**:163-171.

Haber JE. (2000) Partners and pathways repairing a double-strand break. *Trends Genet.* **16**:259-264.

Hall PA, McKee PH, Menage HD, Dover R, Lane DP. (1993) High levels of p53 protein in UV-irradiated normal human skin. *Oncogene.* **8**:203-207.

Hammond EM, Giaccia AJ. (2005) The role of p53 in hypoxia-induced apoptosis. *Biochem Biophys Res Commun*. **331**:718-725.

Hanahan D, Weinberg RA. (2000) The hallmarks of cancer. *Cell*. **100**:57-70.

Hanawalt PC, Ford JM, Lloyd DR. (2003) Functional characterization of global genomic DNA repair and its implications for cancer. *Mutat Res*. **54**:107-114.

Hannon GJ, Beach D. (1994) p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature.* **371**:257-261.

Harley CB, Futcher AB, Greider CW. (1990) Telomeres shorten during ageing of human fibroblasts. *Nature*. **345**:458-460.

Harms K, Nozell S, Chen X. (2004) The common and distinct target genes of the p53 family transcription factors. *Cell Mol Life Sci.* **61**:822-842.

Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell.* **75**:805-816.

Hartmann A, Blaszyk H, Cunningham JS, McGovern RM, Schroeder JS, Helander SD, Pittelkow MR, Sommer SS, Kovach JS. (1996) Overexpression and mutations of p53 in metastatic malignant melanomas. *Int J Cancer.* **67**:313-317.

Hartwell LH, Weinert TA. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science*. **246**:629-634.

Haupt Y, Maya R, Kazaz A, Oren M. (1997) Mdm2 promotes the rapid degradation of p53. *Nature.* **387**:296-299.

Hayakawa T, Haraguchi T, Masumoto H, Hiraoka Y. (2003) Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase. *J Cell Sci*.**116**:3327-3338.

Hayflick L. (1965) The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res*. **37**:614-636.

He LZ, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V, Cattoretti G, Pandolfi PP. (1997) Acute leukemia with promyelocytic features in PML/RARalpha transgenic mice. *Proc Natl Acad Sci U S A*. **94**:5302-5307.

He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A, Pandolfi PP. (1998) Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. *Nat Genet.* **18**:126-135.

He LZ, Merghoub T, Pandolfi PP. (1999) In vivo analysis of the molecular pathogenesis of acute promyelocytic leukemia in the mouse and its therapeutic implications. *Oncogene.* **18**:5278-5292.

Herbig UW, Jobling A, Chen BP, Chen DJ, Sedivy JM. (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell.* **14**:501-513.

Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, Thiagalingam S, Kinzler KW, Vogelstein B. (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol Cell*. **1**:3-11.

Herrera JE, Savkur R, Olson MO. (1995) The ribonuclease activity of nucleolar protein B23. *Nucleic Acid Res.* **23**:3974-3979.

Herrera JE, Correia JJ, Jones AE, Olson MO. (1996) Sedimentation analysis of the salt- and divalent metal ion-induced oligomerization of nucleolar protein B23. *Biochemistry*. **35**:2668-2673.

Hickman MJ, Samson LD. (1999) Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents. *Proc Natl Acad Sci U S A*. **96**:10764-10769.

Hinchcliffe EH, Li C, Thompson EA, Maller JL, Sluder G. (1999) Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in Xenopus egg extracts. *Science.* **283**:851-854.

Hingorani K, Szebeni A, Olson MO. (2000) Mapping the functional domains of nucleolar protein B23. *J Biol Chem.* **275**: 24451-24457.

Hiscox JA. (2002) The nucleolus - a gateway to viral infection? *Arch Virol.* **147**:1077-1089.

Hoffman WH, Biade S, Zilfou JT, Chen J, Murphy M. (2002) Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J Biol Chem.* **277**:3247-3257.

Hollstein M, Sidransky D, Vogelstein B, Harris CC. (1991) p53 mutations in human cancers. *Science.* **253**:49-53.

Honda R, Tanaka H, Yasuda H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett. **420**:25-27.

Honda R, Yasuda H. (1999) Association of p19(ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. *EMBO J.* **18**:22-27.

Honda R, Yasuda H. (2000) Activity of MDM2, a ubiquitin ligase, toward p53 or itself is dependent on the RING finger domain of the ligase. *Oncogene*. **19**:1473-1476.

Hoogstraten D, Nigg AL, Heath H, Mullenders LH, van Driel R, Hoeijmakers JH, Vermeulen W, Houtsmuller AB. (2002) Rapid switching of TFIIH between RNA polymerase I and II transcription and DNA repair in vivo. *Mol Cell.* **10**:1163-1174.

Hsieh JK, Yap D, O´Connor DJ, Fogal V, Fallis L, Chan F, Zhong S, Lu X. (2002) Novel function of the cyclin A binding site of E2F in regulating p53-induced apoptosis in response to DNA damage. *Mol Cell Biol.* **22**:78-93.

Hsu CY, Yung BY. (1998) Down-regulation of nucleophosmin/B23 during retinoic acid-induced differentiation of human promyelocytic leukemia HL-60 cells. *Oncogene*. **16**:915-923.

Hsu CY, Yung BY. (2000) Over-expression of nucleophosmin/B23 decreases the susceptibility of human leukemia HL-60 cells to retinoic acid-induced differentiation and apoptosis. *Int J Cancer.* **88**:392-400.

Huang W, Sun GL, Li XS, Cao Q, Lu Y, Jang GS, Zhang FQ, Chai JR, Wang ZY, Waxman S, et al. (1993) Acute promyelocytic leukemia: clinical relevance of two major PML-RAR alpha isoforms and detection of minimal residual disease by retrotranscriptase/polymerase chain reaction to predict relapse. *Blood.* **82**:1264-1269.

Huang P (1998) Excision of mismatched nucleotides from DNA: a potential mechanism for enhancing DNA replication fidelity by the wildtype p53 protein. *Oncogene.* **17**:261-270.

Hupp TR, Meek DW, Midgley CA, Lane DP. (1992) Regulation of the specific DNA binding function of p53. *Cell*. **71**:875-886.

Hupp TR, Lane DP. (1994) Allosteric activation of latent p53 tetramers. *Curr Biol.* **4**:865-875

Hupp TR. (1999) Regulation of p53 protein function through alterations in protein-folding pathways. *Cell Mol Life Sci.* **55**:88-95.

Hwang BJ, Ford JM, Hanawalt PC, Chu G. (1999) Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc Natl Acad Sci U S A.* **96**:424-428.

Inga A, Storici F, Darden TA, Resnick MA. (2002) Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol Cell Biol.* **22**:8612-8625.

Inoue T, Geyer RK, Yu ZK, Maki CG. (2001) Downregulation of MDM2 stabilizes p53 by inhibiting p53 ubiquitination in response to specific alkylating agents. *FEBS Lett.* **490**:196-201.

Insinga A, Monestiroli S, Ronzoni S, Carbone R, Pearson M, Pruneri G, Viale G, Appella E, Pelicci P, Minucci S. (2004) Impairment of p53 acetylation, stability and function by an oncogenic transcription factor. *EMBO J.* **23**:1144-1154.

Irwin MS, Kaelin WG. (2001) p53 family update: p73 and p63 develop their own identities. *Cell Growth Differ.* **12**:337-349.

Ishov AM, Maul GG. (1996) The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. *J Cell Biol*. **134**:815-826.

Ishov AM, Stenberg RM, Maul GG. (1997) Human cytomegalovirus immediate early interaction with host nuclear structures: definition of an immediate transcript environment. *J Cell Biol.* **138**:5-16.

Ishov AM, Sotnikov AG, Negorev D, Vladimirova OV, Neff N, Kamitani T, Yeh ET, Strauss JF 3rd, Maul GG. (1999) PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. *J Cell Biol.* **147**:221-234.

Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, Pramanik A, Selivanova G. (2004) Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nat Med*. **10**:1321-1328.

Ithana K, Dimri G, Campisi J. (2001) Regulation of cellular senescence by p53. *Eur J Biochem*. **268**:2784-2791.

Itahana K, Bhat KP, Jin A, Itahana Y, Hawke D, Kobayashi R, Zhang Y. (2003) Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. *Mol Cell.* **12**:1151-1164.

Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, Yao TP. (2001) p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. *EMBO J*. **20**:1331-1340.

Ito A, Kawaguchi Y, Lai CH, Kovacs JJ, Higashimoto Y, Appella E, Yao TP. (2002) MDM2-HDAC1-mediated deacetylation of p53 is required for its degradation. *EMBO J.* **21**:6236-6245.

Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. (1994) Tumor spectrum analysis in p53-mutant mice. *Curr Biol*. **4**:1-7.

Janus F, Albrechtsen N, Knippschild U, Wiesmuller L, Grosse F, Deppert W. (1999) Different regulation of the p53 core domain activities 3'-to-5' exonuclease and sequence-specific DNA binding. *Mol Cell Biol.* **19**:2155-2168.

Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, MacLean KH, Han J, Chittenden T, Ihle JN, McKinnon PJ, Cleveland JL, Zambetti GP. (2003) Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. *Cancer Cell*. **4**:321-328.

Jeffrey PD, Gorina S, Pavletich NP. (1995) Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science.* **267**:1498-1502.

Jensen K, Shiels C, Freemont PS. (2001) PML protein isoforms and the RBCC/TRIM motif. *Oncogene*. **20**:7223-7233.

Jiang PS, Yung BY. (1999) Down-regulation of nucleophosmin/B23 mRNA delays the entry of cells into mitosis. *Biochem Biophys Res Commun.* **257**:865-870.

Jin S, Martinek S, Joo WS, Wortman JR, Mirkovic N, Sali A, Yandell MD, Pavletich NP, Young MW, Levine AJ. (2000) Identification and characterization of a p53 homologue in Drosophila melanogaster. P*roc Natl Acad Sci U S A*. **97**:7301-7306.

Jin A, Itahana K, O'Keefe K, Zhang Y. (2004) Inhibition of HDM2 and activation of p53 by ribosomal protein L23. *Mol Cell Biol.* **24**:7669-7680.

Jiricny J. (2000) Mediating mismatch repair. *Nat Genet*. **24**: 6-8.

Jones SN, Roe AE, Donehower LA, Bradley A. (1995) Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature.* **378**:206-208.

Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A. (1998) Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci.* **95**:15608-15612.

Joseph TW, Zaika A, Moll UM. (2003) Nuclear and cytoplasmic degradation of endogenous p53 and HDM2 occurs during down-regulation of the p53 response after multiple types of DNA damage. *FASEB J.* **17**:1622-1630.

Kaeser MD, Iggo RD. (2004) Promoter-specific p53-dependent histone acetylation following DNA-damage. *Oncogene.* **23**:4007-4013.

Kakizuka A, Miller WH Jr, Umesono K, Warrell RP Jr, Frankel SR, Murty VV, Dmitrovsky E, Evans RM. (1991) Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell*. **66**:663-674.

Kalantry S, Delva L, Gaboli M, Gandini D, Giorgio M, Hawe N, He LZ, Peruzzi D, Rivi R, Tribioli C, Wang ZG, Zhang H, Pandolfi PP. (1997) Gene rearrangements in the molecular pathogenesis of acute promyelocytic leukemia. *J Cell Physiol.* **173**:288-296.

Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. (1998) Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc Natl Acad Sci U S A*. **95**:8292-8297.

Kamitani T, Nguyen HP, Kito K, Fukuda-Kamitani T, Yeh ET. (1998a) Covalent modification of PML by the sentrin family of ubiquitin-like proteins. *J Biol Chem.* **273**:3117-3120.

Kamitani T, Kito K, Nguyen HP, Wada H, Fukuda-Kamitani T, Yeh ET. (1998b) Identification of three major sentrinization sites in PML. *J Biol Chem.* **273**:26675-26682.

Kapoor M, Hamm R, Yan W, Taya Y, Lozano G. (2000) Cooperative phosphorylation at multiple sites is required to activate p53 in response to UV radiation. *Oncogene.* **19**:358-364.

Kastner P, Perez A, Lutz Y, Rochette-Egly C, Gaub MP, Durand B, Lanotte M, Berger R, Chambon P. (1992) Structure, localization and transcriptional properties of two classes of retinoic acid receptor alpha fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. *EMBO J.* **11**:629-642.

Kataoka H, Bonnefin P, Vieyra D, Feng X, Hara Y, Miura Y, Joh T, Nakabayashi H, Vaziri H, Harris CC, Riabowol K. (2003) ING1 represses transcription by direct DNA binding and through effects on p53. *Cancer Res.* **63**:5785-5792.

Kawai H, Wiederschain D, Kitao H, Stuart J, Tsai KK, Yuan ZM. (2003) DNA damage-induced MDMX degradation is mediated by MDM2. *J Biol Chem.* **278**:45946-45953.

Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B. (1991) Identification of p53 as a sequence-specific DNA-binding protein. *Science*. **252**:1708-1711.

Khan MM, Nomura T, Kim H, Kaul SC, Wadhwa R, Shinagawa T, Ichikawa-Iwata E, Zhong S, Pandolfi PP, Ishii S. (2001a) Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. *Mol Cell.* **7**:1233-1243.

Khan MM, Nomura T, Kim H, Kaul SC, Wadhwa R, Zhong S, Pandolfi PP, Ishii S. (2001b) PML-RARalpha alleviates the transcriptional repression mediated by tumor suppressor Rb.*J Biol Chem*. **276**:43491-43494.

Khanna KK, Keating KE, Kozlov S, Scott S, Gatei M, Hobson K, Taya Y, Gabrielli B, Chan D, Lees-Miller SP, Lavin MF. (1998) ATM associates with and phosphorylates p53: mapping the region of interaction. *Nat Genet.* **20**:398-400.

Khosravi R, Maya R, Gottlieb T, Oren M, Shiloh Y, Shkedy D. (1999) Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage.*Proc Natl Acad Sci U S A*. **96**:14973-14977.

Kielbassa C, Roza L, Epe B. (1997) Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis*. **18**:811-816.

Kiesslich A, von Mikecz A, Hemmerich P. (2002) Cell cycle-dependent association of PML bodies with sites of active transcription in nuclei of mammalian cells. *J Struct Biol.* **140**:167-179.

Kim YH, Choi CY, Kim Y. (1999) Covalent modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. *Proc Natl Acad Sci U S A*. **96**:12350-12355.

Kim YE, Kim DY, Lee JM, Kim ST, Han TH, Ahn JH. (2005) Requirement of the coiled-coil domain of PML-RARalpha oncoprotein for localization, sumoylation, and inhibition of monocyte differentiation. *Biochem Biophys Res Commun.* **330**:746-754.

Kim E, Deppert W. (2006) The versatile interactions of p53 with DNA: when flexibility serves specificity. *Cell Death Differ*. **13**:885-889.

Klibanov SA, O'Hagan HM, Ljungman M. (2001) Accumulation of soluble and nucleolar-associated p53 proteins following cellular stress. *J Cell Sci*. **114**:1867-1873.

Ko, LJ, Prives C. (1996) p53: puzzle and paradigm. *Genes Dev.* **10**:1054-1072.

Kobet E, Zeng X, Zhu Y, Keller D, Lu H. (2000) MDM2 inhibits p300-mediated p53 acetylation and activation by forming a ternary complex with the two proteins. *Proc Natl Acad Sci U S A*. **97**:12547-12552.

Kogan SC, Hong SH, Shultz DB, Privalsky ML, Bishop JM. (2000) Leukemia initiated by PMLRARalpha: the PML domain plays a critical role while retinoic acid-mediated transactivation is dispensable. *Blood.* **95**:1541-1550.

Kogan SC, Brown DE, Shultz DB, Truong BT, Lallemand-Breitenbach V, Guillemin MC, Lagasse E, Weissman IL, Bishop JM. (2001) BCL-2 cooperates with promyelocytic leukemia retinoic acid receptor alpha chimeric protein (PMLRARalpha) to block neutrophil differentiation and initiate acute leukemia. *J Exp Med.* **193**:531-543.

Koken MH, Puvion-Dutilleul F, Guillemin MC, Viron A, Linares-Cruz G, Stuurman N, de Jong L, Szostecki C, Calvo F, Chomienne C, et al. (1994) The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion.*EMBO J.* **13**:1073-1083.

Koken MH, Linares-Cruz G, Quignon F, Viron A, Chelbi-Alix MK, Sobczak-Thepot J, Juhlin L, Degos L, Calvo F, de The H. (1995) The PML growth-suppressor has an altered expression in human oncogenesis. *Oncogene.* **10**:1315-1324.

Kondo T, Minamino N, Nagamura-Inoue T, Matsumoto M, Taniguchi T, Tanaka N. (1997) Identification and characterization of nucleophosmin/B23/numatrin which binds the anti-oncogenic transcription factor IRF-1 and manifests oncogenic activity. *Oncogene.* **15**:1275-1281.

Korgaonkar C, Hagen J, Tompkins V, Frazier AA, Allamargot C, Quelle FW, Quelle DE. (2005) Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function. *Mol Cell Biol.* **25**:1258-1271.

Krummel KA, Lee CJ, Toledo F, Wahl GM. (2005) The C-terminal lysines fine-tune P53 stress responses in a mouse model but are not required for stability control or transactivation. *Proc Natl Acad Sci U S A.* **102**:10188-10193.

Kubbutat MH, Jones SN, Vousden KH. (1997) Regulation of p53 stability by Mdm2. *Nature*. **387**:299-303.

Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, Pavletich NP. (1996) Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science.* **274**:948-953.

Kuo ML, den Besten W, Bertwistle D, Roussel MF, Sherr CJ. (2004) N-terminal polyubiquitynation and degradation of the ARF tumor suppressor. *Genes Dev.* **18**:1862-1874.

Kwek SS, Derry J, Tyner AL, Shen Z, Gudkov AV. (2001) Functional analysis and intracellular localization of p53 modified by SUMO-1. *Oncogene*. **20**:2587-2599.

Lacey KR, Jackson PK, Stearns T. (1999) Cyclin-dependent kinase control of centrosome duplication. *Proc Natl Acad Sci U S A.* **96**:2817-2822.

Lai Z, Auger KR, Manubay CM, Copeland RA. (2000) Thermodynamics of p53 binding to hdm2(1-126): effects of phosphorylation and p53 peptide length. *Arch Biochem Biophys.* **381**:278-284.

Lai Z, Ferry KV, Diamond MA, Wee KE, Kim YB, Ma J, Yang T, Benfield PA, Copeland RA, Auger KR. (2001) Human mdm2 mediates multiple monoubiquitination of p53 by a mechanism requiring enzyme isomerization. *J Biol Chem.* **276**:31357-31367.

Lallemand-Breitenbach V, Zhu J, Puvion F, Koken M, Honore N, Doubeikovsky A, Duprez E, Pandolfi PP, Puvion E, Freemont P, de The H. (2001) Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment, and As2O3-induced PML or PML/retinoic acid receptor alpha degradation. *J Exp Med.* **12**:1361-1371.

LaMorte VJ, Dyck JA, Ochs RL, Evans RM. (1998) Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body. *Proc Natl Acad Sci U S A*. **95**:4991-4996.

Lane DP. (1992) p53, guardian of the genome. *Nature.* **358**:15-16.

Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, El-Naggar AK, Lozano G. (2004) Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell.* **119**:861-872.

Lanotte M, Martin-Thouvenin V, Najman S, Balerini P, Valensi F, Berger R. (1991) NB4, a maturation inducible cell line with t(15;17) marker isolated from a human acute promyelocytic leukemia (M3). *Blood.* **77**:1080-1086.

Latonen L, Taya Y, Laiho M. (2001) UV-radiation induces dose-dependent regulation of p53 response and modulates p53-HDM2 interaction in human fibroblasts. *Oncogene*. **20**:6784-6793.

Latonen L, Kurki S, Pitkanen K, Laiho M. (2003) p53 and MDM2 are regulated by PI-3-kinases on multiple levels under stress induced by UV radiation and proteasome dysfunction. *Cell Signal.* **15**:95-102.

Latonen L, Laiho M. (2005) Cellular UV damage responses--functions of tumor suppressor p53. *Biochim Biophys Acta*. **1755**:71-89.

Lavau C, Marchio A, Fagioli M, Jansen J, Falini B, Lebon P, Grosveld F, Pandolfi PP, Pelicci PG, Dejean A. (1995) The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene*. **11**:871-876.

Lavin MF, Gueven N. (2006) The complexity of p53 stabilization and activation. *Cell Death Differ*. **13**:941-950.

Lavoie JN, L`Allemain G, Brunet A, Muller R, Pouyssegur J (1996) Cyclin D1 expression is regulated positively by the p42/p44MAPK and negatively by the p38/HOGMAPK pathway. *J Biol Chem.* **271**: 20608-20616.

Le XF, Yang P, Chang KS. (1996) Analysis of the growth and transformation suppressor domains of promyelocytic leukemia gene, PML. *J Biol Chem.* **271**:130-135.

Le XF, Vallian S, Mu ZM, Hung MC, Chang KS. (1998) Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. *Oncogene*.**16**:1839-1849.

Lee S, Elenbaas B, Levine A, Griffith J. (1995) p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. *Cell.* **81**:1013-1020.

Lee C, Smith BA, Bandyopadhyay K, Gjerset RA. (2005) DNA damage disrupts the p14ARF-B23(nucleophosmin) interaction and triggers a transient subnuclear redistribution of p14ARF. *Cancer Res.* **65**:9834-9842.

Lee SY, Park JH, Kim S, Park EJ, Yun Y, Kwon J. (2005) A proteomics approach for the identification of nucleophosmin and heterogeneous nuclear ribonucleoprotein C1/C2 as chromatin-binding proteins in response to DNA double-strand breaks. *Biochem J*. **388**:7-15.

Leng RP, Lin Y, Ma W, Wu H, Lemmers B, Chung S, Parant JM, Lozano G, Hakem R, Benchimol S. (2003) Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell*. **112**:779-791.

Leu JI, Dumont P, Hafey M, Murphy ME, George DL. (2004) Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol.* **6**:443-450.

Leung AK, Trinkle-Mulcahy L, Lam YW, Andersen JS, Mann M, Lamond AI. (2006) NOPdb: Nucleolar Proteome Database. *Nucleic Acids Res*. **34**:D218-D220.

Levine AJ, Momand J, Finlay CA. (1991) The p53 tumour suppressor gene. *Nature.* **315**:453-455.

Levine AJ.(1997) p53, the cellular gatekeeper for growth and division. *Cell*. **88**:323-331.

Levy L, Broad S, Diekmann D, Evans RD, Watt FM. (2000) beta1 integrins regulate keratinocyte adhesion and differentiation by distinct mechanisms. *Mol Biol Cell.* **11**:453-466.

Levy L, Hill CS. (2006) Alterations in components of the TGF-beta superfamily signaling pathways in human cancer. *Cytokine Growth Factor Rev.* **17**:41-58.

Li FP, Fraumeni JF, Jr. (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome ? *Ann Intern Med.* **71**: 747-752.

Li YP. (1997) Protein B23 is an important human factor for the nucleolar localization of the human immunodeficiency virus protein Tat. *J Virol.* **71**:4098-4102.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell.* **91**:479-489.

Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ, Chen JD. (2000a) Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. *Mol Cell Biol*. **20**:1784-1796.

Li H, Chen JD. (2000b) PML and the oncogenic nuclear domains in regulating transcriptional repression. *Curr Opin Cell Biol*.**12**:641-644.

Li M, Luo J, Brooks CL, Gu W. (2002) Acetylation of p53 inhibits its ubiquitination by Mdm2. *J Biol Chem*. **277**:50607-50611.

Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W. (2003) Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. *Science.* **302**:1972-1975.

Li J, Zhang X, Sejas DP, Bagby GC, Pang Q. (2004) Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. *J Biol Chem.* **279**:41275-41279.

Li J, Zhang X, Sejas DP, Pang Q. (2005) Negative regulation of p53 by nucleophosmin antagonizes stress-induced apoptosis in human normal and malignant hematopoietic cells. *Leuk Res*. **29**:1415-1423.

Lill NL, Grossman SR, Ginsberg D, DeCaprio J, Livingston DM. (1997) Binding and modulation of p53 by p300/CBP coactivators. *Nature.* **387**:823-827.

Lin J, Chen J, Elenbaas B, Levine AJ. (1994) Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5E1B 55-kD protein. *Genes Dev*. **8**:1235-1246.

Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM. (1998) Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature.* **391**:811-814.

Lin RJ, Evans RM. (2000) Acquisition of oncogenic potential by RAR chimeras in acute promyelocytic leukemia through formation of homodimers. *Mol Cell*. **5**:821-830.

Lin HK, Bergmann S, Pandolfi PP. (2004) Cytoplasmic PML function in TGF-beta signalling. *Nature.* **431**:205-211.

Lindahl T, Wood RD. (1999) Quality control by DNA repair. *Science*. **286**:1897-1905.

Linke SP, Sengupta S, Khabie N, Jeffries BA, Buchhop S, Miska S, Henning W, Pedeux R, WangXW, Hofseth LJ, Yang Q, Garfield SH, Sturzbecher HW, Harris CC. (2003) p53 interacts with hRad51 and hRad54, and directly modulates homologous recombination. *Cancer Res.* **63**:2596-2605.

Liu JH, Mu ZM, Chang KS. (1995) PML suppresses oncogenic transformation of NIH/3T3 cells by activated neu. *J Exp Med.* **181**:1965-1973.

Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD, Berger SL. (1999) p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. *Mol Cell Biol.* **19**:1202-1209.

Liu Y, Lagowski JP, Vanderbeek GE, Kulesz-Martin MF. (2004) Facilitated search for specific genomic targets by p53 C-terminal basic DNA binding domain. *Cancer Biol Ther.* **3**:1102-1108.

Liu Y, Kulesz-Martin MF. (2006) Sliding into home: facilitated p53 search for targets by the basic DNA binding domain. *Cell Death Differ.* **13**:881-884.

Ljungman M, Zhang F. (1996) Blockage of RNA polymerase as a possible trigger for u.v. light-induced apoptosis. *Oncogene*.**13**:823-831.

Ljungman M, Zhang F, Chen F, Rainbow AJ, McKay BC. (1999) Inhibition of RNA polymerase II as a trigger for the p53 response. *Oncogene.***18**:583-592.

Ljungman M, O'Hagan HM, Paulsen MT. (2001) Induction of ser15 and lys382 modifications of p53 by blockage of transcription elongation. *Oncogene.* **20**:5964-5971.

Llanos S, Clark PA, Rowe J, Peters G. (2001) Stabilization of p53 by p14ARF without relocation of MDM2 to the nucleolus. *Nat Cell Biol.* **3**:445-452.

Lloyd DR, Hanawalt PC. (2000) p53-dependent global genomic repair of benzo[a]pyrene-7,8-diol-9,10-epoxide adducts in human cells. *Cancer Res*. **60**:517-521.

Lloyd DR, Hanawalt PC. (2002) p53 controls global nucleotide excision repair of low levels of structurally diverse benzo(g)chrysene-DNA adducts in human fibroblasts. *Cancer Res.* **62**:5288-5294.

Lohrum MA, Ashcroft M, Kubbutat MH, Vousden KH. (2000) Identification of a cryptic nucleolar-localization signal in MDM2. *Nat Cell Biol.* **2**:179-181.

Lohrum MA, Ludwig RL, Kubbutat MH, Hanlon M, Vousden KH. (2003) Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell*. **3**:577-587.

Lombard DB, Guarente L. (2000) Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres. *Cancer Res.* **60**:2331-2334.

Loughran O and La Thangue NB. (2002) E2F proteins. *Curr Biol.* **12**:R377.

Louria-Hayon I, Grossman T, Sionov RV, Alsheich O, Pandolfi PP, Haupt Y. (2003) The promyelocytic leukemia protein protects p53 from Mdm2-mediated inhibition and degradation. *J Biol Chem.* **278**:33134-33141.

Lu X, Lane DP. (1993) Differential induction of transcriptionally active p53 following UV or ionizing radiation: defects in chromosome instability syndromes? *Cell.* **75**:765-778.

Lu X, Nguyen TA, Appella E, Donehower LA. (2004) Homeostatic regulation of base excision repair by a p53-induced phosphatase: linking stress response pathways with DNA repair proteins. *Cell Cycle*. **3**:1363-1366.

Lukas J, Lukas C, Bartek J. (2004) Mammalian cell cycle checkpoints: signalling pathways and their organization in space and time. *DNA Repair (Amst)*. **3**:997-1007.

Luo J, Li M, Tang Y, Laszkowska M, Roeder RG, Gu W. (2004) Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. Proc *Natl Acad Sci U S A.* **101**:2259-2264.

Maiguel DA, Jones L, Chakravarty D, Yang C, Carrier F. (2004) Nucleophosmin sets a threshold for p53 response to UV radiation. *Mol Cell Biol*. **24**:3703-3711.

Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J, Lukas J. (2000) Rapid destruction of human Cdc25A in response to DNA damage. *Science.* **288**:1425-1429.

Maki CG, Huibregtse JM, Howley PM. (1996) In vivo ubiquitination and proteasome-mediated degradation of p53(1). *Cancer Res*.**56**:2649-2654.

Maki CG, Howley PM. (1997) Ubiquitination of p53 and p21 is differentially affected by ionizing and UV radiation. *Mol Cell Biol*. **17**:355-363.

Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH et al., (1990) Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science*. **250**:1233-1238.

Maltzman W, Czyzyk L. (1984) UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. *Mol Cell Biol*. **4**:1689-1694.

Mantovani F, Gostissa M, Collavin L, Del Sal G. (2004) KeePin´ the p53 family in good shape. *Cell Cycle*. **3**:905-911.

Marcchal V, Elenbaas B, Piette J, Nicolas JC, Levine AJ. (1994) The ribosomal L5 protein is associated with mdm-2 and mdm-2-p53 complexes. *Mol Cell Biol*. **14**:7414-7420.

Marchenko ND, Zaika A, Moll UM. (2000) Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J Biol Chem*. **275**:16202-16212.

Marechal V, Elenbaas B, Piette J, Nicolas JC, Levine AJ. (1994) The ribosomal L5 protein is associated with mdm-2 and mdm-2-p53 complexes. *Mol Cell Biol.* **14**:7414-7420.

Marine JC, Jochemsen AG. (2005) Mdmx as an essential regulator of p53 activity. *Biochem Biophys Res Commun.* **331**:750-760.

Marmorstein LY, Ouchi T, Aaronson SA. (1998) The BRCA2 gene product functionally interacts with p53 and Rad51. *Proc Natl Acad Sci USA*. **95**: 13869-13874.

Martin K, Trouche D, Hagemeier C, Sorensen TS, La Thangue NB, Kouzarides T. (1995) Stimulation of E2F1/DPI transcriptional activity by MDM2 oncoprotein. *Nature*. **375**:691-694.

Mathonnet G, Leger C, Desnoyers J, Drouin R, Therrien JP, Drobetsky EA. (2003) UV wavelength-dependent regulation of transcription-coupled nucleotide excision repair in p53-deficient human cells. *Proc Natl Acad Sci U S A*. **100**:7219-7224.

Matsushime H, Roussel MF, Ashmun RA, Sherr CJ. (1991) Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell.* **65**:701-713.

Matthews DA. (2001) Adenovirus protein V induces redistribution of nucleolin and B23 from the nucleolus to cytoplasm. *J Virol.* **75**:1031-1038.

Mattsson K, Pokrovskaja K, Kiss C, Klein G, Szekely L. (2001) Proteins associated with the promyelocytic leukemia gene product (PML)-containing nuclear body move to the nucleolus upon inhibition of proteasome-dependent protein degradation. *Proc Natl Acad Sci USA*. **98**:1012-1017.

Maul GG, Guldner HH, Spivack JG. (1993) Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). *J Gen Virol*. **74**:2679-90.

Maul GG, Everett RD. (1994) The nuclear location of PML, a cellular member of the C3HC4 zinc-binding domain protein family, is rearranged during herpes simplex virus infection by the C3HC4 viral protein ICP0. *J Gen Virol*. **75**:1223-1233.

Maul GG, Yu E, Ishov AM, Epstein AL. (1995) Nuclear domain 10 (ND10) associated proteins are also present in nuclear bodies and redistribute to hundreds of nuclear sites after stress. *J Cell Biochem.* **59**:498-513.

Maul GG, Ishov AM, Everett RD. (1996) Nuclear domain 10 as preexisting potential replication start sites of herpes simplex virus type-1.*Virology*. **217**:67-75.

Maul GG, Negorev D, Bell P, Ishov AM. (2000) properties and assembly mechanisms of ND10, PML bodies, or PODs. *J Struct Biol.***129**:278-287.

Maya R, Balass M, Kim ST, Shkedy D, Leal JF, Shifman O, Moas M, Buschmann T, Ronai Z, Shiloh Y, Kastan MB, Katzir E, Oren M. (2001) ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. *Genes Dev.* **15**:1067-1077.

Mayer C, Bierhoff H, Grummt I. (2005) The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. *Genes Dev.* **19**:933-941.

Mayer C, Grummt I. (2005) Cellular stress and nucleolar function. *Cell Cycle.* **4**:1036-1038.

Mayo LD, Donner DB. (2001) A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci U S A*. **98**:11598-11603.

Mayo LD, Dixon JE, Durden DL, Tonks NK, Donner DB. (2002) PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. *J Biol Chem*. **277**:5484-5489.

McKay BC, Ljungman M, Rainbow AJ. (1998) Persistent DNA damage induced by ultraviolet light inhibits p21waf1 and bax expression: implications for DNA repair, UV sensitivity and the induction of apoptosis. *Oncogene.* **17**:545-55.

McKay BC, Ljungman M, Rainbow AJ. (1999) Potential roles for p53 in nucleotide excision repair. *Carcinogenesis.* **20**:1389-1396.

McKay BC, Ljungman M. (1999) Role for p53 in the recovery of transcription and protection against apoptosis induced by ultraviolet light. *Neoplasia*. **1**:276-284.

McKay BC, Chen F, Perumalswami CR, Zhang F, Ljungman M. (2000) The tumor suppressor p53 can both stimulate and inhibit ultraviolet light-induced apoptosis. *Mol Biol Cell.* **11**:2543-2551.

McKinney K, Mattia M, Gottifredi V, Prives C. (2004) p53 linear diffusion along DNA requires its C terminus. *Mol Cell.* **16**:413-424.

McLure KG, Lee PW. (1998) How p53 binds DNA as a tetramer. *EMBO J.* **17**:3342-3350.

Meani N, Minardi S, Licciulli S, Gelmetti V, Coco FL, Nervi C, Pelicci PG, Muller H, Alcalay M. (2005) Molecular signature of retinoic acid treatment in acute promyelocytic leukemia. *Oncogene*. **24**:3358-3368.

Medema RH, Bos JL. (1993) The role of p21ras in receptor tyrosine kinase signaling. *Crit Rev Oncog*. **4**:615-661.

Meek DW, Knippschild U. (2003) Posttranslational modification of MDM2. *Mol Cancer Res*. **1**:1017-1026.

Mekeel KL, Tang W, Kachnik LA, Luo CM, DeFrank JS, Powell SN. (1997) Inactivation of p53 results in high rates of homologous recombination. *Oncogene*. **14**:1847-1857.

Melchior F, Hengst L. (2002) SUMO-1 and p53. *Cell Cycle*.**1**:245-249.

Mello JA, Lippard SJ, Essigmann JM. (1995) DNA adducts of cis-diamminedichlorop latinum(II) and its trans isomer inhibit RNA polymerase II differentially in vivo. *Biochemistry.* **34**:14783-14791.

Melnick A, Licht JD. (1999) Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood.* **93**:3167-3215.

Mendez J, Stillman B. (2000) Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol Cell Biol.* **20**:8602-8612.

Mendrysa SM, Perry ME. (2000) The p53 tumor suppressor protein does not regulate expression of its own inhibitor, MDM2, except under conditions of stress. *Mol Cell Biol*. **20**:2023-2030.

Midgley CA, Desterro JM, Saville MK, Howard S, Sparks A, Hay RT, Lane DP. (2000) An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination in vitro and can activate p53 in vivo. *Oncogene*. **19**:2312-2323.

Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll UM. (2003) p53 has a direct apoptogenic role at the mitochondria. *Mol Cell.* **11**:577-590.

Migliorini D, Lazzerini Denchi E, Danovi D, Jochemsen A, Capillo M, Gobbi A, Helin K, Pelicci PG, Marine JC. (2002) Mdm4 (Mdmx) regulates p53-induced growth arrest and neuronal cell death during early embryonic mouse development. *Mol Cell Biol.* **22**:5527-5538.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science.* **266**:66-71.

Minsky N, Oren M. (2004) The RING domain of Mdm2 mediates histone ubiquitylations and transcriptional repression. *Mol Cell.* **16**:631-639.

Minucci S, Maccarana M, Cioce M, De Luca P, Gelmetti V, Segalla S, Di Croce L, Giavara S, Matteucci C, Gobbi A, Bianchini A, Colombo E, Schiavoni I, Badaracco G, Hu X, Lazar MA, Landsberger N, Nervi C, Pelicci PG. (2000) Oligomerization of RAR and AML1 transcription factors as a novel mechanism of oncogenic activation. *Mol Cell*. **5**:811-820.

Mirza A, Wu Q, Wang L, McClanahan T, Bishop WR, Gheyas F, Ding W, Hutchins B, Hockenberry T, Kirschmeier P, Greene JR, Liu S. (2003) Global transcriptional program of p53 target genes during the process of apoptosis and cell cycle progression. *Oncogene.* **22**:3645-5364.

Mirzayans R, Enns L, Dietrich K, Barley RD, Paterson MC. (1996) Faulty DNA polymerase delta/epsilon-mediated excision repair in response to gamma radiation or ultraviolet light in p53-deficient fibroblast strains from affected members of a cancer-prone family with Li-Fraumeni syndrome. *Carcinogenesis.***17**:691-698.

Mishina Y, Duguid EM, He C. (2006) Direct reversal of DNA alkylation damage. *Chem Rev*.**106**:215-232.

Mitchell JR, Hoeijmakers JH, Niedernhofer LJ. (2003) Divide and conquer: nucleotide excision repair battles cancer and ageing. *Curr Opin Cell Biol*. **15**:232-240.

Miyashita T, Reed JC. (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell.* **80**:293-299.

Miyazaki Y, Takamatsu T, Nosaka T, Fujita S, Martin TE, Hatanaka M. (1995) The cytotoxicity of human immunodeficiency virus type 1 Rev: implications for its interaction with the nucleolar protein B23. *Exp Cell Res*. **219**:93-101.

Moll UM, Petrenko O. (2003) The MDM2-p53 interaction. *Mol Cancer Res.* **1**:1001-1008.

Momand J, Zambetti GP, Olson DC, George D, Levine AJ. (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell*. **69**:1237-1245.

Mone MJ, Volker M, Nikaido O, Mullenders LH, van Zeeland AA, Verschure PJ, Manders EM, van Driel R. (2001) Local UV-induced DNA damage in cell nuclei results in local transcription inhibition. *EMBO Rep.* **2**:1013-1017.

Montes de Oca Luna R, Wagner DS, Lozano G. (1995) Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature.* **378**:203-206.

Moroni MC, Hickman ES, Lazzerini Denchi E, Caprara G, Colli E, Cecconi F, Muller H, Helin K. (2001) Apaf-1 is a transcriptional target for E2F and p53. *Nat Cell Biol.* **3**:552-558.

Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT. (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science.* 263:1281-1284.

Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS. (1994) PML, a growth suppressor disrupted in acute promyelocytic leukemia. *Mol Cell Biol*. **14**:6858-6867.

Mu ZM, Le XF, Glassman AB, Chang KS. (1996) The biologic function of PML and its role in acute promyelocytic leukemia. *Leuk Lymphoma.* **23**:277-285.

Mu ZM, Le XF, Vallian S, Glassman AB, Chang KS. (1997) Stable overexpression of PML alters regulation of cell cycle progression in HeLa cells. *Carcinogenesis.* **18**:2063-2069.

Muller S, Matunis MJ, Dejean A. (1998) Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *EMBO J.* **17**:61-70.

Mummenbrauer T, Janus F, Muller B, Wiesmuller L, Deppert W, Grosse F (1996) p53 exhibits 3´-to 5´-exonuclease activity. *Cell.* **85**:1089-1099.

Muppidi JR, Tschopp J, Siegel RM. (2004) Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity*. **21**:461-465.

Nagashima M, Shiseki M, Miura K, Hagiwara K, Linke SP, Pedeux R, Wang XW, Yokota J, Riabowol K, Harris CC. (2001) DNA damage-inducible gene p33ING2 negatively regulates cell proliferation through acetylation of p53. *Proc Natl Acad Sci USA*. **98**:9671-9676.

Nagashima M, Shiseki M, Pedeux RM, Okamura S, Kitahama-Shiseki M, Miura K, Yokota J, Harris CC. (2003) A novel PHD-finger motif protein p47ING3, modulates p53-mediated transcription, cell cycle control, and apoptosis. *Oncogene*. **22**:343-350.

Nakamura S, Roth JA, Mukhopadhyay T. (2000) Multiple lysine mutations in the C-terminal domain of p53 interfere with MDM2-dependent protein degradation and ubiquitination . *Mol Cell Biol.* **20**:9391-9398.

Nakano K, Vousden KH. (2001) PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell.* **7**:683-694.

Nefkens I, Negorev DG, Ishov AM, Michaelson JS, Yeh ET, Tanguay RM, Muller WE, Maul GG. (2003) Heat shock and Cd2+ exposure regulate PML and Daxx release from ND10 by independent mechanisms that modify the induction of heat-shock proteins 70 and 25 differently. *J Cell Sci.* **116**:513-524.

Negorev D, Maul GG. (2001) Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. *Oncogene*. **20**:7234-7242.

Nozawa Y, Van Belzen N, Van der Made AC, Dinjens WN, Bosman FT. (1996) Expression of nucleophosmin/B23 in normal and neoplastic colorectal mucosa. *J Pathol.* **178**:48-52.

Nurse P. (2000) A long twentieth century of the cell cycle and beyond. *Cell*. **100**:71-78.

Nyberg KA, Michelson RJ, Putnam CW, Weinert TA. (2002) Toward maintaining the genome: DNA damage and replication checkpoints. *Annu Rev Genet.* **36**:617-656.

Nylander K, Bourdon JC, Bray SE, Gibbs NK, Kay R, Hart I, Hall PA. (2000) Transcriptional activation of tyrosinase and TRP-1 by p53 links UV irradiation to the protective tanning response. *J Pathol.* **190**:39-46.

Oberosler P, Hloch P, Ramsperger U, Stahl H. (1993) p53-catalyzed annealing of complementary single-stranded nucleic acids. *EMBO J.* **12**:2389-2396.

O´Connor DJ, Lu X. (2000) Stress signals induce transcriptionally inactive E2F-1 independently of p53 and Rb. *Oncogene.* **19**:2369-2376.

Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T, Tanaka N. (2000) Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science.* **288**:1053-1058.

Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y. (2000) p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell*. **102**:849-862.

Offer H, Wolkowicz R, Matas D, Blumenstein S, Livneh Z, Rotter V. (1999) Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery. *FEBS Lett.* **450**:197-204.

Offer H, Milyavsky M, Erez N, Matas D, Zurer I, Harris CC, Rotter V. (2001) Structural and functional involvement of p53 in BER in vitro and in vivo. *Oncogene.* **20**:581-589.

O'Hagan HM, Ljungman M. (2004) Nuclear accumulation of p53 following inhibition of transcription is not due to diminished levels of MDM2. *Oncogene*.**23**:5505-5512.

Okamoto K, Li H, Jensen MR, Zhang T, Taya Y, Thorgeirsson SS, Prives C. (2002) Cyclin G recruits PP2A to dephosphorylate Mdm2. *Mol Cell*. **9**:761-771.

Okuda M, Horn HF, Tarapore P, Tokuyama Y, Smulian AG, Chan PK, Knudsen ES, Hofmann IA, Snyder JD, Bove KE, Fukasawa K. (2000) Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell.* **103**:127-140.

Okuda M. (2003) The role of nucleophosmin in centrosome duplication. *Oncogene*. **21**:6170-6174.

Okuwaki M, Matsumoto K, Tsujimoto M, Nagata K. (2001) Function of nucleophosmin/B23, a nucleolar acidic protein, as a histone chaperone. *FEBS Lett.* **506**:272-276.

Okuwaki M, Tsujimoto M, Nagata K. (2002) The RNA binding activity of a ribosome biogenesis factor, nucleophosmin/B23, is modulated by phosphorylation with a cell cycle-dependent kinase and by association with its subtype. *Mol Biol Cell.* **13**:2016-2030.

Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature.* **358**:80-83.

Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. (1993) Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature.* **362**:857-860.

Olive KP, Tuveson DA, Ruhe ZC, Yin B, Willis NA, Bronson RT, Crowley D, Jacks T. (2004) Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell*. **119**:847-860.

Ollmann M, Young LM, Di Como CJ, Karim F, Belvin M, Robertson S, Whittaker K, Demsky M, Fisher WW, Buchman A, Duyk G, Friedman L, Prives C, Kopczynski C. (2000) Drosophila p53 is a structural and functional homolog of the tumor suppressor p53. *Cell.* **101**:91-101.

Olney HJ, Le Beau MM. (2002) The myelodysplastic syndromes, Pathobiology and clinical management (ed. Bennet JM). Marcel Dekker, New York, 2002: 89-120.

Olson MO, Wallace MO, Herrera AH, Marshall-Carlson L, Hunt RC. (1986) Preribosomal ribonucleoprotein particles are major component of a nucleolar matrix fraction. *Biochemistry.* **25**:484-491.

Olson MO, Dundr M, Szebeni A. (2000) The nucleolus: an old factory with unexpected capabilities. *Trends Cell Biol*. **10**:189-196.

Olson MO, Hingorani K, Szebeni A. (2002) Conventional and nonconventional roles of the nucleolus. *Int Rev Cytol*. **219**:199-266.

Olson MO. (2004) Sensing cellular stress: another new function for the nucleolus? *Sci STKE.* **2004**:pe10.

Olson MO, Dundr M. (2005) The moving parts of the nucleolus. *Histochem. Cell Biol*. **123**:203-216.

Pagano M, Pepperkok R, Verde F, Ansorge W, Draetta G. (1992) Cyclin A is required at two points in the human cell cycle. *EMBO J.* **11**:961-971.

Palmero I, Pantoja C, Serrano M. (1998) p19ARF links the tumour suppressor p53 to Ras. *Nature.* **395**:125-126.

Pan Y, Chen J. (2003) MDM2 promotes ubiquitination and degradation of MDMX. *Mol Cell Biol*. **23**:5113-5121.

Pandolfi PP, Grignani F, Alcalay M, Mencarelli A, Biondi A, LoCoco F, Grignani F, Pelicci PG. (1991) Structure and origin of the acute promyelocytic leukemia myl/RAR alpha cDNA and characterization of its retinoid-binding and transactivation properties.*Oncogen*e. **6**:1285-1292.

Pandolfi PP. (2001) In vivo analysis of the molecular genetics of acute promyelocytic leukemia. *Oncogene*. **20**:5726-5735.

Pang Q, Christianson TA, Koretsky T, Carlson H, David L, Keeble W, Faulkner GR, Speckhart A, Bagby GC. (2003) Nucleophosmin interacts with and inhibits the catalytic function of eukaryotic initiation factor 2 kinase PKR. *J Biol Chem.* **278**:41709-41717.

Panse SL, Masson C, Heliot L, Chassery JM, Junera HR, Hernandez-Verdun D. (1999) 3-D organization of ribosomal transcription units after DRB inhibition of RNA polymerase II transcription. *J Cell Sci.***112**:2145-2154.

Parant J, Chavez-Reyes A, Little NA, Yan W, Reinke V, Jochemsen AG, Lozano G. (2001) Rescue of embryonic lethality in Mdm4-null mice by loss of Trp53 suggests a nonoverlapping pathway with MDM2 to regulate p53. *Nat Genet*. **29**:92-95.

Pardee AB. (1989) G1 events and regulation of cell proliferation. *Science.* **246**:603-608.

Pavletich NP. (1999) Mechanism of cyclin-dependent kinase regulation: structures of Cdks, their cyclin activators, and Cip and INK4 inhibitors. *J Mol Biol.* **287**:821-828.

Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. (2000) PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. *Nature.* **406**:207-210.

Pearson M, Pelicci PG. (2001) PML interaction with p53 and its role in apoptosis and replicative senescence. *Oncogene.* **20**:7250-7256.

Pedeux R, Sengupta S, Shen JC, Demidov ON, Saito S, Onogi H, Kumamoto K, Wincovitch S, Garfield SH, McMenamin M, Nagashima M, Grossman SR, Appella E, Harris C. (2005) ING2 regulates the onset of replicative senescence by induction of p300-dependent p53 acetylation. *Mol Cell Biol*. **25**:6639-6678.

Pendle AF, Clark AF, Boon R, Lewandowska D, Lam YW, Andersen J, Mann M, Lamond AI, Brown W, Shaw PJ. (2005) Proteomic analysis of the Arabidopsis nucleolus suggests novel nucleolar functions. *Mol Biol Cell*. **16**:260-269.

Perez A, Kastner P, Sethi S, Lutz Y, Reibel C, Chambon P. (1993) PMLRAR homodimers: distinct DNA binding properties and heteromeric interactions with RXR. *EMBO J.* **12**:3171-3182.

Perry ME, Piette J, Zawadzki JA, Harvey D, Levine AJ. (1993) The mdm-2 gene is induced in response to UV light in a p53-dependent manner. *Proc Natl Acad Sci U S A*. **90**:11623-11627.

Perry ME, Mendrysa SM, Saucedo LJ, Tannous P, Holubar M. (2000) p76(MDM2) inhibits the ability of p90(MDM2) to destabilize p53. *J Biol Chem*. **275**:5733-5738.

Pestov DG, Strezoska Z, Lau LF. Evidence for p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G(1)/S transition. *Mol Cell Biol.* **21**:4246-4255.

Peterson CL, Cote J. (2004) Cellular machineries for chromosomal DNA repair. *Genes Dev*.**18**:602-616.

Petit C, Sancar A (1999) Nucleotide excision repair: from E. coli to man. *Biochimie.* **81**:15-25.

Petrini JH, Stracker TH. (2003) The cellular response to DNA double-strand breaks: defining the sensors and mediators. *Trends Cell Biol*. **13**:458-462.

Petros AM, Gunasekera A, Xu N, Olejniczak ET, Fesik SW. (2004) Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. *FEBS Lett.* **559**:171-174.

Phair RD, Misteli T. (2000) High mobility of proteins in the mammalian cell nucleus. *Nature.* **404**:604-609.

Pines J. (1997) Cyclin-dependent kinase inhibitors: the age of crystals. *Biochim Biophys Acta.* **1332**: M39-M42.

Pomerantz J, Schreiber-Agus N, Liegeois NJ, Silverman A, Alland L, Chin L, Potes J, Chen K, Orlow I, Lee HW, Cordon-Cardo C, DePinho RA. (1998) The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53.*Cell*. **92**:713-723.

Prestayko AW, Klomp GR, Schmoll DJ, Busch H. (1974) Comparison of proteins of ribosomal subunits and nucleolar preribosomal particles from Novkoff hepatoma ascites cells by two-dimensional polyacrylamide gel electrophoresis. *Biochemistry.* **13**:1945-1951.

Price B, Hughes-Davies L, Park S. (1995) cdk2 kinase phosphorylates serine315 of human p53 in vitro. *Oncogene.* **11**:73-80.

Prives C, Hall PA. (1999) The p53 pathway. *J Pathol*.**187**:112-126.

Quelle DE, Zindy F, Ashmun RA, Sherr CJ. (1995)Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. *Cell*. **83**:993-1000.

Quignon F, De Bels F, Koken M, Feunteun J, Ameisen JC, de The H. (1998) PML induces a novel caspase-independent death process. *Nat Genet*. **20**:259-265.

Rabut G, Ellenberg J. (2005) Photobleaching techniques to study mobility and molecular dynamics of proteins in live cells: FRAP, iFRAP and FLIP. In: Live Cell Imaging- A Laboratory manual. Edited by Goldman RD, Spector DL. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 2005: 101-126.

Radfar A, Unnikrishnan I, Lee HW, DePinho RA, Rosenberg N. (1998) p19(Arf) induces p53-dependent apoptosis during abelson virus-mediated pre-B cell transformation. *Proc Natl Acad Sci U S A*. **95**:13194-13199.

Raimondi SC, Dube ID, Valentine MB, Mirro J Jr, Watt HJ, Larson RA, Bitter MA, Le Beau MM, Rowley JD. (1989) Clinicopathologic manifestations and breakpoints of the t(3;5) in patients with acute nonlymphocytic leukemia. *Leukemia.* **3**:42-47.

Ramanathan B, Smerdon MJ. (1986) Changes in nuclear protein acetylation in u.v.-damaged human cells. *Carcinogenesis.* **7**:1087-1094.

Ravanat JL, Douki T, Cadet J. (2001) Direct and indirect effects of UV radiation on DNA and its components. *J Photochem Photobiol B.* **63**:88-102.

Redner RL. (2002) Variations on a theme: the alternate translocations in APL. *Leukemia.***16**:1927-1932.

Reed M, Woelker B, Wang P, Wang Y, Anderson ME, Tegtmeyer P. (1995) The C-terminal domain of p53 recognizesDNA damaged by ionizing radiation. *Proc Natl Acad Sci USA.* **92**:9455-9459.

Rego EM, Wang ZG, Peruzzi D, He LZ, Cordon-Cardo C, Pandolfi PP. (2001) Role of promyelocytic leukemia (PML) protein in tumor suppression. *J Exp Med.* **193**:521-529.

Rego EM, Ruggero D, Tribioli C, Cattoretti G, Kogan S, Redner RK, Pandolfi PP. (2006) Leukemia with distinct phenotypes in transgenic mice expressing PML/RARα, PLZF/RARα or NPM/RARα. *Oncogene.* **25**:1974-1979.

Reinke V, LozanoG. (1997) Differential activation of p53 targets in cells treated with ultraviolet radiation that undergo both apoptosis and growth arrest. *Radiat Res.* **148**:115-122.

Restle A, Janz C, Wiesmuller L. (2005) Differences in the association of p53 phosphorylated on serine 15 and key enzymes of homologous recombination. *Oncogene*. **24**:4380-4387.

Ries S, Biederer C, Woods D, Shifman O, Shirasawa S, Sasazuki T, McMahon M, Oren M, McCormick F. (2000) Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. *Cell.* **103**:321-330.

Rodriguez MS, Desterro JM, Lain S, Midgley CA, Lane DP, Hay RT. (1999) SUMO-1 modification activates the transcriptional response of p53. *EMBO J.* **18**:6455-6461.

Rodriguez MS, Desterro JM, Lain S, Lane DP, Hay RT. (2000) Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. *Mol Cell Biol.* **20**:8458-8467.

Romanova LY, Willers H, Blagosklonny Mv, Powell SN. (2004) The interaction of p53 with replication protein A mediates suppression of homologous recombination. *Oncogene.* **23**:9025-9033.

Roth J, Dobbelstein M, Freedman DA, Shenk T, Levine AJ. (1998) Nucleo-cytoplasmic shuttling of the hdm2 oncoprotein regulates the levels of the p53 protein via a pathway used by the human immunodeficiency virus rev protein. *EMBO J.* **17**:554-564.

Royds JA, Iacopetta B. (2006) p53 and disease: when the guardian angel fails. *Cell Death Differ.* **13**:1017-1026.

Rubbi CP, Milner J. (2000) Non-activated p53 co-localizes with sites of transcription within both the nucleoplasm and the nucleolus. *Oncogene.* **19**:85-96.

Rubbi CP, Milner J. (2003a) p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. *EMBO J.* **22**:975-986.

Rubbi CP, Milner J. (2003b) Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* **22**:6068-6077.

Rush EA, Schlesinger KW, Watkins SC, Redner RL. (2006) The NPM-RAR fusion protein associated with the t(5;17) variant of APL does not interact with PML. *Leuk Res.* **30**:979-986.

Ryan KM, Ernst MK, Rice NR, Vousden KH. (2000) Role of NF-kappaB in p53-mediated programmed cell death. *Nature.* **404**:892-897.

Saavedra HI, Maiti B, Timmers C, Altura R, Tokuyama Y, Fukasawa K, Leone G. (2003) Inactivation of E2F3 results in centrosome amplification. *Cancer Cell.* **3**:333-346.

Sabbatini P, McCormick F. (2002) MDMX inhibits the p300/CBP-mediated acetylation of p53. *DNA Cell Biol.* **21**:519-525.

Saintigny Y, Rouillard D, Chaput B, Soussi T, Lopez BS (1999) Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G1 checkpoint. *Oncogene.* **18**:3553-3565.

Saito S, Yamaguchi H, Higashimoto Y, Chao C, Xu Y, Fornance AJ Jr, Appella E, Anderson CW. (2003) Phosphorylation site interdependence of human p53 post-translational modifications in response to stress. *J Biol Chem.* **278**:37536-37544.

Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E. (1998) DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* **12**:2831-2841.

Salomoni P, Pandolfi PP. (2002) The role of PML in tumor suppression. *Cell.* **108**:165-170.

Salomoni P, Bernardi R, Bergmann S, Changou A, Tuttle S, Pandolfi P. (2005) The promyelocytic leukemia protein PML regulates c-Jun function in response to DNA damage. *Blood*. **105**:3686-3690.

Samuels-Lev Y, O´Connor DJ, Bergamaschi D, Trigiante G, Hsieh JK, Zhong S, Campargue I, Naumovski L, Crook T, Lu X. (2001) ASPP proteins specifically stimulate the apoptotic function of p53. *Mol Cell*. **8**:781-794.

Sato K, Hayami R, Wu W, Nishikawa T, Nishikawa H, Okuda Y, Ogata H, Fukuda M, Ohta T. (2004) Nucleophosmin/B23 is a candidate substrate for the BRCA1-BARD1 ubiquitin ligase. *J Biol Chem.* **279**:30919-30922.

Saucedo LJ, Myers CD, Perry ME. (1999) Multiple murine double minute gene 2 (MDM2) proteins are induced by ultraviolet light. *J Biol Chem.* **274**:8161-8168.

Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh Y, Rotman G. (1995) The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum Mol Genet.* **4**:2025-2032.

Savkur RS, Olson MO. (1998) Preferential cleavage in pre-ribosomal RNA byprotein B23 endoribonuclease. *Nucleic Acids Res*. **26**:4508-4515.

Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. (1990) The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell*. **63**:1129-1136.

Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. (1993) The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell*. **75**:495-505.

Scherer SJ, Maier SM, Seifert M, Hanselmann RG, Zang KD, Muller-Hermelink HK, Angel P, Welter C, Schartl M. (2000) p53 and c-Jun functionally synergize in the regulation of the DNA repair gene hMSH2 in response to UV . *J Biol Chem.* **275**:37469-37473.

Schmidt-Zachmann MS, Hugle-Dorr B, Franke WW (1987) A constitutive nucleolar protein identified as a member of the nucleoplasmin family. *EMBO J.* **6**:1881-1890.

Schmidt-Zachmann MS, Franke WW. DNA cloning and amino acid sequence determination of a major constituent protein of mammalian nucleoli. Correspondence of the nucleoplasmin-related protein NO38 to mammalian protein B23. *Chromosoma.* **96**:417-426.

Schmitt CA, Fridman JS, Yang M, Baranov E, Hoffman RM, Lowe SW. (2002) Dissecting p53 tumor suppressor functions in vivo. *Cancer Cell*. **1**:289-298.

Schmitt CA, Fridman JS, Yang M, Lee E, Baranov E, Hoffman RM, Lowe SW. (2002b) A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. *Cell.* **109**:335-346.

Schnapp A, Pfleiderer C, Rosenbauer H, Grummt I. (1990) A growth-dependent transcription initiation factor (TIF-IA) interacting with RNA polymerase I regulates mouse ribosomal RNA synthesis. *EMBO J.* **9**:2857-2863.

Schon O, Friedler A, Bycroft M, Freund SM, Fersht AR. (2002) Molecular mechanism of the interaction between MDM2 and p53. *J Mol Biol.* **323**:491-501.

Seeler JS, Marchio A, Sitterlin D, Transy C, Dejean A. (1998) Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. *Proc Natl Acad Sci U S A*. **95**:7316-7321.

Segalla S, Rinaldi L, Kilstrup-Nielsen C, Badaracco G, Minucci S, Pelicci PG, Landsberger N. (2003) Retinoic acid receptor alpha fusion to PML affects its transcriptional and chromatin-remodeling properties. *Mol Cell Biol*. **23**:8795-8808.

Sengupta S, Harris CC. (2005) p53: traffic cop at the crossroads of DNA repair and recombination.*Nat Rev Mol Cell Biol.* **6**:44-55.

Seker H, Rubbi C, Linke SP, Bowman ED, Garfield S, Hansen L, Borden KL, Milner J, Harris CC. (2003) UV-C-induced DNA damage leads to p53-dependent nuclear trafficking of PML. *Oncogene.* **22**:1620-1628.

Selby CP, Drapkin R, Reinberg D, Sancar A. (1997) RNA polymerase II stalled at a thymine dimer: footprint and effect on excision repair. *Nucleic Acids Res.* **25**:787-793.

Seo YR, Fishel ML, Amundson S, Kelley MR, Smith ML. (2002) Implication of p53 in base excision DNA repair: in vivo evidence. *Oncogene.* **21**:731-737.

Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell.* **88**:593-602.

Sesto A, Navarro M, Burslem F, Jorcano JL. (2002) Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc Natl Acad Sci USA*. **99**:2965-2970.

Shao W, Fanelli M, Ferrara FF, Riccioni R, Rosenauer A, Davison K, Lamph WW, Waxman S, Pelicci PG, Lo Coco F, Avvisati G, Testa U, Peschle C, Gambacorti-Passerini C, Nervi C, Miller WH Jr. (1998) Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells. *J Natl Cancer Inst.* **90**:124-133.

Sharpless NE, DePinho RA. (1999) The INK4A/ARF locus and its two gene products. Curr Opin *Genet Dev.* **9**:22-30.

Shaulian E, Karin M. (2002) AP-1 as a regulator of cell life and death. *Nat Cell Biol.* **4**:E131-E136.

Shaulsky G, Goldfinger N, Ben-Zeév A, Rotter V. (1990) Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol Cell Biol.* **10**:6565-6577.

Shaulsky G, Goldfinger N, Tosky MS, Levine AJ, Rotter V. (1991) Nuclear localization is essential for the activity of p53 protein. *Oncogene.* **6**:2055-2065.

Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH, Zipori D. (2005) Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. *Mol Biol Cell.* **16**:2395-2413.

Shay JW, Roninson IB. (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene*. **23**:2919-2933.

Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY. (1997) Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood.* **89**:3354-3360.

Shen ZX, Shi ZZ, Fang J, Gu BW, Li JM, Zhu YM, Shi JY, Zheng PZ, Yan H, Liu YF, Chen Y, Shen Y, Wu W, Tang W, Waxman S, De The H, Wang ZY, Chen SJ, Chen Z. (2004) All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A.* **101**:5328-5335.

Sherr CJ, Roberts JM (1999) CDK inhibitors: positive and negative regulators of the G1-phase progression. *Genes Dev.* **13**:1501-1512.

Sherr CJ, Weber JD. (2000) the ARF/p53 pathway. *Curr Opin Genet Dev.* **10**:94-99.

Sherr CJ. (2001) The INK4a/ARF network in tumour suppression. *Nat Rev Mol Cell Biol.* **2**:731-737.

Shibue T, Takeda K, Oda E, Tanaka H, Murasawa H, Takaoka A, Morishita Y, Akira S, Taniguchi T, Tanaka N. (2003) Integral role of Noxa in p53-mediated apoptotic response. *Genes Dev.* **17**:2233-2238.

Shieh SY, Ikeda M, Taya Y, Prives C. (1997) DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell.* **91**:325-334.

Shields LB, Gercel-Taylor C, Yashar CM, Wan TC, Katsanis WA, Spinnato JA, Taylor DD. (1997) Induction of immune responses to ovarian tumor antigens by multiparity. *J Soc Gynecol Investig.* **4**:298-304.

Shiels C, Islam SA, Vatcheva R, Sasieni P, Sternberg MJ, Freemont PS, Sheer D. (2001) PML bodies associate specifically with the MHC gene cluster in interphase nuclei. *J Cell Sci.* **114**:3705-3716.

Shiloh Y. (2003) ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer.* **3**:155-168.

Shimizu H, Burch LR, Smith AJ, Dornan D, Wallace M, Ball KL, Hupp TR. (2002) The conformationally flexible S9-S10 linker region in the core domain of p53 contains a novel MDM2 binding site whose mutation increases ubiquitination of p53 in vivo. *J Biol Chem.* **277**:28446-28458.

Shirangi TR, Zaika A, Moll UM. (2002) Nuclear degradation of p53 occurs during down-regulation of the p53 response after DNA damage. *FASEB J*. **16**:420-422

Shiseki M, Nagashima M, Pedeux RM, Kitahama-Shiseki M, Miura K, Okamura S, Onogi H, Higashimoto Y, Appella E, Yokota J, Harris CC. (2003) p29ING4 and p28ING5 bind to p53 and p300, and enhance p53 activity. *Cancer Res.* **63**:2373-2378.

Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, Kastan MB. (1997) DNA damage induces phosphorylation of the amino terminus of p53. *Genes Dev*.**11**:3471-3481.

Skaar TC, Prasad SC, Sharareh S, Lippman ME, Brunner N, Clarke R. (1998) Two-dimensional gel electrophoresis analyses identify nucleophosmin as an estrogen regulated protein associated with acquired estrogen-independence in human breast cancer cells. *J Steroid Biochem Mol Biol.* **67**:391-402.

Skalski V, Lin ZY, Choi BY, Brown KR. (2000) Substrate specificity of the p53-associated 3´-5´exonuclease. *Oncogene.* **19**:3321-3329.

Smerdon MJ, Lieberman MW. (1978) Nucleosome rearrangement in human chromatin during UV-induced DNA- reapir synthesis. *Proc Natl Acad Sci U S A*. **75**:4238-4241.

Smith ML, Chen IT, Zhan Q, O´Connot PM, Fornace AJ Jr. (1995) Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage. *Oncogene.* **10**:1053-1059.

Smith ML, Bortnick RA, Sheikh MS, Fornace AJ Jr. (1998) Chromatin relaxation by overexpression of mutant p53, HPV16-E6, or cyclin G transgenes. *Exp Cell Res.* **242**:235-243.

Smith ML, Ford JM, Hollander MC, Bortnick RA, Amundson SA, Seo YR, Deng CX, Hanawalt PC, Fornace AJ Jr. (2000) p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol Cell Biol.* **20**:3705-3714.

Smits VA, Medema RH. (2001) Checking out the G(2)/M transition. *Biochim Biophys Acta.* **1519**:1-12.

Song X, Sheppard HM, Norman AW, Liu X. (1999) Mitogen-activated protein kinase is involved in the degradation of p53 protein in the bryostatin-1-induced differentiation of the acute promyelocytic leukemia NB4 cell line. *J Biol Chem.* **274**:1677-1682.

Soussi T, Caron de Fromentel C, May P. (1990) Structural aspects of the p53 protein in relation to gene evolution. *Oncogene.* **5**:945-952.

Spector DL, Ochs RL, Busch H. (1984) Silver staining, immunofluorescence, and immunoelectron microscopic localization of nucleolar phosphoprotein B23 and C23. *Chromosoma.* **90**:139-148.

Spierings D, McStay G, Saleh M, Bender C, Chipuk J, Maurer U, Green DR. (2005) Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. *Science.* **310**:66-67.

Spivak G, Itoh T, Matsunaga T, Nikaido O, Hanawalt P, Yamaizumi M. (2002) Ultraviolet-sensitive syndrome cells are defective in transcription-coupled repair of cyclobutane pyrimidine dimers. *DNA Repair (Amst)*.**1**:629-643.

Spivak G. (2005) UV-sensitive syndrome. *Mutat Res.* **577**:162-169.

Stadler M, Chelbi-Alix MK, Koken MH, Venturini L, Lee C, Saib A, Quignon F, Pelicano L, Guillemin MC, Schindler C, de Thė H. (1995) Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. *Oncogene.* **11**:2565-2573.

Stallmach A, Giese T, Pfister K, Wittig BM, Kunne S, Humphries M, Zeitz M, Meuer SC. (2001) Activation of beta(1) integrins mediates proliferation and inhibits apoptosis of intestinal CD4-positive lymphocytes. *Eur J Immunol.* **31**:1228-1238.

Sternsdorf T, Jensen K, Will H. (1997)Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. *J Cell Biol.* **139**:1621-1634.

Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ, Wahl GM. (1999) A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of the subcellular localization and p53 activity by NES masking. *EMBO J.* **18**:1660-1672.

Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden KH, Peters G. (1998) The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. *EMBO J.* **17**:5001-5014.

Stuurman N, de Graaf A, Floore A, Josso A, Humbel B, de Jong L, van Driel R. (1992) A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies. *J Cell Sci.* **101**:773-784.

Subong EN, Shue MJ, Epstein JI, Briggman JV, Chan PK, Partin AW. (1999) Monoclonal antibody to prostate cancer nuclear matrix protein (PRO:4-216) recognizes nucleophosmin/B23. *Prostate*. **39**:298-304.

Sugasawa K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH. (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell.* **2**:223-232.

Sugimoto M, Kuo ML, Roussel MF, Sherr CJ. (2003) Nucleolar Arf tumor suppressor inhibits ribosomal RNA processing. *Mol Cell*. **11**:415-424.

Sun Y, Durrin LK, Krontiris TG. (2003) Specific interaction of PML bodies with the TP53 locus in Jurkat interphase nuclei. *Genomics*.**82**:250-252.

Symonds H, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T, Van Dyke T. (1994) p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell*. **78**:703-711.

Swaminathan V, Kishore AH, Febitha KK, Kundu TK. (2005) Human histone chaperone nucleophosmin enhances acetylation-dependent chromatin transcription. *Mol Cell Biol.* **25**:7534-7545.

Szebeni A, Olson MO. (1999) Nucleolar protein B23 has molecular chaperone activities. *Protein Sci*. **8**:905-912.

Szekely L, Pokrovskaja K, Jiang WQ, de The H, Ringertz N, Klein G. (1996) The Epstein-Barr virus-encoded nuclear antigen EBNA-5 accumulates in PML-containing bodies. *J Virol.* **70**:2562-2568.

Szostecki C, Guldner HH, Netter HJ, Will H. (1990) Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. *J Immunol.* **145**:4338-4347.

Takagi M, Absalon MJ, McLure KG, Kastan MB. (2005) Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell*. **123**:49-63.

Takahashi Y, Lallemand-Breitenbach V, Zhu J, de The H. (2004) PML nuclear bodies and apoptosis. *Oncogene.* **23**:2819-2824.

Tan T, Chu G. (2002) p53 binds and activates the xeroderma pigmentosum DDB2 gene in humans but not in mice. *Mol Cell Biol*. **22**:3247-3254.

Tanaka M, Sasaki H, Kino I, Sugimura T, Terada M. (1992) Genes preferentially expressed in embryo stomach are predominantly expressed in gastric cancer. *Cancer Res*. **52**:3372-3377.

Tang J, Chu G. (2002) Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein. *DNA Repair (Amst)*.**1**:601-616.

Tao W, Levine AJ. (1999a) Nucleocytoplasmic shuttling of oncoprotein Hdm2 is required for Hdm2-mediated degradation of p53. *Proc Natl Acad Sci U S A*. **96**:3077-3080.

Tao W, Levine AJ. (1999b) P19(ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. *Proc Natl Acad Sci U S A.* **96**:6937-6941.

Tarapore P, Fukasawa K. (2002) Loss of p53 and centrosome hyperamplification. *Oncogene.* **21**:6234-6240.

Tarapore P, Okuda M, Fukasawa K. (2002) A mammalian in vitro centriole duplication system: evidence for involvement of CDK2/cyclin E and nucleophosmin/B23 in centrosome duplication. *Cell Cycle*. **1**:75-81.

Taylor WR, Stark GR. (2001) Regulation of the G2/M transition by p53. *Oncogene*. **20**:1803-1815.

Terris B, Baldin V, Dubois S, Degott C, Flejou JF, Henin D, Dejean A. (1995) PML nuclear bodies are general targets for inflammation and cell proliferation. *Cancer Res*. **55**:1590-1597.

Therrien JP, Drouin R, Baril C, Drobetsky EA. (1999) Human cells compromised for p53 function exhibit defective global and transcription-coupled nucleotide excision repair, whereas cells compromised for pRb function are defective only in global repair. *Proc Natl Acad Sci U S A.* **96**:15038-15043.

Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. (2006) Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood.***107**:4011-4020.

Thoma F. (1999) Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. *EMBO J.* **18**:6585-6598.

Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. (2000) Recognition of the polyubiquitin proteolytic signal. *EMBO J.* **19**:94-102.

Tijsterman M, de Pril R, Tasseron-de Jong JG, Brouwer J. (1999) RNA polymerase II transcription suppresses nucleosomal modulation of UV-induced (6-4) photoproduct and cyclobutane pyrimidine dimer repair in yeast. *Mol Cell Biol.* **19**:934-940.

Tokuyama Y, Horn HF, Kawamura K, Tarapore P, Fukasawa K. (2001) Specific phosphorylation of nucleophosmin on Thr(199) by cyclin-dependent kinase 2-cyclin E and its role in centrosome duplication. *J Biol Chem.* **276**:21529-21537.

Toledo F, Krummel KA, Lee CJ, Liu CW, Rodewald LW, Tang M, Wahl GM. (2006) A mouse p53 mutant lacking the proline-rich domain rescues Mdm4 deficiency and provides insight into the Mdm2-Mdm4-p53 regulatory network. *Cancer Cell*. **9**:273-285.

Torii S, Egan DA, Evans RA, Reed JC. (1999) Human Daxx regulates Fas-induced apoptosis from nuclear PML oncogenic domains (PODs). *EMBO J.* **18**:6037-49.

Tornaletti S, Hanawalt PC. (1999) Effect of DNA lesions on transcription elongation. *Biochimie.* **81**:139-146.

Tsai LH, Lees E, Faha B, Harlow E, Riabowol K. (1993) The cdk2 kinase is required for the G1-to-S transition in mammalian cells. *Oncogene.* **8**:1593-1602.

Tsui KH, Cheng AJ, Chang PL, Pan TL, Yung BY. (2004) Association of nucleophosmin/B23 mRNA expression with clinical outcome in patients with bladder carcinoma. *Urology.* **64**: 839-844.

Tyrrell RM. (1994) The molecular and cellular pathology of solar ultraviolet radiation. *Mol Aspects Med.* **15**:1-77.

Umekawa H, Chang JH, Correia JJ, Wang D, Wingfield PT, Olson MO. (1993) Nucleolar protein B23: bacterial expression, purification, oligomerization and secondary structures of two isoforms. *Cell Mol Biol Res.* **39**:635-645.

Unger T, Juven-Gershon T, Moallem E, Berger M, Vogt Sionov R, Lozano G, Oren M, Haupt Y. (1999) Critical role for Ser20 of human p53 in the negative regulation of p53 by Mdm2. *EMBO J.* **18**:1805-1814.

Vahdat L, Maslak P, Miller WH Jr, Eardley A, Heller G, Scheinberg DA, Warrell RP Jr. (1994) Early mortality and the retinoic acid syndrome in acute promyelocytic leukemia: impact of leukocytosis, low-dose chemotherapy, PML/RAR-alpha isoform, and CD13 expression in patients treated with all-trans retinoic acid. *Blood.* **84**:3843-3849.

Valery C, Grob JJ, Verrando P. (2001) Identification by cDNA microarray technology of genes modulated by artificial ultraviolet radiation in normal human melanocytes: relation to melanocarcinogenesis. *J Invest Dermatol.* **117**:1471-1482.

Vallian S, Chin KV, Chang KS. (1998) The promyelocytic leukemia protein interacts with Sp1 and inhibits its transactivation of the epidermal growth factor receptor promoter. *Mol Cell Biol.* **18**:7147-7156.

Varley JM. (2003) Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat*. **21**:313-320.

Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. **303**:844-848.

Vaziri H, West MD, Allsopp RC, Davison TS, Wu YS, Arrowsmith CH, Poirier GG, Benchimol S. (1997) ATM-dependnet telomere loss in aging human diploid fibroblasts and DNA damage lead to the post-translational activation of p53 protein involving poly(ADP-ribose) polymerase. *EMBO J.* **16**:6018-6033.

Villa R, Morey L, Raker VA, Buschbeck M, Gutierrez A, De Santis F, Corsaro M, Varas F, Bossi D, Minucci S, Pelicci PG, Di Croce L. (2006) The methyl-CpG binding protein MBD1 is required for PML-RARalpha function. *Proc Natl Acad Sci U S A.* **103**:1400-1405.

Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, Adams JM, Strasser A. (2003) p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science.* **302**:1036-1038.

Vlatkovic N, Guerrera S, Li Y, Linn S, Haines DS, Boyd MT. (2000) MDM2 interacts with the C-terminus of the catalytic subunit of DNA polymerase ε. *Nucleic Acids Res*. **28**:3581-3586.

Volker M, Mone MJ, Karmakar P, van Hoffen A, Schul W, Vermeulen W, Hoeijmakers JH, van Driel R, van Zeeland AA, Mullenders LH. (2001) Sequential assembly of the nucleotide excision repair factors in vivo. *Mol Cell.* **8**:213-224.

von Mikecz A, Zhang S, Montminy M, Tan EM, Hemmerich P. (2000) CREB-binding protein (CBP)/p300 and RNA polymerase II colocalize in transcriptionally active domains in the nucleus. *J Cell Biol*.**150**:265-273.

Vousden KH, Lu X. (2002) Live or let die: the cell's response to p53. *Nat Rev Cancer.* **2**:594-604.

Wahl GM, Carr AM. (2001) The evolution of diverse biological responses to DNA damage: insights from yeast and p53. *Nat Cell Biol.* **3**:E277-E286.

Wakasugi M, Kawashima A, Morioka H, Linn S, Sancar A, Mori T, Nikaido O, Matsunaga T. (2002) DDB accumulates at DNA damage sites immediately after UV irradiation and directly stimulates nucleotide excision repair. *J Biol Chem.* **277**:1637-1640.

Wang D, Umekawa H, Olson MO. (1993) Expression and subcellular locations of two isoforms of nucleolar protein B23 in rat tissues and cells. *Cell Mol Biol Res.* **39**:33-42.

Wang D, Baumann A, Szebeni A, Olson MO. (1994) The nucleic acid binding activity of nucleolar protein B23.1 resides in its carboxyl-terminal end. *J Biol Chem.* **269**:30994-30998.

Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, Wang Z, Freidberg EC, Evans MK, Taffe BG, et al. (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nat Genet.* **10**:188-95.

Wang ZG, Delva L, Gaboli M, Rivi R, Giorgio M, Cordon-Cardo C, Grosveld F, Pandolfi PP.(1998a) Role of PML in cell growth and the retinoic acid pathway. *Science.* **279**:1547-1551.

Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R, Pandolfi PP. (1998b) PML is essential for multiple apoptotic pathways. *Nat Genet.* **20**:266-272.

Wang XW, Zhan Q, Coursen JD, Khan MA, Kontny HU, Yu L, Hollander MC, O'Connor PM, Fornace AJ Jr, Harris CC. (1999) GADD45 induction of a G2/M cell cycle checkpoint. *Proc Natl Acad Sci U S A.* **96**:3706-3711.

Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, Wang Z, Freidberg EC, Evans MK, Taffe BG, Bohr VA, Weeda G, Hoeijimakers JHJ, Forrester K, Harris CC. (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nat Genet.* **10**:188-195.

Wang XW, Tseng A, Ellis NA, Spillare EA, Linke SP, Robles AI, Seker H, Yang Q, Hu P, Beresten S, Bemmels NA, Garfiled S, Harris CC. (2001) Functional interaction of p53 and BLM DNA helicase in apoptosis. *J Biol Chem.* **276**:32948-32955.

Wang X, Michael D, de Murcia G, Oren M. (2002) p53 Activation by nitric oxide involves down-regulation of Mdm2. *J Biol Chem.* **277**:15697-15702.

Wang HY, Liu SX, Zhang M. (2003) Gene expression profile changes in NB4 cells induced by arsenic trioxide. *Acta Pharmacol Sin*. **24**:646-650.

Wang J, Shiels C, Sasieni P, Wu PJ, Islam SA, Freemont PS, Sheer D. (2004) Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. *J Cell Biol.* **164**:515-526.

Wang X, Taplick J, Geva N, Oren M. (2004) Inhibition of p53 degradation by Mdm2 acetylation. *FEBS Lett.* **561**:195-201.

Wang W, Budhu A, Forgues M, Wang XW. (2005) Temporal and spatial control of nucleophosmin by the Ran-Crm1 complex in centrosome duplication. *Nature Cell Biol*. **7**:823-830.

Wang J, Chin MY, Li G. (2006) The novel tumor suppressor p33ING2 enhances nucleotide excision repair via inducement of histone H4 acetylation and chromatin relaxation. *Cancer Res.* **66**:1906-1911.

Wani MA, Zhu Q, El-Mahdy M, Venkatachalam S, Wani AA. (2000) Enhanced sensitivity to anti-benzo(a)pyrene-diol-epoxide DNA damage correlates with decreased global genomic repair attributable to abrogated p53 function in human cells. *Cancer Res.* **60**:2273-2280.

Ward P, Packman S, Loughman W, Sparkes M, Sparkes R, McMahon A, Gregory T, Ablin A. (1984) Location of the retinoblastoma susceptibility gene(s) and the human esterase D locus. *J Med Genet*. **21**:92-95.

Warnick CT, Dabbas B, Ford CD, Strait KA. (2001) Identification of a p53 response element in the promoter region of the hMSH2 gene required for expression in A2780 ovatian cancer cells. *J Biol Chem.* **276**:27363-27370.

Warrell RP Jr, Frankel SR, Miller WH Jr, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jakubowski A, et al. (1991) Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). *N Engl J Med*. **324**:1385-1393.

Waterman JL, Shenk JL, Halazonetis TD. (1995) The dihedral symmetry of the p53 tetramerization domain mandates a conformational switch upon DNA binding. *EMBO J.* **14**:512-519.

Weber JD, Taylor LJ, Roussel MF, Sherr CJ, Bar-Sagi D. (1999) Nucleolar Arf sequesters Mdm2 and activates p53. *Nat Cell Biol.* **1**:20-26.

Webley K, Bond JA, Jones CJ, Blaydes JP, Craig A, Hupp T, Wynford-Thomas D. (2000) Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. *Mol Cell Biol.* **20**:2803-2808.

Weinberg RA (1995) The retinoblastoma protein and cell cycle control. *Cell*. **81**:323-330.

Wei X, Yu ZK, Ramalingam A, Grossman SR, Yu JH, Bloch DB, Maki CG. (2003) Physical and functional interactions between PML and MDM2. *J Biol Chem*. **278**:29288-29297.

Weis K, Rambaud S, Lavau C, Jansen J, Carvalho T, Carmo-Fonseca M, Lamond A, Dejean A. (1994) Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. *Cell.* **76**:345-356.

Webley K, Bond JA, Jones CJ, Blaydes JP, Craig A, Hupp T, Wynford-Thomas D. (2000) Posttranslational modifications of p53 in replicative senescence overlapping but distinct from those induced by DNA damage. *Mol Cell Biol.* **20**:2803-2808.

Wiesmuller L, Cammenga J, Deppert W. (1996) In vivo assay of p53 function in homologous recombination between Simian Virus 40 chromosomes. *J Virol.* **70**:737-744.

Willis TG, Dyer MJS. (2000) The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. *Blood*. **96**:808-822.

Wood RD, Mitchell M, Sgouros J, Lindahl T. (2001) Human DNA repair genes. *Science*. **291**: 1284-1289.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. (1995) Identification of the breast cancer susceptibility gene BRCA2. *Nature.* **378**:789-92.

Wu X, Bayle JH, Olson D, Levine AJ. (1993) The p53-mdm-2 autoregulatory feedback loop. *Genes Dev.* **7**:1126-1132.

Wu MH, Lam CY, Yung BY. (1995) Translocation of nucleophosmin from nucleoli to nucleoplasm requires ATP. *Biochem J*. **305**:987-992.

Wu L, Levine AJ. (1997) Differential regulation of the p21/WAF-1 and mdm2 genes after high-dose UV irradiation: p53-dependent and p53-independent regulation of the mdm2 gene. *Mol Med.* **3**:441-451.

Wu HL, Hsu CY, Liu WH, Yung BY. (1999) Berberine-induced apoptosis of human leukemia HL-60 cells is associated with down-regulation of nucleophosmin/B23 and telomerase activity. *Int J Cancer.* **81**:923-929.

Wu WS, Vallian S, Seto E, Yang WM, Edmondson D, Roth S, Chang KS. (2001) The growth suppressor PML represses transcription by functionally and physically interacting with histone deacetylases. *Mol Cell Biol.* **21**:2259-2268.

Wu MH, Yung BY. (2002) UV stimulation of nucleophosmin/B23 expression is an immediate-early gene response induced by damaged DNA. *J Biol Chem.* **277**:48234-48240.

Wu MH, Chang JH, Yung B. (2002) Resistance to UV-induced cell-killing in nucleophosmin/B23 overexpressed NIH 3T3 fibroblasts: enhancement of DNA repair and up-regulation of PCNA in association with nucleophosmin/B23 over-expression. *Carcinogenesis*. **23**:93-100.

Wulf GM, Liou YC, Ryo A, Lee SW, Lu KP. (2002) Role of Pin1 in the regulation of p53 stability and p21 transactivation, and cell cycle checkpoints in response to DNA damage. *J Biol Chem.* **277**:47976-47979.

Xiao H, Pearson A, Coulombe B, Truant R, Zhang S, Regier JL, Triezenberg SJ, Reinberg D, Flores O, Ingles CJ, et al. (1994) Binding of basal transcription factor TFIIH to the acidic activation domains of VP16 and p53. *Mol Cell Biol.* **14:**7013-7024.

Xiao ZX, Chen J, Levine AJ, Modjtahedi N, Xing J, Sellers WR, Livingston DM. (1995) Interaction between the retinoblastoma protein and the oncoprotein MDM2. *Nature.* **375**:694-698.

Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. (1993) p21 is a universal inhibitor of cyclin kinases. *Nature.* **366**:701-704.

Xirodimas DP, Stephen CW, Lane DP. (2001) Cocompartmentalization of p53 and Mdm2 is a major determinant for Mdm2-mediated degradation of p53. *Exp Cell Res.* **270**:66-77.

Xu D, Wang Q, Gruber A, Bjorkholm M, Chen Z, Zaid A, Selivanova G, Peterson C, Wiman KG, Pisa P. (2000) Downregulation of telomerase reverse transcriptase mRNA expression by wild type p53 in human tumor cells. *Oncogene.* **19**:5123-5133.

Xu Y. (2003) Regulation of p53 responses by post-translational modifications. *Cell Death Differ.* **10**:400-403.

Xu ZX, Timanova-Atanasova A, Zhao RX, Chang KS. (2003) PML colocalizes with and stabilizes the DNA damage response protein TopBP1. *Mol Cell Biol.* **23**:4247-4256.

Xu ZX, Zou WX, Lin P, Chang KS. (2005) A role for PML3 in centrosome duplication and genome stability. *Mol Cell.* **17**:721-732.

Yamaizumi M, Sugano T. (1994) U.v.-induced nuclear accumulation of p53 is evoked through DNA damage of actively transcribed genes independent of the cell cycle. *Oncogene.* **9**:2775-2784.

Yanamadala S, Ljungman M. (2003) Potential role of MLH1 in the induction of p53 and apoptosis by blocking transcription on damaged DNA templates. *Mol Cancer Res.* **1**:747-754.

Yang A, Kaghad M, Caput D, McKeon F. (2002) On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet.* **18**:90-95.

Yang Y, Ludwig RL, Jensen JP, Pierre SA, Medaglia WV, Davydov IV, Safiran YJ, Oberoi P, Kenten JH, Philips AC, Weissman AM, Vousden KH. (2005) Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell.* **7**:547-559.

Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. (2002) SMC1 is a downstream effector in Atm-dependent and independent responses to DNA damage. *Genes Dev.* **16**:571-582.

Ye K. (2006) Nucleophosmin/B23, a multifunctional protein that can regulate apoptosis. *Cancer Biol Ther*. **4**:918-923.

Yoneda-Kato N, Look AT, Kirstein MN, Valentine MB, Raimondi SC, Cohen KJ, Carroll AJ, Morris SW. (1996) The t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. *Oncogene.***12**:265-275.

You BJ, Huang IJ, Liu WH, Hung YB, Chang JH, Yung BY. (1999) Decrease in nucleophosmin/B23 mRNA and telomerase activity during indomethacin-induced apoptosis of gastric KATO-III cancer cells.*Naunyn Schmiedebergs Arch Pharmacol.* **360**:683-690.

Yu J, Zhang L. (2005) The transcriptional targets of p53 in apoptosis control. *Biochem Biophys Res Commun.* **331**:851-858.

Yu GW, Rudiger S, Veprintsev D, Freund S, Fernandez-Fernandez MR, Fersht AR. (2005) The central region of HDM2 provides a second binding site for p53. *Proc Natl Acad Sci.* **103**:1227-1232.

Yuan X, Zhou Y, Casanova E, Chai M, Kiss E, Grone HJ, Schutz G, Grummt I. (2005) Genetic inactivation of the transcription factor TIF-IA leads to nucleolar disruption, cell cycle arrest, and p53-mediated apoptosis. *Mol Cell.* **19**:77-87.

Yung BY, Busch H, Chan PK. (1985) Translocation of nucleolar phosphoprotein B23 (37 kDa/pI 5.1) induced by selective inhibitors of ribosome synthesis. *Biochim Biophys Acta*. **826**:167-173.

Yung BY, Bor AM, Chan PK. (1990) Short exposure to actinomycin D induces "reversible" translocation of protein B23 as well as "reversible" inhibition of cell growth and RNA synthesis in HeLa cells. *Cancer Res.* **50**:5987-5991.

Zacchi P, Gostissa M, Uchida T, Salvagno C, Avolio F, Volinia S, Ronai Z, Blandino G, Schneider C, Del Sal G. (2002) The prolyl isomerase Pin1 reveals a mechanism to control p53 functions after genotoxic insult. *Nature.* **419**:853-857.

Zatsepina OV, Rousselet A, Chan PK, Olson MO, Jordan EG, Bornens M. (1999) The nucleolar phosphoprotein B23 redistributes in part to the spindle poles during mitosis. *J Cell Sci.* **112**:455-466.

Zeller KI, Haggerty TJ, Barrett JF, Guo Q, Wonsey DR, Dang CV. (2001) Characterization of nucleophosmin (B23) as a Myc target by scanning chromatin immunoprecipitation. *J Biol Chem.* **276**:48285-48291.

Zhang H, Somasundra K, Peng Y, Tian H, Zhang H, Bi D, Weber BL, El-Deiry WS. (1998) BRCA1 physically associates with p53 and stimulates its transcriptional activity. *Oncogene.* **16**:1713-1721.

Zhang Y, Xiong Y, Yarbrough WG. (1998) ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell***. 92**:725-734.

Zhang Y, Xiong Y. (1999) Mutations in human ARF exon 2 disrupt its nucleolar localization and impair its ability to block nuclear export of MDM2 and p53. *Mol Cell.* **3**:579-591.

Zhang Y, Xiong Y. (2001) A p53 amino-terminal nuclear export signal inhibited by DNA damage-induced phosphorylation. *Science.* **292**:1910-1915.

Zhang Y, Wolf GW, Bhat K, Jin A, Allio T, Burkhart WA, Xiong Y. (2003) Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. *Mol Cell Biol*. **23**:8902-8912.

Zhang H, Shi X, Paddon H, Hampong M, Dai W, Pelech S. (2004) B23/nucleophosmin serine 4 phosphorylation mediates mitotic functions of polo-like kinase 1. *J Biol Chem.* **279**:35726-34

Zheng PZ, Wang KK, Zhang QY, Huang QH, Du YZ, Zhang QH, Xiao DK, Shen SH, Imbeaud S, Eveno E, Zhao CJ, Chen YL, Fan HY, Waxman S, Auffray C, Jin G, Chen SJ, Chen Z, Zhang J. (2005) Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia. *Proc Natl Acad Sci U S A.* **102**:7653-7658.

Zhong S, Hu P, Ye TZ, Stan R, Ellis NA, Pandolfi PP. (1999) A role for PML and the nuclear body in genomic stability. *Oncogene*. **18**:7941-7947.

Zhong S, Muller S, Ronchetti S, Freemont PS, Dejean A, Pandolfi PP. (2000a) Role of SUMO-1-modified PML in nuclear body formation. *Blood*. **95**:2748-52.

Zhong S, Salomoni P, Pandolfi PP. (2000b) The transcriptional role of PML and the nuclear body. *Nat Cell Biol.* **2**:E85-90.

Zhong S, Salomoni P, Ronchetti S, Guo A, Ruggero D, Pandolfi PP. (2000c) Promyelocytic leukemia protein (PML) and Daxx participate in a novel nuclear pathway for apoptosis. *J Exp Med.* **191**:631-640.

Zhou BB, Elledge SJ. (2000)The DNA damage response: putting checkpoints in perspective. *Nature*. **408**:433-439.

Zhu J, Koken MH, Quignon F, Chelbi-Alix MK, Degos L, Wang ZY, Chen Z, de The H. (1997) Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. *Proc Natl Acad Sci U S A.* **94**:3978-3983.

Zhu J, Zhou W, Jiang J, Chen X. (1998) Identification of a novel p53 functional domain that is necessary for mediating apoptosis. *J Biol Chem.* **273**:13030-13036.

Zhu J, Gianni M, Kopf E, Honore N, Chelbi-Alix M, Koken M, Quignon F, Rochette-Egly C, de The H. (1999) Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins.*Proc Natl Acad Sci U S A.* **96**:14807-14812.

Zhu J, Zhang S, Jiang J, Chen X. (2000) Definition of the p53 functional domains necessary for inducing apoptosis. *J Biol Chem*. **275**:39927-39934.

Zhu Q, Wani MA, El-Mahdy M, WaniAA. (2000) Decreased DNA repair efficiency by loss or disruption of p53 function preferentially affects removal of cyclobutane pyrimidine dimers from non-transcribed strand and slow repair sites in transcribed strand. *J Biol Chem.* **275**: 11492-11497.

Zhu J, Lallemand-Breitenbach V, de The H. (2001) Pathways of retinoic acid- or arsenic trioxide-induced PML/RARalpha catabolism, role of oncogene degradation in disease remission. *Oncogene*. **20**:7257-7265.

Zhu Q, Yao J, Wani G, Wani MA, Wani AA. (2001) Mdm2 mutant defective in binding p300 promotes ubiquitination but not degradation of p53: evidence for the role of p300 in integrating ubiquitination and proteolysis. *J Biol Chem.* **276**:29695-29701.

Zhu J, Chen Z, Lallemand-Breitenbach V, de The H. (2002) How acute promyelocytic leukaemia revived arsenic. *Nat Rev Cancer*. **2**:705-713.

Zhu H, Wu L, Maki CG. (2003) MDM2 and promyelocytic leukemia antagonize each other through their direct interaction with p53. *J Biol Chem.* **278**:49286-49292.

Zhu J, Zhou J, Peres L, Riaucoux F, Honore N, Kogan S, de The H. (2005) A sumoylation site in PML/RARA is essential for leukemic transformation. *Cancer Cell.* **7**:143-153.

Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, Remington L, Jacks T, Brash DE. (1994) Sunburn and p53 in the onset of skin cancer. *Nature.* **372**:773-776.

Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, Sherr CJ, Roussel MF. (1998) Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev.* **12**:2424-2433.

Zirwes RF, Kouzmenko AP, Peters JM, Franke WW, Schmidt-Zachmann MS. (1997) Topogenesis of a nucleolar protein: determination of molecular segments directing nucleolar association. *Mol Biol Cell.* **8**:231-248.

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. (1997) Apaf-1, a human protein homologous to *C.elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell.* **90**:405-413.

Zou T, Rao JN, Liu L, Marasa BS, Keledjian KM, Zhang AH, Xiao L, Bass BL, Wang JY. (2005) Polyamine depletion induces nucleophosmin modulating stability and transcriptional activity of p53 in intestinal epithelial cells. A*m J Physiol Cell Physiol.* **289**:C686-696.

Zurer I, Hofseth LJ, Cohen Y, Xu-Welliver M, Hussain SP, Harris CC, Rotter V. (2004) The role of p53 in base excision repair following genotoxic stress. *Carcinogenesis.* **25**:11-19.