IDENTIFICATION AND CHARACTERIZATION OF CIP2A AS A NOVEL ONCOGENIC INHIBITOR OF PP2A

by

Minna Niemelä

From the Department of Medical Biochemistry and Genetics, University of Turku, Turku Centre for Biotechnology, University of Turku and Åbo Akademi University and Turku Doctoral Programme of Biomedical Sciences (TuBS), University of Turku, Turku, Finland

Supervised by

Professor Jukka Westermarck, MD, PhD
Turku Centre for Biotechnology
University of Turku and Åbo Akademi University
Department of Pathology
University of Turku
Turku, Finland

Reviewed by

Professor Pirjo Laakkonen, MD, PhD
Research Programs Unit, Molecular Cancer Biology
Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

and

Professor Lea Sistonen, PhD
Cell biology
Åbo Akademi University
Turku, Finland

Opponent

Professor Lars-Gunnar Larsson, PhD
Department of Microbiology, Tumor and Cell Biology (MTC)
Karolinska Institutet
Stockholm, Sweden

To Alma

Minna Niemelä

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From the Department of Medical Biochemistry and Genetics, University of Turku, Turku Centre for Biotechnology, University of Turku and Åbo Akademi University and Turku Doctoral Programme of Biomedical Sciences (TuBS), University of Turku, Turku, Finland

ABSTRACT

Inhibition of the tumor suppressor protein phosphatase 2A (PP2A) activity has been identified as one of the five key alterations required for human cell transformation. Regardless of this crucial role in human cancer development, the detailed mechanisms by which PP2A inhibition occurs in human cancers remain largely uncharacterized. PP2A regulates a plethora of cellular signaling cascades. One of the targets of PP2A is Myc oncoprotein, which is destabilized and degraded in response to PP2A-mediated dephosphorylation of Myc serine 62.

In this study we identify Cancerous Inhibitor of PP2A (CIP2A) as a previously uncharacterized endogenous inhibitor of PP2A in human cancer cells. CIP2A inhibits PP2A activity leading to subsequent stabilization of the Myc protein. CIP2A promotes malignant growth of cancer cells *in vitro* and xenograft tumor formation *in vivo* and is overexpressed in cancer. Moreover, we explored the effect of CIP2A on global transcriptional profiles and validated a CIP2A-dependent transcriptional signature. Analysis of the CIP2A signature revealed both Myc-dependent and -independent functions for CIP2A. Importantly, we demonstrate that the CIP2A signature has clinical relevance in human breast cancer subtypes. Finally, we identify the genes potentially mediating the long-term growth suppression in CIP2A depleted cancer cells. Taken together, this work identifies CIP2A as a novel human oncoprotein and describes its function in cancer cells. These results may open novel possibilities for patient stratification and therapeutic intervention of cancer.

KEY WORDS: Protein phosphatase 2A, Cancerous inhibitor of PP2A, Myc, breast cancer, cancer

Minna Niemelä
Uuden syöpää edistävän PP2A-inhibiittorin, CIP2A:n, tunnistaminen ja karakterisointi

Lääketieteellinen biokemia ja genetiikka, Turun yliopisto, Turun biotekniikan keskus, Turun yliopisto ja Åbo Akademi, Turun biolääketieteellinen tutkijakoulu (TuBS), Turun yliopisto

TIIVISTELMÄ

Proteiinifosfataasi 2A (PP2A) on kasvunestäjäproteiini (tuumorisupressori), jonka aktiivisuuden esto on todettu yhdeksi viidestä välttämättömästä edellytyksestä ihmisen solujen muuttuessa syöpäsoluiksi. Huolimatta sen keskeisestä merkityksestä syövän kehityksessä, PP2A:n toiminnan eston mekanismeja syövissä ei tunneta yksityiskohtaisesti. PP2A säätelee lukuisia solun signalointireittejä. Yksi PP2A:n kohdeproteiineista on Myc-onkoproteiini. PP2A defosforyloi Myc:n seriini 62:n, joka johtaa Myc:n epävakauteen ja hajotukseen. Tässä tutkimuksessa kuvattiin ennalta tuntematon endogeeninen PP2A:n syöpäspesifinen inhibiittori, CIP2A. CIP2A:n osoitettiin estävän PP2A:n aktiivisuutta, mikä johtaa Myc-proteiinin stabiilisuuteen. CIP2A edistää syöpäsolujen pahanlaatuista kasvua *in vitro* ja ihonalaisten kasvainten muodostumista *in vivo* hiirimallissa. CIP2A:n osoitettiin myös yli-ilmentyvän ihmisen syövissä. Lisäksi tutkittiin CIP2A:n vaikutusta genominlaajuiseen geenien ilmentymiseen ja validoitiin CIP2A-riippuvainen geenien ilmentymisprofiili. Tämän ilmentymisprofiilin jatkotutkimukset osoittivat, että CIP2A toimii sekä Myc-riippuvaisesti että -riippumattomasti syöpäsoluissa. Lisäksi osoitettiin, että tunnistettu CIP2A-riippuivanen ilmentymisprofiili on kliinisesti merkittävä ihmisen rintasyövän alatyypeissä. Lopuksi tässä tutkimuksessa tunnistettiin geenejä, jotka mahdollisesti välittävät CIP2A:n poistosta aiheutuvaa pitkäkestoista kasvunestoa syöpäsoluissa. Tässä työssä olemme tunnistaneet uuden ihmisen onkoproteiinin, CIP2A:n, ja kuvanneet sen toimintaa syöpäsoluissa. Nämä tulokset voivat tarjota uusia terapeuttisia mahdollisuuksia syövän hoitoon.

AVAINSANAT: Proteiinifosfataasi 2A, CIP2A, Myc, rintasyöpä, syöpä

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ABBREVIATIONS

AML   Acute myeloid leukemia

ATP   Adenosine triphosphate

BET   Bromodomain and extraterminal

BLBC   Basal-like breast cancer

BRCA1   Breast cancer protein 1

DAPk   Death-associated protein kinase

CDK5   Cyclin dependent kinase 5

ChIP   Chromatin immunoprecipitation

CIP2A   Cancerous inhibitor of PP2A

CML   Chronic myeloid leukemia

CTG   Cell Titer Glo

DBD   DNA-binding domain

DNA   Deoxyribonucleic acid

EGFR   Epidermal growth factor receptor

ECM   Extra cellular matrix

EMT   Epithelial mesenchymal transition

ER   Estrogen receptor

ERK   Extra cellular signal regulated kinase

GSK3   Glycogen synthase kinase 3

HER2   Human epidermal growth factor receptor 2

HIF	Hypoxia inducible factor
HNSCC	Head and neck squamous cell carcinoma
HPV	human papilloma virus
hTERT	Telomerase reverse transcriptase
Huwe1	HECT, UBA and WWE domain containing 1
IHC	Immunohistochemistry
JNK	Jun N-terminal kinase
KO	Knock out
Leu	Leucine
LT	Large T antigen
MB	Myc box
MEF	Mouse embryonal fibroblast
MEK	Mitogen activated/ Extracellular signal regulated kinase kinase
miRNA	micro-RNA
Miz-1	Myc-interacting zinc finger 1
mRNA	Messenger ribonucleic acid
OA	Okadaic acid
OCCC	Ovarian clear cell carcinoma
PI3K	Phosphatidylinositol 3 kinase
PP2A	Protein phosphatase 2A
PR	Progesterone receptor

PTM	Post-translational modification
PTPA	Phosphotyrosyl phosphatase activator
Rb	Retinoblastoma protein
RTK	Receptor tyrosine kinase
S	Serine
SD	Standard deviation
SEM	Standard error of mean
Ser	Serine
siRNA	Short interfering RNA
SKP2	S-phase kinase associated protein 2
SNP	Single nucleotide polymorphism
ST	Small T antigen
SV40	Simian virus 40
T	Threonine
TAD	Trans-activating domain
TAP	Tandem affinity purification
TF	Transcription factor
TGFβ	Transforming growth factor beta
Thr	Threonine
TNFα	Tumor necrosis factor alpha
TNBC	Triple negative breast cancer
USP28	Ubiquitin-specific protease 28

LIST OF ORIGINAL PUBLICATIONS

I.   Junttila MR, Puustinen P, <u>Niemelä M</u>, Ahola R, Arnold H, Böttzauw T, Ala-aho R, Nielsen C, Ivaska J, Taya Y, Lu SL, Lin S, Chan EK, Wang XJ, Grènman R, Kast J, Kallunki T, Sears R, Kähäri VM, Westermarck J. (2007).
CIP2A inhibits PP2A in human malignancies.
Cell 130:51-62

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CIP2A signature reveals the Myc dependency of CIP2A-regulated phenotypes and its clinical association with breast cancer subtypes.
Oncogene. Jan 16, (online publication ahead of print)

III.   <u>Niemelä M</u>, Saarela J, Hautaniemi S, Wennerberg K, Kallioniemi OP, Westermarck J. (2012).
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1. INTRODUCTION

Cancer is one of the leading causes of disease-related deaths worldwide. According to worldwide statistics, in 2008, over 12,7 million new cancer cases emerged and cancer was the cause of 7,6 million deaths (13% of all deaths) (Globocan 2008 http://globocan.iarc.fr). Cancer is a common term used for various types of malignant tumors and neoplasms, which may occur in any organ or tissue of the body. Vast amounts of work and monetary efforts have been directed towards cancer research, in order to establish preventative measures as well as develop novel therapeutics for the treatment of cancer. However, these efforts have not lead to decreases in cancer related deaths.

Previous studies have identified five key alterations required for the malignant transformation of human cells. These prerequisites include activation of oncogenic Ras, active telomerase, suppression of tumor suppressor proteins retinoblastoma (Rb) and p53 and inhibition of protein phosphatase 2A (PP2A) activity. PP2A is a serine/threonine phosphatase accounting for majority of the serine/threonine phosphatase activity in the cell. PP2A functions as a tumor suppressor by virtue of its capacity to dephosphorylate several critical signaling proteins involved in malignant cell behavior. PP2A is a negative regulator of a large number of oncoproteins, such as Myc, Akt, β-catenin and Bcl-2. One specific mechanism by which PP2A prevents cellular transformation and restrains cancer cell malignancy is the dephosphorylation-mediated destabilization of the Myc oncoprotein. Thus, reactivation of PP2A function presents an attractive approach to developing novel therapeutic applications for cancer treatment.

This study identifies and characterizes Cancerous Inhibitor of PP2A (CIP2A) as a novel PP2A interacting protein in human cancers. On the molecular level, we assess the role of CIP2A in PP2A inhibition and Myc stabilization. Furthermore, the role of CIP2A in cancer cell growth, tumor formation and in human tumors is evaluated. In addition, we identify and explore CIP2A regulated transcriptional signature and study its Myc-dependence and clinical relevance in breast cancer.

2. REVIEW OF THE LITERATURE

2.1. Cancer

Cancer is the leading course of death worldwide. However, the word cancer does not refer to a single disease but to a collection of malignancies with diverse characteristics. The progression of cancer requires various alterations, which may occur on both genetic and epigenetic level. Inherited mutations may act as a predisposition to cancer formation. However, the majority of cancers form via the accumulation of mutations over a period of years.

Proto-oncogenes, such as Myc and Ras, are genes which in normal cells regulate cellular growth and proliferation. When deregulated they turn into oncogenes and promote malignant growth. These alterations may occur via various processes such as chromosomal translocations, gene amplification or changes in transcription. Tumor suppressor genes, such as p53 and Rb, in turn, restrain the cellular growth. If the tumor suppressor function is inhibited and oncogene activity enhanced, cells become hyperproliferative. In normal cells, a fine tuned balance exists between proto-oncogenes and tumor suppressors and alterations in both are required for cancer occurrence.

Over a decade ago, Hanahan and Weinberg defined the "Hallmarks of cancer", six key alterations, which allow human cancer development. These crucial changes include self-sufficiency in growth signals, insensitivity to growth inhibitory signals, ability to escape programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000). These features are considered commonalities in all cancers. Recently, the same authors revised these hallmarks and suggested two additions: reprogramming of energy metabolism and evading destruction by the immune system (Hanahan and Weinberg, 2011). Genomic instability forms the basis of all the hallmarks of cancer by providing the alterations in genetic material (Hanahan and Weinberg, 2011; Negrini et al., 2010).

Due to recent developments in high-throughput technologies, large amount of genes have been implicated in cancer. However, the exact number of real "cancer genes" remains unknown. Genes implicated in cancers can be divided into drivers and passengers (Haber and Settleman, 2007). Alterations in driver genes are able to contribute to transformation or cancer progression, whereas alterations in passenger genes occur as a result of cancer progression. Distinguishing driver genes from the passengers would be crucial for the identification of potential therapeutical targets in cancer.

Cancer cells within the same tumor may vary highly in their phenotypic qualities. This phenotypic heterogeneity of cancer has been thought to be due to the genetic diversity resulting from Darwinian-like clonal evolution. However, the cancer stem cell (CSC) hypothesis suggests that this heterogeneity may also be of non-genetic origin (Marusyk et al., 2012). According to this model, only a small subpopulation of cells within a tumor is tumorigenic and capable to metastasize. It is suggested that these cancer stem cells undergo epigenetic changes resembling normal cell differentiation. These epigenetic changes lead to formation on phenotypically different, non-tumorigenic cell populations, which go on to form the majority of the tumor cells (Shackleton et al., 2009)

The most lethal feature of cancer is the ability of the primary tumor to metastasize, invade new territories and grow new tumors. Tumors reside within tissues and organs and not as a separate entity. The surrounding normal cells and the extracellular matrix (ECM), form the tumor microenvironment, which potentially plays an important role in tumor progression and metastasis (Negrini et al., 2010). In order to succeed in metastasis, tumors need to acquire blood vessels by a process called angiogenesis. Epithelial-mesenchymal transition (EMT) consists of multiple biochemical changes, which epithelial cells undergo to adopt a mesenchymal phenotype (Kalluri, 2009; Kalluri and Neilson, 2003). In EMT, epithelial cells gain capacity to migrate and invade, resistance to apoptotic signaling and increase the production of ECM components (Kalluri and Weinberg, 2009). Cancer cells undergo EMT in order to disseminate from the primary tumor and metastasize (Kalluri, 2009).

2.1.1 Requirements for human cell transformation

In the process of malignant transformation, cells need to acquire qualities enabling them to escape the normal cellular control mechanisms. The genetic changes underlying the transformation process were first delineated in primary rodent cells in which a combination of at least two mutations in cooperating oncogenes, such as Ras and Myc, were sufficient to transform the cells (Land et al., 1983).

Interestingly, the subsequent transformation studies in human cells demonstrated that the same requirements do not apply between species. In human cells four key alterations are needed for immortalization: the expression of active telomerase, the inhibition of tumor suppressor proteins p53 and Rb, and the activation of Ras oncogene (Hahn et al., 1999; Hahn and Weinberg, 2002; Yu et al., 2001) (Figure 1). Of these, telomerase is a reverse transcriptase enzyme responsible for the maintenance of telomere, which promotes limitless replication of cells. p53 is a tumor suppressor controlling cell cycle, apoptosis and DNA repair. Tumor suppressor Rb controls the G1/S transition in cell cycle by negatively regulating the E2F family of transcription factors (Burkhart and Sage, 2008; Zhu, 2005). Ras proteins (H-Ras, K-Ras and N-Ras) are small GTPases mediating receptor tyrosine kinase (RTK) signaling into various cellular pathways.

Even though introduction of these four genetic changes is sufficient to immortalize normal human cells, the full transformation of human cells was achieved only after introduction of the simian virus 40 (SV40) early region product small T antigen (ST) to the immortalized cells (Hahn et al., 2002; Yu et al., 2001). The SV40 ST inactivates tumor suppressor protein phosphatase 2A (PP2A). Thus, PP2A inhibition has been established as one of the prerequisites for human cell transformation and as a critical step from an immortalized cell to a fully transformed cancer cell (Figure 1).
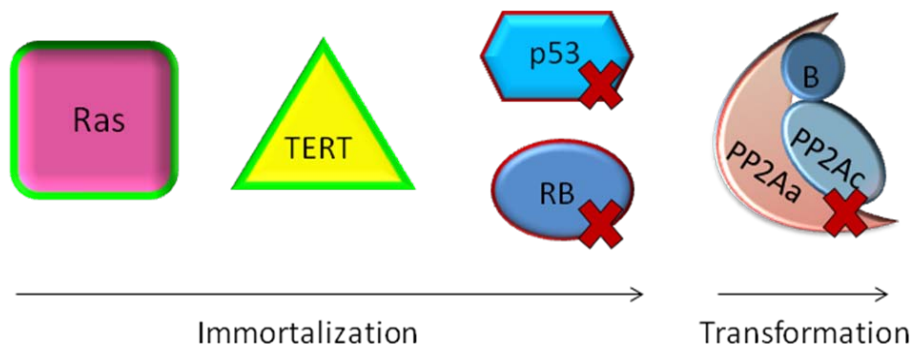
Figure 1. Requirements for human cell transformation.
Active oncogenic Ras and active telomerase enzyme in addition to inhibition of tumor suppressors p53 and Rb are needed for the immortalization of human cells. In addition to these, the inhibition of PP2A is required for the complete transformation of the immortalized cells.

2.1.2. Cancer types

Cancer is a common nominator for many distinct malignancies. Cancers are divided into different types based on their origin. Carcinomas are malignant tumors, which arise from epithelial cells. Majority (~90%) of common human cancers, such as lung, breast, and colon cancers are carcinomas. Adenocarcinomas originate from glandular tissue. Cancers of non-epithelial origin include sarcomas, hematological cancers, neuronal cancers, myelomas and melanomas. Sarcomas originate from mesenchymal tissues, such as muscle, fat, bone, cartilage or vasculature. Sarcomas are much more infrequent than carcinomas, comprising only ~1% of all cancers. Hematological cancers originate from the hematopoietic cells and are divided into leukemias and lymphomas. Neuronal cancers include gliomas, neuroblastomas, schwannomas and medulloblastomas, which all arise from different cell types. Cancers are also classified by name of the organ where the tumor occurs. The human cancer types studied in this thesis work, head and neck squamous cell carcinoma (HNSCC) and breast cancer, will be described in a more detailed manner in the next two chapters.

2.1.3 HNSCC

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer by incidence (Kamangar et al., 2006). The incidence is two times higher in males than in females. HNSCCs locate in the oral cavity, oropharynx, larynx or hypopharynx. Over 95% of all head and neck cancers are squamous cell carcinomas.

A distinct feature in the HNSCC pathogenesis is the formation of genetically altered mucosal preneoplastic fields where the carcinomas form (Leemans et al., 2011). HNSCCs are often not entirely excised in surgery, which may cause local recurrence. The initial metastasis of the HNSCC occurs to the lymph nodes of the neck. Many studies have focused on the role of matrix metalloproteinases (MMP) in HNSCC metastasis (Rosenthal and Matrisian, 2006). Gene expression profiles of the HNSCC tumors are able to predict the lymph node metastasis, but the underlying cancer genes remain to be elucidated (Lallemant et al., 2010).

It is well established that the main exogenous factors contributing to HNSCC development are alcohol consumption and tobacco smoking. Also, human papilloma virus (HPV) infection has been shown to contribute to the formation of a subclass of HNSCCs (Gillison et al., 2000). HPV-positive HNSCCs form a distinct molecular and clinicopathological HNSCC group with good prognosis (Gillison et al., 2000). A recent study also suggests that smoking and HPV status may be significant prognostic features of oropharyngeal cancers (Ang et al., 2010). The classification of HNSCCs into disease stages has traditionally been performed by using the tumor, node, metastasis (TNM) system. Also gene expression patterns have been established for the classification of the HNSCC (Chung et al., 2004).

HNSCC is a heterogeneous collection of diseases, especially on the gene expression level. However, the inactivation of p53 and Rb pathways seems to be a commonality in HNSCC. p53 has been reported to be mutated in 50–80% of HNSCC tumors (Balz et al., 2003; Poeta et

al., 2007; van Houten et al., 2002). The Rb pathway inactivation is obtained by inactivating mutations of p16, hypermethylation of the promoter or a loss of heterozygosity, which have been reported in 75% of HNSCC (Gonzalez-Zulueta et al., 1995; Olshan et al., 1997). The disruption of the p53 and Rb signaling leads to perturbed cell cycle regulation and immortalization (Leemans et al., 2011). Immortalization of mucosal keratinocytes is acquired by overexpression of a dominant-negative p53 mutant in combination with cyclin D1 overexpression or p16 loss (Opitz et al., 2001; Rheinwald et al., 2002). These observations further emphasize the importance of p53 and Rb inactivation in the HNSCC development.

In addition to p53 and Rb, other signaling pathways are frequently perturbed in HNSCC. A subgroup of HNSCC possesses somatic changes in the epidermal growth factor receptor (EGFR) pathway that provides them independence of growth factor stimuli (Leemans et al., 2011). Many HNSCCs harbor mutations in the key genes of the transforming growth factor-β (TGF-β) tumor suppressor pathway (Leemans et al., 2011). A HNSCC mouse model, in which TGFβRII-/- mice are treated with carcinogenic 7,12-dimethylbenz(a)anthracene (DBMA), which induces oncogenic Ras mutations, develops tumors with pathology indistinguishable from human the HNSCC (Lu et al., 2006). Importantly, a recent study reveals that the knockout of Smad4, one of TGF-β downstream effectors, is sufficient to form spontaneuos HNSCC in mouse (Bornstein et al., 2009). Gene amplifications and mutations in the MET oncogene have been reported in HNSCCs (Leemans et al., 2011). MET promotes HNSCCs growth, motility and angiogenesis by activating AKT and Ras signaling pathways (Engelman, 2009; Leemans et al., 2011). Myc amplification has also been reported in HNSCC (Bhattacharya et al., 2005; Rodrigo et al., 1996). However, there are no reported mutations of PP2A in HNSCC.

2.1.4 Breast cancer

Breast cancer is the most common type of tumor in women and the second most common cancer worldwide (Globocan 2008 http://globocan.iarc.fr). The etiology of breast cancer is not comprehensively understood, but family history is an important risk factor. Well studied germline mutations in breast cancer susceptibility genes, such as BRCA1 and BRCA2, account for ~25% of the risk increase (Easton et al., 2007). The expression status of hormone receptors, estrogen receptor (ER) and progesterone receptor (PR), and human epidermal growth factor receptor type 2 (HER2), are predictive markers in breast cancer.

Extensive gene expression profile studies of large human breast tumor data sets have lead to the formation of five molecular subtypes of breast cancer: normal breast-like, basal-like, luminal A, luminal B and HER2+ (Hu et al., 2006; Perou et al., 2000; Sorlie et al., 2001). These tumor subtypes have distinct therapy responses and clinical outcome expectancies. Majority of the luminal-subtype tumors are ductal carcinomas, whereas medullary cancers form a subgroup of basal-like breast cancers (BLBC) (Bertucci et al., 2006; Charafe-Jauffret et al., 2005). Luminal A and B tumors are ER positive whilst normal breast-like, BLBC and HER2+ tumors are ER-negative.

Triple negative breast cancer (TNBC) refers to breast tumors which in immunohistochemical staining do not show significant levels of HER2, ER and PR (Reis-Filho and Tutt, 2008). TNBCs do not form a group of single uniform disease, but comprise various types of tumors, majority of which are basal-like tumors (Carey et al., 2010).

Breast cancer prognosis varies between the tumor subtypes. The ER-positive Luminal A group has good prognosis and the ER-negative HER2+ and BLBC have poor prognosis (Sorlie et al., 2001). Treatment of BLBC is specifically challenging. The lack of ER and HER2+ expression makes them refractory to hormonal therapy and HER2 targeting antibody trastuzumab (Reis-Filho and Tutt, 2008).

A recent meta-analysis of 25 studies revealed that 82% of breast cancer cases exhibited telomerase activity, whereas only 18% of benign lesions had active telomerase (Winnikow et al., 2012). Twenty-five percent of human breast cancers have been reported to harbor p53 mutations (Olivier et al., 2010; Petitjean et al., 2007) p53 mutation status strongly correlates with basal-like and HER2+ breast cancer subtypes and is linked with poor prognosis (Langerød et al., 2007).

2.2. Protein phosphatase 2 A

2.2.1. Structure and function

Protein phosphatase 2A (PP2A) is highly conserved and ubiquitinously expressed serine-threonine phosphatase (Janssens and Goris, 2001). It is a heterotrimeric protein which has a variety of substrates and functions in many cellular processes, such as cell cycle, apoptosis and adhesion.

The multitude and diversity of cellular functions PP2A is involved in is due to the variety of possibilities in the PP2A holoenzyme composition. In fact, the term PP2A does not refer to a single protein, but to a family of trimeric phosphatase complexes. The possible combinations of subunits could result in over 70 distinct trimeric holoenzymes with different targets and functions. In addition, approximately 150 other proteins may associate with PP2A, emphasizing its regulatory potential. PP2A has been shown to be involved in most of the fundamental cellular processes and it accounts for the majority of serine/threonine phosphatase activity in the cell. The ubiquitous expression and the plethora of cellular functions exerted by PP2A emphasize the importance of this sophisticated enzyme. However, the specific PP2A holoenzymes regulating distinct cellular processes still remain to be comprehensively defined. (Eichhorn et al., 2009; Janssens and Goris, 2001; Walter and Ruediger, 2012)

The PP2A heterodimeric core enzyme is comprised of a ~65kDa scaffolding A subunit and a ~36 kDa catalytic C subunit. Even though the dimeric PP2A core complexes (AC) exist as such in cells, the heterotrimeric holoenzyme (ABC) is the predominant form in most cells. The regulatory B subunit accounts for the cellular localization and substrate specificity of PP2A (Figure 2A).
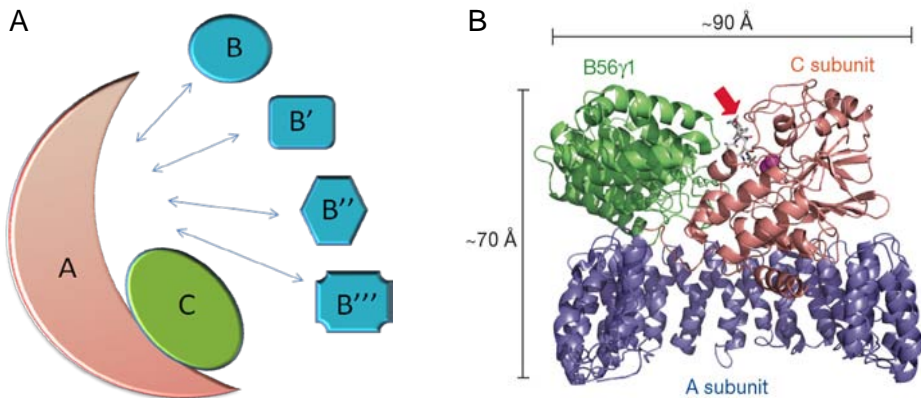
Figure 2.
A) Schematic representation of the trimeric PP2A holoenzyme assembly. A is the scaffolding subunits, C is the catalytic subunit and the four regulatory subunit families are represented by B, B', B'' and B'''.
B) Crystal structure of the trimeric PP2A complex. The red arrow indicates the PP2A active site (Cho and Xu, 2007). Reprinted with permission from Macmillan Publishers Ltd: Nature (Cho and Xu, 2007), copyright 2007.

2.2.1.1 PP2A A-subunit

The PP2A A subunit (PR65/PPP2R1) is a crescent shaped scaffolding unit, which is structurally needed for the holoenzyme composition and enzyme function. Recent identification of the crystal structure of PP2A heterotrimeric holoenzyme further clarified its structural composition and showed that, in the trimeric form, the A subunit is bent into a more

closed horseshoe like shape (Chen et al., 2007; Cho and Xu, 2007; Xu et al., 2006) (Fig. 2B). In mammals, the A subunit has two closely related isoforms (α and β) encoded by two distinct genes which are 87% homologous (Eichhorn et al., 2009; Hemmings et al., 1990; Zhou et al., 2003). The α and β isoforms differ in their B-subunit binding properties (Zhou et al., 2003). Aα is a significantly more prominent isoform in adult tissues, whereas Aβ is more abundant in early developmental stages of vertebrates (Bosch et al., 1995; Hendrix et al., 1993). In addition, Aα overexpression is not sufficient for reversing Aβ depletion mediated transformation (Sablina and Hahn, 2007). Aβ containing PP2A complexes have been shown to specifically dephosphorylate and inactivate RalA, a Ras GTPase family member (Sablina and Hahn, 2007). These results show that Aα and Aβ exert non-redundant functions.

The A subunit is composed almost solely of 15 HEAT-repeats (present in Huntingtin, EF3, PP2A A and TOR), which are capable of mediating protein-protein interactions. The C subunit has been shown to interact with the carboxy-terminal HEAT repeats 11-15 and the B-subunits with amino-terminal repeats 2-8 of the A subunit (Cho and Xu, 2007; Xu et al., 2006).

2.2.1.2 PP2A C-subunit

The catalytic subunit C (PPP2C) accounts for the enzymatic activity of PP2A. In mammals, two closely related (97% homology) isoforms (α and β) of the C-subunit exist (Arino et al., 1988). They are both ubiquitously expressed, even though the expression level of Cα isoform is 10-fold higher than that of the Cβ. This fold difference may be explained by the differences in promoter strength (Janssens and Goris, 2001). Crystal structure of PP2A heterotrimeric holoenzyme widened the understanding of the C subunit function by implicating that the C-terminal tail of the C-subunit is crucial for B subunit recruitment and thus also for the heterotrimer assembly (Cho et al., 2007). The C-subunit may also form an alternate heterodimer by binding B cell receptor-associated protein

alpha4 (Tap42 /α4) instead of the A subunit (Murata et al., 1997). In addition, the knock out (KO) of the catalytic subunit Cα results in delayed embryonic lethality in mice at day 5-6 (Gotz et al., 1998). These results accentuate the indispensable role of PP2A in mammals.

2.2.1.3 PP2A B-subunit

The regulatory B-subunits are divided into four families: B/B55/PR55/PPP2R2, B'/B56/PR61/PPP2R5, B''/PR72/PPP2R3 and B'''/PR93/SG2NA/PR110/Striatin (Table 1). Although all the B-subunits can recognize and bind similar A subunit sites, there is a lack of sequence homology between the B-subunit families (Janssens and Goris, 2001). The B-subunits are indicated to be responsible for PP2A substrate specificity and subcellular localization. Also, the expression of some B-subunits is tissue specific, for example PPP2R2B/B55β is highly enriched in brain (Janssens and Goris, 2001). KO mice lacking PP2A subunit PPP2R5D/B56δ are viable and without any obvious phenotype at young age (Louis et al., 2011). However, they develop a neuronal phenotype: spatially restricted tauopathy, which was shown to be mediated by the deregulation of cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase 3 β (GSK3β) (Louis et al., 2011). Neuronal phenotype could be anticipated for the KO of PPP2R5D/B56δ, since it is expressed at the highest level in brain (Martens et al., 2004). The substrate specificity of the distinct B-subunit containing holoenzymes has not been clearly defined as yet. However, only a subset of B-subunits is thought to be involved in the tumor suppressor activity of PP2A. The PP2A-PPP2R5A/B56α complexes have been indicated in the dephosphorylation and destabilization of Myc (Arnold and Sears, 2006). Recently, PPP2R5A/B56α containing PP2A complexes have been linked to regulation of oncogene-induced senescence in melanocytic cells (Mannava et al., 2012). In turn, PPP2R5C/B56γ has been implicated in the DNA damage induced dephosphorylation of p53 (Li et al., 2007; Shouse et al., 2008).

Table 1. PP2A B-subunits		
Family	Name	Gene name
B	B55α	PPP2R2A
	B55β	PPP2R2B
	B55γ	PPP2R2C
	B55δ	PPP2R2D
B'	B56α	PPP2R5A
	B56β	PPP2R5B
	B56γ	PPP2R5C
	B56δ	PPP2R5D
	B56ε	PPP2R5E
B''	PR72/130	PPP2R3A
	PR48	PPP2R3B
	G5PR	PPP2R3C
B'''	PR93/SG2NA	STRN3
	PR110/Striatin	STRN

2.2.2 Regulation of PP2A

PP2A is involved in a plethora of physiological processes and in order to maintain cellular homeostasis, the activity of PP2A must be carefully controlled. Yet, the mechanisms regulating PP2A *in vivo* remain elusive. The holoenzyme composition with the abundance of possible combinations forms a level of regulation *per se*. Various PP2A binding proteins, both cellular and viral, may also affect its activity. For example, the phosphotyrosyl phosphatase activator (PTPA), which was first considered as a B-subunit (Cayla et al., 1990), is able to reactivate the Ser/Thr phosphatase activity of inactive PP2A (Fellner et al., 2003). PP2A activity is regulated also by post-translational modifications. Leucine 309 (Leu309) of the PP2A C-subunit is carboxymethylated by leucine carboxyl methyltransferase 1 (LCMT1) and demethylated by phosphatase methylesterase PME-1 (Lee and Stock, 1993; Ogris et al., 1999). The leu309 demethylation inactivates PP2A. The C-subunit can also be phosphorylated in Tyr307 or Tyr304. Tyr307 phosphorylation

leads to the inhibition of PP2A activity (Chen et al., 1992), due to inhibition of PPP2R2B/B55β and PPP2R5ABE/B56αβε recruitment. The B56 subunit containing PP2A holoenzymes have been implicated in dephosphorylation and inhibition of ERK activity (Letourneux et al., 2006). In turn, ERK can phosphorylate the B56 subunits, leading to the PP2A holoenzyme disassembly (Letourneux et al., 2006).

In addition to SV40 and other transforming DNA viruses, other human viruses may also affect PP2A activity. Human papilloma virus (HPV) protein E7 has been shown to interact with PP2A and specifically inhibit Akt dephosphorylation, leading to constitutive activation of phosphatidylinositol 3 kinase (PI3K) signaling (Pim et al., 2005). Also Epstein-Barr virus (EBV) has been proposed to bind and inhibit PP2A and thus prevent apoptosis (Garibal et al., 2007). HIV-1 protein Vpr has also been indicated to bind PP2A (Godet et al., 2010), however its effect on PP2A activity remains unclear.

2.2.3. PP2A in cancer

The first implications of the involvement of PP2A in tumor suppression emerged from okadaic acid studies. Okadaic acid is a cytotoxin polyether first isolated from marine sponge (Tachibana, 1981). It is a selective but not specific inhibitor of PP2A and was shown to induce tumor formation in mouse skin (Fujiki et al., 1991; Suganuma et al., 1988). Further indications of the tumor suppression capabilities of PP2A arose with the observations that PP2A activity could be inhibited by several tumor-promoting viruses such as polyoma small T and middle T antigens and SV40 ST (Eichhorn et al., 2009).

Originally SV40 ST was shown to directly interact with the PP2A A subunit and more specifically with its amino-terminus, whereas the C subunit binds to the carboxy-terminus of the A subunit and interacts with ST via the A subunit (Ruediger et al., 1992; Yang et al., 1991). The binding site for PP2A B subunits was shown to overlap with the ST

binding site in the N-terminus of the A subunit (Ruediger et al., 1994). This observation suggested that ST may be able to displace the B subunit from the A subunit. Also other studies supported this model (Chen et al., 2004; Sontag et al., 1993). However, recent structural studies indicate that due to its lower binding affinity, ST is not capable to displace the B subunit from the A subunit (Chen et al., 2007; Cho et al., 2007). Instead, ST was shown to inhibit the phosphatase activity of PP2A through its N-terminal J domain, probably by competing with PP2A substrates for the active site binding (Chen et al., 2007).

The tumor suppressor role of PP2A was further confirmed by showing that the suppression of the Aβ subunit of PP2A enables the transformation of immortalized human cells (Sablina et al., 2007). However, not all PP2A complexes are considered tumor suppressors. Rather only those which contain B-subunits targeting key oncogenes or tumor suppressors are considered. Suppression of either PPP2R5A/B56α, PPP2R5C/B56γ, PPP2R3A/PR72/130 or PTPA, could replace SV40 ST expression in a human cell transformation assay (Sablina et al., 2010), suggesting that PP2A complexes containing these subunits would be specifically important in transformation.

Cancer specific, low frequency mutations have been observed in both Aα and Aβ isoforms of PP2A in human cancers, such as breast, colon and lung cancer, and melanoma (Calin et al., 2000; Wang et al., 1998). Importantly, cancer specific point mutations of the A subunit were shown to inhibit the interaction between the A and C subunits (Ruediger et al., 2001). PP2A structural analysis indicated that these mutations indeed are located in the C-subunit binding site (Xing et al., 2008). PP2A Aα mutations have also been observed in ovarian clear cell carcinomas (OCCC) and in endometrial carcinomas (Jones et al., 2010; McConechy et al., 2011). Missense mutations of the Aβ have been reported in colorectal cancers (Takagi et al., 2000). A Point mutation in PPP2R5C/B56γ in lung cancer was reported to impair p53-dependent tumor suppression ability of PP2A (Shouse et al., 2010). PPP2R2B/B55β silencing by promoter methylation has been reported in human colorectal cancer (Tan et al., 2010). A single nucleotide polymorphism (SNP) in PPP2R5E/B56ε has been associated with onset, risk and survival of soft tissue sarcoma (Grochola et al., 2009). In addition, a recent report

indicated that micro-RNA-31 is able to repress PPP2R2A/B55α in human and mouse lung cancer (Liu et al., 2010).

Despite the accumulating evidence on PP2A mutations in human cancers, in general they occur at low frequency and are not enough to explain PP2A inhibition in all cancers. The next section will concentrate on another level of PP2A regulation: inhibitory proteins.

2.2.3.1 Inhibitors of PP2A

In addition to okadaic acid, other chemical inhibitors and viral proteins, endogenous cellular proteins are also able to inhibit PP2A activity. Three such proteins, SET, PME1 and CIP2A will be discussed in more detail below.

2.2.3.1.1 SET

SET was first identified as myeloid leukemia associated SET-CAN fusion gene and later shown to inhibit PP2A activity (Adachi et al., 1994; Li et al., 1995; Li et al., 1996; von Lindern et al., 1992). SET overexpression has been reported mainly in hematological cancers such as chronic myelogenous leukemia (CML), where it was indicated to operate downstream of the oncogenic BCR-ABL kinase (Neviani et al., 2005). A recent study indicates SET overexpression as a predictor for poor prognosis in B-cell chronic lymphocytic leukemia and non-Hodgkin's lymphoma (Christensen et al., 2011). In solid tumors, SET has been reported to be overexpressed in Wilm's tumors (Carlson et al., 1998). However, according to the Oncomine gene expression database (www.oncomine.org), SET overexpression is detected also in several other cancer types (Westermarck and Hahn, 2008). SET has been implicated in regulating many cellular processes such as cell cycle and cell growth (Canela et al., 2003; Carujo et al., 2006; Seo et al., 2001). However, it still remains elusive which specific cancer-related pathways are affected by SET deregulation.

2.2.3.1.2 PME-1

PME-1 demethylates the C-terminal Leu309 of PP2AC, thus inhibiting PP2A activity (Longin et al., 2004; Ogris et al., 1999). The methylating enzyme of PP2AC 309 is LCMT (Janssens et al., 2008; Lee and Stock, 1993). PME-1 binds the inactive form of the PP2A C subunit and the stabilization of the inactive pool of PP2A has been suggested as the main function of PME-1 (Longin et al., 2004; Longin et al., 2008). The LCMT1-PME-1 regulated PP2A methylation has a crucial role in neuroblastoma cell differentiation (Sontag et al., 2010). PME-1 expression has been shown to promote the activity of the MEK-ERK pathway and to correlate with glioblastoma progression (Puustinen et al., 2009). A recent structural study of PME-1-PP2A complex revealed that PME-1 binds directly to the PP2AC active site. This indicates that in addition to demethylation, PME-1 could also directly inhibit PP2A activity (Longin et al., 2008; Xing et al., 2008).

2.2.2.1.3 CIP2A

The identification of the cancerous inhibitor of PP2A (CIP2A) as an endogenous inhibitor of PP2A is one of the findings in this thesis work and will be discussed in more detail in the Results and Discussion sections. Before this work began, only very limited information was available regarding CIP2A. It was first discovered by the identification of autoantigens against a 90 kDa protein in hepatocellular carcinoma (HCC) and named as p90 for its molecular weight (Soo Hoo et al., 2002). In the same study p90 was cloned and shown to be overexpressed in six out of 11 gastric cancer samples (Soo Hoo et al., 2002).

Even though these results indicated a potential role for CIP2A in human cancer, nothing was known about its function. In this work, we identified CIP2A as a novel endogenous inhibitor of PP2A, which targets the Myc S62 specific dephosphorylation activity of PP2A thus stabilizing the Myc protein (Study I). We also show that CIP2A promotes human cell

transformation and malignant growth of cancer cells (Study I). In addition, CIP2A is shown to be overexpressed in colon cancer and head and neck squamous cell carcinoma (Study I).

After the initial characterization of CIP2A, accumulating studies have further demonstrated the role of CIP2A in human cancers, such as gastric, breast, lung, colon, lung, and prostate cancers, squamous cell carcinomas of the head and neck and acute myeloid leukemia (Come et al., 2009; Dong et al., 2011; Junttila et al., 2007; Katz et al., 2010; Khanna et al., 2009; Li et al., 2008; Qu et al., 2012; Vaarala et al., 2010; Wang et al., 2011)

2.3. Myc

The first *Myc* gene, *v-Myc*, was identified in avian retroviruses for its capability to induce *my*eloma*c*ytomatosis in birds (Alitalo et al., 1983; Sheiness et al., 1978). Later the cellular homologue of *v-Myc, Myc,* was identified (Vennstrom et al., 1982). In fact, Myc protein (previously called c-myc) with N-myc and L-myc form a family of transcription factors, which control the growth, proliferation, and differentiation of normal cells. The *MYC* genes are closely related and evolutionarily conserved (DePinho et al., 1991; Vennstrom et al., 1982). Human *MYC* has two major isoforms p64 and p67, which display distinct expression patterns, p64 being the prominent form (Hann and Eisenman, 1984). Recently a transcriptionally inactive cytoplasmic form of Myc, Myc-nick was identified (Conacci-Sorrell et al., 2010). It is a calpain cleaved form of Myc that lacks the nuclear localization signal and DNA-binding domain (DBD) (Conacci-Sorrell et al., 2010). Myc-nick has been shown to to induce α-tubulin acetylation, alter cell morphology and drive muscle cell differentiation (Conacci-Sorrell et al., 2010).

Myc is a basic helix-loop-helix-zipper (bHLHZ) protein, which belongs to the large Myc/Max/Mad transcription factor protein family. DBD is located at the C-terminus of Myc, whereas the N-terminal 143 residues of Myc contain the transactivation domain (TAD). Within the TAD, two highly conserved sequence domains, Myc box 1 and 2 (MB1, MB2), are indicated to play a role in Myc degradation, transactivation, and to control its oncogenic activities (Flinn et al., 1998; Grandori et al., 2000) (Figure 3).
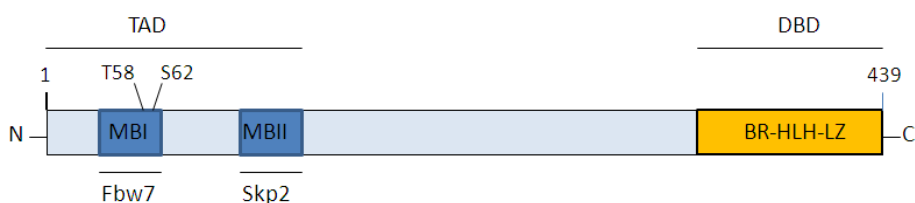

Figure 3. Schematic presentation of the Myc protein.
The transactivation domain (TAD) is located at the N-terminus of Myc. Within the TAD, are located Myc box I and II (MBI and MBII) which are binding sites for ubiquitin ligases Fbw7 and Skp2. The two amino acid residues indicated important for Myc stability (T58 and S62) are located in MB1. The basic helix-loop-helix-leucine zipper (BR-HLH-LZ) forms the DNA binding domain (DBD) of Myc.

2.3.1 Biological role of Myc

The importance of Myc for cellular functions is probably best described by the estimation that 10-15% of all human genes are regulated by Myc (Dang et al., 2006; Meyer and Penn, 2008). Myc plays an important role in normal cell-cycle progression, inhibition of terminal differentiation and induction of apoptosis. In non-transformed cells, expression of *MYC* is dependent on mitogenic signaling and both its mRNA and protein expression levels are low in quiescent cells. However, proliferating cells

are shown to express high levels of Myc (Armelin et al., 1984), implicating an important role in cell proliferation. During development, the expression of *MYC* family genes is highest at the embryonic stage, but downregulated in differentiated tissues (Zimmerman et al., 1986). Differentiated tissues in general are in growth arrest and exert minimal level of cell proliferation. Accordingly, depletion of Myc in HL60 cells was shown to induce cell differentiation (Eckhardt et al., 1994). More recently, Myc and N-myc have been shown to be important for the maintenance of pluripotency and self-renewal in embryonic stem (ES) cells (Cartwright et al., 2005; Takahashi and Yamanaka, 2006), neuronal stem and progenitor cells (Betschinger et al., 2006; Knoepfler et al., 2002) and proliferation and maintenance of hematopoietic stem cells (Laurenti et al., 2008). Recently, Myc protein stability was shown to indicate hematopoietic stem cell quiescence and Fbw7 ubiquitin ligase was shown to be important in Myc degradation in this system (Reavie et al., 2010). These observations emphasize the role of Myc in promoting proliferation and inhibiting differentiation. Many reports have also shown an emerging role for Myc in DNA damage response, but the overall contribution of Myc to this mechanism remains unclear.

An important part of the widespread role of Myc is its function in both activation and repression of transcription. The activation of transcription occurs when Myc is dimerized with the Myc-associated factor X (Max), also a bHLHZ-protein, and binds to the canonical Myc E-box sequence 5'-CACGTG-3' or to the non-canonical E-box sequences (Blackwood and Eisenman, 1991; Eilers and Eisenman, 2008; Grandori et al., 1996; Solomon et al., 1993). The Myc-Max dimerization and subsequent DNA-binding occurs via the bHLHZ-domains. Yet another group of bHLHZ proteins, called the Mad protein, can antagonize Myc in Max binding (Rottmann and Luscher, 2006). Myc-Max dimers can associate with other transcription factors, such as NF-Y or Miz-1, which interfere with the dimer function (Izumi et al., 2001; Mao et al., 2003; Seoane et al., 2002; Staller et al., 2001). Myc-Max association with Myc-interacting zinc finger 1 (Miz-1) leads to the inhibition of Miz-1 transcriptional activity and subsequent transcriptional repression (Kleine-Kohlbrecher et al., 2006). Myc is able to displace the Miz-1 coactivator p300 and thus repress the transcription of Miz-1 target genes (Staller et al., 2001).

2.3.2 Myc target genes

Myc may induce cell proliferation, apoptosis or senescence, depending on the cellular context. Myc has been shown to collaborate with other oncogenic proteins in cell transformation (Pelengaris et al., 2002). Considering these functions, it is plausible that Myc regulates distinct target genes in different contexts. Chromatin immunoprecipitation (ChIP) studies have identified Myc-binding sites in many genes (Fernandez et al., 2003; Patel et al., 2004). However, not all of these genes are regulated by Myc (Fernandez et al., 2003; Patel et al., 2004). Despite the various transcriptional targets and cofactors identified for Myc (Boon et al., 2001; Coller et al., 2000; O'Hagan et al., 2000; Pelengaris et al., 2002), it remains to be clarified whether they contribute to Myc functions in physiological and pathological conditions. The mapping of Myc binding sites in serum-stimulated fibroblasts resulted in identification of 298 Myc-dependent serum response (MDSR) genes (Perna et al., 2012). Most of these genes were shown to be direct transcriptional targets of Myc, but they represented only 5.5% of all promoters bound by Myc (Perna et al., 2012). These results further indicate that Myc controls distinct target subgroups depending on the cellular context.

2.3.3 Regulation of Myc

The activity of Myc is regulated at multiple levels, including gene amplification, transcription, translation, protein stability, and cellular localization. The early studies on Myc regulation focused on transcriptional regulation of *MYC* gene expression. Various signaling pathways including the WNT, ERK, JAK/STAT, TGF-β, and NFk-β have been indicated in deregulation of the Myc promoter (Liu and Levens, 2006). These tumor associated alterations mainly enhance the expression of Myc and affect the expression of the Myc target genes.

In non-transformed cells, the transcription of *MYC* proto-oncogene is tightly regulated. In addition to various transcription factors, non-B DNA structures are also known to contribute to *MYC* regulation (Michelotti et al., 1996). Recent advances in micro-RNA (miRNA) studies have identified Myc mRNA as a target for miRNA mediated repression (Christoffersen et al., 2010; Sachdeva et al., 2009). Myc is also able to negatively autoregulate itself. Myc autoregulation was first identified in non-transformed cells, in which the overexpression of activated Myc or v-Myc resulted in downregulation of endogenous Myc at the level of transcription initiation (Penn et al., 1990). The autoregulation mechanism is sensitive to the overall cellular level of Myc. The autosuppression returns Myc to a level which corresponds to Myc protein levels found in non-transformed cells, thus homeostatically maintaining the appropriate level of Myc signaling and hence restricting cellular proliferation. This negative feedback loop functions only in non-transformed cells. Cancer cells have managed to escape this growth-limiting mechanism.

Myc protein is degraded rapidly in non-transformed cells, with the half-life of only ~20 min (Cole, 1986; Gregory and Hann, 2000). Myc stability is regulated by post-translational modification (PTM) mechanisms. Several different types of post-translational modifications have been found on Myc including phosphorylation, ubiquitination, O-linked glycosylation, and acetylation. Myc protein has also been shown to undergo ubiquitynation and degradation by the proteasome (Ciechanover et al., 1991; Salghetti et al., 1999).

Myc can be phosphorylated at multiple sites spanning the whole length of the protein. The phosphorylation of two specific amino acid residues, serine 62 (S62) and threonine 58 (T58), both located in the MB1 are considered crucial for the Myc stability and activity (Lutterbach and Hann, 1994; Sears et al., 1999). In response to mitogenic stimuli, S62 is phosphorylated by Ras activation mediated mechanism, most likely by ERK (Sears et al., 2000). Also other kinases, such as Jun N-terminal kinase (JNK) and CDKs have been implicated in Myc S62 phosphorylation. The phosphorylation of S62 has been shown to stabilize Myc protein levels (Yeh et al., 2004). PP2A has been identified as the phosphatase dephosphorylating the S62 residue, leading to

destabilization of Myc protein (Arnold and Sears, 2006; Yeh et al., 2004). The phosphorylation of T58 is mediated by GSK3 (Welcker et al., 2004; Yada et al., 2004).

2.3.4 Myc in cancer

In contrast to the tight regulation of Myc in normal cells, expression of Myc is often deregulated in cancer cells. In cancer, the regulation of Myc can be perturbed at different levels: gene amplification, transcription, translation, and post-translational modification. Overexpression of Myc has been observed in many human cancer types and it correlates with advanced tumor stage and poor prognosis (Pelengaris et al., 2002; Vogelstein and Kinzler, 2004). *MYC* gene amplifications have been reported in many human cancers such as lung, breast, colorectal, HNSCC, and osteosarcoma (Al-Kuraya et al., 2007; Bhattacharya et al., 2005; Rodriguez-Pinilla et al., 2007). Interestingly, genetic alterations, such as amplifications, are not the main cause underlying Myc overexpression since they account for only ~20% of the cases (Arnold and Sears, 2006). Thus, regulation of Myc activity in cancer must include an additional level of control.

In addition to its oncogenic properties, the overexpression of Myc has been shown to trigger tumor suppression programs (Murphy et al., 2008). Accordingly, the overexpression of Myc can sensitize cells to apoptosis (Meyer and Penn, 2008). In primary MEFs, the overexpression of Myc has been shown to induce apoptosis by signaling through the p19Arf and subsequently activating p53 (Zindy et al., 1998). The apoptosis induction by Myc may also occur via activation of tumor suppressor p14Arf, which in turn binds MDM2 and activates p53 (Cotter, 2009). Myc has been shown to trigger apoptosis also by repressing the expression of the anti-apoptotic BCL-2 family members, leading to activation of the caspase cascade (Eischen et al., 2001). It has been suggested, that different thresholds define whether the output of Myc expression is oncogenic or apoptotic (Murphy et al., 2008). At lower

levels Myc expression leads to proliferation but higher expression shifts the signaling into tumor suppression (Murphy et al., 2008).

Myc has been shown to be able to suppress oncogene induced senescence. In melanoma cells, Myc inhibits BRAF induced senescence (Zhuang et al., 2008). In rat primary fibroblast, Myc has been shown able to inhibit Ras-induced senescence (Hydbring et al., 2010). This function is dependent on the cyclin dependent kinase 2 (CDK2) mediated phosphorylation of Myc S62 (Hydbring et al., 2010). Ras, in turn, is able to suppress Myc induced apoptosis. The cooperation between these two major oncogenes facilitates the escape of cells from the two major barriers in tumorigenesis, apoptosis and senescence. In contrast, Myc has been shown to induce senescence in cells depleted of Werner syndrome protein (Grandori et al., 2003). These seemingly contradictory results of the Myc contribution to the oncogene-induced senescence may be partly explained by the possible mechanistic differences in Ras and Myc induced senescence.

In mouse fibroblasts lacking CDK2, Myc expression initially triggers a proliferation response, which is then substituted by induction of senescence (Campaner et al., 2010a). This observation indicates cdk2 as a senescence specific suppressor of Myc. Eμ-Myc is a B-cell lymphoma mouse model, in which *MYC* is expressed under the control of the immunoglobulin enhancer (Adams et al., 1985). Studies in this model have indicated a role for Myc in senescence induction, which leads to inhibition of lymphomagenesis (Campaner et al., 2010a; Campaner et al., 2010b; Post et al., 2010).

Myc protein has been shown to undergo ubiquitylation and degradation by the proteasome (Ciechanover et al., 1991; Salghetti et al., 1999). F-box and WD repeat domain-containing 7 (Fbw7), is one of the ubiquitin ligases implicated in directing Myc for proteasomal degradation (Welcker et al., 2004; Yada et al., 2004). Fbw7 is often deleted or mutated in human cancers and acts like a tumor suppressor (Rajagopalan et al., 2004; Welcker and Clurman, 2008). A fairly complex model has been proposed to lead to Fbw7-mediated Myc degradation. First Myc is phosphorylated at S62, which then serves as a priming site for T58 phosphorylation by GSK3 kinase (Lutterbach and Hann, 1994). Next the

S62 is dephosphorylated in a process involving the prolyl isomerase Pin1 and PP2A (Arnold and Sears, 2006; Escamilla-Powers and Sears, 2012). Recently, Axin1 has also been indicated to promote Myc degradation by forming a scaffold for GSK3, PP2A and Pin1 (Arnold et al., 2009). Thereafter, Fbw7 recognizes the T58 phosphorylated Myc and targets it for proteasomal degradation (Yeh et al., 2004). This model is also contradictory, since in general, Fbw7 is thought to bind double phosphorylated substrates (Orlicky et al., 2003). Accordingly, *in vitro*, Fbw7 was shown to recognize a Myc peptide phosphorylated in both T58 and S62 (Yada et al., 2004). However, *in vivo*, Fbw7 was shown to recognize both mono- and bi-phosphorylated form of its other substrate, cyclin E (Welcker and Clurman, 2007). Consistent with its role in Myc protein stability, the phosho-site T58 is frequently mutated in lymphoma (Bahram et al., 2000). But this mutation has not been found thus far in solid tumors. T58A mutation significantly increases the half-life of Myc up to 110 min, emphasizing its importance in Myc stability (Salghetti et al., 1999). The deletion of Myc MB1 (including T58 and S62) only increased protein stability to 45 min (Herbst et al., 2004). These results could be explained by the opposing roles of T58 and S62 phosphorylation. However, these results also suggest that other sites besides the MB1 phosphodegron may be important in the regulation of Myc stability.

Regardless of the specific mechanism of how Fbw7 binds Myc, its role in regulating Myc degradation has been established. Depletion of Fbw7 increases both Myc protein level and its activity (Yada et al., 2004). Interestingly, ubiquitin-specific protease 28 (USP28), has been shown to counteract the Fbw7 dependent ubiquitination of Myc and rescue it from proteasomal degradation (Popov et al., 2007). Supporting this proposed oncogenic function of USP28, it was observed to be upregulated in human colon and breast cancers (Popov et al., 2007). Recently, another ubiquitin ligase β-TRcP was also reported to antagonize the Fbw7 mediated Myc degradation and shown to be required for Myc-induced apoptosis (Popov et al., 2010).

Another E3 ubiquitin ligase S-phase kinase associated protein 2 (SKP2) has been shown to mediate the ubiquitination of Myc by binding to the MB2 and HLHZ domains. SKP2 has also been implicated as a transcriptional cofactor of Myc, for its capability to increase the

transcriptional activity of Myc (Kim et al., 2003; von der Lehr et al., 2003). In accordance with these results, SKP2 has been indicated to function as an oncogene (Frescas and Pagano, 2008). In addition, the SKP2 gene is regulated by Myc, indicating a feedback loop between Myc and SKP2 (Bretones et al., 2011).

HECT, UBA and WWE domain containing 1 (Huwe1) is a ubiquitin ligase, which binds both endogenous Myc and N-myc, but the exact binding site has not been identified (Adhikary et al., 2005; Muller and Eilers, 2008). Instead of Myc degradation, HectH9/Huwe1 has been linked to its activity (Adhikary et al., 2005). HectH9/Huwe1 is overexpressed in many human tumors and has been shown to promote cancer cell proliferation (Adhikary et al., 2005). However, in neural stem/progenitor cells, HectH9/Huwe1 has been shown to destabilize N-Myc and thus promote differentiation (Zhao et al., 2008).

2.3.4.1 Myc in breast cancer

There is accumulating evidence of the involvement of Myc in the induction and progression of breast cancer. The occurrence of *MYC* gene amplification has been observed in 13-22 % of breast cancer cases and has been associated with poor prognosis (Aulmann et al., 2006; Berns et al., 1996; Berns et al., 1992; Corzo et al., 2006). Meta-analysis of 29 studies indicated that the average frequency of *MYC* amplification in breast cancer is 15.7% (Deming et al., 2000; Xu et al., 2010). However, gene amplification does not seem to correlate with Myc mRNA (22-35%) (Bieche et al., 1999; Scorilas et al., 1999) or protein (41.5-45%) (Chrzan et al., 2001; Naidu et al., 2002) expression levels in breast cancer. All tumors harboring *MYC* amplification do not overexpress Myc at the protein level and *vice versa*. Tumors overexpressing Myc protein do not necessarily harbor amplification of the gene (Bruggers et al., 1998; Pertschuk et al., 1993). These observations indicate that yet again Myc regulation involves various levels of control.

Breast cancers are divided into five subtypes. Basal-like subtype breast tumors have the worst prognosis and the luminal A type the best (Sorlie et al., 2001). In general, Myc is highly expressed in basal- (50%) and normal-like tumors (38%) (Sorlie et al., 2001). The transcriptional core signature of Myc was shown to associate with basal-like breast cancer (Chandriani et al., 2009). In addition, *MYC* amplifications have been observed at high levels (15.9%) in medullary cancers (Al-Kuraya et al., 2004), which fall into the basal-like subgroup. *MYC* amplification has also been associated with BRCA1 deregulated breast cancers (Grushko et al., 2004).

2.3.4.2 Myc as a drug target

Myc is a key orchestrator of various cellular processes and signaling cascades essential for cell proliferation, presenting Myc as an attractive target for the development of therapeutic interventions for cancer. Considering its crucial role in growth and proliferation of many cancers, Myc could be seen as the perfect target for cancer therapy. However, there are few obstacles. Myc has no active site and thus, no site for binding of a conventional small molecule inhibitor. Myc is needed for the proliferation of all cells, not only cancer cells, so serious adverse effects would be expected in Myc inhibiting therapy. Recent studies have demonstrated that Myc also possesses an important role in the regulation of the stem cell compartment, suggesting that inhibition of Myc may result in damaging of renewing tissues. Several approaches have been proposed for targeting Myc in cancer, both to inhibit the oncogenic function of Myc and to promote its apoptotic functions.

As Myc has no active site, most studies have focused on interrupting the interaction of Myc-Max dimer. Dimerization can be targeted with inhibitory peptides or small molecules. OmoMyc is a dominant negative Myc bHLHZip mutant, which dimerizes with Myc, inhibiting it from dimerizing with Max (Soucek et al., 1998). This leads to inhibition of Myc-Max E-box binding and inhibition of Myc-dependent transactivation (Soucek et al., 1998; Soucek et al., 2002). However, OmoMyc does not

affect Myc-dependent transcriptional gene repression, which is an E-box independent function (Soucek et al., 2002). OmoMyc has been shown to enhance Myc-induced apoptosis (Soucek et al., 2002) and to inhibit Myc-induced skin papillomas (Soucek et al., 2004).

Transactivation of Myc target genes is associated with increased histone lysine acetylation (Frank et al., 2003; Vervoorts et al., 2003). Bromodomain and extraterminal (BET) family proteins bind acetylated histone lysines and can recruit transcriptional activators (Alderton, 2011). BET bromodomain proteins have been shown to regulate the transcription of Myc and a small-molecule BET inhibitor, JQ1, selectively downregulates Myc and its target genes (Delmore et al., 2011). Importantly, JQ1 was shown to inhibit myeloma cell proliferation (Delmore et al., 2011). BET inhibitor treatment induced antitumor activity in Burkitt's lymphoma and acute myeloid leukemia (AML) xenograft models (Mertz et al., 2011).

G-quadruplexes are four-stranded non-B-DNA structures suggested as good targets for small molecule drugs (Brooks et al., 2010; Sun et al., 1997). A purine-rich strand in the Myc promoter has been shown to be able to form a G-quadruplex structure (Siddiqui-Jain et al., 2002). However, the G-quadruplexes are not well studied *in vivo* and their potential role in targeting Myc needs further validation.

3. AIMS OF THE STUDY

When this study started, our research group had just identified CIP2A as a novel PP2A interacting protein by TAP-purification. However, the mechanisms of CIP2A function and its significance in cancer remained uncharacterized.

The specific aims of this study were:

1. To characterize the novel interaction between PP2A and CIP2A and asses the role of CIP2A in cancer cell growth both *in vitro* and *in vivo.*

2. To identify CIP2A regulated transcriptional signature and use it to characterize novel CIP2A function in cancer cells.

3. To study the gene expression changes mediating the long-term effect of CIP2A depletion in cancer cell growth.

4. MATERIALS AND METHODS

Detailed information on the Materials and Methods is found in the original publications (I-III)

Experimental procedures

Method	Used in
Adhesion assay	II
Colony formation assay on plastic	I, II
Colony growth on soft agar	I
Co-immumoprecipitation	I
Ingenuity analysis	II, III
Incucyte growth assay	III
IHC	I, II
Lentiviral infection	I
Luciferase assay	II
Microarray	I, II, III
Mouse xenografts	I
Phosphatase assay	I
RNA extraction	I, II, III
RT–qPCR	I, II, III
siRNA screen	III

siRNA transfection	I, II, III
Tandem affinity purification	I
Transwell migration assay	II
Wound healing assay	II

Cell lines

Cell line	Cell type	Used in
3T3	Mouse fibroblast	I
AGS	Human gastric cancer	II
HeLa	Human cervical cancer	I, II, III
HEK	Human epidermal keratinocytes	I
HEK-Terv	Human epidermal keratinocytes	I
HEK-293	Human embryonic kidney	I
HT-1080	Human fibrosarcoma	I
MDA-MB-321	Human mammary adenocarcinoma	II
MCF-7	Human mammary epithelial cancer	II
PC-3	Human prostate cancer	III
PNT2	Human normal prostate	III
UT-SCC7	HNSCC	I
UT-SCC9	HNSCC	I

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Materials

DMBA	I
Cycloheximide	I
Okadaic acid	I, II

Antibodies

CIP2A	Rabbit polyclonal	I, II
CIP2A	Mouse monoclonal	II, III
Flag	Mouse monoclonal	I
GST	Mouse monoclonal	I
MDM2	Mouse monoclonal	I
Myc	Mouse monoclonal	I, II, III
Myc	Rabbit polyclonal	I
p-Myc S62	Rabbit polyclonal	I
β-actin	Mouse monoclonal	I, II, III
JNK2	Mouse monoclonal	II
PARP	Rabbit polyclonal	I
PP2Ac	Mouse monoclonal	I
PP2Ac	Rabbit polyclonal	I
PR65	Rabbit polyclonal	I
p-MEK	Rabbit polyclonal	I
p53	Mouse monoclonal	I
V5	Rabbit polyclonal	I

5. RESULTS

5.1. CIP2A is an endogenous inhibitor of PP2A which promotes Myc activity (I, II)

5.1.1 Identification of CIP2A as a novel PP2A interacting protein (I)

In order to discover novel binding partners for PP2A in cancer cells, we used tandem affinity purification (TAP) method (Puig et al., 2001; Westermarck et al., 2002) TAP-purification was performed for cytoplasmic extracts of HT-1080 fibrosarcoma cells stably transfected with TAP-tagged PP2A scaffolding subunit Aα/PR65α vector and mock transfected control cells. The purified proteins were separated in SDS-PAGE gel and silver stained (I Fig. 1B). The proteins co-purifying only with Aα/PR65α as compared to the control, were identified by mass spectrometric peptide sequencing.

The identification of PP2Ac and PPP2R2A/B55α subunits amongst the co-purified proteins showed that we were able to pull down trimeric PP2A, thus validating the TAP approach (I Fig. 1B). In addition to the PP2A subunits, a previously unknown interacting partner, CIP2A (KIAA1524, p90), was co-purified with the Aα/PR65α (I Fig. 1B). This novel interaction was verified in Hela cells by endogenous co-immunoprecipitation of Aα/PR65α, CIP2A and PP2Ac (I Fig. 1C). In addition, confocal microscopy of endogenous CIP2A and PR65α revealed colocalization of these proteins in the cytoplasm and in the perinuclear area of HeLa cells (I Fig. 1D). These results indentified and verified the novel endogenous interaction between PP2A and CIP2A. Other proteins, which were purified with the PP2A complex, have been described since (Puustinen et al., 2009).

5.1.2. CIP2A stabilizes Myc (I, II)

PP2A has been shown to be crucial for cell transformation and it regulates Myc and p53, two transcription factors implicated in cell transformation. Therefore, we analyzed the global transcriptional changes induced by CIP2A depletion and compared them with Myc (http://www.Myc-cancer-gene.org/) and p53 (http://p53.bii.a-star.edu.sg/aboutp53/targetgene/index.php) target databases. Only 1/76 CIP2A affected genes was identified as a p53 target, whereas 12/76 were Myc target genes. This finding and the established Myc regulating role of PP2A indicated that CIP2A might be involved in Myc regulation.

To demonstrate this, we showed that CIP2A depletion by two distinct siRNAs led to downregulation of Myc protein levels 72h after transfection (I Fig. 2A and 2B). Importantly, this effect was rescued by the expression of siRNA-resistant CIP2A cDNA (I Fig. 2A), confirming the specificity of the siRNA. The effect of CIP2A depletion on Myc levels was further confirmed in subsequent studies using HeLa cells (II, Fig. 3A and Suppl. Fig. 2A; III, Fig. 1A). The same effect was also shown in three different low-passage human HNSCC cell lines (I, Fig. 7A). However, CIP2A depletion did not have a significant effect on Myc mRNA expression levels (I Fig. 2D). This was further shown in Study II by microarray analysis of CIP2A depleted cells (II, Suppl. Fig. 2B). These results indicate that CIP2A is involved in the post-transcriptional regulation of Myc. Importantly, PP2A has also been shown to regulate Myc post-transcriptionally (Yeh et al., 2004). Therefore, we analyzed the half-life of Myc protein in CIP2A or scrambled siRNA transfected HeLa cells, treated with cyclohexamide (I Fig. 2E). Myc protein levels were significantly lower in the CIP2A depleted cells 1-2 h after cycloheximide treatment (I Fig. 2F). Taken together, these results show that CIP2A increases Myc protein stability in cancer cells.

5.1.3 CIP2A inhibits the PP2A mediated dephosphorylation of Myc S62 (I)

PP2A has been shown to dephosphorylate Myc S62 and thus destabilize Myc protein (Yeh et al., 2004). Our above described results demonstrated that CIP2A interacts with PP2A and stabilizes Myc protein. Therefore, we next studied whether CIP2A is able to inhibit the activity of PP2A towards Myc. CIP2A depletion downregulated more efficiently the S62 phosphorylated Myc than total Myc protein in HeLa cells (I, Fig. 3A). In order to study the role of PP2A in this event, we immunoprecipitated endogenous Myc from CIP2A or scrambled siRNA treated HeLa cells followed by the measurement of PP2A activity of the immunoprecipitates using 6,8-difluoro-4-methylumbelliferyl substrate (Pastula et al., 2003). Myc immunoprecipitates from CIP2A siRNA treated HeLa cells showed increase in PP2A activity relative to the scrambled siRNA treated control cells (I, Fig 3B). This result was further confirmed by similar experiments in two HNSCC cell lines (I, Fig. 7B). Importantly, the increment in phosphatase activity was rescued by the expression of a siRNA resistant CIP2A cDNA in HeLa cells (I, Fig. 3B). Interestingly, this effect was not detected in the immunorecipitates of MDM2, another PP2A substrate, from the same HeLa extracts (I, Fig 3B). These results indicated that CIP2A selectively inhibits the Myc associated PP2A activity. The specificity of the phosphatase assay for detecting PP2A activity was confirmed by showing that PP2A inhibitor okadaic acid abolished the activity of purified PP2Ac-PR65 core dimer and inhibited the phosphatase activity associated with the Myc immunoprecipitate (I, Fig. S2). Next, we studied whether CIP2A interferes with the interaction between Myc and PP2A. To this end, we analyzed the PP2Ac levels from CIP2A depleted Myc immunoprecipitates (I, Fig. 3C). The depletion of CIP2A did not affect the amount of PP2Ac co-immunoprecipitated with Myc, demonstrating that CIP2A does not affect the PP2A-Myc interaction (I, Fig. 3C). Taken together these results confirm that CIP2A inhibits PP2A activity towards Myc.

5.2. Oncogenic properties of CIP2A (I, II, III)

5.2.1. CIP2A promotes cancer cell proliferation, cell transformation and tumor growth (I, II, III)

It is well established that inhibition of PP2A and activity of Myc increase the malignant properties of human cells. Therefore, we assessed the effect of CIP2A depletion on HeLa cell proliferation. In a thymidine incorporation assay, siRNA mediated depletion of CIP2A resulted in significantly decreased cell proliferation compared to the scrambled siRNA treated control cells (I, Fig. 4A).

CIP2A depletion also significantly inhibited HeLa cell colony formation on plastic (I, Fig 4E and II Fig 4A and 4B). In Study III, we show that CIP2A depletion inhibited the growth of HeLa cells measured as cell confluency (III, Fig. 1A). Also, the depletion of CIP2A significantly inhibited the cell viability of PC-3 prostate cancer cells as compared to normal prostate cells measured by Cell Titer Glo (CTG) assay (Promega) (III, Suppl.Table 1). CTG-assay determines the number of metabolically active viable cells based on quantitation of the adenosine triphosphate (ATP) present in the cells. In addition, CIP2A depletion significantly impaired the ability of anchorage independent growth of both HeLa and HNSCC cells in soft agar assay (I, Fig.4F and Fig. 7D & 7E, respectively). These results indicate that CIP2A promotes the malignant properties of cancer cells. Next, we assessed whether the depletion of CIP2A induces apoptosis. In flow cytometric cell cycle analysis of unsynchronized HeLa cells, CIP2A depletion did not induce increase in sub-G1 fraction (I, Fig 4B). Western blot analysis of lysates of CIP2A or scrambled siRNA treated HeLa cells did not show differences in poly ADP-ribose polymerase (PARP) cleavage, which indicates caspase activity (I, Fig. 4C). These results suggested that depletion of CIP2A does not induce apoptosis in HeLa cells.

To study the role of CIP2A in tumor growth in vivo, we subcutaneously injected athymic nude mice with CIP2A or scrambled siRNA treated HeLa cells and followed the tumor growth for 27 days. CIP2A depletion resulted in a growth delay of tumor xenografts (I, Fig 4G). Importantly, CIP2A depletion led to a significant decrease in tumor weight at the end of the experiment at day 27 (I, Fig. 4H). This effect was further studied in the HNSCC cell lines UT-SCC7 and UT-SCC9, which were transfected with either CIP2A or scrambled siRNA and injected subcutaneously in severe combined immunodeficiency mice (SCID) and the tumor growth was followed for 65 days. Interestingly, 3/5 and 2/6 mice injected with CIP2A depleted UT-SCC7 or UT-SCC9 cells, respectively, did not develop palpable tumors during the 65 days of observation (I, Figure 7F). In addition, depletion of CIP2A reduced the tumor weights in both UT-SCC cell lines (I, Figure 7F). These results demonstrate that CIP2A promotes tumor growth *in vivo*.

Finally, we used an established human cell transformation model to study the role of CIP2A in cell transformation. The HEK-TERV cells harbor inactivated p53 and Rb tumor suppressors and express oncogenic Ras and the catalytic subunit of telomerase (hTERT). These cells are immortalized but not transformed. For complete transformation, they require inhibition of PP2A (Hahn et al., 1999; Hahn et al., 2002). Lentiviral overexpression of CIP2A in HEK-TERV cells was able to transform them, as measured in soft agar growth assay (I, Fig. 5B).

5.2.2. CIP2A is overexpressed in human malignancies

Since the above described results implicated oncogenic properties for CIP2A, we wished to assess the expression of CIP2A in normal and cancerous tissues. For this purpose, we used qRT-PCR to analyze CIP2A mRNA expression in 21 normal human tissues. Except for bone marrow, prostate, testis, cerebellum and brain, CIP2A was expressed at low levels (I, Fig. 6A). In addition, western blot analysis of CIP2A protein levels showed that cancer cell lines HT-1080 and HeLa expressed high levels of CIP2A protein, whereas spontaneously immortalized human

epidermal keratinocytes (HEK) and spontaneously immortalized NIH3T3 mouse fibroblasts expressed very low levels of CIP2A protein (I, Fig. 6B). CIP2A mRNA levels were also significantly higher in HNSCC cell lines than in normal human epidermal keratinocytes (I, Fig. 6C). Immunostaining of human tissue samples showed that 11/14 HNSCC tumors were CIP2A positive, whereas none of the nine normal control tissues showed positive CIP2A staining (I, Fig 6D). To further substantiate the role of CIP2A in HNSCC, we studied its expression in the HNSCC mouse model (Lu et al., 2006). In this model, treatment of the TGF-βRII-/- mice with DMBA, induces oncogenic Ras mutation leading to development on HNSCC tumors with similar pathology to human HNSCC (Lu et al., 2006). In immunohistochemical staining of wild type and mutant mouse tissues, CIP2A overexpression was observed in 62.5% (5/8) of hyperplastic tissues and in 83% (10/12) of HNSCC, whereas wild type tissues were all CIP2A negative (I, Fig. 6E). In order to study the role of CIP2A in another human cancer, we next concentrated on colon cancer as it has also been associated with inhibition of TGF-β signaling and active Ras (Grady and Markowitz, 2002). In a sample panel of 43 human colon cancers and five controls, CIP2A mRNA levels were significantly higher in tumor samples (I, Fig. 6F). Taken together, these results demonstrate that CIP2A is overexpressed in human malignancies.

5.3. CIP2A-driven transcriptional signatures (II, III)

5.3.1 Characterization of the CIP2A signature (II)

The role of CIP2A as a novel PP2A inhibiting and Myc promoting oncogene was established in study I. In study II, we aimed to define a reliable CIP2A regulated gene expression signature. We used Illumina platform to analyze the transcriptional changes in HeLa cells, 3 and 5 days after CIP2A or scrambled siRNA transfection. In order to create a high-confidence signature, the experiment was repeated five times and the fold change for each CIP2A/scrambled pair was calculated. Only the

genes that passed the threshold of 1.3 in all five experiments were considered differentially expressed in response to CIP2A depletion. The CIP2A signature constructed by these criteria consists of 134 genes in total (II, Suppl. Table 1). 112 genes were differentially regulated only at day 3, nine genes only at day 5 and 13 on both days (II, Fig 1B). Majority of the signature genes were upregulated in response to CIP2A depletion (II, Fig. 1C; Suppl. Table 1). In order to validate the signature, we chose 12 signature genes and determined their expression level in the CIP2A depleted cells by qRT-PCR. In HeLa cells, all 12 qRT-PCR tested signature genes were regulated similarly as in the microarray (II, Fig. 1D). In the MCF7 breast cancer cells, 9/12 and in AGS gastric cancer cells, 8/12 of these genes were expressed. All expressed genes were regulated as in HeLa cells (II, Suppl. Fig. 1B and 1C). These results validated the CIP2A signature as a high confidence list of CIP2A regulated genes.

5.3.2 Network analysis of the CIP2A signature (II)

In order to characterize the cell signaling networks associated with the CIP2A regulated genes, we performed Ingenuity analysis of the CIP2A signature. At day 3, the most significantly altered network included many genes implicated in cell migration (II, Fig. 2A; Table 1). In turn, the top network at day 5 was centered in Myc (II, Fig. 2B; Table 1). At day 3 the Myc network was ranked as number 7 by the Ingenuity score (II, Table 1). It should be noted that Myc itself was not found in the CIP2A signature. In accordance with observations from study I, the analysis of the microarray samples revealed that CIP2A depletion did not alter Myc mRNA expression (II, Suppl. Fig. 2B), whereas Myc protein expression was downregulated (II, Suppl. Fig. 2A).

The Ingenuity analysis results were further confirmed by analysis of the CIP2A signature genes by gene set enrichment analysis (GSEA) (www.broadinstitute.org/gsea/). Both 3- and 5-day signature genes, which were upregulated in response to CIP2A depletion, were significantly (p-values $5.58e^{-9}$ and $7.77e^{-7}$, respectively) associated with "Genes downregulated by Myc, according to the Myc Target Gene

Database". In addition, GSEA associated the CIP2A signature with focal adhesion phenotype (p-value 4.8e-5), further indicating a role in cell migration.

The analysis of molecular and cellular functions associated with the CIP2A signatures showed that the 3-day signature was most significantly linked with cellular movement and the 5-day signature with cellular growth and proliferation (II, Table 2).

5.3.3. Myc-dependency of the CIP2A signature (II)

As the above in silico analysis of the CIP2A signature genes indicated a strong association of the 3-day signature with cell migration, we next assessed the role of CIP2A in cell migration. CIP2A depletion in HeLa cells did not have significant effect in scratch wound healing or in cell adhesion assays (II, Suppl. Fig. 5A and 5B). However, in the Boyden chamber transwell migration assay, CIP2A depletion significantly inhibited HeLa cell migration (II, Fig. 3A and Suppl. Fig. 5C). In accordance with a previous report, Myc depletion did not have significant effect on migration in this assay (II, Fig. 3A) (Cappellen et al., 2007). To further assess the Myc-independent aspect of CIP2A function, we concentrated on JNK2, one of the CIP2A signature genes linked to Myc independent functions in the Ingenuity analysis (II, Table 1 and 2). We showed that CIP2A depletion downregulated JNK2 mRNA and protein levels, whereas Myc depletion did not affect JNK2 mRNA levels (II, Fig. 3E). Taken together, we identified transwell migration and JNK2 regulation as Myc-independent CIP2A functions.

To assess the Myc-dependent function of the CIP2A signature, we studied the Myc-dependency of the qRT-PCR validated signature genes (II, Fig. 5A). Four of these were previously known Myc target genes (*PLAUR, SERPINE2, SLC22A4* and *CAV1*). *PLAUR, SERPINE2,* and *SLC22A4* were similarly regulated in response to either CIP2A or Myc depletion (II, Fig. 5A). *CAV1* expression was only very moderately upregulated in response to MYC depletion and downregulated in

response to CIP2A depletion (II, Fig. 5A). Also five previously unknown Myc targets (FAP, SAT1, COL8A1, LAMA1 and MFAP5) were identified as Myc-dependent CIP2A targets (II, Fig. 5A). Based on the Ingenuity analysis, the cellular growth and proliferation functions linked to the CIP2A signature included Myc associated genes (II, Table 1 and 2). Therefore, we studied the role of CIP2A and Myc in HeLa cell colony formation on plastic. The depletion of either CIP2A or Myc by siRNA led to a similar degree of inhibition in colony formation indicating that the enhancing effect of CIP2A in HeLa cell colony formation is Myc mediated (II, Fig. 4A and B).

5.3.4 CIP2A signature in breast cancer (II)

To assess the clinical relevance of the CIP2A signature, we compared it with two published breast cancer microarray signatures (Enerly et al., 2011; Miller et al., 2005). In both breast cancer signatures, the CIP2A signature clustered together with the basal-like and HER2+ breast cancers subtypes (II, Fig. 6A and Suppl. Fig. 7). To further study the breast cancer subtype specific role of CIP2A, we immunostained CIP2A in a series of 1028 human breast cancer tissue samples (Joensuu et al., 2003). CIP2A was overexpressed in 45% of all studied samples and significantly more frequently in basal-like and HER2-positive tumors (II, Fig 6B and Suppl. Fig. 8A). In addition, analysis of the CIP2A mRNA expression levels in a panel of 40 breast cancer cell lines showed that the seven most CIP2A expressing cell lines were all of basal-like origin (II, Fig. 6D).

5.3.5 Identification of genes involved in long-term growth suppression in CIP2A depleted cancer cells (III)

In order to identify the genes underlying the long-term growth suppression induced by CIP2A depletion (Come et al., 2009; Junttila et al., 2007), we used Illumina platform to study the global transcriptional changes 7 days after CIP2A siRNA transfection (III, Fig. 1B). Fourteen

(14) genes were further validated as CIP2A regulated by qRT-PCR (III, Fig. 1C). Since *SNAR-A1* encodes a small non-coding RNA, a qRT-PCR assay for its detection could not be designed and it was not included in the validation experiment (III, Fig. 1C). Ingenuity network analysis of the microarray hits indicated a link to TGF-β and TNF signaling (III, Fig. 2A and 2B). In order to identify the genes which impact specifically on cancer cell growth, we performed RNAi screen in both PC-3 prostate cancer and PNT2 normal prostate cells and evaluated the effect of the siRNAs on cell viability by CTG-assay Promega (III, Suppl.Table 1). PC-3 and PNT2 cells had been previously validated in the RNAi screen and for that reason selected to be used in this experiment. Only those genes, inhibition of which by 2/3 probed siRNAs significantly (p-value < 0.05) inhibited cancer cell growth more in PC-3 cells as compared to the PNT2 cells, were considered to have cancer specific growth effect. In addition to CIP2A itself, six other genes passed this limit: *COL15, GNAT3, RPRM, SCIN, SVEP1* and *TPST1* (III, Fig 3). Interestingly, three of these genes were downregulated and three upregulated by CIP2A depletion (III, Fig. 1B and 1C). As we wished to concentrate on potential siRNA therapy targets, we further studied those genes that were downregulated by CIP2A depletion: *RPRM, SCIN* and *SVEP1*. In order to study cell morphology after the depletion of RPRM, SCIN or SVEP1, HeLa cells were transfected with corresponding siRNAs and imaged for 72 hours by Incucyte. Interestingly, SVEP1 or SCIN depletion caused an EMT-like phenotype in HeLa cells (III, Fig. 4C), whereas this was not observed in CIP2A or RPRM depleted cells (III, Fig. 4C). However, depletion of either CIP2A or RPRM decreased cell growth as measured by cell confluency (III, Fig. 4B), indicating that RPRM may be a potential target in mediating the CIP2A effect on cancer cell growth. SCIN depletion did not have a clear effect on HeLa cell confluency whereas SVEP1 depletion increased the cell confluency (III, Fig. 4B).

6. DISCUSSION

6.1. CIP2A as a PP2A inhibitor and promoter of Myc activity (I, II)

Myc is a well established and widely studied human oncogene. The phosphorylation of Myc S62 has been shown to stabilize Myc protein (Lutterbach and Hann, 1994; Yeh et al., 2004). PP2A dephosphorylates Myc S62 and thus destabilizes Myc protein (Arnold and Sears, 2006).

Before this study, CIP2A was called p90 due to its molecular weight of 90 kDa and it had been shown to be overexpressed in gastric cancer samples (Soo Hoo et al., 2002). Importantly, nothing was known about the cellular function of CIP2A. In this study, we demonstrate that CIP2A is a novel endogenous inhibitor of PP2A that inhibits the S62 dephosphorylation of Myc and thus stabilizes Myc. The depletion of CIP2A increased the Myc-associated activity of PP2A in phosphatase assay *in vitro* and decreased the levels of S62 phosphorylated and total Myc protein. CIP2A depletion did not disrupt the interaction between PP2A and Myc, but enhanced PP2A activity towards Myc S62. Importantly, CIP2A depletion did not cause changes in other PP2A targets (pMEK and MDM2). This indicates that CIP2A targets, at least somewhat selectively, Myc-associated PP2A activity. These results were further confirmed in gastric cancer cells, where CIP2A was shown to promote Myc protein stability in AGS and MKN-28 cells (Khanna et al., 2009). In addition, the depletion of CIP2A was shown to decrease the Myc S62 phosphorylation (Khanna et al., 2009). Also, in lung cancer cells lines A549 and H1299, CIP2A depletion caused downregulation in Myc protein levels (Dong et al., 2011). Further support to our results was provided by a recent report, which shows that CIP2A depletion inhibits Myc S62 phosphorylation, decreases total Myc protein levels and increases PP2A activity in chronic myeloid leukemia (CML) cells (Lucas et al., 2011).

The close association of the CIP2A signature with Myc-regulated signaling and the significant effect of CIP2A depletion on direct Myc

target gene expression further confirmed that CIP2A promotes MYC activity. Our results of colony formation assays in HeLa cells indicate that the cancer cell growth promoting properties of CIP2A are at least partly Myc mediated. Interestingly, in gastric cancer cells a positive feedback loop mechanism between CIP2A and Myc has been identified, further strengthening the close relationship between CIP2A and Myc (Khanna et al., 2009).

Myc is involved in a myriad of cellular processes. Even though we show that CIP2A stabilizes Myc protein, it is not plausible that CIP2A would regulate all the functions of Myc. This view is supported by a number of results presented here. The levels of co-purified CIP2A in endogenous Myc immunoprecipitates were low compared to input, indicating that only part of the cellular CIP2A is bound to Myc and *vice versa*. Based on the CIP2A signature, CIP2A also affects Myc-independent gene expression. Importantly, CIP2A seemed to mainly regulate Myc repressed genes. As Myc exerts its transcription factor role in the nucleus and CIP2A stabilizes Myc, it is expected that CIP2A would also be localized in the nucleus. Confocal microscopy images of HeLa cells indicated that CIP2A localizes mainly in cytoplasm and perinuclear region. However, immunohistochemical analysis of human and mouse HNSCC tumor samples showed CIP2A staining also in nucleus. Nuclear CIP2A staining has been shown by other groups in IHC analyses of HNSCC, ovarian cancer and renal cell carcinoma tumor samples (Bockelman et al., 2011a; Bockelman et al., 2011b; Katz et al., 2010; Ren et al., 2011). The exact cellular localization of CIP2A remains to be defined in more detail. Even though CIP2A seems to stabilize only part of cellular Myc, our results indicate that the CIP2A regulated Myc pool is important for cancer cell growth. Taken together, the results presented here and in subsequent studies, convincingly show that CIP2A is a novel inhibitor of PP2A which exerts its function by preventing PP2A from dephosphorylating Myc S62.

6.2. Myc- and PP2A-dependency of CIP2A functions (I, II)

In study I, CIP2A was characterized as a PP2A inhibitor which promotes Myc activity. To further explore CIP2A functions, we identified and characterized the CIP2A transcriptional signature, 3 and 5 days post CIP2A depletion. The reliability and repeatability of microarray results are of general concern. Taking this into consideration, the microarray analysis of CIP2A depleted cells was performed in five independent sample pairs and only genes surpassing the fold change threshold (1.3) in all sample pairs were included in the signature. Also a panel of signature genes was further validated in four different cancer cell lines by qRT-PCR. Therefore, it can be concluded that we have identified a high confidence CIP2A regulated transcriptional signature.

As discussed in the above section, characterization of the CIP2A signature further confirmed the role of CIP2A in regulating Myc signaling. Importantly, the analysis of the CIP2A signature also revealed novel Myc-independent functions for CIP2A. Various *in silico* analysis of the CIP2A signature associated networks and cellular and molecular functions indicated a role for CIP2A in cell movement and migration. However, we did not observe significant changes in wound healing and cell adhesion assays after CIP2A depletion in HeLa cells. Consistently, Come and coworkers have previously shown that CIP2A depletion does not affect the migration of MDA-MB-231 cells in the wound healing assay (Come et al., 2009). Interestingly, CIP2A depletion significantly inhibited transwell migration of HeLa cells. Furthermore, this phenotype was independent of Myc, but PP2A dependent. In contrast to our results, CIP2A depletion does not affect the matrigel invasion capacity of lung cancer cells (Dong et al., 2011). The transwell migration and martigel invasion assay results cannot be directly compared, since the mechanisms involved in cell migration and invasion are not identical. Transwell migration assay measures the ability of the cells to migrate through membrane pores towards higher serum concentration whereas in matrigel invasion assay, cells need the ability to sever the ECM in order to invade. Interestingly, in renal cell carcinoma patient samples CIP2A expression correlated positively with lymph node metastasis and

tumor invasion and metastasis (Ren et al., 2011). In addition, in breast cancer CIP2A expression has been linked to lymph node positivity (Come et al., 2009). These results argue that in human cancer, CIP2A would promote invasion and metastasis.

The role of Myc in metastasis and cancer cell invasion has not been clearly defined. Stable depletion of Myc has been shown to inhibit lung metastasis of MDA-MB-231 breast cancer cells *in vivo* (Wolfer et al., 2010). In contrast, in a recent report the overexpression of Myc in MDA-MB-231 cells resulted in larger primary xenograft tumors, but inhibited lung metastasis (Liu et al., 2012). PP2A has been implicated to be involved in the suppression of non-small-cell lung cancer metastasis (Li et al., 2012). In addition, low expression of PPP2R5A/B56α was detected predominantly in metastatic melanoma as compared to primary melanomas (Mannava et al., 2012). These results are in accordance with our results, that CIP2A regulates transwell migration Myc-independently but PP2A dependently. However, further experimentation is required in order to define the relevance for CIP2A in cell adhesion, migration, invasion and metastasis.

The regulation of JNK2 expression was also shown to be a Myc-independent and PP2A-dependent function of CIP2A. Moreover, CIP2A has also been implicated in regulation of Akt and death-associated protein kinase (DAPk). Ectopic expression of CIP2A in hepatocellular carcinoma (HCC) cells decreased PP2A activity towards Akt (Chen et al., 2011). In HEK293T cells, CIP2A was shown to inhibit DAPk dephosphorylation by PP2A (Guenebeaud et al., 2010). It is plausible that future studies will reveal yet more Myc-independent functions for CIP2A. Our results and other published results have not provided any clear indications of PP2A-independent roles for CIP2A, leaving this possibility to be defined in the future.

One interesting observation in Study II was that the majority of the CIP2A signature genes were upregulated in response to CIP2A depletion, indicating that CIP2A would mainly repress gene expression. This was also seen in Study III. In addition, CIP2A seemed to regulate mainly Myc repressed genes. Interestingly, many genes repressed by

Myc are negative regulators of cell proliferation and Myc mediated gene repression may be important in cell transformation (Herkert et al., 2010).

Taken together, we report the first analysis of the effects of CIP2A on global gene expression events in cancer cells. The identified CIP2A signature revealed both Myc-dependent and -independent functions for CIP2A and offers an important resource to better comprehend the function of CIP2A in cancer.

6.2.1. Potential mediators of long-term growth suppression in CIP2A depleted cancer cells

In Study I, we observed that CIP2A depletion by single transfection of 21-mer siRNA induced prolonged inhibition of tumor growth *in vivo*. The same effect was also seen in xenografts of MDA-MB-231 breast cancer cells and in lung cancer cells (Come et al., 2009; Dong et al., 2011).

In order to delineate the underlying transcriptional changes, we identified and verified gene expression changes 7 days after CIP2A depletion. Ingenuity analysis indicated a connection between the microarray hits and TGF-β and TNF tumor suppressor signaling.

By siRNA screen of the microarray hits, we identified six genes, depletion of which mRNA product significantly inhibited cancer cell growth as compared to normal cell. Since we wished to find possible targets for siRNA mediated therapy, we concentrated on to studying those genes which were both downregulated by CIP2A depletion and exhibited cancer cell specific growth effects (*RPRM, SCIN* and *SVEP1*). Accordingly, RPRM depletion inhibited HeLa cell growth in a cell confluency assay, indicating that it may mediate the effect of CIP2A in cancer cell growth.

SCIN depletion had no effect on HeLa cell confluency, whereas SVEP1 depletion increased it. Interestingly, morphological examination revealed that depletion of either SCIN or SVEP1 resulted in morphological changes resembling EMT. The more spread form of SVEP1 depleted

Hela cells probably accounts for the increase in cell confluency, indicating that SVEP1 depletion would not increase HeLa cell growth. Further experiments are needed in order to define whether the phenotype observed is indeed EMT. Neither SVEP1 nor SCIN has been previously linked to EMT. SCIN is an actin-binding protein, which have been linked to chondrocyte differentiation (Marcu et al., 1994; Nurminsky et al., 2007) and tumor resistance to cytolytic T lymphocyte mediated killing (Abouzahr et al., 2006). SVEP1 is a cell adhesion molecule (CAM), shown to be involved in adhesion of mesenchymal osteogenic cells (Shur et al., 2006) and linked to cell differentiation (Shefer and Benayahu, 2010). SVEP1 is regulated by TNF and estradiol (Glait-Santar and Benayahu, 2012) and it expressed in breast cancer cell lines (Shur et al., 2007). Importantly, there is no published data about the function of SVEP1 in cancer cells.

Cancer cells undergo EMT in order to disseminate and metastasize from the primary tumor. Ingenuity analysis indicated a connection between the CIP2A regulated microarray hits and TGF-β and TNF tumor suppressor signaling. TNF and TGF-B have both been linked to EMT. Considering CIP2A depletion as cancer therapy, the here observed EMT-like change in response to SCIN and SVEP1 is alarming. However, CIP2A depletion itself does not induce EMT-like phenotype, indicating, that some other CIP2A regulated genes are counteracting the effect of SCIN and SVEP1 depletion. This indicates that targeting of CIP2A itself would be better than targeting individual CIP2A regulated genes. Further investigation of other genes regulated by CIP2A depletion is also needed. In both study II and study III, CIP2A depletion caused more gene induction than repression, indicating that CIP2A would mainly cause gene repression in cancer. Interestingly, it has been reported that most of the genes that have significant survival effect are repressed in glioblastoma (Ovaska et al., 2010). CIP2A repressed genes were not functionally studied in this work, but their examination should be done in the future.

Taken together, we have identified 14 genes as possible mediators of the long-term growth effects of CIP2A depletion in cancer cells. Of these, we show that SCIN and SVEP1 affect cell morphology and RPRM

promotes cancer cell viability. However, further experimentation is required for elucidating if and how these CIP2A regulated genes contribute to cancer cell malignancy.

6.3. CIP2A as a human oncoprotein (I, II, III)

The role of PP2A as a tumor suppressor is well established and the inhibition of PP2A activity has been identified as one of the five prerequisites for human cell transformation (Hahn, 2002; Hahn et al., 2002; Hahn and Weinberg, 2002). Even though mutations in PP2A have been described in human cancers, the observed mutation frequencies are low in general and thus not enough to account for the overall inhibition of PP2A in human cancers. Endogenous inhibitors of PP2A, such as PME-1 and SET, have been previously identified. However, their role in human cancers has not been well established. Considering this, identification of CIP2A as an oncogenic inhibitor of PP2A, is a major advance in understanding how PP2A is inhibited in human cancers. PME-1 has been shown to be overexpressed in glioma (Puustinen et al., 2009), whereas no indications of CIP2A or SET overexpression in glioma has been published. This indicates that PME-1 function may not overlap with CIP2A and SET. CIP2A and SET overexpression has been both linked with many human cancer types and seem to overlap at least in leukemia (Christensen et al., 2011; Coenen et al., 2011; Cristobal et al., 2011; Li et al., 1996; Lucas et al., 2011; Westermarck and Hahn, 2008). This suggests that CIP2A and SET may play a more general role in PP2A inhibition in human cancers as compared to PME-1. However, CIP2A seems to mainly regulate Myc stability whereas SET has been mainly imlicated in the regulation of BRC-ABL mediated signaling. This indicates that even though CIP2A and SET expression may overlap in human cancers, they may regulate different PP2A targets.

Our results show, that CIP2A depletion inhibited HeLa cell proliferation and growth. In addition, CIP2A depletion in HeLa and HNSCC cells inhibits colony formation on plastic and anchorage independent growth in soft agar. This was further confirmed in breast, lung and gastric cancer cells (Come et al., 2009; Dong et al., 2011; Khanna et al., 2009;

Li et al., 2008). Importantly, CIP2A promoted both HeLa and HNSCC tumor xenograft growth. These results were also later confirmed in MDA-MB-231 breast cancer cells and in lung cancer cells (Come et al., 2009; Dong et al., 2011).

In study I, expression of CIP2A induced cell transformation of immortalized HEK-TERV cells and enhanced Ras-mediated transformation of spontaneously immortalized MEFs. Supporting our results, the expression of CIP2A was sufficient for transformation of JNK-/- MEFs, which are defective in Ras transformation (Mathiasen et al., 2012). In a mouse model of human HNSCC, induced by inhibition of the TGF-β tumor suppressor pathway and expression of oncogenic RAS, CIP2A expression was detected already in premalignant tissue. These results indicate that CIP2A acts as an enhancer of cellular transformation, in collaboration with other oncogenes. Since PP2A has been shown to be crucial in transforming immortalized human cells, these results further emphasize the role of CIP2A as cancerous inhibitor of PP2A.

In study I, CIP2A depletion did not affect apoptosis or cell cycle progression. Also, *in silico* analysis of CIP2A regulated genes did not indicate a clear role in apoptosis. However, one of the molecular and cellular functions indicated in the 5-day signature was cell death. A recent study indicates, that CIP2A expression protects HEK293T cells from Death-Associated-Protein kinase (DAPk) induced apoptosis (Guenebeaud et al., 2010). CIP2A inhibits PP2A activity towards DAPk, which remains in an inactive autophosphorylated state (Guenebeaud et al., 2010). CIP2A inhibition has also been suggested to result in enhanced radiation induced apoptosis (Huang et al., 2012). Even though these results are in contrast to our results, it should be noted that we only studied the effect of CIP2A depletion on apoptosis in Hela cells by analyzing PARP-cleavage and sub-G1 populations. Huang and coworkers studied PARP-cleavage in SiHa cells (Huang et al., 2012). Even though HeLa and SiHa cells are both of cervical cancer origin, they may still differ sufficiently to explain the contradicting results reported. Taken together, more thorough studies are needed to clarify the role of CIP2A in apoptosis.

CIP2A was already overexpressed in neoplastic lesions in HNSCC mouse model. This mouse model develops HNSCC in response to TGFβRII knockout and oncogenic Ras mutation, indicating that CIP2A expression could be regulated by TGFβ- or Ras-mediated signaling. Moreover, TGFβ-signaling was associated with CIP2A-regulated genes in study III. The study of the possible connection between CIP2A and TGFβ-signaling could give further insight to CIP2A function and regulation. Several mechanisms have been indicated to regulate CIP2A. In gastric cancer, Myc has been shown to positively regulate CIP2A expression (Khanna et al., 2009). Also, Ras activated MEK1/2-signaling has been reported to induce CIP2A expression via ETS1 transcription factor (Khanna et al., 2011; Zhao et al., 2010). In MEFs, ATF2 transcription factor was indicated in the regulation CIP2A in a Ras- and JNK-dependent manner (Mathiasen et al., 2012). Moreover, HPV 16 E7 oncoprotein has been reported to upregulate CIP2A expression in cervical cancer (Liu et al., 2011).

Even though CIP2A was identified and characterized only recently (Junttila et al., 2007; Soo Hoo et al., 2002), there are already many reports confirming the expression of CIP2A in human cancers. In study I, CIP2A was overexpressed in HNSCC and colon cancer, whereas its expression in normal tissues was low in general. Further studies have shown CIP2A overexpression in various malignancies such as gastric, breast, colon, lung and prostate cancers, and squamous cell carcinomas of the head and neck (Come et al., 2009; Dong et al., 2011; Junttila et al., 2007; Katz et al., 2010; Khanna et al., 2009; Li et al., 2008; Qu et al., 2012; Vaarala et al., 2010). In addition, CIP2A overexpression has been shown to correlate with poor prognosis in gastric, lung, ovarian, renal, tongue and hematological cancers (Bockelman et al., 2011a; Bockelman et al., 2011b; Dong et al., 2011; Khanna et al., 2009; Lucas et al., 2011; Ren et al., 2011). CIP2A expression correlated positively with lymph node metastasis in breast cancer and with node metastasis, tumor invasion and metastasis in renal cancer (Come et al., 2009; Ren et al., 2011). Also, CIP2A expression has been shown to associate with aggressive disease characteristics in breast cancer, high proliferation in lung cancer, and high grade in colon, breast, and renal cancer (Bockelman et al., 2012; Come et al., 2009; Dong et al., 2011; Ren et al., 2011). Interestingly, a translocation forming Mixed Lineage Leukemia

(MLL) and CIP2A gene fusion has been found in one case of infant acute myeloid leukemia (AML) (Coenen et al., 2011). Our results of CIP2A signature correlation and protein expression in breast cancer revealed a strong connection between CIP2A expression and basal-type breast cancer. Basal-type breast cancers have the worst prognosis of the breast cancer sub-types. In study I, we showed that CIP2A expression is already upregulated in premalignant neoplastic HNSCC lesions. In addition, CIP2A inhibits PP2A and stabilizes Myc protein, events which have been shown to be important for human cell transformation. Based on these results, upregulation of CIP2A is an early event in cancer development and is associated with more aggressive and advanced cancers. This suggests that CIP2A may function as a driver in cancer development and progression. Taken together, CIP2A is a novel clinically relevant human oncoprotein, which promotes tumor growth and is overexpressed at high frequency in human cancers.

6.3.1. CIP2A signature is clinically relevant in breast cancer

CIP2A and Myc have both been associated with human breast cancer (Come et al., 2009; Xu et al., 2010). CIP2A was shown to be overexpressed in 39% of 33 human breast cancer samples and CIP2A expression correlated with aggressive tumor properties (Come et al., 2009). High expression of CIP2A also correlated with poor 5-year patient prognosis, indicating a prognostic role for CIP2A in breast cancer (Come et al., 2009). In this study, we identified the CIP2A regulated transcriptional signature, which was compared with two human breast cancer microarray signatures (Enerly et al., 2011; Miller et al., 2005). We show that the CIP2A signature clustered together with both basal-like and HER2+ breast tumors. In addition, in immunohistochemical staining of human breast tumors, CIP2A expression significantly associated with basal-like and HER2+ subtypes. Moreover, breast cancer cell lines of basal-like origin showed highest CIP2A mRNA expression. These results

show, that CIP2A is strongly associated with basal-like subtype and involved in HER2+ breast cancers.

The role of Myc in breast cancer has been vastly studied (Xu et al., 2010). Myc overexpression has been linked mainly to the basal-like breast cancers (Sorlie et al., 2001). Protein stability of Myc has been indicated as being important for the oncogenic properties of Myc in breast cancer (Xu et al., 2010). However, the mechanisms behind Myc stabilization in breast cancers remain unclear. Axin1 has been reported to promote Myc degradation by acting as a scaffold for proteins involved in Myc degradation (Arnold et al., 2009). Interestingly, Axin1 downregulation in breast cancer was proposed as a mechanism for Myc stabilization in breast cancer (Zhang et al., 2012). However, no analysis of Axin1 expression in different breast cancer subtypes has been done. High CIP2A expression and association of CIP2A signature to basal-like and HER+ breast cancers (described in this study) may offer an explanation to the increased Myc protein levels and activity in these breast cancers.

Alterations in PP2A subunits have been linked to breast cancers, but they seem to occur at low frequency (Calin et al., 2000; Dupont et al., 2010; Esplin et al., 2006). Interestingly, a very recent study of 2000 breast tumors identifies the PPP2R2A/B55α deletion as a potential driver mutation of breast cancer (Curtis et al., 2012). However, this deletion occurs predominantly in luminal B subtype and not in basal-like or HER2+ breast cancers (Curtis et al., 2012). Considering this, it is tempting to hypothesize that as PP2R2A is deleted in luminal B breast cancers there is no need for increased CIP2A activity, whereas in basal-type and HER2+ breast cancers CIP2A could be predominantly responsible for the inhibition of PP2A and subsequent stabilization of Myc. However, in HER2+ breast cancers, Tyr307 phosphorylation of PP2A could account at least partly for its decreased activity (Wong et al., 2009). It would also be interesting to study whether there are differences in, for example, Axin downregulation or SET expression between the distinct breast cancer subtypes. This would help to define whether CIP2A alone is responsible for the PP2A inhibition in basal-type breast cancer or whether it is achieved by collaboration.

Basal-type breast cancers are known to have worse prognosis as compared to other breast cancer subtypes. Basal-type cancers are often also triple negative (i.e. they do not express ER, PR of HER2). Due to the lack of receptors, they generally do not response to hormone therapies commonly used in breast cancer treatment. Considering this, the development of CIP2A targeted therapy could be an important advancement in basal-like breast cancer treatment.

Taken together, this is the first report linking CIP2A expression to specific breast cancer subtypes. It is obvious, that further validation of the role of CIP2A in these breast cancer subtypes is needed. Also, functional analysis of Myc-dependent CIP2A signature genes in breast cancer could provide more detailed information on how Myc and CIP2A collaborate in basal-like and HER2+ breast cancers. This information may be useful for the development of novel breast cancer patient stratification strategies for future CIP2A- of Myc-targeted therapies.

7. SUMMARY

Prior to this study, inhibition of PP2A had been identified as one of the five prerequisites for human cell transformation. However, the mechanisms mediating this inhibition in human cancer remained elusive. This study describes CIP2A as a novel endogenous inhibitor of PP2A, which promotes the stability of Myc oncoprotein. Importantly, CIP2A was identified as a novel human oncogene which promotes malignant growth of cancer cells and is overexpressed in human cancers (Fig. 4). Moreover we delineate the CIP2A regulated transcriptional signature in cancer cells and demonstrate its clinical relevance in breast cancer subtypes. Considering this, this work has provided substantial novel insight in the regulation of PP2A and Myc in cancer. Importantly, the identification of CIP2A as a novel human oncogene has opened new lines in cancer research.
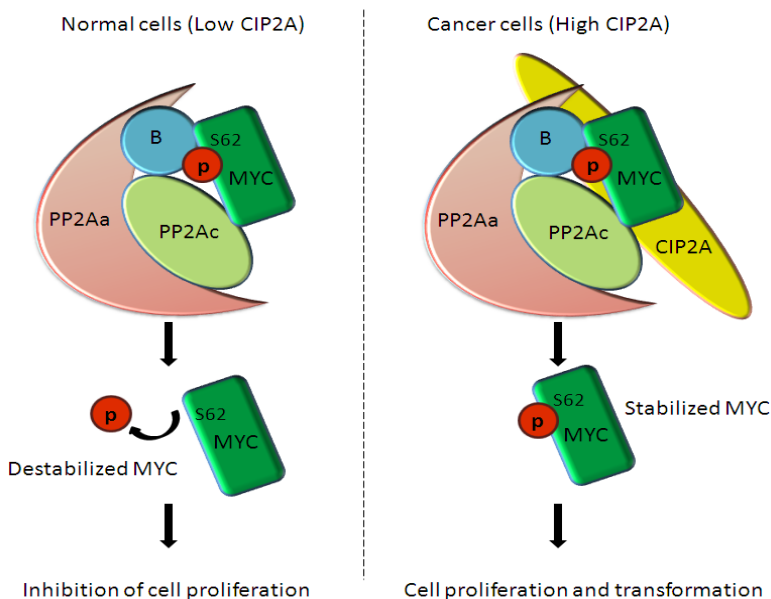
Figure 4. Schematic representation of CIP2A function in cancer cells. In normal cells, the level of CIP2A is low, and PP2A is able to dephosphorylate Myc S62, which leads to destabilization and degradation of Myc and subsequent inhibition of cell proliferation. In turn, cancer cells express high levels of CIP2A, leading to inhibition of PP2A activity towards Myc S62. Stabilized Myc promotes cell proliferation and transformation. Adapted from (Junttila and Westermarck, 2008).

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REFERENCES

Abouzahr, S., G. Bismuth, C. Gaudin, O. Caroll, P. Van Endert, A. Jalil, J. Dausset, I. Vergnon, C. Richon, A. Kauffmann, J. Galon, G. Raposo, F. Mami-Chouaib, and S. Chouaib. 2006. Identification of target actin content and polymerization status as a mechanism of tumor resistance after cytolytic T lymphocyte pressure. *Proc Natl Acad Sci U S A*. 103:1428-1433.

Adachi, Y., G.N. Pavlakis, and T.D. Copeland. 1994. Identification and characterization of SET, a nuclear phosphoprotein encoded by the translocation break point in acute undifferentiated leukemia. *J Biol Chem*. 269:2258-2262.

Adams, J.M., A.W. Harris, C.A. Pinkert, L.M. Corcoran, W.S. Alexander, S. Cory, R.D. Palmiter, and R.L. Brinster. 1985. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature*. 318:533-538.

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

Adhikary, S., F. Marinoni, A. Hock, E. Hulleman, N. Popov, R. Beier, S. Bernard, M. Quarto, M. Capra, S. Goettig, U. Kogel, M. Scheffner, K. Helin, and M. Eilers. 2005. The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation. *Cell*. 123:409-421.

Al-Kuraya, K., H. Novotny, P. Bavi, A.K. Siraj, S. Uddin, A. Ezzat, N.A. Sanea, F. Al-Dayel, H. Al-Mana, S.S. Sheikh, M. Mirlacher, C. Tapia, R. Simon, G. Sauter, L. Terracciano, and L. Tornillo. 2007. HER2, TOP2A, CCND1, EGFR and C-MYC oncogene amplification in colorectal cancer. *J Clin Pathol*. 60:768-772.

Al-Kuraya, K., P. Schraml, J. Torhorst, C. Tapia, B. Zaharieva, H. Novotny, H. Spichtin, R. Maurer, M. Mirlacher, O. Kochli, M. Zuber, H. Dieterich, F. Mross, K. Wilber, R. Simon, and G. Sauter. 2004. Prognostic relevance of gene amplifications and coamplifications in breast cancer. *Cancer Res*. 64:8534-8540.

Alderton, G.K. 2011. Therapy: Targeting MYC? You BET. *Nat Rev Cancer*. 11:693-693.

Alitalo, K., J.M. Bishop, D.H. Smith, E.Y. Chen, W.W. Colby, and A.D. Levinson. 1983. Nucleotide sequence to the v-myc oncogene of avian retrovirus MC29. *Proc Natl Acad Sci U S A*. 80:100-104.

Ang, K.K., J. Harris, R. Wheeler, R. Weber, D.I. Rosenthal, P.F. Nguyen-Tan, W.H. Westra, C.H. Chung, R.C. Jordan, C. Lu, H. Kim, R. Axelrod, C.C. Silverman, K.P. Redmond, and M.L. Gillison. 2010. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 363:24-35.

Arino, J., C.W. Woon, D.L. Brautigan, T.B. Miller, and G.L. Johnson. 1988. Human liver phosphatase 2A: cDNA and amino acid sequence of two catalytic subunit isotypes. *Proc Natl Acad Sci U S A*. 85:4252-4256.

Armelin, H.A., M.C. Armelin, K. Kelly, T. Stewart, P. Leder, B.H. Cochran, and C.D. Stiles. 1984. Functional role for c-myc in mitogenic response to platelet-derived growth factor. *Nature*. 310:655-660.

Arnold, H.K., and R.C. Sears. 2006. Protein phosphatase 2A regulatory subunit B56alpha associates with c-myc and negatively regulates c-myc accumulation. *Mol Cell Biol*. 26:2832-2844.

Arnold, H.K., X. Zhang, C.J. Daniel, D. Tibbitts, J. Escamilla-Powers, A. Farrell, S. Tokarz, C. Morgan, and R.C. Sears. 2009. The Axin1 scaffold protein promotes formation of a degradation complex for c-Myc. *EMBO J*. 28:500-512.

Aulmann, S., N. Adler, J. Rom, B. Helmchen, P. Schirmacher, and H.P. Sinn. 2006. c-myc amplifications in primary breast carcinomas and their local recurrences. *J Clin Pathol*. 59:424-428.

Bahram, F., N. von der Lehr, C. Cetinkaya, and L.G. Larsson. 2000. c-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover. *Blood*. 95:2104-2110.

Balz, V., K. Scheckenbach, K. Gotte, U. Bockmuhl, I. Petersen, and H. Bier. 2003. Is the p53 inactivation frequency in squamous cell carcinomas of the head and neck underestimated? Analysis of p53 exons 2-11 and human papillomavirus 16/18 E6 transcripts in 123 unselected tumor specimens. *Cancer Res*. 63:1188-1191.

Berns, E.M., J.G. Klijn, M. Smid, I.L. van Staveren, M.P. Look, W.L. van Putten, and J.A. Foekens. 1996. TP53 and MYC gene alterations independently predict poor prognosis in breast cancer patients. *Genes Chromosomes Cancer*. 16:170-179.

Berns, E.M., J.G. Klijn, W.L. van Putten, I.L. van Staveren, H. Portengen, and J.A. Foekens. 1992. c-myc amplification is a better prognostic factor than HER2/neu amplification in primary breast cancer. *Cancer Res*. 52:1107-1113.

Bertucci, F., P. Finetti, N. Cervera, E. Charafe-Jauffret, E. Mamessier, J. Adelaide, S. Debono, G. Houvenaeghel, D. Maraninchi, P. Viens, C. Charpin, J. Jacquemier, and D. Birnbaum. 2006. Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. *Cancer Res*. 66:4636-4644.

Betschinger, J., K. Mechtler, and J.A. Knoblich. 2006. Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. *Cell*. 124:1241-1253.

Bhattacharya, N., M.G. Sabbir, A. Roy, A. Dam, S. Roychoudhury, and C.K. Panda. 2005. Approximately 580 Kb surrounding the MYC gene is amplified in head and neck squamous cell carcinoma of Indian patients. *Pathol Res Pract*. 201:691-697.

Bieche, I., I. Laurendeau, S. Tozlu, M. Olivi, D. Vidaud, R. Lidereau, and M. Vidaud. 1999. Quantitation of MYC gene expression in sporadic breast

tumors with a real-time reverse transcription-PCR assay. *Cancer Res.* 59:2759-2765.

Blackwood, E.M., and R.N. Eisenman. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science.* 251:1211-1217.

Bockelman, C., J. Hagstrom, L.K. Makinen, H. Keski-Santti, V. Hayry, J. Lundin, T. Atula, A. Ristimaki, and C. Haglund. 2011a. High CIP2A immunoreactivity is an independent prognostic indicator in early-stage tongue cancer. *Br J Cancer.* 104:1890-1895.

Bockelman, C., S. Koskensalo, J. Hagstrom, M. Lundin, A. Ristimaki, and C. Haglund. 2012. CIP2A overexpression is associated with c-Myc expression in colorectal cancer. *Cancer Biol Ther.* 13:289-295.

Bockelman, C., H. Lassus, A. Hemmes, A. Leminen, J. Westermarck, C. Haglund, R. Butzow, and A. Ristimaki. 2011b. Prognostic role of CIP2A expression in serous ovarian cancer. *Br J Cancer.* 105:989-995.

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

Bornstein, S., R. White, S. Malkoski, M. Oka, G. Han, T. Cleaver, D. Reh, P. Andersen, N. Gross, S. Olson, C. Deng, S.L. Lu, and X.J. Wang. 2009. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. *J Clin Invest.* 119:3408-3419.

Bosch, M., X. Cayla, C. Van Hoof, B.A. Hemmings, R. Ozon, W. Merlevede, and J. Goris. 1995. The PR55 and PR65 subunits of protein phosphatase 2A from Xenopus laevis. molecular cloning and developmental regulation of expression. *Eur J Biochem.* 230:1037-1045.

Bretones, G., J.C. Acosta, J.M. Caraballo, N. Ferrandiz, M.T. Gomez-Casares, M. Albajar, R. Blanco, P. Ruiz, W.C. Hung, M.P. Albero, I. Perez-Roger, and J. Leon. 2011. SKP2 oncogene is a direct MYC target gene and MYC down-regulates p27(KIP1) through SKP2 in human leukemia cells. *J Biol Chem.* 286:9815-9825.

Brooks, T.A., L.H. Hurley, and hurley@pharmacy.arizona.edu. 2010. Targeting MYC Expression through G-Quadruplexes. *Genes & Cancer.* 1:641-649.

Bruggers, C.S., K.F. Tai, T. Murdock, L. Sivak, K. Le, S.L. Perkins, C.M. Coffin, and W.L. Carroll. 1998. Expression of the c-Myc protein in childhood medulloblastoma. *J Pediatr Hematol Oncol.* 20:18-25.

Burkhart, D.L., and J. Sage. 2008. Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nat Rev Cancer.* 8:671-682.

Calin, G.A., M.G. di Iasio, E. Caprini, I. Vorechovsky, P.G. Natali, G. Sozzi, C.M. Croce, G. Barbanti-Brodano, G. Russo, and M. Negrini. 2000. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene.* 19:1191-1195.

Campaner, S., M. Doni, P. Hydbring, A. Verrecchia, L. Bianchi, D. Sardella, T. Schleker, D. Perna, S. Tronnersjo, M. Murga, O. Fernandez-Capetillo,

M. Barbacid, L.G. Larsson, and B. Amati. 2010a. Cdk2 suppresses cellular senescence induced by the c-myc oncogene. *Nat Cell Biol.* 12:54-59.

Campaner, S., M. Doni, A. Verrecchia, G. Faga, L. Bianchi, and B. Amati. 2010b. Myc, Cdk2 and cellular senescence: Old players, new game. *Cell Cycle.* 9:3655-3661.

Canela, N., A. Rodriguez-Vilarrupla, J.M. Estanyol, C. Diaz, M.J. Pujol, N. Agell, and O. Bachs. 2003. The SET protein regulates G2/M transition by modulating cyclin B-cyclin-dependent kinase 1 activity. *J Biol Chem.* 278:1158-1164.

Cappellen, D., T. Schlange, M. Bauer, F. Maurer, and N.E. Hynes. 2007. Novel c-MYC target genes mediate differential effects on cell proliferation and migration. *EMBO Rep.* 8:70-76.

Carey, L., E. Winer, G. Viale, D. Cameron, and L. Gianni. 2010. Triple-negative breast cancer: disease entity or title of convenience? *Nat Rev Clin Oncol.* 7:683-692.

Carlson, S.G., E. Eng, E.G. Kim, E.J. Perlman, T.D. Copeland, and B.J. Ballermann. 1998. Expression of SET, an inhibitor of protein phosphatase 2A, in renal development and Wilms' tumor. *J Am Soc Nephrol.* 9:1873-1880.

Cartwright, P., C. McLean, A. Sheppard, D. Rivett, K. Jones, and S. Dalton. 2005. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. *Development.* 132:885-896.

Carujo, S., J.M. Estanyol, A. Ejarque, N. Agell, O. Bachs, and M.J. Pujol. 2006. Glyceraldehyde 3-phosphate dehydrogenase is a SET-binding protein and regulates cyclin B-cdk1 activity. *Oncogene.* 25:4033-4042.

Cayla, X., J. Goris, J. Hermann, P. Hendrix, R. Ozon, and W. Merlevede. 1990. Isolation and characterization of a tyrosyl phosphatase activator from rabbit skeletal muscle and Xenopus laevis oocytes. *Biochemistry.* 29:658-667.

Chandriani, S., E. Frengen, V.H. Cowling, S.A. Pendergrass, C.M. Perou, M.L. Whitfield, and M.D. Cole. 2009. A core MYC gene expression signature is prominent in basal-like breast cancer but only partially overlaps the core serum response. *PLoS One.* 4:e6693.

Charafe-Jauffret, E., C. Ginestier, F. Monville, S. Fekairi, J. Jacquemier, D. Birnbaum, and F. Bertucci. 2005. How to best classify breast cancer: conventional and novel classifications (review). *Int J Oncol.* 27:1307-1313.

Chen, J., B.L. Martin, and D.L. Brautigan. 1992. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science.* 257:1261-1264.

Chen, K.F., H.C. Yu, C.Y. Liu, H.J. Chen, Y.C. Chen, D.R. Hou, P.J. Chen, and A.L. Cheng. 2011. Bortezomib Sensitizes HCC Cells to CS-1008, an Antihuman Death Receptor 5 Antibody, through the Inhibition of CIP2A. *Molecular Cancer Therapeutics.* 10:892-901.

Chen, W., R. Possemato, K.T. Campbell, C.A. Plattner, D.C. Pallas, and W.C. Hahn. 2004. Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell*. 5:127-136.

Chen, Y., Y. Xu, Q. Bao, Y. Xing, Z. Li, Z. Lin, J.B. Stock, P.D. Jeffrey, and Y. Shi. 2007. Structural and biochemical insights into the regulation of protein phosphatase 2A by small t antigen of SV40. *Nat Struct Mol Biol*. 14:527-534.

Cho, U.S., S. Morrone, A.A. Sablina, J.D. Arroyo, W.C. Hahn, and W. Xu. 2007. Structural basis of PP2A inhibition by small t antigen. *PLoS Biol*. 5:e202.

Cho, U.S., and W. Xu. 2007. Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. *Nature*. 445:53-57.

Christensen, D.J., Y. Chen, J. Oddo, K.M. Matta, J. Neil, E.D. Davis, A.D. Volkheimer, M.C. Lanasa, D.R. Friedman, B.K. Goodman, J.P. Gockerman, L.F. Diehl, C.M. de Castro, J.O. Moore, M.P. Vitek, and J.B. Weinberg. 2011. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin lymphoma: a predictor of aggressive disease and a new treatment target. *Blood*. 118:4150-4158.

Christoffersen, N.R., R. Shalgi, L.B. Frankel, E. Leucci, M. Lees, M. Klausen, Y. Pilpel, F.C. Nielsen, M. Oren, and A.H. Lund. 2010. p53-independent upregulation of miR-34a during oncogene-induced senescence represses MYC. *Cell Death Differ*. 17:236-245.

Chrzan, P., J. Skokowski, A. Karmolinski, and T. Pawelczyk. 2001. Amplification of c-myc gene and overexpression of c-Myc protein in breast cancer and adjacent non-neoplastic tissue. *Clin Biochem*. 34:557-562.

Chung, C.H., J.S. Parker, G. Karaca, J. Wu, W.K. Funkhouser, D. Moore, D. Butterfoss, D. Xiang, A. Zanation, X. Yin, W.W. Shockley, M.C. Weissler, L.G. Dressler, C.G. Shores, W.G. Yarbrough, and C.M. Perou. 2004. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. *Cancer Cell*. 5:489-500.

Ciechanover, A., J.A. DiGiuseppe, B. Bercovich, A. Orian, J.D. Richter, A.L. Schwartz, and G.M. Brodeur. 1991. Degradation of nuclear oncoproteins by the ubiquitin system in vitro. *Proc Natl Acad Sci U S A*. 88:139-143.

Coenen, E.A., C.M. Zwaan, C. Meyer, R. Marschalek, R. Pieters, L.T. van der Veken, H.B. Beverloo, and M.M. van den Heuvel-Eibrink. 2011. KIAA1524: A novel MLL translocation partner in acute myeloid leukemia. *Leuk Res*. 35:133-135.

Cole, M.D. 1986. The myc oncogene: its role in transformation and differentiation. *Annu Rev Genet*. 20:361-384.

Coller, H.A., C. Grandori, P. Tamayo, T. Colbert, E.S. Lander, R.N. Eisenman, and T.R. Golub. 2000. Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proc Natl Acad Sci U S A*. 97:3260-3265.

Come, C., A. Laine, M. Chanrion, H. Edgren, E. Mattila, X. Liu, J. Jonkers, J. Ivaska, J. Isola, J.M. Darbon, O. Kallioniemi, S. Thezenas, and J.

Westermarck. 2009. CIP2A is associated with human breast cancer aggressivity. *Clin Cancer Res.* 15:5092-5100.

Conacci-Sorrell, M., C. Ngouenet, and R.N. Eisenman. 2010. Myc-nick: a cytoplasmic cleavage product of Myc that promotes alpha-tubulin acetylation and cell differentiation. *Cell.* 142:480-493.

Corzo, C., J.M. Corominas, I. Tusquets, M. Salido, M. Bellet, X. Fabregat, S. Serrano, and F. Sole. 2006. The MYC oncogene in breast cancer progression: from benign epithelium to invasive carcinoma. *Cancer Genet Cytogenet.* 165:151-156.

Cotter, T.G. 2009. Apoptosis and cancer: the genesis of a research field. *Nat Rev Cancer.* 9:501-507.

Cristobal, I., L. Garcia-Orti, C. Cirauqui, M.M. Alonso, M.J. Calasanz, and M.D. Odero. 2011. PP2A impaired activity is a common event in acute myeloid leukemia and its activation by forskolin has a potent anti-leukemic effect. *Leukemia.* 25:606-614.

Curtis, C., S.P. Shah, S.-F. Chin, G. Turashvili, O.M. Rueda, M.J. Dunning, D. Speed, A.G. Lynch, S. Samarajiwa, Y. Yuan, S. Graf, G. Ha, G. Haffari, A. Bashashati, R. Russell, S. McKinney, A. Langerod, A. Green, E. Provenzano, G. Wishart, S. Pinder, P. Watson, F. Markowetz, L. Murphy, I. Ellis, A. Purushotham, A.-L. Borresen-Dale, J.D. Brenton, S. Tavare, C. Caldas, and S. Aparicio. 2012. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature.* 486:346-352.

Dang, C.V., K.A. O'Donnell, K.I. Zeller, T. Nguyen, R.C. Osthus, and F. Li. 2006. The c-Myc target gene network. *Semin Cancer Biol.* 16:253-264.

Delmore, J.E., G.C. Issa, M.E. Lemieux, P.B. Rahl, J. Shi, H.M. Jacobs, E. Kastritis, T. Gilpatrick, R.M. Paranal, J. Qi, M. Chesi, A.C. Schinzel, M.R. McKeown, T.P. Heffernan, C.R. Vakoc, P.L. Bergsagel, I.M. Ghobrial, P.G. Richardson, R.A. Young, W.C. Hahn, K.C. Anderson, A.L. Kung, J.E. Bradner, and C.S. Mitsiades. 2011. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell.* 146:904-917.

Deming, S.L., S.J. Nass, R.B. Dickson, and B.J. Trock. 2000. C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. *Br J Cancer.* 83:1688-1695.

DePinho, R.A., N. Schreiber-Agus, and F.W. Alt. 1991. myc family oncogenes in the development of normal and neoplastic cells. *Adv Cancer Res.* 57:1-46.

Dong, Q.Z., Y. Wang, X.J. Dong, Z.X. Li, Z.P. Tang, Q.Z. Cui, and E.H. Wang. 2011. CIP2A is overexpressed in non-small cell lung cancer and correlates with poor prognosis. *Ann Surg Oncol.* 18:857-865.

Dupont, W.D., J.P. Breyer, K.M. Bradley, P.A. Schuyler, W.D. Plummer, M.E. Sanders, D.L. Page, and J.R. Smith. 2010. Protein phosphatase 2A subunit gene haplotypes and proliferative breast disease modify breast cancer risk. *Cancer.* 116:8-19.

Easton, D.F., K.A. Pooley, A.M. Dunning, P.D.P. Pharoah, D. Thompson, D.G. Ballinger, J.P. Struewing, J. Morrison, H. Field, R. Luben, N. Wareham,

S. Ahmed, C.S. Healey, R. Bowman, K.B. Meyer, C.A. Haiman, L.K. Kolonel, B.E. Henderson, L. Le Marchand, P. Brennan, S. Sangrajrang, V. Gaborieau, F. Odefrey, C.-Y. Shen, P.-E. Wu, H.-C. Wang, D. Eccles, D.G. Evans, J. Peto, O. Fletcher, N. Johnson, S. Seal, M.R. Stratton, N. Rahman, G. Chenevix-Trench, S.E. Bojesen, B.G. Nordestgaard, C.K. Axelsson, M. Garcia-Closas, L. Brinton, S. Chanock, J. Lissowska, B. Peplonska, H. Nevanlinna, R. Fagerholm, H. Eerola, D. Kang, K.-Y. Yoo, D.-Y. Noh, S.-H. Ahn, D.J. Hunter, S.E. Hankinson, D.G. Cox, P. Hall, S. Wedren, J. Liu, Y.-L. Low, N. Bogdanova, P. Schurmann, T. Dork, R.A.E.M. Tollenaar, C.E. Jacobi, P. Devilee, J.G.M. Klijn, A.J. Sigurdson, M.M. Doody, B.H. Alexander, J. Zhang, A. Cox, I.W. Brock, G. MacPherson, M.W.R. Reed, F.J. Couch, E.L. Goode, J.E. Olson, H. Meijers-Heijboer, A. van den Ouweland, A. Uitterlinden, F. Rivadeneira, R.L. Milne, G. Ribas, A. Gonzalez-Neira, J. Benitez, J.L. Hopper, M. McCredie, M. Southey, G.G. Giles, C. Schroen, C. Justenhoven, H. Brauch, U. Hamann, Y.-D. Ko, A.B. Spurdle, J. Beesley, X. Chen, A. Mannermaa, V.-M. Kosma, V. Kataja, J. Hartikainen, N.E. Day, et al. 2007. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*. 447:1087-1093.

Eckhardt, S.G.S., A.A. Dai, K.K.K. Davidson, B.J.B. Forseth, G.M.G. Wahl, and D.D.D.V. Hoff. 1994. Induction of differentiation in HL60 cells by the reduction of extrachromosomally amplified c-myc. *Proc Natl Acad Sci U S A*. 91:6674.

Eichhorn, P.J., M.P. Creyghton, and R. Bernards. 2009. Protein phosphatase 2A regulatory subunits and cancer. *Biochim Biophys Acta*. 1795:1-15.

Eilers, M., and R.N. Eisenman. 2008. Myc's broad reach. *Genes Dev*. 22:2755-2766.

Eischen, C.M., D. Woo, M.F. Roussel, and J.L. Cleveland. 2001. Apoptosis triggered by Myc-induced suppression of Bcl-X(L) or Bcl-2 is bypassed during lymphomagenesis. *Mol Cell Biol*. 21:5063-5070.

Enerly, E., I. Steinfeld, K. Kleivi, S.K. Leivonen, M.R. Aure, H.G. Russnes, J.A. Ronneberg, H. Johnsen, R. Navon, E. Rodland, R. Makela, B. Naume, M. Perala, O. Kallioniemi, V.N. Kristensen, Z. Yakhini, and A.L. Borresen-Dale. 2011. miRNA-mRNA integrated analysis reveals roles for miRNAs in primary breast tumors. *PLoS One*. 6:e16915.

Engelman, J.A. 2009. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer*. 9:550-562.

Escamilla-Powers, J.R., and R.C. Sears. 2012. A Conserved Pathway That Controls c-Myc Protein Stability through Opposing Phosphorylation Events Occurs in Yeast. *J Biol Chem*. 282:5432-5442.

Esplin, E.D., P. Ramos, B. Martinez, G.E. Tomlinson, M.C. Mumby, and G.A. Evans. 2006. The glycine 90 to aspartate alteration in the Abeta subunit of PP2A (PPP2R1B) associates with breast cancer and causes a deficit in protein function. *Genes Chromosomes Cancer*. 45:182-190.

Fellner, T., D.H. Lackner, H. Hombauer, P. Piribauer, I. Mudrak, K. Zaragoza, C. Juno, and E. Ogris. 2003. A novel and essential mechanism determining specificity and activity of protein phosphatase 2A (PP2A) in vivo. *Genes Dev*. 17:2138-2150.

Fernandez, P.C., S.R. Frank, L. Wang, M. Schroeder, S. Liu, J. Greene, A. Cocito, and B. Amati. 2003. Genomic targets of the human c-Myc protein. *Genes Dev*. 17:1115-1129.

Flinn, E.M., C.M. Busch, and A.P. Wright. 1998. myc boxes, which are conserved in myc family proteins, are signals for protein degradation via the proteasome. *Mol Cell Biol*. 18:5961-5969.

Frank, S.R., T. Parisi, S. Taubert, P. Fernandez, M. Fuchs, H.M. Chan, D.M. Livingston, and B. Amati. 2003. MYC recruits the TIP60 histone acetyltransferase complex to chromatin. *EMBO Rep*. 4:575-580.

Frescas, D., and M. Pagano. 2008. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. *Nat Rev Cancer*. 8:438-449.

Fujiki, H., M. Suganuma, S. Yoshizawa, S. Nishiwaki, B. Winyar, and T. Sugimura. 1991. Mechanisms of action of okadaic acid class tumor promoters on mouse skin. *Environ Health Perspect*. 93:211-214.

Garibal, J., E. Hollville, A.I. Bell, G.L. Kelly, B. Renouf, Y. Kawaguchi, A.B. Rickinson, and J. Wiels. 2007. Truncated form of the Epstein-Barr virus protein EBNA-LP protects against caspase-dependent apoptosis by inhibiting protein phosphatase 2A. *J Virol*. 81:7598-7607.

Gillison, M.L., W.M. Koch, R.B. Capone, M. Spafford, W.H. Westra, L. Wu, M.L. Zahurak, R.W. Daniel, M. Viglione, D.E. Symer, K.V. Shah, and D. Sidransky. 2000. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*. 92:709-720.

Glait-Santar, C., and D. Benayahu. 2012. Regulation of SVEP1 gene expression by 17beta-estradiol and TNFalpha in pre-osteoblastic and mammary adenocarcinoma cells. *J Steroid Biochem Mol Biol*. 130:36-44.

Godet, A.N., J. Guergnon, A. Croset, X. Cayla, P.B. Falanga, J.H. Colle, and A. Garcia. 2010. PP2A1 binding, cell transducing and apoptotic properties of Vpr(77-92): a new functional domain of HIV-1 Vpr proteins. *PLoS One*. 5:e13760.

Gonzalez-Zulueta, M., C.M. Bender, A.S. Yang, T. Nguyen, R.W. Beart, J.M. Van Tornout, and P.A. Jones. 1995. Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res*. 55:4531-4535.

Gotz, J., A. Probst, E. Ehler, B. Hemmings, and W. Kues. 1998. Delayed embryonic lethality in mice lacking protein phosphatase 2A catalytic subunit Calpha. *Proc Natl Acad Sci U S A*. 95:12370-12375.

Grady, W.M., and S.D. Markowitz. 2002. Genetic and epigenetic alterations in colon cancer. *Annu Rev Genomics Hum Genet*. 3:101-128.

Grandori, C., S.M. Cowley, L.P. James, and R.N. Eisenman. 2000. The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu Rev Cell Dev Biol*. 16:653-699.

Grandori, C., J. Mac, F. Siebelt, D.E. Ayer, and R.N. Eisenman. 1996. Myc-Max heterodimers activate a DEAD box gene and interact with multiple E box-related sites in vivo. *EMBO J.* 15:4344-4357.

Grandori, C., K.J. Wu, P. Fernandez, C. Ngouenet, J. Grim, B.E. Clurman, M.J. Moser, J. Oshima, D.W. Russell, K. Swisshelm, S. Frank, B. Amati, R. Dalla-Favera, and R.J. Monnat, Jr. 2003. Werner syndrome protein limits MYC-induced cellular senescence. *Genes Dev.* 17:1569-1574.

Gregory, M.A., and S.R. Hann. 2000. c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. *Mol Cell Biol.* 20:2423-2435.

Grochola, L.F., A. Vazquez, E.E. Bond, P. Wurl, H. Taubert, T.H. Muller, A.J. Levine, and G.L. Bond. 2009. Recent natural selection identifies a genetic variant in a regulatory subunit of protein phosphatase 2A that associates with altered cancer risk and survival. *Clin Cancer Res.* 15:6301-6308.

Grushko, T.A., J.J. Dignam, S. Das, A.M. Blackwood, C.M. Perou, K.K. Ridderstrale, K.N. Anderson, M.J. Wei, A.J. Adams, F.G. Hagos, L. Sveen, H.T. Lynch, B.L. Weber, and O.I. Olopade. 2004. MYC is amplified in BRCA1-associated breast cancers. *Clin Cancer Res.* 10:499-507.

Guenebeaud, C., D. Goldschneider, M. Castets, C. Guix, G. Chazot, C. Delloye-Bourgeois, A. Eisenberg-Lerner, G. Shohat, M. Zhang, V. Laudet, A. Kimchi, A. Bernet, and P. Mehlen. 2010. The dependence receptor UNC5H2/B triggers apoptosis via PP2A-mediated dephosphorylation of DAP kinase. *Mol Cell.* 40:863-876.

Haber, D.A., and J. Settleman. 2007. Cancer: Drivers and passengers. *Nature.* 446:145-146.

Hahn, W.C. 2002. Immortalization and transformation of human cells. *Mol Cell.* 13:351-361.

Hahn, W.C., C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks, and R.A. Weinberg. 1999. Creation of human tumour cells with defined genetic elements. *Nature.* 400:464-468.

Hahn, W.C., S.K. Dessain, M.W. Brooks, J.E. King, B. Elenbaas, D.M. Sabatini, J.A. DeCaprio, and R.A. Weinberg. 2002. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Mol Cell Biol.* 22:2111-2123.

Hahn, W.C., and R.A. Weinberg. 2002. Rules for making human tumor cells. *N Engl J Med.* 347:1593-1603.

Hanahan, D., and R.A. Weinberg. 2000. The Hallmarks of Cancer. *Cell.* 100:57-70.

Hanahan, D., and Robert A. Weinberg. 2011. Hallmarks of Cancer: The Next Generation. *Cell.* 144:646-674.

Hann, S.R., and R.N. Eisenman. 1984. Proteins encoded by the human c-myc oncogene: differential expression in neoplastic cells. *Mol Cell Biol.* 4:2486-2497.

Hemmings, B.A., C. Adams-Pearson, F. Maurer, P. Muller, J. Goris, W. Merlevede, J. Hofsteenge, and S.R. Stone. 1990. alpha- and beta-forms

of the 65-kDa subunit of protein phosphatase 2A have a similar 39 amino acid repeating structure. *Biochemistry*. 29:3166-3173.

Hendrix, P., P. Turowski, R.E. Mayer-Jaekel, J. Goris, J. Hofsteenge, W. Merlevede, and B.A. Hemmings. 1993. Analysis of subunit isoforms in protein phosphatase 2A holoenzymes from rabbit and Xenopus. *J Biol Chem*. 268:7330-7337.

Herbst, A., S.E. Salghetti, S.Y. Kim, and W.P. Tansey. 2004. Multiple cell-type-specific elements regulate Myc protein stability. *Oncogene*. 23:3863-3871.

Herkert, B., M. Eilers, and martin.eilers@biozentrum.uni-wuerzburg.de. 2010. Transcriptional Repression The Dark Side of Myc. *Genes & Cancer*. 1:580-586.

Hu, Z., C. Fan, D.S. Oh, J. Marron, X. He, B.F. Qaqish, C. Livasy, L.A. Carey, E. Reynolds, L. Dressler, A. Nobel, J. Parker, M.G. Ewend, L.R. Sawyer, J. Wu, Y. Liu, R. Nanda, M. Tretiakova, A.R. Orrico, D. Dreher, J.P. Palazzo, L. Perreard, E. Nelson, M. Mone, H. Hansen, M. Mullins, J.F. Quackenbush, M.J. Ellis, O.I. Olopade, P.S. Bernard, and C.M. Perou. 2006. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics*. 7:96.

Huang, C.Y., C.C. Wei, K.C. Chen, H.J. Chen, A.L. Cheng, and K.F. Chen. 2012. Bortezomib enhances radiation-induced apoptosis in solid tumors by inhibiting CIP2A. *Cancer Lett*. 317:9-15.

Hydbring, P., F. Bahram, Y. Su, S. Tronnersjö, K. Högstrand, N. von der Lehr, H.R. Sharifi, R. Lilischkis, N. Hein, S. Wu, J. Vervoorts, M. Henriksson, A. Grandien, B. Lüscher, L.-G. Larsson, and lars-gunnar.larsson@ki.se. 2010. Phosphorylation by Cdk2 is required for Myc to repress Ras-induced senescence in cotransformation. *Proc Natl Acad Sci U S A*. 107:58-63.

Izumi, H., C. Molander, L.Z. Penn, A. Ishisaki, K. Kohno, and K. Funa. 2001. Mechanism for the transcriptional repression by c-Myc on PDGF beta-receptor. *J Cell Sci*. 114:1533-1544.

Janssens, V., and J. Goris. 2001. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J*. 353:417-439.

Janssens, V., S. Longin, and J. Goris. 2008. PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail). *Trends Biochem Sci*. 33:113-121.

Jones, S., T.-L. Wang, I.-M. Shih, T.-L. Mao, K. Nakayama, R. Roden, R. Glas, D. Slamon, L.A. Diaz, B. Vogelstein, K.W. Kinzler, npapado1@jhmi.edu, V.E. Velculescu, npapado1@jhmi.edu, N. Papadopoulos, and npapado1@jhmi.edu. 2010. Frequent Mutations of Chromatin Remodeling Gene ARID1A in Ovarian Clear Cell Carcinoma. *Science*. 330:228-231.

Junttila, M.R., P. Puustinen, M. Niemela, R. Ahola, H. Arnold, T. Bottzauw, R. Ala-aho, C. Nielsen, J. Ivaska, Y. Taya, S.L. Lu, S. Lin, E.K. Chan, X.J. Wang, R. Grenman, J. Kast, T. Kallunki, R. Sears, V.M. Kahari, and J.

Westermarck. 2007. CIP2A inhibits PP2A in human malignancies. *Cell*. 130:51-62.

Junttila, M.R., and J. Westermarck. 2008. Mechanisms of MYC stabilization in human malignancies. *Cell Cycle*. 7:592-596.

Kalluri, R. 2009. EMT: when epithelial cells decide to become mesenchymal-like cells. *J Clin Invest*. 119:1417-1419.

Kalluri, R., and E.G. Neilson. 2003. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest*. 112:1776-1784.

Kalluri, R., and R.A. Weinberg. 2009. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 119:1420-1428.

Kamangar, F., G.M. Dores, and W.F. Anderson. 2006. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol*. 24:2137-2150.

Katz, J., A. Jakymiw, M.K. Ducksworth, C.M. Stewart, I. Bhattacharyya, S. Cha, and E.K. Chan. 2010. CIP2A expression and localization in oral carcinoma and dysplasia. *Cancer Biol Ther*. 10:694-699.

Khanna, A., C. Bockelman, A. Hemmes, M.R. Junttila, J.P. Wiksten, M. Lundin, S. Junnila, D.J. Murphy, G.I. Evan, C. Haglund, J. Westermarck, and A. Ristimaki. 2009. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. *J Natl Cancer Inst*. 101:793-805.

Khanna, A., J. Okkeri, T. Bilgen, T. Tiirikka, M. Vihinen, T. Visakorpi, and J. Westermarck. 2011. ETS1 mediates MEK1/2-dependent overexpression of cancerous inhibitor of protein phosphatase 2A (CIP2A) in human cancer cells. *PLoS One*. 6:e17979.

Kim, S.Y., A. Herbst, K.A. Tworkowski, S.E. Salghetti, and W.P. Tansey. 2003. Skp2 regulates Myc protein stability and activity. *Mol Cell*. 11:1177-1188.

Kleine-Kohlbrecher, D., S. Adhikary, and M. Eilers. 2006. Mechanisms of transcriptional repression by Myc. *Curr Top Microbiol Immunol*. 302:51-62.

Knoepfler, P.S., P.F. Cheng, and R.N. Eisenman. 2002. N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. *Genes Dev*. 16:2699-2712.

Lallemant, B., A. Evrard, G. Chambon, O. Sabra, S. Kacha, J.G. Lallemant, S. Lumbroso, and J.P. Brouillet. 2010. Gene expression profiling in head and neck squamous cell carcinoma: Clinical perspectives. *Head Neck*. 32:1712-1719.

Land, H., L.F. Parada, and R.A. Weinberg. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature*. 304:596-602.

Langerød, A., H. Zhao, Ø. Borgan, J.M. Nesland, I.R. Bukholm, T. Ikdahl, R. Kåresen, A.-L. Børresen-Dale, and S.S. Jeffrey. 2007. TP53 mutation status and gene expression profiles are powerful prognostic markers of breast cancer. *Breast Cancer Research*. 9.

Laurenti, E., B. Varnum-Finney, A. Wilson, I. Ferrero, W.E. Blanco-Bose, A. Ehninger, P.S. Knoepfler, P.F. Cheng, H.R. MacDonald, R.N.

Eisenman, I.D. Bernstein, and A. Trumpp. 2008. Hematopoietic stem cell function and survival depend on c-Myc and N-Myc activity. *Cell Stem Cell*. 3:611-624.

Lee, J., and J. Stock. 1993. Protein phosphatase 2A catalytic subunit is methyl-esterified at its carboxyl terminus by a novel methyltransferase. *J Biol Chem*. 268:19192-19195.

Leemans, C.R., B.J. Braakhuis, and R.H. Brakenhoff. 2011. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 11:9-22.

Letourneux, C., G. Rocher, and F. Porteu. 2006. B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. *EMBO J*. 25:727-738.

Li, G., X.-D. Ji, H. Gao, J.-S. Zhao, J.-F. Xu, Z.-J. Sun, Y.-Z. Deng, S. Shi, Y.-X. Feng, Y.-Q. Zhu, T. Wang, J.-J. Li, and D. Xie. 2012. EphB3 suppresses non-small-cell lung cancer metastasis via a PP2A/RACK1/Akt signalling complex. *Nat Commun*. 3:667.

Li, H.H., X. Cai, G.P. Shouse, L.G. Piluso, and X. Liu. 2007. A specific PP2A regulatory subunit, B56gamma, mediates DNA damage-induced dephosphorylation of p53 at Thr55. *EMBO J*. 26:402-411.

Li, M., H. Guo, and Z. Damuni. 1995. Purification and characterization of two potent heat-stable protein inhibitors of protein phosphatase 2A from bovine kidney. *Biochemistry*. 34:1988-1996.

Li, M., A. Makkinje, and Z. Damuni. 1996. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. *J Biol Chem*. 271:11059-11062.

Li, W., Z. Ge, C. Liu, Z. Liu, M. Bjorkholm, J. Jia, and D. Xu. 2008. CIP2A is overexpressed in gastric cancer and its depletion leads to impaired clonogenicity, senescence, or differentiation of tumor cells. *Clin Cancer Res*. 14:3722-3728.

Liu, H., D.C. Radisky, D. Yang, R. Xu, E.S. Radisky, M.J. Bissell, and J.M. Bishop. 2012. MYC suppresses cancer metastasis by direct transcriptional silencing of alpha(v) and beta(3) integrin subunits. *Nat Cell Biol*. 14:567-574.

Liu, J., and D. Levens. 2006. Making myc. *Curr Top Microbiol Immunol*. 302:1-32.

Liu, J., X. Wang, G.Y. Zhou, H. Wang, L. Xiang, Y.Z. Cheng, W.J. Liu, Y. Wang, J.H. Jia, and W.M. Zhao. 2011. Cancerous inhibitor of protein phosphatase 2A is overexpressed in cervical cancer and upregulated by human papillomavirus 16 E7 oncoprotein. *Gynecologic Oncology*. 122:430-436.

Liu, X., L.F. Sempere, H. Ouyang, V.A. Memoli, A.S. Andrew, Y. Luo, E. Demidenko, M. Korc, W. Shi, M. Preis, K.H. Dragnev, H. Li, J. Direnzo, M. Bak, S.J. Freemantle, S. Kauppinen, and E. Dmitrovsky. 2010. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. *J Clin Invest*. 120:1298-1309.

Longin, S., J. Jordens, E. Martens, I. Stevens, V. Janssens, E. Rondelez, I. De Baere, R. Derua, E. Waelkens, J. Goris, and C. Van Hoof. 2004. An inactive protein phosphatase 2A population is associated with methylesterase and can be re-activated by the phosphotyrosyl phosphatase activator. *Biochem J*. 380:111-119.

Longin, S., K. Zwaenepoel, E. Martens, J.V. Louis, E. Rondelez, J. Goris, and V. Janssens. 2008. Spatial control of protein phosphatase 2A (de)methylation. *Exp Cell Res*. 314:68-81.

Louis, J.V., E. Martens, P. Borghgraef, C. Lambrecht, W. Sents, S. Longin, K. Zwaenepoel, R. Pijnenborg, I. Landrieu, G. Lippens, B. Ledermann, J. Gotz, F. Van Leuven, J. Goris, and V. Janssens. 2011. Mice lacking phosphatase PP2A subunit PR61/B'delta (Ppp2r5d) develop spatially restricted tauopathy by deregulation of CDK5 and GSK3beta. *Proc Natl Acad Sci U S A*. 108:6957-6962.

Lu, S.L., H. Herrington, D. Reh, S. Weber, S. Bornstein, D. Wang, A.G. Li, C.F. Tang, Y. Siddiqui, J. Nord, P. Andersen, C.L. Corless, and X.J. Wang. 2006. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. *Genes Dev*. 20:1331-1342.

Lucas, C.M., R.J. Harris, A. Giannoudis, M. Copland, J.R. Slupsky, and R.E. Clark. 2011. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. *Blood*. 117:6660-6668.

Lutterbach, B., and S.R. Hann. 1994. Hierarchical phosphorylation at N-terminal transformation-sensitive sites in c-Myc protein is regulated by mitogens and in mitosis. *Mol Cell Biol*. 14:5510-5522.

Mannava, S., A.R. Omilian, J.A. Wawrzyniak, E.E. Fink, D. Zhuang, J.C. Miecznikowski, J.R. Marshall, M.S. Soengas, R.C. Sears, C.D. Morrison, and M.A. Nikiforov. 2012. PP2A-B56alpha controls oncogene-induced senescence in normal and tumor human melanocytic cells. *Oncogene*. 31:1484-1492.

Mao, D.Y., J.D. Watson, P.S. Yan, D. Barsyte-Lovejoy, F. Khosravi, W.W. Wong, P.J. Farnham, T.H. Huang, and L.Z. Penn. 2003. Analysis of Myc bound loci identified by CpG island arrays shows that Max is essential for Myc-dependent repression. *Curr Biol*. 13:882-886.

Marcu, M.G., A. Rodriguez del Castillo, M.L. Vitale, and J.M. Trifaro. 1994. Molecular cloning and functional expression of chromaffin cell scinderin indicates that it belongs to the family of Ca(2+)-dependent F-actin severing proteins. *Mol Cell Biochem*. 141:153-165.

Martens, E., I. Stevens, V. Janssens, J. Vermeesch, J. Gotz, J. Goris, and C. Van Hoof. 2004. Genomic organisation, chromosomal localisation tissue distribution and developmental regulation of the PR61/B' regulatory subunits of protein phosphatase 2A in mice. *J Mol Biol*. 336:971-986.

Marusyk, A., V. Almendro, and K. Polyak. 2012. Intra-tumour heterogeneity: a looking glass for cancer? *Nat Rev Cancer*. 12:323-334.

Mathiasen, D.P., C. Egebjerg, S.H. Andersen, B. Rafn, P. Puustinen, A. Khanna, M. Daugaard, E. Valo, S. Tuomela, T. Bottzauw, C.F. Nielsen,

B.M. Willumsen, S. Hautaniemi, R. Lahesmaa, J. Westermarck, M. Jaattela, and T. Kallunki. 2012. Identification of a c-Jun N-terminal kinase-2-dependent signal amplification cascade that regulates c-Myc levels in ras transformation. *Oncogene*. 31:390-401.

McConechy, M.K., M.S. Anglesio, S.E. Kalloger, W. Yang, J. Senz, C. Chow, A. Heravi-Moussavi, G.B. Morin, A.M. Mes-Masson, G. Australian Ovarian Cancer Study, M.S. Carey, J.N. McAlpine, J.S. Kwon, L.M. Prentice, N. Boyd, S.P. Shah, C.B. Gilks, and D.G. Huntsman. 2011. Subtype-specific mutation of PPP2R1A in endometrial and ovarian carcinomas. *J Pathol*. 223:567-573.

Mertz, J.A., A.R. Conery, B.M. Bryant, P. Sandy, S. Balasubramanian, D.A. Mele, L. Bergeron, and R.J. Sims, 3rd. 2011. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc Natl Acad Sci U S A*. 108:16669-16674.

Meyer, N., and L.Z. Penn. 2008. Reflecting on 25 years with MYC. *Nat Rev Cancer*. 8:976-990.

Michelotti, G.A., E.F. Michelotti, A. Pullner, R.C. Duncan, D. Eick, and D. Levens. 1996. Multiple single-stranded cis elements are associated with activated chromatin of the human c-myc gene in vivo. *Mol Cell Biol*. 16:2656-2669.

Miller, L.D., J. Smeds, J. George, V.B. Vega, L. Vergara, A. Ploner, Y. Pawitan, P. Hall, S. Klaar, E.T. Liu, and J. Bergh. 2005. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A*. 102:13550-13555.

Muller, J., and M. Eilers. 2008. Ubiquitination of Myc: proteasomal degradation and beyond. *Ernst Schering Found Symp Proc*:99-113.

Murata, K., J. Wu, and D.L. Brautigan. 1997. B cell receptor-associated protein alpha4 displays rapamycin-sensitive binding directly to the catalytic subunit of protein phosphatase 2A. *Proc Natl Acad Sci U S A*. 94:10624-10629.

Murphy, D.J., M.R. Junttila, L. Pouyet, A. Karnezis, K. Shchors, D.A. Bui, L. Brown-Swigart, L. Johnson, and G.I. Evan. 2008. Distinct thresholds govern Myc's biological output in vivo. *Cancer Cell*. 14:447-457.

Naidu, R., N.A. Wahab, M. Yadav, and M.K. Kutty. 2002. Protein expression and molecular analysis of c-myc gene in primary breast carcinomas using immunohistochemistry and differential polymerase chain reaction. *Int J Mol Med*. 9:189-196.

Negrini, S., V.G. Gorgoulis, and T.D. Halazonetis. 2010. Genomic instability--an evolving hallmark of cancer. *Nat Rev Mol Cell Biol*. 11:220-228.

Neviani, P., R. Santhanam, R. Trotta, M. Notari, B.W. Blaser, S. Liu, H. Mao, J.S. Chang, A. Galietta, A. Uttam, D.C. Roy, M. Valtieri, R. Bruner-Klisovic, M.A. Caligiuri, C.D. Bloomfield, G. Marcucci, and D. Perrotti. 2005. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell*. 8:355-368.

Nurminsky, D., C. Magee, L. Faverman, and M. Nurminskaya. 2007. Regulation of chondrocyte differentiation by actin-severing protein adseverin. *Dev Biol*. 302:427-437.

O'Hagan, R.C., N. Schreiber-Agus, K. Chen, G. David, J.A. Engelman, R. Schwab, L. Alland, C. Thomson, D.R. Ronning, J.C. Sacchettini, P. Meltzer, and R.A. DePinho. 2000. Gene-target recognition among members of the myc superfamily and implications for oncogenesis. *Nat Genet*. 24:113-119.

Ogris, E., X. Du, K.C. Nelson, E.K. Mak, X.X. Yu, W.S. Lane, and D.C. Pallas. 1999. A protein phosphatase methylesterase (PME-1) is one of several novel proteins stably associating with two inactive mutants of protein phosphatase 2A. *J Biol Chem*. 274:14382-14391.

Olivier, M., M. Hollstein, and P. Hainaut. 2010. TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use. *Cold Spring Harbor Perspectives in Biology*. 2.

Olshan, A.F., M.C. Weissler, H. Pei, K. Conway, S. Anderson, D.B. Fried, and W.G. Yarbrough. 1997. Alterations of the p16 gene in head and neck cancer: frequency and association with p53, PRAD-1 and HPV. *Oncogene*. 14:811-818.

Opitz, O.G., Y. Suliman, W.C. Hahn, H. Harada, H.E. Blum, and A.K. Rustgi. 2001. Cyclin D1 overexpression and p53 inactivation immortalize primary oral keratinocytes by a telomerase-independent mechanism. *J Clin Invest*. 108:725-732.

Orlicky, S., X. Tang, A. Willems, M. Tyers, and F. Sicheri. 2003. Structural basis for phosphodependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase. *Cell*. 112:243-256.

Ovaska, K., M. Laakso, S. Haapa-Paananen, R. Louhimo, P. Chen, V. Aittomaki, E. Valo, J. Nunez-Fontarnau, V. Rantanen, S. Karinen, K. Nousiainen, A.M. Lahesmaa-Korpinen, M. Miettinen, L. Saarinen, P. Kohonen, J. Wu, J. Westermarck, and S. Hautaniemi. 2010. Large-scale data integration framework provides a comprehensive view on glioblastoma multiforme. *Genome Med*. 2:65.

Pastula, C., I. Johnson, J.M. Beechem, and W.F. Patton. 2003. Development of fluorescence-based selective assays for serine/threonine and tyrosine phosphatases. *Comb Chem High Throughput Screen*. 6:341-346.

Patel, J.H., Y. Du, P.G. Ard, C. Phillips, B. Carella, C.J. Chen, C. Rakowski, C. Chatterjee, P.M. Lieberman, W.S. Lane, G.A. Blobel, and S.B. McMahon. 2004. The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. *Mol Cell Biol*. 24:10826-10834.

Pelengaris, S., M. Khan, and G. Evan. 2002. c-MYC: more than just a matter of life and death. *Nat Rev Cancer*. 2:764-776.

Penn, L.J., E.M. Laufer, and H. Land. 1990. C-MYC: evidence for multiple regulatory functions. *Semin Cancer Biol*. 1:69-80.

Perna, D., G. Faga, A. Verrecchia, M.M. Gorski, I. Barozzi, V. Narang, J. Khng, K.C. Lim, W.K. Sung, R. Sanges, E. Stupka, T. Oskarsson, A. Trumpp, C.L. Wei, H. Muller, and B. Amati. 2012. Genome-wide mapping of Myc

binding and gene regulation in serum-stimulated fibroblasts. *Oncogene*. 31:1695-1709.

Perou, C.M., T. Sorlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, C.A. Rees, J.R. Pollack, D.T. Ross, H. Johnsen, L.A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S.X. Zhu, P.E. Lonning, A.-L. Borresen-Dale, P.O. Brown, and D. Botstein. 2000. Molecular portraits of human breast tumours. *Nature*. 406:747-752.

Pertschuk, L.P., J.G. Feldman, D.S. Kim, K. Nayeri, K.B. Eisenberg, A.C. Carter, W.T. Thelmo, Z.T. Rhong, P. Benn, and A. Grossman. 1993. Steroid hormone receptor immunohistochemistry and amplification of c-myc protooncogene. Relationship to disease-free survival in breast cancer. *Cancer*. 71:162-171.

Petitjean, A., E. Mathe, S. Kato, C. Ishioka, S.V. Tavtigian, P. Hainaut, and M. Olivier. 2007. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat*. 28:622-629.

Pim, D., P. Massimi, S.M. Dilworth, and L. Banks. 2005. Activation of the protein kinase B pathway by the HPV-16 E7 oncoprotein occurs through a mechanism involving interaction with PP2A. *Oncogene*. 24:7830-7838.

Poeta, M.L., J. Manola, M.A. Goldwasser, A. Forastiere, N. Benoit, J.A. Califano, J.A. Ridge, J. Goodwin, D. Kenady, J. Saunders, W. Westra, D. Sidransky, and W.M. Koch. 2007. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med*. 357:2552-2561.

Popov, N., C. Schulein, L.A. Jaenicke, and M. Eilers. 2010. Ubiquitylation of the amino terminus of Myc by SCF(beta-TrCP) antagonizes SCF(Fbw7)-mediated turnover. *Nat Cell Biol*. 12:973-981.

Popov, N., M. Wanzel, M. Madiredjo, D. Zhang, R. Beijersbergen, R. Bernards, R. Moll, S.J. Elledge, and M. Eilers. 2007. The ubiquitin-specific protease USP28 is required for MYC stability. *Nat Cell Biol*. 9:765-774.

Post, S.M., A. Quintas-Cardama, T. Terzian, C. Smith, C.M. Eischen, and G. Lozano. 2010. p53-dependent senescence delays Emu-myc-induced B-cell lymphomagenesis. *Oncogene*. 29:1260-1269.

Puig, O., F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, and B. Seraphin. 2001. The tandem affinity purification (TAP) method: a general procedure of protein complex purification. *Methods*. 24:218-229.

Puustinen, P., M.R. Junttila, S. Vanhatupa, A.A. Sablina, M.E. Hector, K. Teittinen, O. Raheem, K. Ketola, S. Lin, J. Kast, H. Haapasalo, W.C. Hahn, and J. Westermarck. 2009. PME-1 protects extracellular signal-regulated kinase pathway activity from protein phosphatase 2A-mediated inactivation in human malignant glioma. *Cancer Res*. 69:2870-2877.

Qu, W., W. Li, L. Wei, L. Xing, X. Wang, and J. Yu. 2012. CIP2A is overexpressed in esophageal squamous cell carcinoma. *Med Oncol*. 29:113-118.

Rajagopalan, H., P.V. Jallepalli, C. Rago, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, and C. Lengauer. 2004. Inactivation of hCDC4 can cause chromosomal instability. *Nature*. 428:77-81.

Reavie, L., G. Della Gatta, K. Crusio, B. Aranda-Orgilles, S.M. Buckley, B. Thompson, E. Lee, J. Gao, A.L. Bredemeyer, B.A. Helmink, J. Zavadil, B.P. Sleckman, T. Palomero, A. Ferrando, and I. Aifantis. 2010. Regulation of hematopoietic stem cell differentiation by a single ubiquitin ligase-substrate complex. *Nat Immunol*. 11:207-215.

Reis-Filho, J.S., and A.N. Tutt. 2008. Triple negative tumours: a critical review. *Histopathology*. 52:108-118.

Ren, J., W. Li, L. Yan, W. Jiao, S. Tian, D. Li, Y. Tang, G. Gu, H. Liu, and Z. Xu. 2011. Expression of CIP2A in renal cell carcinomas correlates with tumour invasion, metastasis and patients' survival. *Br J Cancer*. 105:1905-1911.

Rheinwald, J.G., W.C. Hahn, M.R. Ramsey, J.Y. Wu, Z. Guo, H. Tsao, M. De Luca, C. Catricala, and K.M. O'Toole. 2002. A two-stage, p16(INK4A)- and p53-dependent keratinocyte senescence mechanism that limits replicative potential independent of telomere status. *Mol Cell Biol*. 22:5157-5172.

Rodrigo, J.P., P.S. Lazo, S. Ramos, I. Alvarez, and C. Suarez. 1996. MYC amplification in squamous cell carcinomas of the head and neck. *Arch Otolaryngol Head Neck Surg*. 122:504-507.

Rodriguez-Pinilla, S.M., R.L. Jones, M.B. Lambros, E. Arriola, K. Savage, M. James, S.E. Pinder, and J.S. Reis-Filho. 2007. MYC amplification in breast cancer: a chromogenic in situ hybridisation study. *J Clin Pathol*. 60:1017-1023.

Rosenthal, E.L., and L.M. Matrisian. 2006. Matrix metalloproteases in head and neck cancer. *Head Neck*. 28:639-648.

Rottmann, S., and B. Luscher. 2006. The Mad side of the Max network: antagonizing the function of Myc and more. *Curr Top Microbiol Immunol*. 302:63-122.

Ruediger, R., M. Hentz, J. Fait, M. Mumby, and G. Walter. 1994. Molecular model of the A subunit of protein phosphatase 2A: interaction with other subunits and tumor antigens. *J Virol*. 68:123-129.

Ruediger, R., H.T. Pham, and G. Walter. 2001. Alterations in protein phosphatase 2A subunit interaction in human carcinomas of the lung and colon with mutations in the A beta subunit gene. *Oncogene*. 20:1892-1899.

Ruediger, R., D. Roeckel, J. Fait, A. Bergqvist, G. Magnusson, and G. Walter. 1992. Identification of binding sites on the regulatory A subunit of protein phosphatase 2A for the catalytic C subunit and for tumor antigens of simian virus 40 and polyomavirus. *Mol Cell Biol*. 12:4872-4882.

Sablina, A.A., W. Chen, J.D. Arroyo, L. Corral, M. Hector, S.E. Bulmer, J.A. DeCaprio, and W.C. Hahn. 2007. The tumor suppressor PP2A Abeta regulates the RalA GTPase. *Cell*. 129:969-982.

Sablina, A.A., and W.C. Hahn. 2007. The role of PP2A A subunits in tumor suppression. *Cell Adh Migr*. 1:140-141.

Sablina, A.A., M. Hector, N. Colpaert, and W.C. Hahn. 2010. Identification of PP2A complexes and pathways involved in cell transformation. *Cancer Res*. 70:10474-10484.

Sachdeva, M., S. Zhu, F. Wu, H. Wu, V. Walia, S. Kumar, R. Elble, K. Watabe, Y.-Y. Mo, and ymo@siumed.edu. 2009. p53 represses c-Myc through induction of the tumor suppressor miR-145. *Proc Natl Acad Sci U S A*. 106:3207-3212.

Salghetti, S.E., S.Y. Kim, and W.P. Tansey. 1999. Destruction of Myc by ubiquitin-mediated proteolysis: cancer-associated and transforming mutations stabilize Myc. *EMBO J*. 18:717-726.

Scorilas, A., T. Trangas, J. Yotis, C. Pateras, and M. Talieri. 1999. Determination of c-myc amplification and overexpression in breast cancer patients: evaluation of its prognostic value against c-erbB-2, cathepsin-D and clinicopathological characteristics using univariate and multivariate analysis. *Br J Cancer*. 81:1385-1391.

Sears, R., G. Leone, J. DeGregori, and J.R. Nevins. 1999. Ras enhances Myc protein stability. *Mol Cell*. 3:169-179.

Sears, R., F. Nuckolls, E. Haura, Y. Taya, K. Tamai, and J.R. Nevins. 2000. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev*. 14:2501-2514.

Seo, S.B., P. McNamara, S. Heo, A. Turner, W.S. Lane, and D. Chakravarti. 2001. Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell*. 104:119-130.

Seoane, J., H.V. Le, and J. Massague. 2002. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature*. 419:729-734.

Shackleton, M., E. Quintana, E.R. Fearon, and S.J. Morrison. 2009. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell*. 138:822-829.

Shefer, G., and D. Benayahu. 2010. SVEP1 is a novel marker of activated pre-determined skeletal muscle satellite cells. *Stem Cell Rev*. 6:42-49.

Sheiness, D., L. Fanshier, and J.M. Bishop. 1978. Identification of nucleotide sequences which may encode the oncogenic capacity of avian retrovirus MC29. *J Virol*. 28:600-610.

Shouse, G.P., X. Cai, and X. Liu. 2008. Serine 15 phosphorylation of p53 directs its interaction with B56gamma and the tumor suppressor activity of B56gamma-specific protein phosphatase 2A. *Mol Cell Biol*. 28:448-456.

Shouse, G.P., Y. Nobumori, and X. Liu. 2010. A B56gamma mutation in lung cancer disrupts the p53-dependent tumor-suppressor function of protein phosphatase 2A. *Oncogene*. 29:3933-3941.

Shur, I., R. Socher, M. Hameiri, A. Fried, and D. Benayahu. 2006. Molecular and cellular characterization of SEL-OB/SVEP1 in osteogenic cells in vivo and in vitro. *J Cell Physiol*. 206:420-427.

Shur, I., E. Zemer-Tov, R. Socher, and D. Benayahu. 2007. SVEP1 expression is regulated in estrogen-dependent manner. *J Cell Physiol*. 210:732-739.

Siddiqui-Jain, A., C.L. Grand, D.J. Bearss, and L.H. Hurley. 2002. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc Natl Acad Sci U S A*. 99:11593-11598.

Solomon, D.L., B. Amati, and H. Land. 1993. Distinct DNA binding preferences for the c-Myc/Max and Max/Max dimers. *Nucleic Acids Res*. 21:5372-5376.

Sontag, E., S. Fedorov, C. Kamibayashi, D. Robbins, M. Cobb, and M. Mumby. 1993. The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. *Cell*. 75:887-897.

Sontag, J.M., V. Nunbhakdi-Craig, M. Mitterhuber, E. Ogris, and E. Sontag. 2010. Regulation of protein phosphatase 2A methylation by LCMT1 and PME-1 plays a critical role in differentiation of neuroblastoma cells. *J Neurochem*. 115:1455-1465.

Soo Hoo, L., J.Y. Zhang, and E.K. Chan. 2002. Cloning and characterization of a novel 90 kDa 'companion' auto-antigen of p62 overexpressed in cancer. *Oncogene*. 21:5006-5015.

Sorlie, T., C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, J.C. Matese, P.O. Brown, D. Botstein, P.E. Lonning, and A.L. Borresen-Dale. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 98:10869-10874.

Soucek, L., M. Helmer-Citterich, A. Sacco, R. Jucker, G. Cesareni, and S. Nasi. 1998. Design and properties of a Myc derivative that efficiently homodimerizes. *Oncogene*. 17:2463-2472.

Soucek, L., R. Jucker, L. Panacchia, R. Ricordy, F. Tato, and S. Nasi. 2002. Omomyc, a potential Myc dominant negative, enhances Myc-induced apoptosis. *Cancer Res*. 62:3507-3510.

Soucek, L., S. Nasi, and G.I. Evan. 2004. Omomyc expression in skin prevents Myc-induced papillomatosis. *Cell Death Differ*. 11:1038-1045.

Staller, P., K. Peukert, A. Kiermaier, J. Seoane, J. Lukas, H. Karsunky, T. Moroy, J. Bartek, J. Massague, F. Hanel, and M. Eilers. 2001. Repression of p15INK4b expression by Myc through association with Miz-1. *Nat Cell Biol*. 3:392-399.

Suganuma, M., H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada, and T. Sugimura. 1988. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proc Natl Acad Sci U S A*. 85:1768-1771.

Sun, D., B. Thompson, B.E. Cathers, M. Salazar, S.M. Kerwin, J.O. Trent, T.C. Jenkins, S. Neidle, and L.H. Hurley. 1997. Inhibition of human telomerase by a G-quadruplex-interactive compound. *J Med Chem*. 40:2113-2116.

Tachibana, K. 1981. Okadaic acid, a cytotoxic polyether from two marine sponges of the genus Halichondria. J Am Chem Soc. 103:2469-2471.

Takagi, Y., M. Futamura, K. Yamaguchi, S. Aoki, T. Takahashi, and S. Saji. 2000. Alterations of the PPP2R1B gene located at 11q23 in human colorectal cancers. *Gut*. 47:268-271.

Takahashi, K., and S. Yamanaka. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 126:663-676.

Tan, J., P.L. Lee, Z. Li, X. Jiang, Y.C. Lim, S.C. Hooi, and Q. Yu. 2010. B55β-Associated PP2A Complex Controls PDK1-Directed Myc Signaling and Modulates Rapamycin Sensitivity in Colorectal Cancer. *Cancer Cell*. 18:459-471.

Vaarala, M.H., M.R. Vaisanen, and A. Ristimaki. 2010. CIP2A expression is increased in prostate cancer. *J Exp Clin Cancer Res*. 29:136.

van Houten, V.M., M.P. Tabor, M.W. van den Brekel, J.A. Kummer, F. Denkers, J. Dijkstra, R. Leemans, I. van der Waal, G.B. Snow, and R.H. Brakenhoff. 2002. Mutated p53 as a molecular marker for the diagnosis of head and neck cancer. *J Pathol*. 198:476-486.

Vennstrom, B., D. Sheiness, J. Zabielski, and J.M. Bishop. 1982. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. *J Virol*. 42:773-779.

Vervoorts, J., J.M. Luscher-Firzlaff, S. Rottmann, R. Lilischkis, G. Walsemann, K. Dohmann, M. Austen, and B. Luscher. 2003. Stimulation of c-MYC transcriptional activity and acetylation by recruitment of the cofactor CBP. *EMBO Rep*. 4:484-490.

Vogelstein, B., and K.W. Kinzler. 2004. Cancer genes and the pathways they control. *Nat Med*. 10:789-799.

von der Lehr, N., S. Johansson, S. Wu, F. Bahram, A. Castell, C. Cetinkaya, P. Hydbring, I. Weidung, K. Nakayama, K.I. Nakayama, O. Soderberg, T.K. Kerppola, and L.G. Larsson. 2003. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. *Mol Cell*. 11:1189-1200.

von Lindern, M., S. van Baal, J. Wiegant, A. Raap, A. Hagemeijer, and G. Grosveld. 1992. Can, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3' half to different genes: characterization of the set gene. *Mol Cell Biol*. 12:3346-3355.

Walter, G., and R. Ruediger. 2012. Mouse model for probing tumor suppressor activity of protein phosphatase 2A in diverse signaling pathways. *Cell Cycle*. 11:451-459.

Wang, J., W. Li, L. Li, X. Yu, J. Jia, and C. Chen. 2011. CIP2A is over-expressed in acute myeloid leukaemia and associated with HL60 cells proliferation and differentiation. *International Journal of Laboratory Hematology*. 33:290-298.

Wang, S.S., E.D. Esplin, J.L. Li, L. Huang, A. Gazdar, J. Minna, and G.A. Evans. 1998. Alterations of the PPP2R1B gene in human lung and colon cancer. *Science*. 282:284-287.

Welcker, M., and B.E. Clurman. 2007. Fbw7/hCDC4 dimerization regulates its substrate interactions. *Cell Div*. 2:7.

Welcker, M., and B.E. Clurman. 2008. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer*. 8:83-93.

Welcker, M., A. Orian, J. Jin, J.A. Grim, J.W. Harper, R.N. Eisenman, and B.E. Clurman. 2004. The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. *Proc Natl Acad Sci U S A*. 101:9085-9090.

Westermarck, J., and W.C. Hahn. 2008. Multiple pathways regulated by the tumor suppressor PP2A in transformation. *Trends Mol Med*. 14:152-160.

Westermarck, J., C. Weiss, R. Saffrich, J. Kast, A.M. Musti, M. Wessely, W. Ansorge, B. Séraphin, M. Wilm, B.C. Valdez, and D. Bohmann. 2002. The DEXD/H-box RNA helicase RHII/Gu is a co-factor for c-Jun-activated transcription. *EMBO J*. 21:451-460.

Winnikow, E.P., L.R. Medeiros, M.I. Edelweiss, D.D. Rosa, M. Edelweiss, P.W. Simoes, F.R. Silva, B.R. Silva, and M.I. Rosa. 2012. Accuracy of telomerase in estimating breast cancer risk: a systematic review and meta-analysis. *Breast*. 21:1-7.

Wolfer, A., B.S. Wittner, D. Irimia, R.J. Flavin, M. Lupien, R.N. Gunawardane, C.A. Meyer, E.S. Lightcap, P. Tamayo, J.P. Mesirov, X.S. Liu, T. Shioda, M. Toner, M. Loda, M. Brown, J.S. Brugge, and S. Ramaswamy. 2010. MYC regulation of a "poor-prognosis" metastatic cancer cell state. *Proc Natl Acad Sci U S A*. 107:3698-3703.

Wong, L.L., C.F. Chang, E.S. Koay, and D. Zhang. 2009. Tyrosine phosphorylation of PP2A is regulated by HER-2 signalling and correlates with breast cancer progression. *Int J Oncol*. 34:1291-1301.

Xing, Y., Z. Li, Y. Chen, J.B. Stock, P.D. Jeffrey, and Y. Shi. 2008. Structural mechanism of demethylation and inactivation of protein phosphatase 2A. *Cell*. 133:154-163.

Xu, J., Y. Chen, and O.I. Olopade. 2010. MYC and Breast Cancer. *Genes Cancer*. 1:629-640.

Xu, Y., Y. Xing, Y. Chen, Y. Chao, Z. Lin, E. Fan, J.W. Yu, S. Strack, P.D. Jeffrey, and Y. Shi. 2006. Structure of the protein phosphatase 2A holoenzyme. *Cell*. 127:1239-1251.

Yada, M., S. Hatakeyama, T. Kamura, M. Nishiyama, R. Tsunematsu, H. Imaki, N. Ishida, F. Okumura, K. Nakayama, and K.I. Nakayama. 2004. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. *EMBO J*. 23:2116-2125.

Yager, J.D., and N.E. Davidson. 2006. Estrogen carcinogenesis in breast cancer. *N Engl J Med*. 354:270-282.

Yang, S.I., R.L. Lickteig, R. Estes, K. Rundell, G. Walter, and M.C. Mumby. 1991. Control of protein phosphatase 2A by simian virus 40 small-t antigen. *Mol Cell Biol*. 11:1988-1995.

Yeh, E., M. Cunningham, H. Arnold, D. Chasse, T. Monteith, G. Ivaldi, W.C. Hahn, P.T. Stukenberg, S. Shenolikar, T. Uchida, C.M. Counter, J.R. Nevins, A.R. Means, and R. Sears. 2004. A signalling pathway

controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat Cell Biol*. 6:308-318.

Yu, J., A. Boyapati, and K. Rundell. 2001. Critical role for SV40 small-t antigen in human cell transformation. *Virology*. 290:192-198.

Zhang, X., A.S. Farrell, C.J. Daniel, H. Arnold, C. Scanlan, B.J. Laraway, M. Janghorban, L. Lum, D. Chen, M. Troxell, and R. Sears. 2012. Mechanistic insight into Myc stabilization in breast cancer involving aberrant Axin1 expression. *Proc Natl Acad Sci U S A*. 109:2790-2795.

Zhao, D., Z. Liu, J. Ding, W. Li, Y. Sun, H. Yu, Y. Zhou, J. Zeng, C. Chen, and J. Jia. 2010. Helicobacter pylori CagA upregulation of CIP2A is dependent on the Src and MEK/ERK pathways. *J Med Microbiol*. 59:259-265.

Zhao, X., J.I. Heng, D. Guardavaccaro, R. Jiang, M. Pagano, F. Guillemot, A. Iavarone, and A. Lasorella. 2008. The HECT-domain ubiquitin ligase Huwe1 controls neural differentiation and proliferation by destabilizing the N-Myc oncoprotein. *Nat Cell Biol*. 10:643-653.

Zhou, J., H.T. Pham, R. Ruediger, and G. Walter. 2003. Characterization of the Aalpha and Abeta subunit isoforms of protein phosphatase 2A: differences in expression, subunit interaction, and evolution. *Biochem J*. 369:387-398.

Zhu, L. 2005. Tumour suppressor retinoblastoma protein Rb: a transcriptional regulator. *Eur J Cancer*. 41:2415-2427.

Zhuang, D., S. Mannava, V. Grachtchouk, W.H. Tang, S. Patil, J.A. Wawrzyniak, A.E. Berman, T.J. Giordano, E.V. Prochownik, M.S. Soengas, and M.A. Nikiforov. 2008. C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. *Oncogene*. 27:6623-6634.

Zimmerman, K.A., G.D. Yancopoulos, R.G. Collum, R.K. Smith, N.E. Kohl, K.A. Denis, M.M. Nau, O.N. Witte, D. Toran-Allerand, C.E. Gee, J.D. Minna, and F.W. Alt. 1986. Differential expression of myc family genes during murine development. *Nature*. 319:780-783.

Zindy, F., C.M. Eischen, D.H. Randle, T. Kamijo, J.L. Cleveland, C.J. Sherr, and M.F. Roussel. 1998. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev*. 12:2424-2433.

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