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Review

TP63 and *TP73* in cancer, an unresolved "family" puzzle of complexity, redundancy and hierarchy

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ABSTRACT

***TP53* belongs to a small gene family that includes, in mammals, two additional paralogs, *TP63* and *TP73*. The p63 and p73 proteins are structurally and functionally similar to p53 and their activity as transcription factors is regulated by a wide repertoire of shared and unique post-translational modifications and interactions with regulatory cofactors. p63 and p73 have important functions in embryonic development and differentiation but are also involved in tumor suppression. The biology of p63 and p73 is complex since both *TP63* and *TP73* genes are transcribed into a variety of different isoforms that give rise to proteins with antagonistic properties, the TA-isoforms that act as tumor-suppressors and DN-isoforms that behave as proto-oncogenes. The p53 family as a whole behaves as a signaling "network" that integrates developmental, metabolic and stress signals to control cell metabolism, differentiation, longevity, proliferation and death. Despite the progress of our knowledge, the unresolved puzzle of complexity, redundancy and hierarchy in the p53 family continues to represent a formidable challenge.**

1. Introduction

In the late 1990s the two related orthologs of *TP53*, *TP63* and *TP73*, were first described [1,2]. The developmental functions of *TP63* (epithelial cell formation) and *TP73* (a role in central nervous system) were elucidated soon in a series of knockout-mouse experiments [3–5]. At the same time, the complex gene expression strategy from alternative promoters and the large number of different functional alternative splicing isoforms described for p63 and p73 [6], and later for p53 [7], has made the full understanding of p53 family members functions in different tissues and physiopathological contexts a difficult and yet unfinished task. The work from different research groups led to define the role of TA-p73 and TAp63 in DNA damage response (DDR) and cancer cells chemiosensitivity in the early 2000s [8,9] but their contribution to tumor suppression was established only later through the analysis of p73 and p63 heterozygous mutation in mice [10] and the generation of mice selectively lacking TAp73 or TA63 isoforms [11,12]. The repertoire of functions, pathways and genes regulated by p53, p63 and p73 has progressively widened well beyond cell fate, tumor suppression and development. p53-family members have been so far involved in reproduction, genomic repair, fidelity and recombination, metabolic processes, longevity, stem cells biology and changes in epigenetic marks. The complexity of p53 family expression and our incomplete understanding of the extent of functional redundancy and operational hierarchy in different physiological and pathological conditions continues to fuel the research efforts but has, at the same time, limited a rapid translation of knowledge into the clinical management of cancer patients. Here we review the current evidence on p73 and p63 and their role in cancer tumor suppression and development with a special focus on the role of TA-p63/p73 in chemioresistance and DNp73/p63 as oncogenes.

2. Molecular structure of p63/p73

The p63 and p73 proteins share with p53 a similar domain organization. Full length p63/p73 (TA-p63/TAp73) contain a N-terminal transactivation domain (TAD), followed by a proline-rich sequence (PR), a central DNA-binding domain (DBD) and a C-termi-

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nal oligomerization domain (OD) that is involved in the formation of active tetramers (Fig. 1). The high sequence homology (>70% of sequence identity) in the DBDs [6,7] accounts for p73 and p63 ability to regulate many known p53 target genes (e.g. p21, PUMA, NOXA, BAX and MDM2) [13], although the full repertoire of common and private target genes regulated by the different members under different physiological and pathological conditions is still to be determined. Oligomerization domains are much less conserved: p73 and p63 form hetero-oligomerize between each other but not, or to a limited extent with p53 [14,15].

3. Molecular complexity of p63/p73

A common feature of all p53 family members is that they can be expressed in a number of different isoforms [6,7,16]. In p73, the use of an internal promoter (P2), the alternative splicing of the first exons or the use of an alternative translation start site, generate several variants with a truncated N-terminus, identified collectively as DN-p73 [6,7]. DNp73 isoforms lack a functional transactivation domain and acquire dominant negative, anti-apoptotic and proproliferative functions over TA-p73 (see below). The C-terminus of the alpha isoforms contains a sterile alpha motif (SAM), and a terminal transcription inhibitory domain, not conserved in p53 [6,7,9,17]. Additional shorter isoforms (β, γ, δ, and the less investigated ε, ζ and η), are generated by C-terminus alternative splicing whose specific functions are still poorly characterized [8,18,19] (Fig. 1).

Relatively little is known regarding the mechanisms responsible for the differential expression of TA and DN isoforms in the different tissues and physio-pathologicals conditions. TAp73 isoforms transcription from the P1 promoter is primarily driven by E2F1 [20–22] but its activity can be also modulated by other factors such as C-EBPa [23], ZEB [24] and Ying Yang 1 (YY1) [25]. The regulation of the P2 promoter is much less clear [26–29]. In preneoplastic cirrhotic livers and hepatocellular carcinomas (HCCs) autocrine activation of epidermal growth factor receptor (EGFR) by its ligand amphiregulin (AR) triggers c-Jun N-terminal kinase-1 (JNK1) activity and inhibits the expression of the splicing regulator Slu7, leading to the selective accumulation of DNp73 transcripts, namely DeltaEx2p73 [30]. Finally, the uncovering of at least twelve p53 protein isoforms produced in normal tissues through alternative initiation of translation, usage of alternative promoters, and alternative splicing and their abnormal expression in cancer cells has uncovered an additional level of complexity to the p53 family [7,16].

4. Regulation of 73 and p63 functions

A complex network of post-translational modifications and protein–protein interactions control the levels and functions of all the members of the p53 family in unstressed and stressed cells.

Several proteins have been reported to regulate p73 and p63 stability: (a) the NEDD4-like ubiquitin ligase Itch binds p73 and p63 trigger their poly-ubiquitination and proteasomal degradation [31,32]; (b) the transcriptional co-activator YAP1 (Yes-associated protein 1), a key member of the Hippo signaling pathway, competes with Itch for the PY motif of p73, thus allowing its stabilization [33]; (c) mouse double minute 2 (MDM2) protein, the main E3

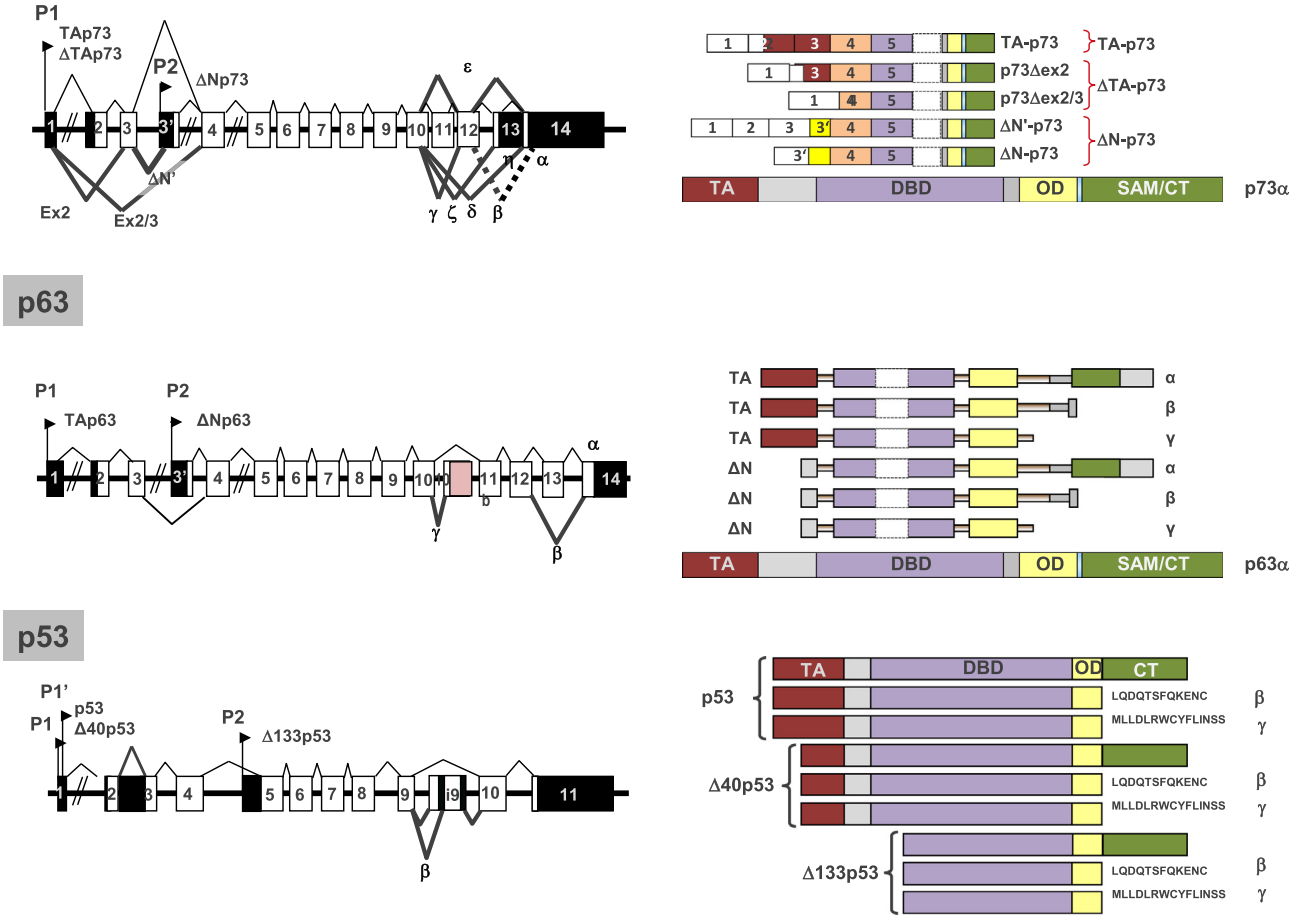

Fig. 1. The p53 family – a complex expression strategy.

ubiquitin ligase controlling p53 stability also binds to p73, interferes with p300/CBP acetylation of p73 and blocks of p73 transcriptional activities without triggering p73 degradation [34,35]. MDM2 does not induce p73 ubiquitination but catalyze p73 neddylation (conjugation of NEDD8 ubiquitin-like protein) which also inhibits p73 transcriptional activity [36]. MDM2 also binds DNp63a and promotes its nuclear export, poly-ubiquitination by the Fbw7/FBXW7 E3-ligase and proteasomal degradation in cells exposed to UV irradiation or adriamycin and upon keratinocyte differentiation [37]; (d) F-box protein FBXO45 binds to and promotes the ubiquitination/degradation of both TA- and DN-p73 isoforms [38]; (e) the U-box-type E3/E4 ubiquitin ligase UFD2a interacts with the SAM domain of TA-p73a, and promotes its ubiquitination-independent proteasomal [39]; (f) NEDL2, a NEDD4-related protein binds and increases p73 stability [40]; (g) the NAD(P)H quinone oxidoreductase 1 NQO1 binds to p73 and prevents its ubiquitin-independent degradation by the 20S proteasome [41]; (h) the peptidyl-prolyl isomerase Pin1 directly binds to and stabilizes TAp63α and ΔNp63α by inhibiting the E3 ligase WWP1 and proteasomal degradation [42,43]. The spectrum of functional interactions between these regulators and the different p63/p73 isoforms has not been fully characterized. However, some factors, such as c-Jun and the p53-induced RING-H2 E3 ligase Pirh2 have been shown to regulate differentially the protein levels of TA-p73 and DN-p73 isoforms [44–46]. Pirh2 also physically interacts with DNp63 that is targeted for polyubiquitination and subsequent proteasomal degradation [47]. Association with ASPP (Ankirin repeats, SH3 domain, proline-rich protein) proteins does not induce post-translational modifications but affects p73 as well as p53 and p63 functions: ASPP1/2 stimulate p73 and p63 transcriptional activity, while iASPP inhibits p73 activation and p73-mediated apoptosis [48–50].

5. p63, a developmental transcription factor

The main proof for a p63 role in human organ development is that dominantly inherited mutations in the p63 gene are found in a number of human ectodermal dysplasias, including ectrodactyly ectodermal dysplasia-cleft lip/palate syndrome (EEC), limb-mammary syndrome (LMS), ankyloblepharon ectodermal dysplasia clefting (AEC) and non-syndromic split-hand/split-foot malformation (SHFM) [51]. These syndromes affect the development of several organs deriving either directly from the ectoderm or from the interaction between developing ectoderm and mesoderm [52]. Specific genotype-phenotype correlations exist. Thus, mutations causing the EEC syndrome are not found in AEC, LMS or SHFM [53]. Moreover, the majority of mutations found in EEC syndrome are missense mutations generating amino acid substitutions in residues predicted to contact DNA [51]. Since the DNA binding domain is present in all p63 isoforms, all isoforms of p63 are affected by these mutations. Whether p63 DNA-binding mutants act as dominant-negative molecules remains, however, to be determined. Mutations in exon 13 and exon 14, affecting only the alpha isoforms of p63, are almost exclusively associated with AEC [51,54].

Mice lacking p63 die soon after birth with several developmental defects, particularly in limb and skin development [3,4] (Fig. 2). Defects in limb morphogenesis in p63 null mice were evident as early as E9.5. In wild type mice, during this interval, p63 is expressed in the surface ectoderm as well as in the ectoderm covering the limb buds and branchial arches.

The main functions of p63 is to maintain the proliferative potential of epidermal progenitor cells [55]. Indeed, in p63 null mice the proliferative compartment of the skin is progressively depleted of cells and this is directly reflected in a severe hypoplasia of the neonatal tissue [4]. p63 null mice activate a program of cellular senescence that leads to accelerated aging [56] (Fig. 2). In particular, TA-p63 prevents premature tissue aging and maintains dermal and epidermal precursors [57] whereas ΔNp63 is required for the initial commitment of keratinocyte progenitors towards differentiation [58]. In addition to maintain progenitor cell proliferation, p63 also impacts on epidermal stratification and keratinocyte differentiation. Although still a matter of debate [59,60], TAp63 isoforms, the first p63 isoforms expressed during epidermal development, are required for the commitment to stratification while they inhibit terminal differentiation [61]. After commitment to stratification has occurred, ΔNp63 isoforms induce the expression of genes that are required for later stages of epidermal morphogenesis [62] but for differentiation to proceed ΔNp63 needs to be subsequently eliminated [63,64]. The depletion of ΔNp63 occurs mainly via proteasome-mediated degradation [31,32], which in turn is controlled by several proteins some of which are transcriptional targets of ΔNp63 [65–67]. In addition, the expression of a p63-specific microRNA (miR203) is also important to induce p63 downregulation during terminal differentiation [68].

p63 regulates transcription by binding to p63-response elements whose repertoire is largely overlapping with p53 elements. Indeed, many of the p53 responsive elements involved in DNA damage-induced cell cycle arrest or apoptosis are constitutively occupied by ΔNp63 in proliferating keratinocytes ([69,70] and AC unpublished results). ΔNp63 controls distinct transcriptional networks depending on the state of maturation of keratinocyte precursors, which in turn is dependent on a variety of extracellular stimuli. In proliferating keratinocytes of the basal layers ΔNp63 controls the expression of basal layer keratins (*K5, K14*) and of molecules required for the formation of the epidermal barrier, such as *Alox12* [71] and inhibit proliferation-induced activation of cell cycle arrest genes by competing with p53 for the same responsive elements. In response to differentiation stimuli, ΔNp63 detaches from the promoter of cell cycle arrest genes (e.g. *14-3-3 sigma* and *p21waf1*), activates genes required for cell cycle exit (*IKKa* and *IRF6*) and re-organizes the transcription of adhesion molecules to allow keratinocytes to leave the basal layer and stratify. Thus, differences in temporal expression, isoform combination, biochemical properties and transcription activity of p63 protein(s) can have a profound impact on the set of genes transcribed, at a given time in a given cell.

A combination of isoform-specific siRNA-mediated downregulation in primary keratinocytes and in vivo, coupled to analysis of knock-out and disease specific knock-in mice, has allowed to determine the key target genes required for epidermal morphogenesis that are involved in pathogenesis of *p63*-linked ectodemal dysplasias [62,65,72,73]. These studies showed that the protein kinase IKKα is a transcriptional target of ΔNp63 and, indeed, ΔNp63 mutants found in ED are unable to activate the *IKKα* expression. IKKα is a component of the IkB kinase complex and is required for correct epidermal development and epithelial-mesenchymal interaction during development. Although NFkB regulates DNp63 [74], IKKa kinase activity is not required for its function in development [75]. IKKα null mice display defects in epidermal, limb and craniofacial development that are fully reverted after the re-expression of *IKKα* in the developing ectoderm [75]. Interestingly, IKKα has been recently found to be a component of the TGFβ pathway in keratinocytes [76,77] and to repress *FGF7* and *FGF8* expression [75]. These observations directly link ΔNp63 function to the control of developmental factors (TGFβ and FGF8) regulating epidermal, limb and craniofacial development.

Similarly, *IRF6*, another ΔNp63 target gene, is involved in both epidermal development and limb/craniofacial development [65,78]. The underlying mechanism relates to the ability of IRF6

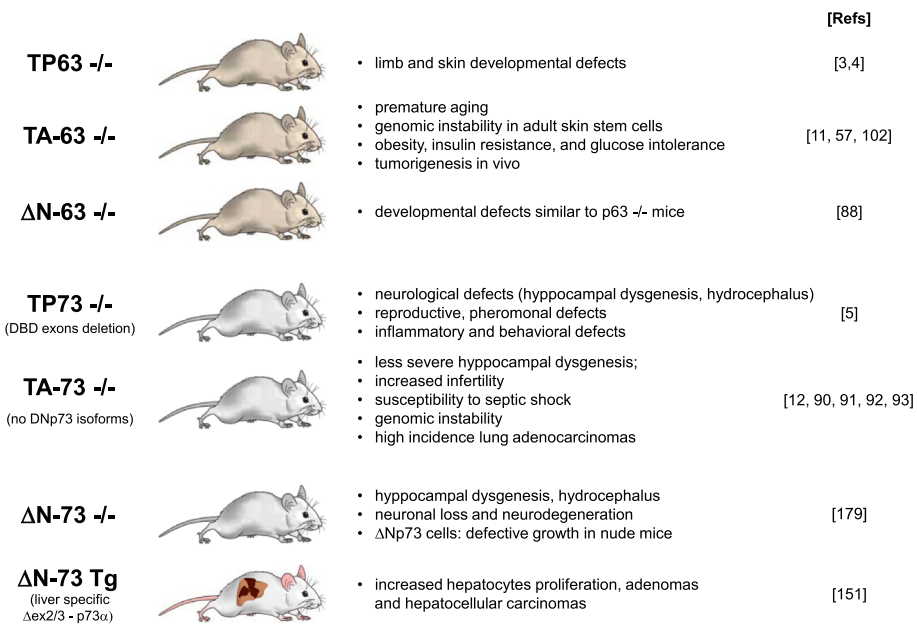

Fig. 2. TP63 and TP73 global and isoform selective knock-out mice. (See above-mentioned reference for further information.)

to modify the stability of the ΔNp63 protein. *IRF6* expression is required at the onset of terminal differentiation to allow proteasome-dependent degradation of ΔNp63. Mutations of *IRF6* cause syndromes characterized by cleft-lip palate and other developmental abnormalities, and *IRF6* knock-in mice carrying the same mutation found in patients display a hyperproliferative epidermis that is unable to terminally differentiate [78]. Finally, members of the BMP/TGFβ and FGF families are to be considered as important soluble mediators of this complex regulatory network [79].

6. TP73 and TP63 as tumor supressors: lessons from animal models

The role of TAp73 and TAp63 as tumor suppressor genes was initially challenged by two orders of evidence. First, no TP73 and TP63 gene deletions were associated to cancer [1,80] and only a very low percentage of human tumors (less than 1%) carry p73 or p63 mutations [81,82]. Second, the phenotype of p73−/− mice lacking all p73 isoforms (DBD exons deletion) did not support a role in cancer (Fig. 2). p73-null mice die at 4–6 weeks of age [5] and display neurological (hydrocephalus), reproductive, pheromonal, inflammatory and behavioral defects. These observations prevailed over the results from in vitro studies (i.e. TAp73 ability to trigger cell cycle arrest, cellular senescence and apoptosis upon DNA damage by promoting the transcription of many p53 target genes [83]; the potentiation of oncogenic RasV12 in the transformation of p53−/− mouse embryonic fibroblasts after TAp73 knockdown [84] and the ex vivo studies in onco-hematologic patients (i.e. P1 promoter hypermethylated and reduced TA-p73 expression in lymphoblastic leukemias and Burkitt's lymphomas [85,86]. The evidence to establish the role of TAp73 and TAp63 as part of an integrated tumor suppressor network with p53 came from the study of p73 and p63 heterozygous mutation in mice and from the generation of mice selectively lacking TAp73 or TA63 isoforms. Flores and coll showed that p73+/− and the p73+/−:p53+/− mice developed a more aggressive tumor phenotype, compared to p73+/+ and p73+/+:p53+/− animals [10]. p63+/− and p63+/−:p53+/− mice are also cancer-prone [10] but this appears to be dependent on the genetic background, as p63+/− mice on a different inbred strain show premature aging but no cancer [56,87]. The analysis of TAp63 selective conditional knockout mice [11,57] showed that TA-p63 prevents premature tissue aging and mantains genomic stability in adult skin stem cells [57] and suppresses tumorigenesis in vivo, irrespective of p53 status and independently from p19(Arf) and p16(Ink4a) [11] (Fig. 2). Selective *ΔNp63* loss [88] results in developmental defects similar to those observed in the original p63−/− mice [4] (Fig. 2). Interestingly, the reduction TAp63 protein levels, due to monoubiquitinated FANCD2 protein deficiency, is responsible for the increased susceptibility to squamous cell neoplasia in Fanconi anemia patients [89]. TA-p73−/− mice, which express DNp73 isoforms, showed less severe hyppocampal dysgenesis, increased infertility and greater mortality by septic shock in response to lipopolysaccharide (LPS) challenge [12,90]. Notably, TAp73(−/−) also had a high incidence of spontaneous tumors, in particular lung adenocarcinomas [12] (Fig. 2). The increased infertility in TA-p73−/− mice is the result of a massive and premature loss of immature germ cells due to disruption of cell–cell adhesions of developing germ cells to Sertoli nurse cells, defective maturation of the germ epithelium and arrested spermiogenesis [91,92]. The impaired resolution of inflammatory responses and the higher susceptibility to septic shock in TAp73−/− mice has been related to an altered macrophage polarization with maintenance of an M1 macrophage effector phenotype together with the elevated production of TNF-α and IL-6 [90]. TA-p73 binds to and regulates the functions of Bub1 and BubR1 in the Spindle Assembly Checkpoint (SAC) [93]. These results, together with previous observations that implicated TAp73 in the control of mitosis [94,95] and aneuploidy [96], clearly assign to TAp73s a crucial role in preventing genomic instability in multiple tissues with a specific role for TAp63s in stratified epithelia. TAp73 have been also show to limit of c-Myc-driven lymphomagenesis in vivo [97,98]. Altogether, these results not only proved the involvement of TA-p73 and TAp63 in tumor suppression but also suggested a role of DNp73 in oncogenesis and the importance of a proper balance between TA- and DN-p73 (and p63) isoforms to maintain genomic integrity in proliferating cells (see below).

Recently, global genomic expression profiling (RNA-Seq) and chromatin immunoprecipitation (ChIP-Seq) experiments have identified revealed an extensive p53-regulated autophagy program

and revealed a key role of specific autophagy genes in p53-dependent apoptosis and suppression of cell transformation but not in cell cycle arrest [99]. Notably, the majority of the identified genes are also by TAp63 and TAp73, suggesting that, similar to their collaborative induction of proapoptotic genes [100], the entire p53 family cooperates in controlling cellular homeostasis and tumor suppression by promoting autophagy.

7. TP73, TP63 and TP53 in normal and cancer cells metabolism

The complexity of the networks engaged by the p53 family proteins/isoforms to execute their physiological functions and how they are recruited for tumorigenesis and tumor suppression is further underlined by the emerging role of p53 and the p53 family members in normal and cancer cells metabolism. p53 has been reported to control several metabolic pathways, including glucose metabolism [repression of insulin receptor (INSR) and the GLUT1 and GLUT4 glucose transporters; activation of TIGAR (TP53-induced glycolysis and apoptosis regulator) and HK II (hexokinase II); degradation of PGM (phosphoglycerate mutase]; the tricarboxylic acid (TCA) cycle [induction of GLS2 (glutaminase 2)]; fatty acid oxidation [activation of GAMT (guanidinoacetate aminotransferase)]; mitochondrial respiration [induction of AIF (apoptosis inducing factor) and SCO2 (synthesis of cytochrome oxidase 2)] [101]. TAp63 accumulates in response to metabolic stress and activates transcription of the two key metabolic regulators, Sirt1 and AMPKα2, resulting in increased fatty acid synthesis and decreased fatty acid oxidation and lowers blood glucose levels in response to metformin [102]. TAp63−/− mice, in addition to show premature aging [57] develop obesity, insulin resistance, and glucose intolerance and restoration of Sirt1 or AMPKα2 in TAp63−/− mice rescued the metabolic defects [102]. TAp73α also regulates liver lipid metabolism in response to nutrient deprivation by direct targeting of the ATG5 gene whose product, autophagy-related protein 5, is required for autophagosome formation and triglyceride hydrolysis into fatty acids (macrolipophagy) [103].

Cancer cells rewire cellular metabolism to satisfy their increased demand of bioenergy, macromolecular biosynthesis and redox maintenance. The metabolic program of these cells is marked by an increased uptake of glucose and glutamine to support cell growth. Most imported glucose is metabolized to lactate through aerobic glycolysis or fueled to the oxidative pentose phosphate pathway (PPP) whereas glutamine serves both as a nitrogen source for the biosynthesis of nucleotides and various non-essential amino acids and as an important carbon source for the replenishment of TCA cycle intermediates [104]. TAp73, but not p53 or p63, drives the transcription of glucose-6-phosphate dehydrogenase (G6PD) [105], the rate-limiting enzyme for the oxidative pentose phosphate pathway (PPP) that controls the production of NADPH and ribose needed for the synthesis of macromolecules and detoxication of reactive oxygen species (ROS). Interestingly, DNp73 and mtp53 do not inhibit G6PD activation by TAp73, suggesting that this mechanism might be operative in proliferating and tumor cells even in the presence of mtp53 or elevated DNp73 levels [105]. TAp73 also targets Cox4i1, a subunit of cytochrome c oxidase in the mitochondrial oxidative phosphorylation chain that promotes oxygen consumption and prevents ROS accumulation and senescence [106]. Although p53 can directly bind to and inhibit G6PD [107], p53 also activates the transcription of TIGAR (tp53-induced glycolysis and apoptosis regulator) [108,109] that degrades fructose-2,6-bisphosphate to limit phosphofructokinase activity and promotes a sustained diversion of glycolytic intermediates to the PPP. Glutaminase type 2 (GLS2) transcription is activated by TAp73 [110,111], TAp63 [112] and p53 [113,114] in normal and cancer cells. In addition to increase ATP production and oxygen consumption, the glutamate produced by GLS2 also regulates the cellular redox balance, by supporting the formation of glutathione (GSH) and NADPH, and affects serine bio-synthesis, by activating the transcription factor ATF4. Serine is a precursor for nucleotides, amino acids and lipids and an allosteric activator of the pyruvate kinase M2 isoform (PKM2) predominantly expressed in cancer cells, thus sustaining aerobic glycolysis and conversion of pyruvate into lactate and cancer cells proliferation [111,115]. The positive effect of TAp73 on the serine biosynthesis and PKM2 activity synergizes with G6PD induction and PPP activation [105]. NADPH production is a rate-limiting step in cell proliferation and NADPH production is tightly controlled by oncogenes, such as K-Ras [116] and tumor suppressors [105,117]. Indeed, TAp73 and G6PD have been shown to supports the proliferation of human and mouse tumor cells [105] but restoration of cell growth in cells lacking TAp73 by G6PD is not complete and cell type dependent [105,118], suggesting that other TAp73 targets might contribute to its proliferative function [119]. TAp63 has also been found to activate cell cycle genes and promote cell proliferation in specific cell contexts [119–121]. The role of TAp73 (and TA63) in cell proliferation is in conflict with the increased tumor formation observed in TAp73- (and TAp63-) deficient mice and their tumor suppressor activity. Although there is no positive evidence, a possible explanation is that any proliferative defects present in these mice is overrun by the extensive genomic instability and the accumulation of oncogenic mutations associated with the early loss of TAp73 and TAp63.

8. p73 and chemiosensitivity

In response to DNA damage and chemiotherapeutic drugs several events converge to build up the TAp73 apoptotic response [122]. As part of the DNA damage response (DDR), E2F1 is acetylated by PCAF [123,124], phosphorylated by the Chk1/2 kinases [125] and directed to the P1 promoter to induce TA-p73 expression [123], whereas E2F1 deacetylation by Sirt1 inhibit of TA-p73 transcription [126]. Similarly, E2F1 methylation by Set9 and its demethylation by LSD1 enzymes regulate E2F1-dependent activation of TA-p73 expression and induction of apoptosis [127]. Multiple kinases activated in the DDR phosphorylate TAp73 proteins leading to its stabilization and accumulation (Fig. 3): (a) the non-receptor tyrosine kinase c-Abl [TA-andDN-p73safterγ-radiation-orcisplatintreatment] [128–130]; (b) ATM (Ataxia Telangiectasia Mutated) serine-protein kinase [after cisplatin treatment] [130]; (c) the downstream effector of ATM Chk2 [131]; (d) protein kinase Cd (PKCδ) [TA-p73β at Ser289 [132]]; JNKs [TA-p73 after cisplatin treatment] [133]; p38 phosphorylation of threonine residues critical for p73 activation by c-Abl [134]. ATM also modulates p73 levels in response to cisplatin by activating cAbl [135] and by phosphorylating IKK-α that accumulates in the nucleus and promotes p73 stabilization [136,137]. Finally, in response to DNA damage Itch levels are downregulated by yet unknown mechanisms to relieve TAp73s from Itch-induced degradation and to allow TAp73 accumulation [2]. Although TAp73 proteins accumulation is expected to translate into a global increase of target genes expression, TAp73 apoptotic capacity is fully activated by p300-mediated acetylation that increases interaction with the YAP1 transcriptional co-factor [138–140] and directs TAp73/YAP complexes to the promoters of apoptotic target genes [138,141]. Notably, both TAp73 conformational changes catalyzed by Pin prolyl isomerase [142] and c-abl-mediated tyrosine phosphorylations are required for TAp73 acetylation by p300 [141,142]. It is important to consider that DN-p73 is, or can be, targeted by many of the phosphorylations associated with the DDR response, as well as by p300-mediated acetylation, resulting in the accumulation of DN-

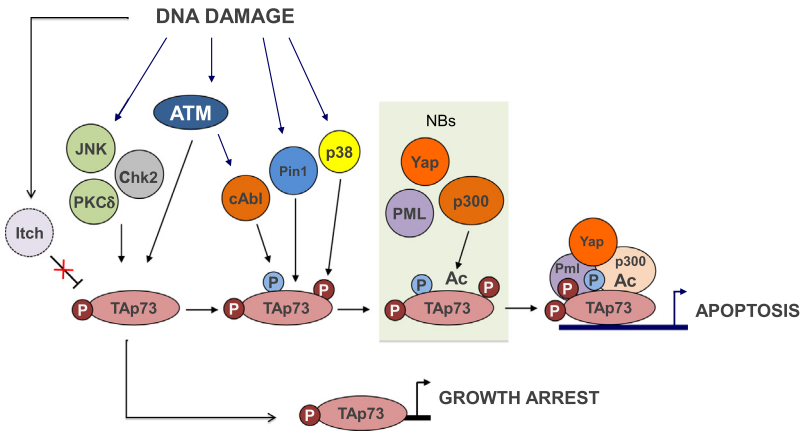

Fig. 3. p73 stabilization and activation in response to DNA genotoxic insults.

p73 proteins. Some factors that regulate differentially the protein levels of TA-p73 and DN-p73 isoforms play an important role in the maintenance of TAp73 apoptotic and tumor suppressor activities. The p73-induced ring-finger domain ubiquitin ligase PIRH2 preferentially degrades DN-p73, thus releasing TA-p73 and triggering apoptosis following DNA damage [46]. c-Jun triggers the proteasome-mediated ubiquitin-independent degradation of DN-p73 in response to DNA damage by inducing the expression of the non-classical polyamine-induced antizyme (Az) [45].

The activation and contribute of TAp63 in the DDR is much less clear. However, a strong link between DN-p63 and chemiosensitivity has been established in head and neck squamous cell carcinomas (HNSCC) where DN-p63 expression in vivo correlates with chemo- and radio-resistance and the experimental knockdown of endogenous p63 in HNSCC cells by RNA interference resulted in induction of TA-p73-dependent apoptosis [143]. Similarly, in cell lines derived from triple negative breast cancers (TNBC, lacking estrogen and progesterone receptors and with Her2 amplification), the activation of the cAbl/TA-p73 axis and apoptosis in response to cisplatin requires the release of TA-p73 from DNp63 [144] and DN-p63 levels are crucial for TNBC chemiosensitivity.

Despite the bulk of evidence that supports the role of TAp73 in chemiosensitivity in cell and animal models, less is know about their contribute to chemio-resistance and chemiosensitivity in human cancers. mt-p53s bind and inhibit TAp73 and TAp63 as part of their GOF activity (“gain of function”).

The presence of a polymorphic site at codon 72 of wt- and mt-p53 that encodes either an arginine (72R) or a proline (72P) [145–147] impacts on p73-dependent chemotherapeutic responses of cancers bearing mutp53. wt-p53 72R induces apoptosis much better than the 72P variant [147] whereas mut-p53 72R confers higher chemoresistance to cancer cells as compared with mut-p53 72P [145,146,148].

9. DN-p73s as a proto-oncogene

The oncogenic role of N-terminally truncated DN-p73 isoforms is supported by many in vitro and in vivo evidence. DN-p73 overexpression in fibroblasts increases their colony formation capacity [149] and cooperates with RAS, cMyc and E1A in promoting transformation and tumorigenicity [84,150]. Liver DN-p73 (in particular Dex2/3-p73α) transgenic mice display increased hepatocytes proliferation and develop both adenomas and hepatocellular carcinomas (HCC) [151] (Fig. 2). Several mechanisms contribute to the oncogenic potential of DN-p73 isoforms. DN-p73s exert a dominant negative effect on both p53 and TA-p73 and TA-p63, by competing for binding to the same target promoters as DN-tetramers and by oligomerizing with them to form transcriptionally ineffective heterocomplexes [143,152]. In addition, DN-p73s promote RB hyper-phosphorylation by cyclin E-Cdk2 and cyclin D-Cdk4/6 kinases, resulting in E2F deregulation and cell cycle progression [151,153,154]. Finally, some C-terminal variants of DN-p73 can up-regulate anti-apoptotic genes independently of p53 [155–157].

More importantly, DN-p73 is overexpressed in several tumors, among them breast [152], ovary [158,159], prostate cancers [160], melanoma [161], neuroblastoma [162] and hepatocellular carcinoma [163,164] and in most cases, DN-p73 expression is associated with therapy failure, chemoresistance, metastasis and vascular invasion [165]. In melanoma xenografts DNp73 expression is associated with upregulation of Slug, downregulation of the actin binding protein EPLIN, activation of the IGF1R-AKT/STAT3 pathway, loss of E-cadherin and a higher ability to invade and metastasize [166].

DN-p73 levels and DN- to TA-p73 ratio determine the net effect of p73 and seem to predict the effectiveness of chemotherapy [8,122,144]. Several signaling and/or oncogenic pathways impact TA- and/or DN-p73 levels by affecting transcription or protein stability. H-RasV12 overexpression in primary fibroblasts down-regulates TA-p73 and increases both DN-p73 expression and anchorage independent cell growth [167]. Importantly, both p53 and TA-p73 bind the internal P2 promoter of TP73, activate the transcription of P2 DN-p73 isoforms [26,28,29] and create a negative feedback loop between DN-p73 and p53/TA-p73 that may self-restrict their transcriptional activities.

10. DNp63 links organ development and tumorigenesis in skin cancer

ΔNp63α is the major p63 isoform expressed in stratified squamous epithelium and in squamous cell carcinomas (SCC) [168,169]. DNp63 contribution to skin tumorigenesis well illustrates the current view of cancer as the result of complex interactions between transformed cells and multiple cell types with the tumor microenvironment and the impact of genes involved in organ development. In this perspective, DNp63 oncogenic potential is related not only to a direct competition with p53, TAp63 and TAp73 on the same p53 Responsive Elements and the consequent inhibition of p53/p73 mediated activation [70] and to its interactions with the other p53 family members and their modulators (i.e. ASPP1/2, HIPK2, mdm2, Pin1, Pirh2 [37,42,43,47,48,170,171] but also to its ability to control transcrip-

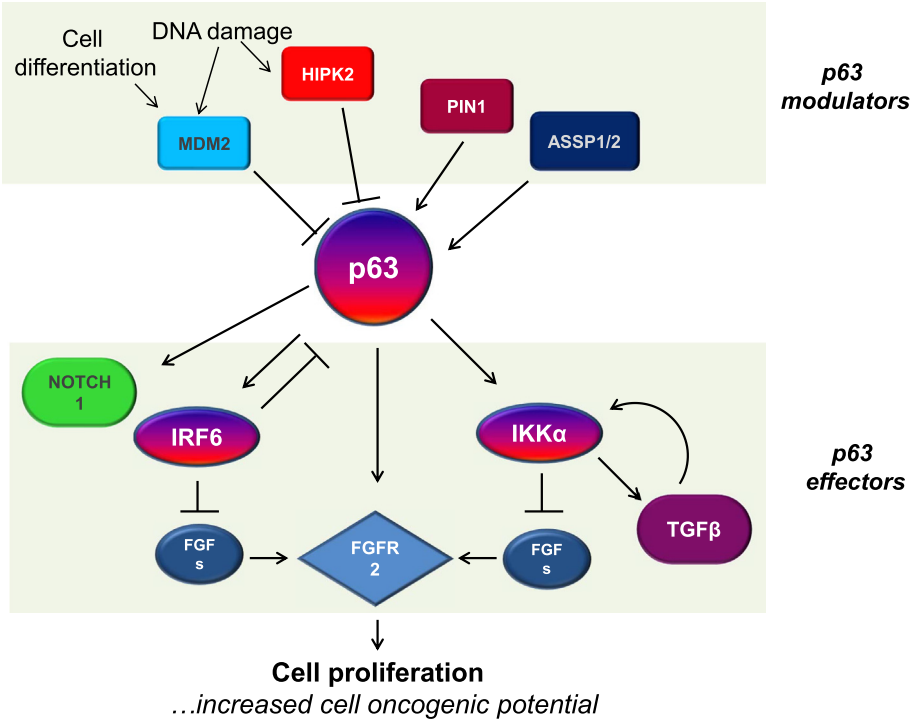

Fig. 4. p63 upstream modulators and effectors.

tion of genes involved in developmental and tumorigenic pathways, such as IRF6, IKKa and FGFR2 [172–174] (Fig. 4). IKKa and IRF6 functions in skin and organ development has been described above. We found that both IKKa and IRF6 are strongly downregulated in SCCs, and that their expression is correlated negatively with cancer differentiation [172–174]. IKKa acts in epithelial cells as a component of the anti-proliferative branch of the TGFbeta signaling pathway, that is frequently inactivated in cancers [76,172,175] but has also another function as repressor of FGFs expression [75,176,177]. IRF6 acts on a partially different pathway, being a mediator of Notch1 anti-cancer activity [178], but also a repressor of cMyc and FGF pathways. Interestingly, recent data indicate in the Receptor 2 for the Fibroblast growth factors (FGFR2) a critical mediator of DNp63 oncogenic functions in SCCs [174], whose inhibition favors tumor regression the same way DNp63 depletion does. FGFR2 is the receptor for FGF7, 8 that are specifically downregulated by IKKa, and IRF6 ([75,177] and AC personal communication). Altogether these informations suggest a model in which DNp63 acts as a regulator of p53 tumor suppressive functions in a cell autonomous way, and as a mediator of activation of FGF signaling pathway in a paracrine way.

References

[1] Kaghad, M., Bonnet, H., Yang, A., et al. (1997) Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 90, 809–819.

[2] Yang, A., Kaghad, M., Wang, Y., et al. (1998) p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing and dominant-negative activities. Mol. Cell 2, 305–316.

[3] Yang, A., Schweitzer, R., Sun, D., et al. (1999) p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 398, 714–718.

[4] Mills, A.A., Zheng, B., Wang, X.J., Vogel, H., et al. (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 398, 708–713.

[5] Yang, A., Walker, N., Bronson, R., et al. (2000) p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. Nature 404, 99–103.

[6] Levrero, M., De Laurenzi, V., Costanzo, A., et al. (2000) The p53/p63/p73 family of transcription factors: overlapping and distinct functions. J. Cell Sci. 113, 1661–1670.

[7] Murray-Zmijewski, F., Lane, D.P. and Bourdon, J.C. (2006) p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ. 3, 962–972.

[8] Lunghi, P., Costanzo, A., Mazzera, L., et al. (2009) The p53 family protein p73 provides new insights into cancer chemosensitivity and targeting. Clin. Cancer Res. 15, 6495–6502.

[9] Stiewe, T. (2007) The p53 family in differentiation and tumorigenesis. Nat. Rev. Cancer 7, 165–168.

[10] Flores, E.R., Sengupta, S., Miller, J.B., et al. (2005) Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell 7, 363–373.

[11] Guo, X., Keyes, W.M., Papazoglu, C., et al. (2009) TAp63 induces senescence and suppresses tumorigenesis in vivo. Nat. Cell Biol. 11, 1451–1457.

[12] Tomasini, R., Tsuchihara, K., Wilhelm, M., et al. (2008) TAp73 knockout shows genomic instability with infertility and tumor suppressor functions. Genes Dev. 22, 2677–2691.

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

[14] Davison, T.S., Vagner, C., Kaghad, M., Ayed, A., et al. (1999) p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. J. Biol. Chem. 274, 18709–18714.

[15] Chikh, A., Matin, R.N., Senatore, V., et al. (2011) IASPP/p63 autoregulatory feedback loop is required for the homeostasis of stratified epithelia. EMBO J. 30, 4261–4273.

[16] Surget, S., Khoury, M.P. and Bourdon, J.C. (2013) Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. Onco Targets Ther. 7, 57–68.

[17] Sauer, M., Bretz, A.C., Beinoraviciute-Kellner, R., et al. (2008) C-terminal diversity within the p53 family accounts for differences in DNA binding and transcriptional activity. Nucleic Acids Res. 36, 1900–1912.

[18] De Laurenzi, V., Costanzo, A., Barcaroli, D., et al. (1998) Two new p73 splice variants, gamma and delta, with different transcriptional activity. J. Exp. Med. 188, 1763–1768.

[19] De Laurenzi, V.D., Catani, M.V., Terrinoni, A., et al. (1999) Additional complexity in p73: induction by mitogens in lymphoid cells and identification of two new splicing variants epsilon and zeta. Cell Death Differ. 6, 389–390.

[20] Irwin, M., Marin, M.C., Phillips, A.C., et al. (2000) Role for the p53 homologue p73 in E2F-1-induced apoptosis. Nature 407, 645–648.

[21] Stiewe, T. and Putzer, B.M. (2000) Role of the p53-homologue p73 in E2F1-induced apoptosis. Nat. Genet. 26, 464–469.

[22] Seelan, R.S., Irwin, M., van der Stoop, P., et al. (2002) The human p73 promoter: characterization and identification of functional E2F binding sites. Neoplasia 4, 195–203.

[23] Marabese, M., Vikhanskaya, F., Rainelli, C., et al. (2003) DNA damage induces transcriptional activation of p73 by removing C-EBPalpha repression on E2F1. Nucleic Acids Res. 31, 6624–6632.
[24] Fontemaggi, G., Gurtner, A., Strano, S., et al. (2001) The transcriptional repressor ZEB regulates p73 expression at the crossroad between proliferation and differentiation. Mol. Cell. Biol. 21, 8461–8470.
[25] Wu, S., Murai, S., Kataoka, K., et al. (2008) Yin Yang 1 induces transcriptional activity of p73 through cooperation with E2F1. Biochem. Biophys. Res. Commun. 365, 75–81.
[26] Grob, T.J., Novak, U., Maisse, C., et al. (2001) Human delta Np73 regulates a dominant negative feedback loop for TAp73 and p53. Cell Death Differ. 8, 1213–1223.
[27] Vossio, S., Palescandolo, E., Pediconi, N., et al. (2002) DN-p73 is activated after DNA damage in a p53-dependent manner to regulate p53-induced cell cycle arrest. Oncogene 21, 3796–3803.
[28] Kartasheva, N.N., Contente, A., Lenz-Stoppler, C., et al. (2002) P53 induces the expression of its antagonist p73 Delta N, establishing an autoregulatory feedback loop. Oncogene 21, 4715–4727.
[29] Nakagawa, T., Takahashi, M., Ozaki, T., et al. (2002) Autoinhibitory regulation of p73 by Delta Np73 to modulate cell survival and death through a p73-specific target element within the Delta Np73 promoter. Mol. Cell. Biol. 22, 2575–2585.
[30] Castillo, J., Goñi, S., Latasa, M.U., et al. (2009) Amphiregulin induces the alternative splicing of p73 into its oncogenic isoform DeltaEx2p73 in human hepatocellular tumors. Gastroenterology 137, 1805–1815 (e1-4).
[31] Rossi, M., De Laurenzi, V., Munarriz, E., et al. (2005) The ubiquitin-protein ligase Itch regulates p73 stability. EMBO J. 24, 836–848.
[32] Rossi, M., Aqeilan, R.I., Neale, M., et al. (2006) The E3 ubiquitin ligase Itch controls the protein stability of p63. Proc. Natl. Acad. Sci. USA 103, 12753–12758.
[33] Levy, D., Adamovich, Y., Reuven, N., et al. (2007) The Yes-associated protein 1 stabilizes p73 by preventing Itch-mediated ubiquitination of p73. Cell Death Differ. 14, 743–751.
[34] Dobbelstein, M., Wienzek, S., Konig, C., et al. (1999) Inactivation of the p53-homologue p73 by the mdm2-oncoprotein. Oncogene 18, 2101–2106.
[35] Zeng, X., Chen, L., Jost, C.A., et al. (1999) MDM2 suppresses p73 function without promoting p73 degradation. Mol. Cell. Biol. 19, 3257–3266.
[36] Watson, I.R., Blanch, A., Lin, D.C., et al. (2006) Mdm2-mediated NEDD8 modification of TAp73 regulates its transactivation function. J. Biol. Chem. 281, 34096–34103.
[37] Galli, F., Rossi, M., D'Alessandra, Y., et al. (2010) MDM2 and Fbw7 cooperate to induce p63 protein degradation following DNA damage and cell differentiation. J. Cell Sci. 123, 2423–2433.
[38] Peschiaroli, A., Scialpi, F., Bernassola, F., et al. (2009) The F-box protein FBXO45 promotes the proteasome-dependent degradation of p73. Oncogene 28, 3157–3166.
[39] Hosoda, M., Ozaki, T., Miyazaki, K., et al. (2005) UFD2a mediates the proteasomal turnover of p73 without promoting p73 ubiquitination. Oncogene 24, 7156–7169.
[40] Miyazaki, K., Ozaki, T., Kato, C., et al. (2003) A novel HECT-type E3 ubiquitin ligase, NEDL2, stabilizes p73 and enhances its transcriptional activity. Biochem. Biophys. Res. Commun. 308, 106–113.
[41] Asher, G., Lotem, J., Sachs, L., et al. (2002) Mdm-2 and ubiquitin-independent p53 proteasomal degradation regulated by NQO1. Proc. Natl. Acad. Sci. USA 99, 13125–13130.
[42] Li, C., Chang, D.L., Yang, Z., Qi, J., et al. (2013) Pin1 modulates p63α protein stability in regulation of cell survival, proliferation and tumor formation. Cell Death Dis. 4, e943 (Dec 5).
[43] Restelli, M., Lopardo, T., Lo Iacono, N., et al. (2014) DLX5, FGF8 and the Pin1 isomerase control ΔNp63α protein stability during limb development: a regulatory loop at the basis of the SHFM and EEC congenital malformations. Hum. Mol. Genet. (Feb 25, Epub ahead of print).
[44] Toh, W.H., Siddique, M.M., Boominathan, L., et al. (2004) C-Jun regulates the stability and activity of the p53 homologue, p73. J. Biol. Chem. 279, 44713–44722.
[45] Dulloo, I., Gopalan, G., Melino, G., et al. (2010) The antiapoptotic DeltaNp73 is degraded in a c-Jun-dependent manner upon genotoxic stress through the antizyme-mediated pathway. Proc. Natl. Acad. Sci. USA 107, 4902–4907.
[46] Sayan, B.S., Yang, A.L., Conforti, F., et al. (2010) Differential control of TAp73 and DeltaNp73 protein stability by the ring finger ubiquitin ligase PIR2. Proc. Natl. Acad. Sci. USA 107, 12877–12882.
[47] Jung, Y.S., Qian, Y., Yan, W., et al. (2013) Pirh2 E3 ubiquitin ligase modulates keratinocyte differentiation through p63. J. Invest. Dermatol. 133, 1178–1187.
[48] Robinson, R.A., Lu, X., Jones, E.Y. and Siebold, C. (2008) Biochemical and structural studies of ASPP proteins reveal differential binding to p53, p63, and p73. Structure 16, 259–268.
[49] Bergamaschi, D., Samuels, Y., Sullivan, A., et al. (2006) IASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. Nat. Genet. 38, 1133–1141.
[50] Bergamaschi, D., Samuels, Y., Jin, B., et al. (2004) ASPP1 and ASPP2: common activators of p53 family members. Mol. Cell. Biol. 24, 1341–1350.
[51] Rinne, T., Hamel, B., van Bokhoven, H., et al. (2006) Pattern of p63 mutations and their phenotypes-update. Am. J. Med. Genet. A 140, 1396–1406.
[52] Guerrini, L., Costanzo, A. and Merlo, G.R. (2011) A symphony of regulations centered on p63 to control development of ectoderm-derived structures. J. Biomed. Biotechnol. 2011, 864904.
[53] van Bokhoven, H. and Brunner, H.G. (2002) Splitting p63. Am. J. Hum. Genet. 71, 1–13.
[54] McGrath, J.A., Duijf, P.H., Doetsch, V., et al. (2001) Hay-Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63. Hum. Mol. Genet. 10, 221–229.
[55] Senoo, M., Pinto, F., Crum, C.P., et al. (2007) P63 Is essential for the proliferative potential of stem cells in stratified epithelia. Cell 129, 523–536.
[56] Keyes, W.M., Wu, Y., Vogel, H., et al. (2005) P63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev. 19, 1986–1999.
[57] Su, X., Paris, M., Gi, Y.J., et al. (2009) TAp63 prevents premature aging by promoting adult stem cell maintenance. Cell Stem Cell 5, 64–75.
[58] Medawar, A., Virolle, T., Rostagno, P., et al. (2008) DeltaNp63 is essential for epidermal commitment of embryonic stem cells. PLoS One 3, e3441.
[59] Laurikkala, J., Mikkola, M.L., James, M., et al. (2006) P63 regulates multiple signalling pathways required for ectodermal organogenesis and differentiation. Development 133, 1553–1563.
[60] Suh, E.K., Yang, A., Kettenbach, A., et al. (2006) P63 protects the female germ line during meiotic arrest. Nature 444, 624–628.
[61] Koster, M.I., Kim, S., Mills, A.A., et al. (2004) P63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev. 18, 126–131.
[62] Koster, M.I., Dai, D., Marinari, B., et al. (2007) p63 induces key target genes required for epidermal morphogenesis. Proc. Natl. Acad. Sci. USA 104, 3255–3260.
[63] King, K.E., Ponnamperuma, R.M., Yamashita, T., et al. (2003) DNp63alpha functions as both a positive and a negative transcriptional regulator and blocks in vitro differentiation of murine keratinocytes. Oncogene 22, 3635–3644.
[64] Nguyen, B.C., Lefort, K., Mandinova, A., et al. (2006) Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. Genes Dev. 20, 1028–1042.
[65] Moretti, F., Marinari, B., Lo Iacono, N., et al. (2010) A regulatory feedback loop involving p63 and IRF6 links the pathogenesis of 2 genetically different human ectodermal dysplasias. J. Clin. Invest. 120, 1570–1577.
[66] Di Costanzo, A., Festa, L., Duverger, O., et al. (2009) Homeodomain protein Dlx3 induces phosphorylation-dependent p63 degradation. Cell Cycle 8, 1185–1195.
[67] Moretti, F. and Costanzo, A. (2009) A feedback regulatory loop between p63 and Dlx3: implications for epidermal differentiation. Cell Cycle 8, 1113.
[68] Yi, R., Poy, M.N., Stoffel, M. and Fuchs, E. (2008) A skin microRNA promotes differentiation by repressing 'stemness'. Nature 452, 225–229.
[69] Hearnes, J.M., Mays, D.J., Schavolt, K.L., et al. (2005) Chromatin immunoprecipitation-based screen to identify functional genomic binding sites for sequence-specific transactivators. Mol. Cell. Biol. 25, 10148–10158.
[70] Papoutsaki, M., Moretti, F., Lanza, M., et al. (2005) A p38-dependent pathway regulates DeltaNp63 DNA binding to p53-dependent promoters in UV-induced apoptosis of keratinocytes. Oncogene 24, 6970–6975.
[71] Ratovitski, E.A. (2013) Phospho-ΔNp63α regulates AQP3, ALOX12B, CASP14 and CLDN1 expression through transcription and microRNA modulation. FEBS Lett. 587, 3581–3586.
[72] Marinari, B., Ballaro, C., Koster, M.I., et al. (2009) IKKalpha is a p63 transcriptional target involved in the pathogenesis of ectodermal dysplasias. J. Invest. Dermatol. 129, 60–69.
[73] Beaudry, V.G., Pathak, N., Koster, M.I. and Attardi, L.D. (2009) Differential PERP regulation by TP63 mutants provides insight into AEC pathogenesis. Am. J. Med. Genet. A 149A, 1952–1957.
[74] Sen, T., Chang, X., Sidransky, D., et al. (2010) Regulation of ΔNp63α by NF-κB. Cell Cycle 9, 24.
[75] Sil, A.K., Maeda, S., Sano, Y., Roop, D.R., et al. (2004) IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. Nature 428, 660–664.
[76] Descargues, P., Sil, A.K., Sano, Y., et al. (2008) IKKalpha is a critical coregulator of a Smad4-independent TGFbeta-Smad2/3 signaling pathway that controls keratinocyte differentiation. Proc. Natl. Acad. Sci. USA 105, 2487–2492.
[77] Marinari, B., Moretti, F., Botti, E., et al. (2008) The tumor suppressor activity of IKKalpha in stratified epithelia is exerted in part via the TGF-beta antiproliferative pathway. Proc. Natl. Acad. Sci. USA 105, 17091–17096.
[78] Thomason, H.A., Zhou, H., Kouwenhoven, E.N., et al. (2010) Cooperation between the transcription factors p63 and IRF6 is essential to prevent cleft palate in mice. J. Clin. Invest. 120, 1561–1569.
[79] Thomason, H.A., Dixon, M.J. and Dixon, J. (2008) Facial clefting in Tp63 deficient mice results from altered Bmp4, Fgf8 and Shh signaling. Dev. Biol. 321, 273–282.
[80] Bagchi, A. and Mills, A.A. (2008) The quest for the 1p36 tumor suppressor. Cancer Res. 68, 2551–2556.
[81] Irwin, M.S. and Kaelin Jr., W.G. (2001) Role of the newer p53 family proteins in malignancy. Apoptosis 6, 17–29.
[82] Melino, G., De Laurenzi, V. and Vousden, K.H. (2002) p73: friend or foe in tumorigenesis. Nat. Rev. Cancer 2, 605–615.
[83] Fontemaggi, G., Kela, I., Amariglio, N., et al. (2002) Identification of direct p73 target genes combining DNA microarray and chromatin immunoprecipitation analyses. J. Biol. Chem. 277, 43359–43368.

[84] Petrenko, O., Zaika, A. and Moll, U.M. (2003) DeltaNp73 facilitates cell immortalization and cooperates with oncogenic Ras in cellular transformation in vivo. Mol. Cell. Biol. 23, 5540–5555.

[85] Corn, P.G., Kuerbitz, S.J., van Noesel, M.M., et al. (1999) Transcriptional silencing of the p73 gene in acute lymphoblastic leukemia and Burkitt's lymphoma is associated with 5′ CpG island methylation. Cancer Res. 59, 3352–3356.

[86] Kawano, S., Miller, C.W., Gombart, A.F., et al. (1999) Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. Blood 94, 1113–1120.

[87] Keyes, W.M., Vogel, H., Koster, M.I., et al. (2006) P63 heterozygous mutant mice are not prone to spontaneous or chemically induced tumors. Proc. Natl. Acad. Sci. USA 103, 8435–8440.

[88] Romano, R.A., Smalley, K., Magraw, C., et al. (2012) ΔNp63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation. Development 4, 772–782.

[89] Park, E., Kim, H., Kim, J.M., Primack, B., Vidal-Cardenas, S., Xu, Y., Price, B.D., Mills, A.A. and D'Andrea, A.D. (2013) FANCD2 activates transcription of TAp63 and suppresses tumorigenesis. Mol. Cell 50, 908–918.

[90] Tomasini, R., Secq, V., Pouyet, L., et al. (2013) TAp73 is required for macrophage-mediated innate immunity and the resolution of inflammatory responses. Cell Death Differ. 20, 293–301.

[91] Holembowski, L., Kramer, D., Riedel, D., et al. (2014) TAp73 is essential for germ cell adhesion and maturation in testis. J. Cell Biol. 204, 1173–1190.

[92] Inoue, S., Tomasini, R., Rufini, A., et al. (2014) TAp73 is required for spermatogenesis and the maintenance of male fertility. Proc. Natl. Acad. Sci. USA 111, 1843–1848.

[93] Tomasini, R., Tsuchihara, K., Tsuda, C., et al. (2009) TAp73 regulates the spindle assembly checkpoint by modulating BubR1 activity. Proc. Natl. Acad. Sci. USA 106, 797–802.

[94] Fulco, M., Costanzo, A., Merlo, P., et al. (2003) P73 is regulated by phosphorylation at the G2/M transition. J. Biol. Chem. 278, 49196–49202.

[95] Merlo, P., Fulco, M., Costanzo, A., et al. (2005) A role of p73 in mitotic exit. J. Biol. Chem. 80, 30354–30360.

[96] Talos, F., Nemajerova, A., Flores, E.R., et al. (2007) P73 suppresses polyploidy and aneuploidy in the absence of functional p53. Mol. Cell 27, 647–659.

[97] Griesmann, H., Schlereth, K., Krause, M., et al. (2009) P53 and p73 in suppression of Myc-driven lymphomagenesis. Int. J. Cancer 124, 502–506.

[98] Nemajerova, A., Petrenko, O., Trumper, L., et al. (2010) Loss of p73 promotes dissemination of Myc-induced B cell lymphomas in mice. J. Clin. Invest. 120, 2070–2080.

[99] Kenzelmann Broz, D., Spano Mello, S., et al. (2013) Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. Genes Dev. 27, 1016–1031.

[100] Flores, E.R., Tsai, K.Y., Crowley, D., et al. (2002) P63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 416, 560–564.

[101] Maddocks, O.D. and Vousden, K.H. (2011) Metabolic regulation by p53. J. Mol. Med. 89, 237–245.

[102] Su, X., Gi, Y.J., Chakravarti, D., et al. (2012) TAp63 is a master transcriptional regulator of lipid and glucose metabolism. Cell Metab. 16, 511–525.

[103] He, Z., Liu, H., Agostini, M., et al. (2013) p73 regulates autophagy and hepatocellular lipid metabolism through a transcriptional activation of the ATG5 gene. Cell Death Differ. 20, 1415–1424.

[104] Cantor, J.R. and Sabatini, D.M. (2012) Cancer cell metabolism: one hallmark, many faces. Cancer Discov. 10, 881–898.

[105] Du, W., Jiang, P., Mancuso, A., et al. (2013) TAp73 enhances the pentose phosphate pathway and supports cell proliferation. Nat. Cell Biol. 15, 991–1000.

[106] Rufini, A., Niklison-Chirou, M.V., Inoue, S., et al. (2012) TAp73 depletion accelerates aging through metabolic dysregulation. Genes Dev. 26, 2009–2014.

[107] Jiang, P., Du, W., Wang, X., et al. (2011) p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. Nat. Cell Biol. 13, 310–316.

[108] Bensaad, K., Tsuruta, A., Selak, M.A., et al. (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126 (1), 107–120.

[109] Cheung, E.C., Athineos, D., Lee, P., et al. (2013) TIGAR is required for efficient intestinal regeneration and tumorigenesis. Dev. Cell 25, 463–477.

[110] Velletri, T., Romeo, F., Tucci, P., et al. (2013) GLS2 is transcriptionally regulated by p73 and contributes to neuronal differentiation. Cell Cycle 12, 3564–3573.

[111] Amelio, I., Markert, E.K., Rufini, A., et al. (2013) p73 regulates serine biosynthesis in cancer. Oncogene, http://dx.doi.org/10.1038/onc.2013.456 (Nov 4, Epub ahead of print).

[112] Giacobbe, A., Bongiorno-Borbone, L., Bernassola, F., et al. (2013) p63 regulates glutaminase 2 expression. Cell Cycle 12, 1395–1405.

[113] Suzuki, S., Tanaka, T., Poyurovsky, M.V., et al. (2010) Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc. Natl. Acad. Sci. USA 107, 7461–7466.

[114] Hu, W., Zhang, C., Wu, R., et al. (2010) Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. Proc. Natl. Acad. Sci. USA 107, 7455–7460.

[115] Maddocks, O.D. and Vousden, K.H. (2011) Metabolic regulation by p53. J. Mol. Med. 2011 (89), 237–245.

[116] Son, J., Lyssiotis, C.A., Ying, H., et al. (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 496, 101–105.

[117] Jiang, P., Du, W., Mancuso, A., Wellen, K.E. and Yang, X. (2013) Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature 493, 689–693.

[118] Jiang, P., Du, W. and Yang, X. (2013) A critical role of glucose-6-phosphate dehydrogenase in TAp73-mediated cell proliferation. Cell Cycle 12, 3720–3726.

[119] Lefkimmiatis, K., Caratozzolo, M.F., Merlo, P., et al. (2009) p73 and p63 sustain cellular growth by transcriptional activation of cell cycle progression genes. Cancer Res. 69, 8563–8571.

[120] Sbisà, E., Mastropasqua, G., Lefkimmiatis, K., et al. (2006) Connecting p63 to cellular proliferation: the example of the adenosine deaminase target gene. Cell Cycle 2, 205–212.

[121] Lau, C.P., Ng, P.K., Li, M.S., et al. (2013) p63 regulates cell proliferation and cell cycle progression-associated genes in stromal cells of giant cell tumor of the bone. Int. J. Oncol. 42, 437–443.

[122] Irwin, M.S., Kondo, K., Marin Jr., M., et al. (2003) Chemosensitivity linked to p73 function. Cancer Cell 3, 403–410.

[123] Pediconi, N., Ianari, A., Costanzo, A., et al. (2003) Differential regulation of E2F1 apoptotic target genes in response to DNA damage. Nat. Cell Biol. 5, 552–558.

[124] Ozaki, T., Okoshi, R., Sang, M., et al. (2009) Acetylation status of E2F-1 has an important role in the regulation of E2F-1-mediated transactivation of tumor suppressor p73. Biochem. Biophys. Res. Commun. 386, 207–211.

[125] Urist, M., Tanaka, T., Poyurovsky, M.V., et al. (2004) P73 induction after DNA damage is regulated by checkpoint kinases Chk1 and Chk2. Genes Dev. 18, 3041–3054.

[126] Pediconi, N., Guerrieri, F., Vossio, S., et al. (2009) HSirT1-dependent regulation of the PCAF-E2F1-p73 apoptotic pathway in response to DNA damage. Mol. Cell. Biol. 29, 1989–1998.

[127] Kontaki, H. and Talianidis, I. (2010) Lysine methylation regulates E2F1-induced cell death. Mol. Cell 39, 152–160.

[128] Agami, R., Blandino, G., Oren, M., et al. (1999) Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 399, 809–813.

[129] Gong, J.G., Costanzo, A., Yang, H.Q., et al. (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 399, 806–809.

[130] Yuan, Z.M., Shioya, H., Ishiko, T., et al. (1999) p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399, 814–817.

[131] Gonzalez, S., Prives, C. and Cordon-Cardo, C. (2003) p73alpha regulation by Chk1 in response to DNA damage. Mol. Cell. Biol. 23, 8161–8171.

[132] Ren, J., Datta, R., Shioya, H., et al. (2002) p73beta is regulated by protein kinase Cdelta catalytic fragment generated in the apoptotic response to DNA damage. J. Biol. Chem. 277, 33758–33765.

[133] Jones, E.V., Dickman, M.J. and Whitmarsh, A.J. (2007) Regulation of p73-mediated apoptosis by c-Jun N-terminal kinase. Biochem. J. 05, 617–623.

[134] Sanchez-Prieto, R., Sanchez-Arevalo, V.J., Servitja, J.M., et al. (2002) Regulation of p73 by c-Abl through the p38 MAP kinase pathway. Oncogene 21, 974–979.

[135] Baskaran, R., Wood, L.D., Whitaker, L.L., et al. (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387, 516–519.

[136] Yoshida, K., Ozaki, T., Furuya, K., et al. (2008) ATM-dependent nuclear accumulation of IKK-alpha plays an important role in the regulation of p73-mediated apoptosis in response to cisplatin. Oncogene 27, 1183–1188.

[137] Furuya, K., Ozaki, T., Hanamoto, T., et al. (2007) Stabilization of p73 by nuclear IkappaB kinase-alpha mediates cisplatin-induced apoptosis. J. Biol. Chem. 282, 18365–18378.

[138] Strano, S., Monti, O., Pediconi, N., et al. (2005) The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA Damage. Mol. Cell 18, 447–459.

[139] Strano, S., Munarriz, E., Rossi, M., et al. (2001) Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J. Biol. Chem. 276, 15164–15173.

[140] Basu, S., Totty, N.F., Irwin, M.S., et al. (2003) Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol. Cell 11, 11–23.

[141] Costanzo, A., Merlo, P., Pediconi, N., et al. (2002) DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. Mol. Cell 9, 175–186.

[142] Mantovani, F., Piazza, S., Gostissa, M., et al. (2004) Pin1 links the activities of c-Abl and p300 in regulating p73 function. Mol. Cell 14, 625–636.

[143] Rocco, J.W., Leong, C.O., Kuperwasser, N., et al. (2006) p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell 9, 45–56.

[144] Leong, C.O., Vidnovic, N., DeYoung, M.P., Sgroi, D. and Ellisen, L.W. (2007) The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. J. Clin. Invest. 117, 1370–1380.

[145] Marin, M.C., Jost, C.A., Brooks, L.A., et al. (2000) A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat. Genet. 25, 47–54.

[146] Bergamaschi, D., Gasco, M., Hiller, L., et al. (2003) p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 3, 387–402.

[147] Dumont, P., Leu, J.I., Della Pietra, A.C., et al. (2003) The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat. Genet. 33, 357–365.

[148] Tada, M., Furuuchi, K., Kaneda, M., et al. (2001) Inactivate the remaining p53 allele or the alternate p73? Preferential selection of the Arg72 polymorphism in cancers with recessive p53 mutants but not transdominant mutants. Carcinogenesis 22, 515–517.
[149] Ishimoto, O., Kawahara, C., Enjo, K., et al. (2002) Possible oncogenic potential of DeltaNp73: a newly identified isoform of human p73. Cancer Res. 62, 636–641.
[150] Stiewe, T., Zimmermann, S., Frilling, A., et al. (2002) Transactivation-deficient DeltaTA-p73 acts as an oncogene. Cancer Res. 62, 3598–3602.
[151] Tannapfel, A., John, K., Mise, N., et al. (2008) Autonomous growth and hepatocarcinogenesis in transgenic mice expressing the p53 family inhibitor DNp73. Carcinogenesis 29, 211–218.
[152] Zaika, A.I., Slade, N., Erster, S.H., et al. (2002) DeltaNp73, a dominant-negative inhibitor of wild-type p53 and TAp73, is up-regulated in human tumors. J. Exp. Med. 196, 765–780.
[153] Stiewe, T., Stanelle, J., Theseling, C.C., et al. (2003) Inactivation of retinoblastoma (RB) tumor suppressor by oncogenic isoforms of the p53 family member p73. J. Biol. Chem. 278, 14230–14236.
[154] Cam, H., Griesmann, H., Beitzinger, M., et al. (2006) p53 family members in myogenic differentiation and rhabdomyosarcoma development. Cancer Cell 10, 281–293.
[155] Toh, W.H., Logette, E., Corcos, L., et al. (2008) TAp73beta and DNp73beta activate the expression of the pro-survival caspase-2S. Nucleic Acids Res. 36, 4498–4509.
[156] Lanza, M., Marinari, B., Papoutsaki, M., et al. (2006) Cross-talks in the p53 family: deltaNp63 is an anti-apoptotic target for deltaNp73alpha and p53 gain-of-function mutants. Cell Cycle 5, 1996–2004.
[157] Kartasheva, N.N., Lenz-Bauer, C., Hartmann, O., et al. (2003) DeltaNp73 can modulate the expression of various genes in a p53-independent fashion. Oncogene 22, 8246–8254.
[158] Becker, K., Pancoska, P., Concin, N., et al. (2006) Patterns of p73 N-terminal isoform expression and p53 status have prognostic value in gynecological cancers. Int. J. Oncol. 29, 889–902.
[159] Concin, N., Becker, K., Slade, N., et al. (2004) Transdominant DeltaTAp73 isoforms are frequently up-regulated in ovarian cancer Evidence for their role as epigenetic p53 inhibitors in vivo. Cancer Res. 64, 2449–2460.
[160] Guan, M. and Chen, Y. (2005) Aberrant expression of DeltaNp73 in benign and malignant tumours of the prostate: correlation with Gleason score. J. Clin. Pathol. 58, 1175–1179.
[161] Tuve, S., Wagner, S.N., Schittek, B., et al. (2004) Alterations of DeltaTA-p 73 splice transcripts during melanoma development and progression. Int. J. Cancer 108, 162–166.
[162] Casciano, I., Mazzocco, K., Boni, L., et al. (2002) Expression of DeltaNp73 is a molecular marker for adverse outcome in neuroblastoma patients. Cell Death Differ. 9, 246–251.
[163] Stiewe, T., Tuve, S., Peter, M., et al. (2004) Quantitative TP73 transcript analysis in hepatocellular carcinomas. Clin. Cancer Res. 10, 626–633.
[164] Muller, M., Schilling, T., Sayan, A.E., et al. (2005) TAp73/Delta Np73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. Cell Death Differ. 12, 1564–1577.
[165] Buhlmann, S. and Putzer, B.M. (2008) DNp73 a matter of cancer: mechanisms and clinical implications. Biochim. Biophys. Acta 1785, 207–216.
[166] Beitzinger, M., Hofmann, L., Oswald, C., et al. (2008) P73 poses a barrier to malignant transformation by limiting anchorage-independent growth. EMBO J. 27, 792–803.
[167] Steder, M., Alla, V., Meier, C., et al. (2013) DNp73 exerts function in metastasis initiation by disconnecting the inhibitory role of EPLIN on IGF1R-AKT/STAT3 signaling. Cancer Cell 24, 512–527.
[168] Westfall, M.D. and Pietenpol, J.A. (2004) p63: molecular complexity in development and cancer. Carcinogenesis 25, 857–864.
[169] Wu, G., Osada, M., Guo, Z.F., et al. (2005) DNp63a up-regulates the Hsp70 gene in human cancer. Cancer Res. 65, 758–766.
[170] Patel, S., George, R., Autore, F., et al. (2008) Molecular interactions of ASPP1 and ASPP2 with the p53 protein family and the apoptotic promoters PUMA and Bax. Nucleic Acids Res. 36, 5139–5151.
[171] Lazzari, C., Prodosmo, A., Siepi, F., et al. (2011) HIPK2 phosphorylates ΔNp63α and promotes its degradation in response to DNA damage. Oncogene 30, 4802–4813.
[172] Botti, E., Spallone, G., Moretti, F., et al. (2011) Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas. Proc. Natl. Acad. Sci. 108, 13710–13715.
[173] Marinari, B., Moretti, F., Botti, E., et al. (2008) The tumor suppressor activity of IKKalpha in stratified epithelia is exerted in part via the TGF-beta antiproliferative pathway. Proc. Natl. Acad. Sci. USA 105, 17091–17096.
[174] Ramsey, M.R., Wilson, C., Ory, B., et al. (2013) FGFR2 signaling underlies p63 oncogenic function in squamous cell carcinoma. J. Clin. Invest. 123, 3525–3538.
[175] Descargues, P., Sil, A.K. and Karin, M. (2008) IKKalpha, a critical regulator of epidermal differentiation and a suppressor of skin cancer. EMBO J. 27, 2639–2647.
[176] Liu, B., Park, E., Zhu, F., et al. (2006) A critical role for I kappaB kinase alpha in the development of human and mouse squamous cell carcinomas. Proc. Natl. Acad. Sci. USA 103, 17202–17207.
[177] Hu, Y., Baud, V., Oga, T., et al. (2001) IKKalpha controls formation of the epidermis independently of NF-kappaB. Nature 410, 710–714.
[178] Restivo, G., Nguyen, B.C., Dziunycz, P., et al. (2011) IRF6 is a mediator of Notch pro-differentiation and tumour suppressive function in keratinocytes. EMBO J. 30, 4571–4585.
[179] Wilhelm, M.T., Rufini, A., Wetzel, M.K., et al. (2010) Isoform-specific **p73** knockout mice reveal a novel role for delta Np73 in the DNA damage response pathway. Genes Dev. 24, 549–560.