

THE ROLE OF P63 IN CANCER, STEM CELLS AND CANCER STEM CELLS

MARTA NEKULOVA¹, JITKA HOLCAKOVA¹, PHILIP COATES²
 and BORIVOJ VOJTESEK^{1*}

¹Masaryk Memorial Cancer Institute, Zluty kopec 7, 65653 Brno, Czech Republic, ²Division of Medical Sciences, University of Dundee, DD1 9SY, UK

Abstract: The transcription factor p63 has important functions in tumorigenesis, epidermal differentiation and stem cell self-renewal. The *TP63* gene encodes multiple protein isoforms that have different or even antagonistic roles in these processes. The balance of p63 isoforms, together with the presence or absence of the other p53 family members, p73 and p53, has a striking biological impact. There is increasing evidence that interactions between p53-family members, whether cooperative or antagonistic, are involved in various cell processes. This review summarizes the current understanding of the role of p63 in tumorigenesis, metastasis, cell migration and senescence. In particular, recent data indicate important roles in adult stem cell and cancer stem cell regulation and in the response of cancer cells to therapy.

Key words: p63, TAp63, ΔNp63, p53 family, Cancer, Stem cells

* Author for correspondence. e-mail: vojtesek@mou.cz

Abbreviations used: APAF-1 – apoptotic protease activating factor 1; ATM – ataxia telangiectasia mutated; BAD – BCL-2 associated agonist of cell death; BAX – BCL2-associated X protein; BRCA2 – breast cancer 2, early onset; c-Abl – Abelson murine leukemia oncogene; Cables1 – CDK and ABL1 enzyme substrate 1; CASP10 – caspase 10; CDHs – cadherins; CLDN1 – claudin 1; CSCs – cancer stem cells; DBD – DNA-binding domain; EGR3 – early growth response 3; FRAS1 – Fraser syndrome 1; HNSCC – squamous cell carcinoma of the head and neck; HOXC4 – homeobox C4; ICAM – inter-cellular adhesion molecule; IER3 – immediate early response 3; IHH – Indian hedgehog; IKK α – inhibitor of nuclear factor kappa-B kinase subunit alpha; JAG1 – jagged 1; MDM2 – murine double minute 2; MEFs – mouse embryonic fibroblasts; MRE11 – meiotic recombination 11 homolog; mTOR – mammalian target of rapamycin; Notch1 – Notch homolog 1, translocation-associated; OD – oligomerization domain; PCNA – proliferating cell nuclear antigen; PERP – p53 apoptosis effector related to PMP-22; RBP – RNA-binding protein; REs – responsive elements; SAM – sterile alpha motif; SHH – sonic hedgehog; STAT3 – signal transducer and activator of transcription 3; SUMO1 – small ubiquitin-related modifier 1; TA – transactivation domain; TRAIL – TNF-related apoptosis-inducing ligand; Tprg – tumor protein p63 regulated; Wnt – wingless-type MMTV integration site family member; YAP – Yes-associated protein; ZEB1 – zinc finger E-box binding homeobox 1

STRUCTURE OF THE *TP63* GENE AND P63 PROTEIN

p63 as a family member

p63 is a member of the p53 family of transcription factors, which have important functions in tumorigenesis and ontogenesis. p53 was for a long time thought to be unique (in structure and function) until the discovery of two new members of this protein family: p73 in 1997 [1] and p63 in 1998 [2]. Phylogenetic analysis indicated that p63 and p73 are evolutionary older than p53, and it was suggested that p63 may be the evolutionary predecessor of both p53 and p73 [2, 3]. Non-vertebrate animals have only one gene of the p53 family, and it has a high degree of similarity to vertebrate p63 [4]. Therefore, p63 and p73 as evolutionarily older family members play different roles in ontogenesis and tumorigenesis, while the later-developed p53 is specifically responsible for tumor suppression.

p53, p63 and p73 share a common protein structure, which arises from their function as transcription factors. They consist of a central DNA-binding domain (DBD), a transactivation (TA) domain, and an oligomerization domain (OD). All three also act as tetramers, and partial homology in the OD results in their potential ability to form heterotetramers. The central DBD domain of p63 is highly homologous with the DBDs of p53 and especially p73, suggesting that p63 is able to bind to the p53 and p73 target genes [2]. However, each homolog also has a specific set of target genes [5-10].

***TP63* gene and p63 protein isoforms**

The human *TP63* gene is localized on chromosome 3, and it consists of 15 exons and contains two promoters (Fig. 1) [2]. Transcription from the first promoter, located upstream of exon 1, gives rise to the full-length protein TAp63. Transcription from the second promoter results in the production of N-terminally truncated protein isoforms, Δ Np63. Due to alternative splicing of the 3' end of *TP63* mRNA, there are also several C-terminal protein isoforms, like α , β , γ and the recently described δ and ϵ . This further contributes to the diversity of the p63 protein products (Fig. 1) [2, 11]. In addition, Δ Np63 α , which seems to be the predominant isoform, contains a sterile alpha motif (SAM) domain [12], known to be involved in protein-protein interactions, and a C-terminal inhibitory domain [13]. This inhibitory domain reduces the activity of p63 α via an intramolecular mechanism, but it is cleaved after the induction of apoptosis by activated caspases [14].

Δ Np63 does not contain the N-terminal TA domain compared to the TAp63 isoform. This short isoform is a dominant negative inhibitor of TAp63 and other transcriptionally active members of the p53 family [2], partly due to its competition for binding to promoters of the target genes (Δ Np63 can bind to the promoter but cannot induce transcription of the target gene) and partly due to the formation of non-functional heterooligomers with TAp63, TAp73 and p53. However, it was recently demonstrated that Δ Np63 also has its own transactivation activity due to the presence of alternative TA domains [15, 16].

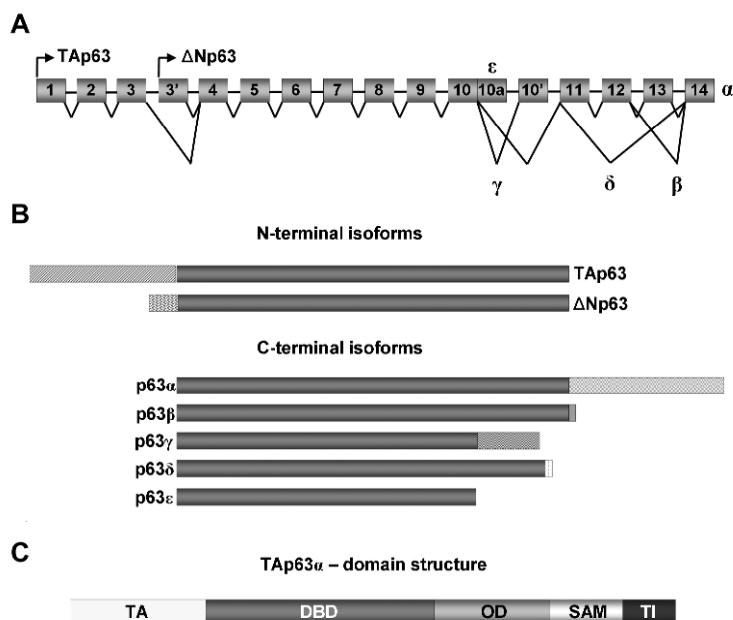


Fig. 1. The structure of the *TP63* gene and p63 protein isoforms. A – The *TP63* gene has two promoters and the mRNA also undergoes alternative splicing on its 3' end, which leads to the broad spectrum of p63 protein isoforms. B – Due to transcription from two promoters of the *TP63* gene, there are two N-terminal isoforms of p63: the full-length TAp63, and N-terminally truncated ΔNp63. The alternative splicing on the 3' end of *TP63* mRNA gives rise to C-terminal protein isoforms of p63, α , β , γ , δ , and ϵ . C – p63 protein consists of the central DNA-binding domain (DBD), oligomerization domain (OD), N-terminal transactivation (TA) domain (only TAp63; ΔNp63 has alternative transactivation domains); the p63 α isoform also contains a sterile alpha motif (SAM) and an inhibitory domain (TI) on its C-terminus.

The expression pattern of p63 in tissues and tumors, p63 protein stability, and target genes

p63 isoforms are characterized by different levels and patterns of expression. ΔNp63 α is the predominant isoform, and is selectively expressed at high levels in the basal cells of stratified and glandular epithelia (Fig. 2). Its expression decreases with cellular differentiation [2, 17, 18].

Yang *et al.* [2] showed strong nuclear staining in the basal cells of the epithelium in paraffin sections of human tissues including the foreskin, cervix, vaginal epithelium, urothelium, and prostate. Di Como *et al.* [19] reported p63 expression restricted to the epithelial cells of stratified epithelia, such as skin, esophagus, ectocervix, tonsil, and bladder, and to certain subpopulations of basal cells in glandular structures of the prostate and breast, as well as in the bronchi. Consistent with the phenotype observed in normal tissues, they found that p63 is expressed predominantly in basal cell and squamous cell carcinomas, and in

transitional cell carcinomas, but not in adenocarcinomas, including those of the breast and prostate. Interestingly, thymomas expressed high levels of p63. Moreover, a subset of non-Hodgkin's lymphoma was also found to express p63.

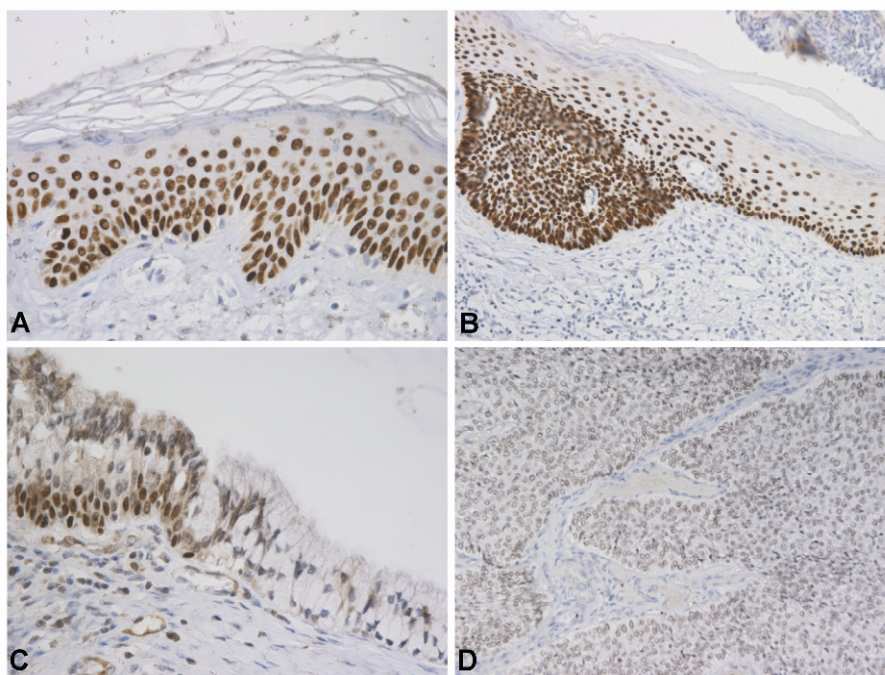


Fig. 2. Immunohistochemical staining of p63 in various tissue samples. A – p63 in normal stratified epithelium of the skin. B – Skin with basal cell carcinoma. C – Endocervical epithelium D – Urothelial carcinoma.

Both of these groups used the same monoclonal antibody, 4A4, which recognizes all known isoforms of p63. To distinguish the N-terminal isoforms of p63, Δ Np63 and TAp63, RT-PCR analyses were performed. In murine tissues, Yang *et al.* [2] revealed the presence of transcripts encoding Δ Np63 isoforms in the kidney, adrenal gland, spleen and thymus, but not in the heart, liver, testes, or brain. The TAp63 transcript was detected in heart, testes, kidney, adrenal gland, thymus, brain and cerebellum. Using isoform-specific RT-PCR, Di Como *et al.* [19] found that thymomas express all isoforms of p63, whereas the non-Hodgkin's lymphoma tended to express the TAp63 isoforms only. They did not detect p63 mRNA/protein in a variety of endocrine tumors, germ cell neoplasms, melanomas or soft tissue sarcomas.

However, only the Δ Np63 N-terminal protein isoform is usually detected in normal and tumor tissues using immunohistochemistry and western blot analysis. Thus, the question arises if this is the real state, or if we are not able to detect the full-length isoform, TAp63.

The differences in the expression pattern of p63 isoforms are linked to the antibodies used for their detection. 4A4, an anti-p63 monoclonal antibody that is the most widely used for immunohistochemical analysis and western blots, identifies both Δ Np63 and TAp63 and also recognizes p73 [20]. Nylander *et al.* [17] suggested that 4A4 does not identify TAp63 by immunohistochemistry, although it recognizes all p63 isoforms in Western blot, as demonstrated by Yang *et al.* [2]. The reason for such different results could be a masked epitope unrecognized in TAp63 when an IHC assay is used. Nylander *et al.* [17] developed a set of polyclonal antibodies directed to each of the two N-terminal isoforms of p63. They showed that TAp63 protein is located suprabasally in stratified epithelia compared to Δ Np63 isoforms, which are more abundantly expressed in the basal cell layer (indicating a switch in expression of p63 isoforms during cellular differentiation). Δ Np63 α was the most widely expressed isoform in squamous cell carcinomas, and it was also restricted to basal cells in the breast and prostate, whereas TAp63 isoforms were more widely expressed in these tissues and in tumors at these sites. TAp63, but not Δ Np63 or p63 α , was detected in the normal colon and in colon carcinoma. In agreement with the data from an mRNA analysis of lymphoid tissue [19], TAp63 isoforms were expressed in the nuclei of a sub-population of lymphoid cells and in some malignant lymphomas, whereas Δ Np63 proteins were not expressed. T-cell regions were essentially negative, suggesting that p63 expression is restricted to B-lymphocytes as confirmed by Hedvat *et al.* [21] in diffuse large cell lymphoma. TAp63 expression was later detected in the ovary and in oocytes in meiotic arrest [22, 23].

In summary, and without distinguishing between N-terminal isoforms, p63 was detected in cervical cancer [24, 25], uterine cancer [26] and pancreatic cancer [27] within the tumor tissue. The N-terminally truncated Δ Np63 isoform is expressed in basal-type breast cancer [28], prostate cancer [29, 30], esophageal cancer [31] and pancreatic neoplasia [32]. The full-length TAp63 only is expressed in some non-Hodgkin's B-cell lymphomas [19]. Both isoforms, Δ Np63 and TAp63, were detected in bladder cancer [33-35], urothelial cancer [36], head and neck cancer [37-39], gastric carcinoma [40], lung cancer [41, 42] and thymomas [19]. Δ Np63 was the predominant isoform.

A possible explanation for the discrepancies between immunohistochemical and RT-PCR results is the protein stability of p63 N-terminal isoforms. TAp63 has a much shorter half-life than Δ Np63 [43] due to the role of the TA domain in the regulation of protein stability in a proteasome-dependent manner [44].

The stability of p63 seems also to be regulated by its C-terminus. The C-terminal inhibitory domain of p63 α , identified by Serber *et al.* [13], binds to the TA domain of TAp63 and consequently masks the sites in the TA domain used by the degradation pathway [44]. The intramolecular folding increases the half-life of the proteins and keeps them in an inactive form. This interaction could be intramolecular, with the inhibitory domain of TAp63 α binding to the TA domain

of the same molecule, or intermolecular, with inhibitory domain of Δ Np63 α binding to the TA domain of TAp63 in heterotetramers.

Additionally, protein stability could be regulated by post-translational modifications. For example, post-translational modification of p63 by the ubiquitin-like molecule SUMO-1 regulates its stability. p63 α (but not p63 β and γ) is sumoylated *in vitro* and *in vivo*, and this modification targets Δ Np63 α for proteasome-mediated degradation [45]. Moreover, phosphorylation and acetylation also seem to be implicated in the regulation of p63 protein stability [46, 47]. In squamous cell carcinoma of the head and neck (HNSCC), after cisplatin treatment, Δ Np63 is phosphorylated, exported from the nucleus into the cytoplasm, and targeted by RACK1 for proteasome degradation [48].

Protein-protein interactions are also critical for the regulation of p63 stability, and are greatly influenced by external stimuli such as DNA damaging agents. Δ Np63 α physically interacts with I κ B kinase, and this interaction decreases its stability and half-life, leading to its ubiquitin-dependent degradation [49]. Conversely, the interaction with Cables1 (Cdk5 and Abl enzyme substrate 1) is responsible for the selective stabilization of the TAp63 isoform, which enables apoptosis of cells after genotoxic stress [50], and interaction with YAP (Yes-associated protein) stabilizes Δ Np63 α in HaCat cells [51].

Mdm2 is the principal regulator of p53 transcriptional activity, and the interaction of Mdm2 with p53 targets this protein for ubiquitin-dependent degradation. However, there are several conflicting results describing the regulation of p63 activity and protein stability by Mdm2. Kadakia *et al.* [52] showed that p63 levels are unaltered by association with Mdm2, although Mdm2 was able to inhibit its transactivation function and drive the translocation of p63 from the nucleus to the cytoplasm. At the same time, Little *et al.* [53] reported that Mdm2 is not able to interact with p63, to repress p63-induced transcription or to affect its half-life. On the contrary, Calabro *et al.* [54] showed that p63 α and p63 γ are able to associate with human Mdm2, and that Mdm2 is able to increase the steady-state level of p63 and enhance transcriptional activity under conditions in which p53 is inhibited. Most recently, Galli *et al.* [55] have shown that Mdm2 induces p63 protein degradation following DNA damage and cell differentiation. They demonstrated that Mdm2 binds Δ Np63 α in the nucleus, promoting its translocation to the cytoplasm, where it is targeted for degradation. Thus, they suggested that Mdm2 regulates the levels of the pro-survival and pro-proliferative Δ Np63 α isoforms following DNA damage and during cellular differentiation. Therefore, p63 proteins are not only regulated by their transcriptional activity, but also by different stability of the various isoforms.

p63 regulates the expression of multiple genes involved in cell adhesion and structural integrity of the skin (*ICAM*, *CDHs*, *PERP*, *FRAS1*), apoptosis (*CASP10*, *BAX*, *APAF1*, *BAD*, *IER3*), transcription (*c-Jun*, *ZEB1*, *EGR3*), cell cycle regulation (*p21^{WAF1}*, *p57*), DNA repair and replication (*RAD51*, *BRCA2*, *MRE11*, *PCNA*), and epithelial development and differentiation (*CLDN1*, *Tprg*, *IKK α* , *HOXC4*) [8, 10, 56-62]. In many but not all cases, TAp63 and Δ Np63

have opposing effects on target gene expression [63]. In addition, the C-terminal isoforms show different transcriptional activities for individual genes and unique targets [15, 64]. In conclusion, the expression pattern of p63 isoforms observed in different tissues is determined not only by the regulation of *TP63* gene transcription, but also by the different protein stability of TAp63 and Δ Np63. The analysis of p63 protein expression has been highly influenced by the sensitivity and specificity of antibodies used for p63 detection, and the precise expression patterns of each isoform are still unclear.

P63 IN TUMORIGENESIS

The homology between p63 and p53 suggests that these proteins might function similarly. However, there is a low rate of mutations of p63 in human malignancies [65-67], which does not support a tumor suppression function for the *TP63* gene. Moreover, there are N- and C-terminal isoforms of p63 with different or even antagonistic functions, implying that p63 expression could be either oncogenic or tumor-suppressive.

Mouse models

The controversy concerning the role of p63 in neoplastic transformation is related to the existence of two p63-deficient mouse models with substantially different susceptibility to cancer [68, 69]. The p63 heterozygous mice constructed by Flores *et al.* [68] were prone to spontaneous tumors; they developed squamous cell carcinomas and histiocytic sarcomas. Mice heterozygous for both p63 and p73 (p63^{+/-} and p73^{+/-}) exhibit a complex tumor phenotype. Loss of p63 may also cooperate with loss of p53 in tumor development, as mice with p63^{+/-} in combination with p53^{+/-} had a more aggressive tumor phenotype than p53^{+/-} mice. Mice carrying p63^{+/-} also exhibit signs of advanced aging.

Conversely, the p63^{+/-} mice constructed by Keyes *et al.* [69] were not tumor prone, and mice heterozygous for both p63 and p53 had fewer tumors than p53^{+/-} mice. Furthermore, the p63^{+/-} mice were not prone to chemically induced tumorigenesis.

The differences in these two models may be linked to the methods used for the generation of these transgenic animals, as they do not guarantee a complete inactivation of p63. It is possible that partially functional p63 proteins with a gain-of function, dominant negative or even hypomorphic function are expressed. The mice used by Keyes *et al.* [69] were heterozygous for p63 alleles that disrupt all p63 isoforms, so it is possible that individual p63 proteins may function in tumor suppression, or that p63 could have a tumor-suppressive role in certain cellular contexts or in cooperation with particular oncogenes.

Additionally, it was shown that loss of p63 mediates cellular senescence and aging. p63^{+/-} mice have a shortened life span and display features of accelerated aging, and p63 deficiency also activates enhanced expression of senescent markers [68, 70]. Early studies showed that upon senescence, Δ Np63 proteins

decreased in abundance, whereas TAp63 isoforms accumulated in serially passaged rat embryo fibroblasts [71]. Recently, Guo *et al.* [72] used a new TAp63-specific mouse model to show that TAp63 isoforms inhibit tumorigenesis *in vivo* and are robust mediators of senescence. Loss of TAp63 enhances sarcoma development in mice lacking p53, whereas gain of TAp63 induces senescence. Therefore, TAp63 isoforms might function as tumor suppressors by regulating senescence through p53-independent pathways. Since senescence is a barrier against tumor progression *in vivo*, activation of this program in tumor cells, especially in those that are resistant to chemotherapy-induced cell death, provides a basis for anti-cancer therapy.

Conversely, Koster *et al.* [73] suggested that TAp63 could also function as an oncogene. They established a gene-switch TAp63 α mouse model and found that deregulated expression of TAp63 α in the epidermis causes hyperproliferation and failure to undergo terminal differentiation [74]. Moreover, ectopic TAp63 α expression in lung epithelia resulted in the development of preneoplastic lesions. They also reported that TAp63 isoforms are reactivated in well-differentiated HNSCC, and that deregulated expression of TAp63 α accelerated skin carcinogenesis and tumor progression in gene-switch TAp63 α mice exposed to a chemical carcinogenesis protocol [73]. The results of various studies of different p63-deficient models have shed some light on p63 function, but more p63 isoform-specific models and a better biochemical understanding of p63 functions will undoubtedly be required to understand the role of p63 in tumorigenesis.

Tumor suppressor or oncogenic role? Crosstalk between p53 family members

Many studies support the hypothesis that p63 can function as a tumor suppressor, especially the full-length TAp63 isoforms. For example, TAp63 overexpression is responsible for the activation of p53 responsive genes, leading to cell cycle arrest and apoptosis [2]. Additionally, it was also found that TAp63 can mediate apoptosis by triggering death receptor complexes (*CD95*, *TRAIL*) and the mitochondrial death pathway (*BAX*, *APAF1*) [58].

On the other hand, Δ Np63 α inhibits death receptor-mediated apoptosis and chemotherapy-induced mitochondrial apoptosis pathways [75]. There is evidence that Δ Np63 actually promotes cancer development and thus functions as an oncogene. Squamous cell carcinomas of the lung or head and neck are characterized by amplification of the p63 locus as well as high levels of expression of the Δ Np63 α isoform [37, 67, 76-78]. Moreover, Δ Np63 α overexpression leads to enhanced cell growth in soft agar and increased tumor size in mice [67]. However, p63 isoforms must be studied in close connection with other p53 family members because of the many possible interactions between p53, p63 and p73 proteins.

Firstly, there is a possibility of heterotetramerization among p53-family members. Δ Np63 isoforms could theoretically affect the transactivation functions of TAp63, p53 and p73 via direct protein-protein interactions. p63 and p73 isoforms can form heterooligomers with each other, but not with wild-type

p53. It was also shown that the oligomerization domain (OD) of p53 does not associate with the OD of either p63 or p73 [3, 79]. On the other hand, mutant p53 can associate *in vitro* and *in vivo* with p63 through its core domain [80, 81]. This interaction impairs the sequence-specific binding of p63. Moreover, in T47D cells carrying the endogenous mutant p53, p63 is unable to recruit some of its target gene promoters [81]. Interaction between mutant p53 and p63 was also confirmed in cell lines that endogenously express both proteins [80]. The inactivation of p63 with mutant p53 might be one of the possible mechanisms of mutant p53 gain of function.

Secondly, the central domain of all p63 variants is highly homologous with the DBDs of p53 and p73 [2], so p63 can interact with p53 target DNA sites. TAp63 is able to transactivate reporter genes containing p53-responsive elements, while the Δ Np63 isoform lacking the N-terminal TA domain can bind to p53 target sites in a competitive manner, and could act as a dominant negative inhibitor of p53, TAp63 or TAp73. Δ Np63 showed a dose-dependent inhibition of p53 and TAp63 transactivation [2], but kept its own transactivation activity.

Thirdly, there is a transcriptional regulation among p53 family members. p63 associates with the promoter of p53, p73 and the p63 gene itself [82]. Thus, p63 may regulate its own expression, and cross-regulate the expression of both p53 and p73. Additionally, Δ Np63 is a positive transcriptional target of p53 [28]. Disruption of p53 activity abolishes the expression of Δ Np63 α . Moreover, Δ Np63 is recruited to and can activate its own promoter, thus providing an auto-regulatory loop of self-regulation [28, 83]. Importantly, loss of p53 leads to the stabilization of TAp63 γ [84]. Consequently, disruption of TAp63 γ expression leads to decreased expression of Δ Np63, and overexpression of TAp63 γ enhances the activity of the Δ Np63 promoter. Thus, TAp63 γ is capable of activating the expression of Δ Np63.

Fourthly, there seems to be cooperation between the p53 family members in cell cycle regulation and apoptosis. In MCF7 cells, p73 and p63, but not p53, are modulated during the cell cycle with a peak in S phase, and their silencing suppresses proliferation [85]. In cycling cells, p73 and p63 are bound to the p53-responsive elements (REs) in the regulatory regions of cell cycle progression genes. However, when the cells are arrested in G₀-G₁, p73 detaches from the REs and is replaced by p53, which represses the expression of these genes. When the cells move into S-phase, p73 is recruited again and p53 is displaced or is weakly bound to the REs. Thus, the elevated concentrations of p73 and p63 found in many cancers could cause the aberrant activation of cell growth progression genes and therefore contribute to cancer initiation or progression.

The interactions among the p53 family are also critical for the control of p73-dependent cisplatin sensitivity in a subset of human breast cancer, where the Δ Np63 α isoform plays a major role [86]. *In vivo*, Δ Np63 and TAp73 isoforms are co-expressed within a subset of triple-negative primary breast cancers that commonly exhibit mutational inactivation of p53. The Δ Np63 α isoform

promoted survival of breast cancer cells by binding TAp73, and thereby inhibiting its pro-apoptotic activity. Breast cancer cells expressing Δ Np63 α and TAp73 exhibited cisplatin sensitivity that was dependent on TAp73. In response to treatment with cisplatin, but not other chemotherapeutic agents, TAp73 underwent c-Abl-dependent phosphorylation, which promoted dissociation of the Δ Np63 α /TAp73 protein complex, TAp73-dependent transcription of pro-apoptotic Bcl-2 family members, and apoptosis. Thus, tumors with this intact pathway would be predicted to be platinum sensitive and to have high levels of Δ Np63 relative to TAp73 to repress TAp73 activity in the absence of cisplatin. The levels of Δ Np63 and TAp73 mRNA were measured in primary tumor samples to monitor the Δ Np63/TAp73 ratio, and it was proved that this ratio is associated with a positive response to cisplatin therapy [87].

Similarly, in HNSCC patients, high levels of Δ Np63 are connected with a good response to platinum-based chemotherapy [39]. In HNSCC cells, Δ Np63 inhibits the activity of TAp73 and suppresses TAp73-dependent apoptosis [88]. TAp73 is necessary for apoptosis following knockdown of Δ Np63, and siRNA-mediated inhibition of TAp73 expression reduces HNSCC cellular sensitivity to cisplatin. Moreover, as shown by immunoprecipitation, Δ Np63 also forms a complex with TAp73 in HNSCC, so it is plausible that there is the same or similar mechanism of regulating tumor cisplatin sensitivity as in triple-negative breast cancer. These results should also be considered in relation to the effects of Δ Np63 in promoting cell survival, which presents an apparent paradox to the sensitivity of p63-expressing breast and HNSCC tumors to cisplatin. Indeed, in HNSCC, siRNA-mediated reduction in the endogenous p63 levels results in more tumor cells being killed by radiation and cisplatin, demonstrating a pro-survival role for p63 [89]. These contradictory data indicate that although the expression of Δ Np63 in tumors leads to enhanced survival, cisplatin is most effective in Δ Np63-positive tumors precisely because this agent targets Δ Np63 for degradation and thereby removes a critical survival factor in addition to acting as a highly genotoxic agent.

These combined data indicate that the cooperative or antagonistic interactions between p53-family members are important for the regulation of the cell cycle and apoptosis, and thus also for tumor development. Moreover, there is substantial evidence that Δ Np63 expression is a predictor of chemosensitivity to cisplatin.

Adhesion, migration, metastasis, invasiveness

There are several reports which suggest that p63 is involved in cell migration and adhesion and thus also in processes connected with these cell abilities, such as metastasis and wound healing. It was shown that p63 regulates adhesion-related genes in HNSCC and contributes to cell invasion and migration in this tumor type [61]. *In vitro* studies performed on squamous cell carcinoma cell lines have demonstrated that disruption of p63 causes upregulation of genes associated with a higher potential to metastasize and invade [90]. Additionally,

in vitro cell migration assays showed that loss of p63 in squamous carcinoma cell lines leads to increased cell migration. Adorno *et al.* [91] also showed that in cells expressing mutant p53, p63 transcriptional activity is inactivated by mutant p53-Smad complex, which is induced by TGF β , and that p63 acts as an antagonist of TGF β -mediated tumor invasiveness and metastasis. Similarly, knockdown of p63 expression caused downregulation of cell adhesion-associated genes, cell detachment and anoikis in mammary epithelial cells and keratinocytes [92]. On the other hand, overexpression of p63 upregulated cell adhesion molecules, increased cellular adhesion and conferred resistance to anoikis. Most recently, Su *et al.* [93] showed that TAp63 suppresses metastasis by regulating microRNA processing complex. These findings uncovered a new role of p63 as a negative regulator of metastasis.

Bamberger *et al.* [94] suggested the role of p63 isoforms in the healing of skin wounds, which is achieved by extensive migration and hyperproliferation of keratinocytes. Δ Np63 variants were found at high levels in basal and suprabasal keratinocytes of the hyperproliferative wound epithelium, TAp63 variants were also expressed in wound keratinocytes. Thurfjell *et al.* [95] compared p63 status in normal oral wounds and in HNSCC, two situations that represent self-limiting and non-self-limiting processes. They found that both processes show upregulation of p63 α and Δ Np63. However, in wounds, there was a downregulation in TAp63 mRNA levels but not in protein expression, indicating post-translational stabilization of TAp63. Thus, the differences in TAp63 expression and protein stability could influence epithelial proliferation and differentiation. In conclusion, p63 isoforms are differentially expressed during wound healing, suggesting that they have specific functions in this process.

P63 AND STEM CELLS

Expression of p63

The existence of stem cells has been demonstrated in various adult tissues including brain, bone marrow and peripheral blood, muscle, skin, breast, lung, kidney, liver, pancreas and thyroid gland [96-108]. However, genes that contribute to the stem cell phenotype and cell differentiation still need to be elucidated. It was suggested that p63 has important functions in stem cells, because of its expression in the basal cells of the epithelium, and because of the phenotype of p63-deficient mice. However, there remains the question of exactly which role it plays in these processes.

Two independent laboratories generated mouse models lacking p63. Although the strategies for generating these models were different, the phenotypes were very similar [109, 110]. However, differences in the interpretation of the phenotypes of these models have led to different views regarding the role of p63 in epidermal morphogenesis and differentiation.

Two distinct hypotheses have been proposed to explain the phenotypes associated with the lack of p63 expression. Mills *et al.* [110] observed

a complete absence of stratified epidermis, whereas Yang *et al.* [109] observed evidence of stratified but disrupted epidermis, and suggested that the embryonic epidermis of p63^{-/-} mice undergoes an unusual process of non-regenerative differentiation. Thus, in the first hypothesis, p63 is proposed to be essential for specification and differentiation of stratified epithelial cells [74, 111]. In the second hypothesis, p63 is proposed to be critical for the proliferation and maintenance of the epithelial progenitor cell populations that give rise to the differentiated stratified epithelial cells, rather than for the differentiation process itself [109, 111]. Evidence exists supporting both of these hypotheses [112].

Indeed, the expression pattern of p63 is consistent with a role in progenitor cell function. p63 expression is highest in the proliferative basal cell layer, where there are thought to be epithelial progenitor cells in stratified epithelia, including the skin, breast, cervix, urogenital tract and prostate [19, 109, 111, 113, 114].

Within the normal prostate epithelium, p63 is selectively expressed in the basal cell layer and is consistently absent in the secretory and neuroendocrine cells [115]. In accordance with observations on other epithelial tissues (e.g. epidermis, breast, and urothelium), $\Delta Np63\alpha$ is the main isoform expressed by basal cells of the prostate. The agenesis of the early prostate suggests that p63 might be required for the formation of prostate stem cells. The early prostate (prostate buds) consist exclusively of p63-positive cells that subsequently differentiate into basal and secretory cells of the mature prostate epithelium, and thus function as progenitor/stem cells. This observation suggests that p63-positive basal cells of the fully developed prostate include stem cells responsible for maintaining and repairing the adult prostate epithelium (i.e. adult stem cells), and p63 knockout mice can be used as a tool to identify the stem cells in the developing prostate [116].

Expression of p63 is also required for maintaining the proliferative potential of stem cells in the thymus epithelium [117]. Additionally, p63 plays an important role in tooth and hair development [118]: it is required for the formation of individual dental and hair placodes, but not for the specification of the dental field.

To test whether p63 is required by epidermal stem cells for extended proliferative potential, Senoo *et al.* [117] used clonal analysis to generate clones labeled as holoclones, meroclones and paraclones from the human epidermis. It was demonstrated previously that cell stemness can be preserved in culture: holoclones have a high proliferative potential, and a single holoclone has the capacity to generate a mature epithelium *in vivo* and to differentiate into distinct cellular lineages. The holoclone-like clones were composed of small, immature cells that all showed intense nuclear staining with the anti-p63 monoclonal antibody. By contrast, the meroclone-like clones contained larger cells toward the clone center that stained weakly with the p63 antibody. The paraclone-like clones consisted of very large cells that generally lacked detectable p63 expression. These data provide a link between p63 expression and proliferative capacity of cells.

Pellegrini *et al.* [111] similarly identified p63 as a keratinocyte stem cell marker using clonal analysis. Within the cornea, p63 is expressed by the basal cells of the limbal epithelium but not by transiently amplifying cells covering the corneal surface. Human keratinocyte stem and transiently amplifying cells were isolated and then grown in culture to form holoclones and paraclones. p63 was abundantly expressed by epidermal and limbal holoclones, but was undetectable in paraclones. This suggests that p63 is a marker of keratinocyte stem cells.

The relative contribution of individual p63 isoforms during ectodermal differentiation and organogenesis is still far from understood. $\Delta Np63$ isoforms dominate throughout the development of the epidermis and its appendages. During embryonic development, $\Delta Np63$ isoforms seem to have independent roles as transcriptional regulators and do not merely act as inhibitors towards transactivating molecules of the p53 family.

Transcription, upstream and downstream regulations connected to stem cell phenotype

Many of the molecular mechanisms underlying self-renewal in stem cells have been elucidated. These signaling pathways are usually implicated in both cancer and stem cells, so they play a role both in stem cell self-renewal and development, and their aberrant expression could be associated with malignant phenotypes. There are several pathways connected to the stem cell phenotype in which p63 is involved.

Notch signaling

The best characterized pathway of Notch activation involves proteolytic cleavage and translocation of the cytoplasmic domain of the receptor to the nucleus, where it associates with the DNA-binding protein RBP, converting it from a repressor into an activator of transcription [119]. Recent studies have implicated aberrant Notch signaling in human breast tumors, melanoma progression, medulloblastoma and ovarian cancers [120-122]. In breast tumors, amplification of Notch receptors correlates with a more aggressive phenotype [120], and breast cancer stem-like cells express elevated Notch induction [123]. Notch activation is generally thought to maintain stem cell potential and inhibit differentiation of mammalian cells, thereby promoting carcinogenesis [124].

However, in specific cell types such as keratinocytes, increased Notch activity causes exit from the cell cycle and commitment to differentiation [125-127], whereas downregulation or loss of Notch function promotes carcinogenesis [128, 129].

p63 is important for maintaining of undifferentiated state of keratinocytes [130]. p63 expression is suppressed by Notch activation in both mouse and human keratinocytes [131]. In turn, elevated p63 expression counteracts the ability of Notch to restrict growth and promote differentiation. Additionally, knockdown of *TP63* upregulates *Notch* expression and causes a reduction in the proliferation and clonogenicity of keratinocytes [132]. Conversely, overexpression of $\Delta Np63\alpha$ leads to decreased level of Notch and increased proliferative potential

of keratinocytes. Therefore, a complex cross-talk between Notch and p63 is involved in the balance between keratinocyte self-renewal and differentiation.

Interestingly, Ma *et al.* [133] recently demonstrated that p63 activates Notch signaling through the stimulation of *JAG1* gene expression. This is a part of a signaling pathway in which mTOR positively regulates Notch through up-regulation of the STAT3/p63/Jagged cascade, and Notch cascade activation impedes cell differentiation. This signaling was shown in MEFs, but also in breast, prostate, lung and other cancer cell lines. mTOR has been reported to be a positive modulator of p53 and a negative regulator of p73. Thus, p63 is a novel effector of mTOR signaling.

The breast epithelium has two major compartments, luminal and basal cells, which are established and maintained by poorly understood mechanisms. The maintenance of basal cell characteristics in primary human breast epithelial cells depends on continued expression of Δ Np63, which is expressed in the basal compartment [134]. Forced expression of Δ Np63 in purified luminal cells confers a basal phenotype. Notch signaling downregulates Δ Np63 expression and mimics Δ Np63 depletion, whereas forced expression of Δ Np63 partially counteracts the effects of Notch. Basal cells in which Notch signaling is active show decreased p63 expression. Both constitutive expression of Δ Np63 and ablation of Notch signaling are incompatible with luminal cell fate. Thus, the balance between basal and luminal cell compartments of the breast is regulated by antagonistic functions of Δ Np63 and Notch.

Recently, using chromatin immunoprecipitation, Ma *et al.* [133] confirmed that Δ Np63 α could directly bind to *Notch1* and activate the Notch pathway. In clinical breast cancer specimens, the expression level of p63 was also found to positively correlate with the expression level of Notch1. Therefore, these data suggest that p63 functions as a selective modulator of Notch1-dependent transcription and function.

Wnt pathway

The Wnt signaling is implicated in tumorigenesis and in the maintenance of stemness. It has been shown to be casual to a variety of tumors, including colon cancer and breast cancer, and it has been shown to be necessary and sufficient to maintain pluripotency in epidermal stem cells [135-139]. After overexpression, Δ Np63 α promotes Wnt-inducible reporter gene activity in human cells [140]. However, in an apparent contradiction to these observations, siRNA-mediated knockdown of endogenous p63 equally enhanced the expression of Wnt-responsive genes. Δ Np63 α was found in a complex with Wnt-responsive transcription factors. On the basis of these findings, it was proposed that Δ Np63 α has a function in recruiting transcriptional repressors to Wnt-responsive genes. Overexpression of p63 may lead to sequestration of such repressors, resulting in a similar effect to the siRNA-mediated removal of p63, i.e. activation of Wnt-responsive genes. The role of p63 as a negative Wnt-regulator thus fits with the frequently

observed downregulation of p63 during tumor progression, when cancer cells adopt a more mesenchymal, invasive phenotype.

SHH signaling

Sonic Hedgehog (SHH) is a morphogen that has been implicated in epithelial stem cell proliferation and in the development of organs [141-145]. Recently, SHH has also been shown to play an important role in the progression of a variety of cancers [146-151], for example, dysregulation of Hedgehog signaling has been implicated in tumorigenesis of prostate cancer and its activation was found in advanced prostate cancer specimens [152]. Moreover, p63 and p73 but not p53 over-expression induces SHH expression in the epidermis [153]. In particular, p63 γ and p63 β (both TA and Δ N isoforms) and TAp63 β induce SHH, TAp63 γ binds to regions within the *SHH* promoter *in vivo*. Additionally, expression of SHH was found to be significantly reduced in mouse embryo fibroblasts obtained from p63 $^{-/-}$ mice and the naturally occurring p63 mutant TAp63 γ (R279H) inhibited the TAp63 γ -mediated transactivation of *SHH* gene. Thus, p63 plays an important role in the regulation of the SHH signaling pathway.

IHH pathway

Hedgehog signaling is also necessary for mammary gland development, and there is evidence of Hedgehog activation in breast cancer [154-156]. Indian Hedgehog (IHH), the other member of the Hedgehog family, induces elaboration and differentiation of mammary progenitors via differential p63 promoter selection [157]. Analysis of Δ Np63 and TAp63 showed segregated expression in mammary stem and progenitor fractions. Hedgehog activation *in vivo* enhanced the elaboration of mammary progenitors and decreased label retention within mammary stem cell-enriched fractions, suggesting that IHH has a mitogenic effect on mammary stem cells. Hedgehog signaling is also enhanced during pregnancy, suggesting that it contributes to expansion of the mammary gland [157]. These studies support a model in which Hedgehog activates the elaboration and differentiation of mammary progenitors via differential p63 promoter selection. In particular, Δ Np63 promotes unlimited proliferative potential and inhibits differentiation, whereas TAp63 promotes differentiation and inhibits proliferative potential.

MicroRNA regulation

It was suggested by Boominathan [158] that p63 can also regulate the microRNA processing complex. MicroRNAs bind to 3'UTR of mRNAs to inhibit their translation or to promote their degradation. MicroRNAs are processed in the nucleus by a processing complex which consists of Drosha-DGCR8 (RNase III endonuclease) and RNA helicases. The pre-microRNAs are then processed in the cytoplasm by protein complex containing Dicer (RNase III endonuclease). Different microRNAs and components of their processing complex have been shown to be dysregulated in multiple cancers [159, 160]. In

breast carcinoma, Dicer expression is downregulated in patients with metastatic relapse [161], suggesting its function as tumor suppressor. Dicer also plays a role in stem cell renewal [162], and its inhibition leads to downregulation of stem cell factors such as Oct4 and Sox2 [163]. Moreover, DGCR8 also plays a role in stem cell renewal and skin development [162]. MicroRNA processing mediated by Dicer seems to be required for the down-regulation of Δ Np63 in the suprabasal cells of the epidermis [164]. It is plausible that p63 and Dicer could regulate each other because there are several p63-responsive elements in the promoter of *Dicer* [158]. Moreover, p63-responsive elements were also found in the promoters of other components of microRNA processing complex [157].

Interestingly, several microRNAs target the expression of p53 family members; p63 expression is inhibited by microRNAs miR-302 [165], miR-203 [166], miR-21 [167] and miR-92 [168]. miR-203 is upregulated upon DNA damage in the HNSCC cells, whereas Δ Np63 is downregulated [166]. In HNSCC, Δ Np63 is downregulated following DNA damage, and Δ Np63 protein levels correlate with the patients' response to cisplatin treatment [39]. It suggests that miR-203 could regulate p63 mRNA and protein levels upon DNA damage in HNSCC.

miR-302 reduces p63 expression in testicular cancer cells and in mature oocytes, and this regulation is probably important in germ cell maturation [165]. Elevated expression of miR-92 is associated with increased proliferation of myeloid cells [168]. miR-92 also down-regulates the expression of Δ Np63, so it might be one of the molecular mechanism of miR-92 function. The levels of miR-21 are increased in glioblastoma [169] and other tumors, so it plays probably an oncogenic role [170, 171]. miR-21 knockdown increases the protein level of TAp63 in glioblastoma cells [167], and it can therefore be a part of the molecular mechanism of its oncogenic function.

Most recently, Su *et al.* [93] showed that TAp63 regulates Dicer by direct binding to its promoter. Moreover, they found that TAp63 coordinately regulates Dicer and miR-130b to suppress metastasis. Metastatic mouse and human tumors deficient in TAp63 expressed Dicer at very low levels and modulation of expression of Dicer and miR-130b significantly affected the metastatic potential of cells lacking TAp63.

DNA repair

One feature of stem cells is their relative resistance to genotoxic stresses. p63 regulates many target genes involved in DNA repair [56]. Δ Np63 (but not the full-length TAp63) binds to the promoters of the *RAD51*, *BRCA2* and *MRE11* genes, which are involved in homologous recombination, one of the most important pathways for repair of double strand breaks. In addition, p63 interacts with ATM, a key kinase involved in the recognition of DNA double-strand breaks [172]. These findings indicate an additional mechanism through which Δ Np63 can enhance cell survival.

Thus, there is overwhelming evidence that p63 has fundamental roles in the regulation of normal stem cells through the transcriptional regulation of genes

involved in maintaining proliferative potential and in the processes of cellular differentiation.

P63 AND CANCER STEM CELLS

It has been proposed that tumors contain rare stem-like cells called cancer stem cells (CSC) or cancer-initiating cells (CIC). They are characterized by unlimited self-renewing capacity, low proliferation rates, the ability to differentiate into proliferating tumor cells, and the ability to withstand cancer therapy [173, 174]. Serial transplantation of CSCs gave rise to tumors that reconstituted the tissue-specific heterogeneous cell types of the parental tumor. Therefore, the CSC model predicts that only this small subpopulation of neoplastic cells with stem-like capacities promote the maintenance and development of the tumor. Current therapies are designed to treat the bulk of neoplastic cells and might not be effective in eradicating the CSCs. Consistent with this notion, tumors often reappear, even after initially successful treatment. Therefore, the CSC model has important clinical implications and its validation could lead to developing new treatment strategies that selectively target the CSCs [175]. Since p63 is involved in several molecular pathways that are implicated in the maintenance of stemness as well as in tumorigenesis, it could be a potential marker of CSCs.

The cell surface glycoprotein CD44 is involved in cell migration and cell adhesion and is widely expressed in normal tissues. CD44 has recently received attention as a marker of cancer stem cells in various epithelial tumors including HNSCC and breast cancer. In breast cancer, the CD44⁺/CD24⁻ population of tumor cells is able to form tumors when xenografted into immunocompromised mice [176], and the CD44⁺ population of HNSCC cells also possesses stem cell properties of self-renewal and differentiation, but CD44⁻ cells do not show self-renewal properties [177]. Using a microarray analysis of HNSCC cells that stably over-express individual Δ Np63 isoforms, Boldrup *et al.* [178] have shown that all three isoforms induce expression of *CD44* (with the Δ Np63 β isoform showing strongest induction). Δ Np63 isoforms specifically upregulate a *CD44* splice variant lacking exon 2, indicating that p63 may play a role in regulating *CD44* mRNA splicing in addition to up-regulating mRNA levels. Data linking CD44 expression with Δ Np63 suggest that Δ Np63 is an important regulator of stem cell phenotypes in HNSCC. The identification of up-regulation and differential splicing of *CD44* following p63 over-expression indicates roles in the regulation of the cancer stem cell phenotype.

It was recently shown that Δ Np63 α induces a stem cell phenotype in the MCF7 breast carcinoma cell line, and that it could be a marker of breast cancer stem cells [179]. Du *et al.* transfected MCF7 cells with Δ Np63 α plasmid, and assayed their cancer stem cell-like features after transfection. Breast cancer stem cells have been identified as CD44⁺/CD24⁻ [179] and the tumor growth depends on their proliferation and self-renewal capacity. Over-expression of Δ Np63 in MCF7 cells increased the percentage of the CD44⁺/CD24⁻ subpopulation and

led to increased cancer cell proliferation, clonogenicity (increased colony formation ability in soft agar), anchorage-independent growth (ability to grow into mammospheres), and the incidence of tumor xenografts formed *in vivo*. In addition, $\Delta Np63\alpha$ over-expressing cells were more drug resistant. These results suggest that $\Delta Np63\alpha$ might be a tumor-initiating transcription factor in breast cancer and that p63 is one of the possible markers of CSCs in epithelial tissues.

CONCLUSION

In summary, the role of p63 in tumorigenesis is highly complex and needs further clarification. Current data suggest that TA and ΔN isoforms might play opposite roles in this process. However, there are also many possible interactions within the p53 protein family, so a complex view is necessary to understand the role of p53 family members in neoplastic transformation, metastasis and response to therapy. In stem cells, p63 is involved in many pathways that take part in regulation of both self-renewal and differentiation processes. Its role both in tumor and stem cell regulation suggests that p63 might be a possible marker of cancer stem cells in a subset of tumor types, and that it may have prognostic and predictive value.

Acknowledgements. This study was supported by RECAMO CZ.1.05/2.1.00/03.0101. Marta Nekulova was supported by MZ0 MOU 2005, Jitka Holcakova was supported by GACR P301/10/P431. We would like to thank Dr. Rudolf Nenutil for his help in immunohistochemical staining.

REFERENCES

1. Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J.C., Valent, A., Minty, A., Chalon, P., Lelias, J.M., Dumont, X., Ferrara, P., McKeon, F. and Caput, D. Monoallelically expressed gene related to p53 at 1p63, a region frequently deleted in neuroblastoma and other human cancers. **Cell** 90 (1997) 809-819.
2. Yang, A.N., Kaghad, M., Wang, Y.M., Gillett, E., Fleming, M.D., Dotsch, V., Andrews, N.C., Caput, D. and McKeon, F. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. **Mol. Cell** 2 (1998) 305-316.
3. Joerger, A.C., Rajagopalan, S., Natan, E., Veprintsev, D.B., Robinson, C.V. and Fersht, A.R. Structural evolution of p53, p63, and p73: Implication for heterotetramer formation. **Proc. Natl. Acad. Sci. USA** 106 (2009) 17705-17710.
4. Stifanic, M., Micic, M., Ramsak, A., Blaskovic, S., Ruso, A., Zahn, R. and Batel, R. p63 in *Mytilus galloprovincialis* and p53 family members in the phylum Mollusca. **Comp. Biochem. Physiol. B. Biochem. Mol. Biol.** 154 (2009) 264-273.

5. Dohn, M., Zhang, S.Z. and Chen, X.B. p63 alpha and Delta Np63 alpha can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. **Oncogene** 20 (2001) 3193-3205.
6. Wu, G., Nomoto, S., Hoque, M., Dracheva, T., Osada, M., Lee, C., Dong, S., Guo, Z., Benoit, N., Cohen, Y., Rechthand, P., Califano, J., Moon, C.S., Ratovitski, E., Jen, J., Sidransky, D. and Trink, B. Delta Np63 alpha and TAp63 alpha regulate transcription of genes with distinct biological functions in cancer and development. **Canc. Res.** 63 (2003) 2351-2357.
7. Osada, M., Park, H.L., Nagakawa, Y., Yamashita, K., Fomenkov, A., Kim, M.S., Wu, G.J., Nomoto, S., Trink, B. and Sidransky D. Differential recognition of response elements determines target gene specificity for p53 and p63. **Mol. Cell. Biol.** 25 (2005) 6077-6089.
8. Testoni, B., Borrelli, S., Tenedini, E., Alotto, D., Castagnoli, C., Piccolo, S., Tagliafico, E., Ferrari, S., Vigano, M.A. and Mantovani R. Identification of new p63 targets in human keratinocytes. **Cell Cycle** 5 (2006) 2805-2811.
9. Yang, A., Zhu, Z., Kapranov, P., McKeon, F., Church, G.M., Gingeras, T.R. and Struhl, K. Relationships between p63 binding, DNA sequence, transcription activity, and biological function in human cells. **Mol. Cell** 24 (2006) 593-602.
10. Vigano, M.A., Lamartine, J., Testoni, B., Merico, D., Alotto, D., Castagnoli, C., Robert, A., Candi, E., Melino, G., Gidrol, X. and Mantovani, R. New p63 targets in keratinocytes identified by a genome-wide approach. **EMBO J.** 25 (2006) 5105-5116.
11. Mangiulli, M., Valletti, A., Caratozzolo, M.F., Tullo, A., Sbisà, E., Pesole, G. and D'Erchia, A.M. Identification and functional characterization of two new transcriptional variants of the human p63 gene. **Nucl. Acid. Res.** 37 (2009) 6092-6104.
12. Thanos, C.D. and Bowie, J.U. p53 Family members p63 and p73 are SAM domain-containing proteins. **Prot. Sci.** 8 (1999) 1708-1710.
13. Serber, Z., Lai, H.C., Yang, A., Ou, H.D., Sigal, M.S., Kelly, A.E., Darimont, B.D., Duijf, P.H.G., van Bokhoven, H., McKeon, F. and Dötsch, V. A C-terminal inhibitory domain controls the activity of p63 by an intramolecular mechanism. **Mol. Cell. Biol.** 22 (2002) 8601-8611.
14. Sayan, B.S., Sayan, A.E., Yang, A.L., Aqeilan, R.I., Candi, E., Coher, G.M., Knight, R.A., Croce, C.M. and Melino, G. Cleavage of the transactivation-inhibitory domain of p63 by caspases enhances apoptosis. **Proc. Natl. Acad. Sci. USA** 104 (2007) 10871-10876.
15. Ghioni, P., Bolognese, F., Duijf, P.H.G., van Bokhoven, H., Mantovani, R. and Guerrini, L. Complex transcriptional effects of p63 isoforms: Identification of novel activation and repression domains. **Mol. Cell. Biol.** 22 (2002) 8659-8668.
16. Helton, E.S., Zhu, J.H. and Chen, X.B. The unique NH2-terminally deleted (Delta N) residues, the PXXP motif, and the PPXY motif are required for

- the transcriptional activity of the Delta N variant of p63. **J. Biol. Chem.** 281 (2006) 2533-2542.
17. Nylander, K., Vojtesek, B., Nenutil, R., Lindgren, B., Roos, G., Wang, Z.X., Sjostrom, B., Dahlqvist, A. and Coates, P.J. Differential expression of p63 isoforms in normal tissues and neoplastic cells. **J. Pathol.** 198 (2002) 417-427.
 18. Reis-Filho, J.S., Torio, B., Albergaria, A. and Schmitt, F.C. p63 expression in normal skin and usual cutaneous carcinomas. **J. Cutan. Pathol.** 29 (2002) 517-523.
 19. Di Como, C.J., Urist, M.J., Babayan, I., Drobnjak, M., Hedvat, C.V., Teruya-Feldstein, J., Pohar, K., Hoos, A. and Cordon-Cardo, C. p63 expression profiles in human normal and tumor tissues. **Clin. Canc. Res.** 8 (2002) 494-501.
 20. Rosenbluth, J.M., Johnson, K., Tang, L.J., Triplett, T. and Pietenpol, J.A. Evaluation of p63 and p73 antibodies for cross-reactivity. **Cell Cycle** 8 (2009) 3702-3706.
 21. Hedvat, C.V., Teruya-Feldstein, J., Puig, P., Capodieci, P., Dudas, M., Pica, N., Qin, J., Cordon-cardo, C. and Di Como, C.J. Expression of p63 in diffuse large B-cell lymphoma. **Appl. Immunohistochem. Mol. Morphol.** 13 (2005) 237-242.
 22. Livera, G., Petre-Lazar, B., Guerin, M.J., Trautmann, E., Coffigny, H. and Habert, R. p63 null mutation protects mouse oocytes from radio-induced apoptosis. **Reproduction** 135 (2008) 3-12.
 23. Suh, E.K., Yang, A., Kettenbach, A., Bamberger, C., Michaelis, A.H., Zhu, Z., Elvin, J.A., Bronson, R.T., Crum, C.P. and McKeon, F. p63 protects the female germ line during meiotic arrest. **Nature** 444 (2006) 624-628.
 24. Nishi, H., Isaka, K., Sagawa, Y., Usuda, S., Fujito, A., Ito, H., Senoo, M., Kato, H. and Takayama, M. Mutation and transcription analyses of the p63 gene in cervical carcinoma. **Int. J. Oncol.** 15 (1999) 1149-1153.
 25. Wang, T.Y., Chen, B.F., Yang, Y.C., Chen, H., Wang, Y., Cviko, A., Quade, B.J., Sun, D., Yang, A., McKeon, F.D. and Crum, C.P. Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: a study of 250 cases. **Hum. Pathol.** 32 (2001) 479-486.
 26. Idrees, M.T., Schlosshauer, P., Li, G. and Burstein, D.E. GLUT1 and p63 expression in endometrial intraepithelial and uterine serous papillary carcinoma. **Histopathology** 49 (2006) 75-81.
 27. Ito, Y., Takeda, T., Wakasa, K., Tsujimoto, M., Sakon, M. and Matsuura, N. Expression of p73 and p63 proteins in pancreatic adenocarcinoma: p73 overexpression is inversely correlated with biological aggressiveness. **Int. J. Mol. Med.** 8 (2001) 67-71.
 28. Harmes, D.C., Bresnick, E., Lubin, E.A., Watson, J.K., Heim, K.E., Curtin, J.C., Suskind, A.M., Lamb, J. and DiRenzo, J. Positive and negative regulation of Delta N-p63 promoter activity by p53 and Delta N-p63-alpha contributes to differential regulation of p53 target genes. **Oncogene** 22 (2003) 7607-7616.

29. Weinstein, M.H., Signoretto, S. and Loda, M. Diagnostic utility of immunohistochemical staining for p63, a sensitive marker of prostatic basal cells. **Mod. Pathol.** 15 (2002) 1302-1308.
30. Chen, B.Y., Liu, J.Y., Chang, H.H., Chang, C.P., Lo, W.Y., Kuo, W.H., Yang, C.R. and Lin, D. Hedgehog is involved in prostate basal cell hyperplasia formation and its progressing towards tumorigenesis. **Biochem. Biophys. Res. Commun.** 357 (2007) 1084-1089.
31. Glickman, J.N., Yang, A., Shahsafaei, A., McKeon, F. and Odze, R.D. Expression of p53-related protein p63 in the gastrointestinal tract and in esophageal metaplastic and neoplastic disorders. **Hum. Pathol.** 32 (2001) 1157-1165.
32. Basturk, O., Khanani, F., Sarkar, F., Levi, E., Cheng, J.D. and Adsay, N.V. DeltaNp63 expression in pancreas and pancreatic neoplasia. **Mod. Pathol.** 18 (2005) 1193-1198.
33. Koga, F., Kawakami, S., Fujii, Y., Saito, K., Ohtsuka, Y., Iwai, A., Ando, N., Takizawa, T., Kageyama, Y. and Kihara, K. Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. **Clin. Cancer Res.** 9 (2003) 5501-5507.
34. Urist, M.J., Di Como, C.J., Lu, M.L., Charytonowicz, E., Verbel, D., Crum, C.P., Ince, T.A., McKeon, F.D. and Cordon-Cardo, C. Loss of p63 expression is associated with tumor progression in bladder cancer. **Am. J. Pathol.** 161 (2002) 1199-1206.
35. Park, B.J., Lee, S.J., Kim, J.I., Lee, S.J., Lee, C.H., Chang, S.G., Park, J.H. and Chi, S.G. Frequent alteration of p63 expression in human primary bladder carcinomas. **Cancer Res.** 60 (2000) 3370-3374.
36. Koga, F., Kawakami, S., Kumagai, J., Takizawa, T., Ando, N., Arai, G., Kageyama, Y. and Kihara, K. Impaired Delta Np63 expression associates with reduced beta-catenin and aggressive phenotypes of urothelial neoplasms. **Br. J. Cancer.** 88 (2003) 740-747.
37. Yamaguchi, K., Wu, L., Caballero, O.L., Hibi, K., Trink, B., Resto, V., Cairns, P., Okami, K., Koch, W.M., Sidransky, D. and Jen, J. Frequent gain of the p40/p51/p63 gene locus in primary head and neck squamous cell carcinoma. **Int. J. Cancer** 86 (2000) 684-689.
38. Thurfjell, N., Coates, P.J., Uusitalo, T., Mahani, D., Dabelsteen, E., Dahlqvist, A., Sjöström, B., Roos, G. and Nylander, K. Complex p63 mRNA isoform expression patterns in squamous cell carcinoma of the head and neck. **Int. J. Oncol.** 25 (2004) 27-35.
39. Zangen, R., Ratovitski, E. and Sidransky, D. DeltaNp63alpha levels correlate with clinical tumor response to cisplatin. **Cell Cycle** 4 (2005) 1313-1315.
40. Tannapfel, A., Schmelzer, S., Benicke, M., Klimpfner, M., Kohlhaw, K., Mössner, J., Engeland, K. and Wittekind, C. Expression of the p53 homologues p63 and p73 in multiple simultaneous gastric cancer. **J. Pathol.** 195 (2001) 163-170.

41. Massion, P.P., Taflan, P.M., Jamshedur Rahman, S.M., Yildiz, P., Shyr, Y., Edgerton, M.E., Westfall, M.D., Roberts, J.R., Pietenpol, J.A., Carbone, D.P. and Gonzalez, A.L. Significance of p63 amplification and overexpression in lung cancer development and prognosis. **Cancer Res.** 63 (2003) 7113-7121.
42. Wang, B.Y., Gil, J., Kaufman, D., Gan, L., Kohtz, D.S. and Burstein, D.E. P63 in pulmonary epithelium, pulmonary squamous neoplasms, and other pulmonary tumors. **Hum. Pathol.** 33 (2002) 921-926.
43. Ying, H., Chang, D.L., Zheng, H., McKeon, F. and Xiao, Z.X. DNA-binding and transactivation activities are essential for TAp63 protein degradation. **Mol. Cell. Biol.** 25 (2005) 6154-6164.
44. Osada, M., Inaba, R., Shinohara, H., Hagiwara, M., Nakamura, M. and Ikawa, Y. Regulatory domain of protein stability of human P51/TAP63, a P53 homologue. **Biochem. Biophys. Res. Commun.** 283 (2001) 1135-1141.
45. Ghioni, P., D'Alessandra, Y., Mansueto, G., Jaffray, E., Hay, R.T., La Mantia, G. and Guerrini, L. The protein stability and transcriptional activity of p63 alpha are regulated by SUMO-1 conjugation. **Cell Cycle** 4 (2005)183-190.
46. Petitjean, A., Ruptier, C., Tribollet, V., Hautefeuille, A., Chardon, F., Cavard, C., Puisieux, A., Hainaut, P. and de Fromental, C.C. Properties of the six isoforms of p63: p53-like regulation in response to genotoxic stress and cross talk with Delta Np73. **Carcinogenesis** 29 (2008) 273-281.
47. MacPartlin, M., Zeng, S., Lee, H., Stauffer, D., Jin, Y., Thayer, M. and Lu, H. p300 regulates p63 transcriptional activity. **J. Biol. Chem.** 280 (2005) 30604-30610.
48. Fomenkov, A., Zangen, R., Huang, Y.P., Osada, M., Guo, Z., Fomenkov, T., Trink, B., Sidransky, D. and Ratovitski, E.A. RACK1 and stratifin target DeltaNp63alpha for a proteasome degradation in head and neck squamous cell carcinoma cells upon DNA damage. **Cell Cycle** 3 (2004) 1285-1295.
49. Chatterjee, A., Chang, X., Sen, T., Ravi, R., Bedi, A. and Sidransky, D. Regulation of p53 family member isoform $\Delta Np63\alpha$ by the nuclear factor- κB targeting kinase I κB kinase β . **Cancer Res.** 70 (2010) 1419-1429.
50. Wang, N., Guo, L., Rueda, B.R. and Tilly, J.L. Cables1 protects p63 from proteasomal degradation to ensure deletion of cells after genotoxic stress. **EMBO J.** 11 (2010) 633-639.
51. Tomlinson, V., Gudmundsdottir, K., Luong, P., Leung, K.-Y., Knebel, A. and Basu, S. JNK phosphorylates Yes-associated protein (YAP) to regulate apoptosis. **Cell Death Dis.** 1, e29 (2010) doi:10.1038/cddis.2010.7.
52. Kadakia, M., Slader, C. and Berberich, S.J. Regulation of p63 function by Mdm2 and MdmX. **DNA Cell Biol.** 20 (2001) 321-330.
53. Little, N.A. and Jochemsen, A.G. Hdmx and Mdm2 can repress transcription activation by p53 but not by p63. **Oncogene** 20 (2001) 4576-4580.
54. Calabro, V., Mansueto, G., Parisi, T., Vivo, M., Calogero, R.A. and La Mantia, G. The human MDM2 oncoprotein increases the transcriptional

- activity and the protein level of the p53 homolog p63. **J. Biol. Chem.** 277 (2002) 2674-2681.
55. Galli, F., Rossi, M., D'Alessandra, Y., De Simone, M., Lopardo, T., Haupt, Y., Alsheich-Bartok, O., Anzi, S., Shaulian, E., Calabro, V., La Mantia, G. and Guerrini, L. MDM2 and Fbw7 cooperate to induce p63 protein degradation following DNA damage and cell differentiation. **J. Cell. Sci.** 123 (2010) 2423-2433.
 56. Lin, Y.L., Sengupta, S., Gurdziel, K., Bell, G.W., Jacks, T. and Flores, E.R. p63 and p73 transcriptionally regulate genes involved in DNA repair. **PLoS Genet.** 5 (2009) e1000680.
 57. Lopardo, T., Lo Iacono, N., Marinari, B., Giustizieri, M.L., Cyr, D.G., Merlo, G., Crosti, F., Costanzo, A. and Guerrini, L. Claudin-1 is a p63 target gene with a crucial role in epithelial development. **PLoS One** 3 (2008) e2715.
 58. Gressner, O., Schilling, T., Lorenz, K., Schulze Schleithoff, E., Koch, A., Schulze-Bergkamen, H., Lena, A.M., Candi, E., Terrinoni, A., Catani, M.V., Oren, M., Melino, G., Krammer, P.H., Stremmel, W. and Müller, M. TAp63alpha induces apoptosis by activating signaling via death receptors and mitochondria. **EMBO J.** 24 (2005) 2458-2471.
 59. Antonini, D., Dentice, M., Mahtani, P., De Rosa, L., Della Gatta, G., Mandinova, A., Salvatore, D., Stupka, E. and Missero, C. Tprg, a gene predominantly expressed in skin, is a direct target of the transcription factor p63. **J. Invest. Dermatol.** 128 (2008) 1676-1685.
 60. Koster, M.I., Dai, D., Marinari, B., Sano, Y., Costanzo, A., Karin, M. and Roop, D.R. p63 induces key target genes required for epidermal morphogenesis. **Proc. Natl. Acad. Sci. USA** 104 (2007) 3255-3260.
 61. Gu, X.L., Coates, P.J., Boldrup, L. and Nylander, K. p63 contributes to cell invasion and migration in squamous cell carcinoma of the head and neck. **Cancer Lett.** 263 (2008) 26-34.
 62. Ihrie, R.A., Marques, M.R., Nguyen, B.T., Horner, J.S., Papazoglu, C., Bronson, R.T., Mills, A.A. and Attardi, L.D. Perp is a p63-regulated gene essential for epithelial integrity. **Cell** 120 (2005) 843-856.
 63. Wu, G., Nomoto, S., Hoque, M.O., Dracheva, T., Osada, M., Lee, C.C., Dong, S.M., Guo, Z., Benoit, N., Cohen, Y., Rechthand, P., Califano, J., Moon, C.S., Ratovitski, E., Jen, J., Sidransky, D. and Trink, B. DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. **Cancer Res.** 63 (2003) 2351-2357.
 64. Boldrup, L., Coates, P.J., Gu, X. and Nylander, K. DeltaNp63 isoforms differentially regulate gene expression in squamous cell carcinoma: identification of Cox-2 as a novel p63 target. **J. Pathol.** 218 (2009) 428-436.
 65. Osada, M., Ohba, M., Kawahara, C., Ishioka, C., Kanamaru, R., Katoh, I., Ikawa, Y., Nimura, Y., Nakagawara, A., Obinata, M. and Ikawa, S. Cloning

- and functional analysis of human p51, which structurally and functionally resembles p53. **Nat. Med.** 4 (1998) 839-843.
66. Sunahara, M., Shishikura, T., Takahashi, M., Todo, S., Yamamoto, N., Kimura, H., Kato, S., Ishioka, C., Ikawa, S., Ikawa, Y. and Nakagawara, A. Mutational analysis of p51A/TAp63gamma, a p53 homolog, in non-small cell lung cancer and breast cancer. **Oncogene** 18 (1999) 3761-3765.
 67. Hibi, K., Trink, B., Patturajan, M., Westra, W.H., Caballero, O.L., Hill, D.E., Ratovitski, E.A., Jen, J. and Sidransky, D. AIS is an oncogene amplified in squamous cell carcinoma. **Proc. Natl. Sci. USA** 97 (2000) 5462-5467.
 68. Flores, E.R., Sengupta, S., Miller, J.B., Newman, J.J., Bronson, R., Crowley, D., Yang, A., McKeon, F. and Jacks, T. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. **Cancer Cell** 7 (2005) 363-373.
 69. Keyes, W.M., Vogel, H., Koster, M.I., Guo, X.C., Qi, Y., Petherbridge, K.M., Roop, D.R., Bradley, A. and Mills, A.A. p63 heterozygous mutant mice are not prone to spontaneous or chemically induced tumors. **Proc. Natl. Acad. Sci. USA** 103 (2006) 8435-8440.
 70. Keyes, W.M., Wu, Y., Vogel, H., Guo, X.C., Lowe, S.W. and Mills, A.A. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. **Genes Dev.** 19 (2005) 1986-1999.
 71. Djelloul, S., Tarunina, M., Barnouin, K., Mackay, A. and Jat, P.S. Differential protein expression, DNA binding and interaction with SV40 large tumour antigen implicate the p63-family of proteins in replicative senescence. **Oncogene** 21 (2002) 981-989.
 72. Guo, X.C., Keyes, W.M., Papazoglu, C., Zuber, J., Li, W.Z., Lowe, S.W., Vogel, H. and Mills, A.A. TAp63 induces senescence and suppresses tumorigenesis in vivo. **Nature Cell Biol.** 11 (2009) 1451-1457.
 73. Koster, M.I., Lu, S.L., White, L.D., Wang, X.J. and Roop, D.R. Reactivation of developmentally expressed p63 isoforms predisposes to tumor development and progression. **Cancer Res.** 66 (2006) 3981-3986.
 74. Koster, M.I., Kim, S., Mills, A.A., DeMayo, F.J. and Roop, D.R. p63 is the molecular switch for initiation of an epithelial stratification program. **Gen. Dev.** 18 (2004) 126-131.
 75. Mundt, H.M., Stremmel, W., Melino, G., Krammer, P.H., Schilling, T. and Müller, M. Dominant negative (DeltaN) p63alpha induces drug resistance in hepatocellular carcinoma by interference with apoptosis signaling pathways. **Biochem. Biophys. Res. Commun.** 396 (2010) 335-341.
 76. Nylander, K., Coates, P.J. and Hall, P.A. Characterization of the expression pattern of p63 alpha and delta Np63 alpha in benign and malignant oral epithelial lesions. **Int. J. Cancer.** 87 (2000) 368-372.
 77. Crook, T., Nicholls, J.M., Brooks, L., O'Nions, J. and Allday, M.J. High level expression of deltaNp63: a mechanism for the inactivation of p53 in

- undifferentiated nasopharyngeal carcinoma (NPC)? **Oncogene** 19 (2000) 3439-3444.
78. Tonon, G., Brennan, C., Protopopov, A., Maulik, G., Feng, B., Zhang, Y., Khattry, D.B., You, M.J., Aguirre, A.J., Martin, E.S., Yang, Z., Ji, H., CHin, L., Wong, K.K. and Depinho, R.A. Common and contrasting genomic profiles among the major human lung cancer subtypes. **Cold Spring Harb. Symp. Quant. Biol.** 70 (2005) 11-24.
79. Davison, T.S., Vagner, C., Kaghad, M., Ayed, A., Caput, D. and Arrowsmith, C.H. p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. **J. Biol. Chem.** 274 (1999) 18709-18714.
80. Gaiddon, C., Lokshin, M., Ahn, J., Zhang and T., Prives, C. A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. **Mol. Cell. Biol.** 21 (2001) 1874-1887.
81. Strano, S., Fontemaggi, G., Costanzo, A., Rizzo, M.G., Monti, O., Baccarini, A., Del Sal, G., Levrero, M., Sacchi, A., Oren, M. and Blandino, G. Physical interaction with human tumor-derived p53 mutants inhibits p63 activities. **J. Biol. Chem.** 277 (2002) 18817-18826.
82. Yang, A., Zhu, Z., Kapranov, P., McKeon, F., Church, G.M., Gingeras, T.R. and Struhl, K. Relationships between p63 binding, DNA sequence, transcription activity, and biological function in human cells. **Mol. Cell** 24 (2006) 593-602.
83. Romano, R.A., Birkaya, B. and Sinha, S. Defining the regulatory elements in the proximal promoter of Delta Np63 in keratinocytes: Potential roles for Sp1/Sp3, NF-Y, and p63. **J. Invest. Dermatol.** 126 (2006) 1469-1479.
84. Li, N., Li, H., Cherukuri, P., Farzan, S., Harmes, D.C. and DiRenzo, J. TA-p63-gamma regulates expression of Delta N-p63 in a manner that is sensitive to p53. **Oncogene** 25 (2006) 2349-2359.
85. Lefkimiatis, K., Caratuzzolo, M.F., Merlo, P., D'Erchia, A.M., Navarro, B., Levrero, M., Sbisà, E. and Tullo, A. p73 and p63 sustain cellular growth by transcriptional activation of cell cycle progression genes. **Cancer Res.** 69 (2009) 8563-8571.
86. Leong, C.O., Vidnovic, N., DeYoung, M.P., Sgroi, D. and Ellisen, L.W. The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. **J. Clin. Invest.** 117 (2007) 1370-1380.
87. Silver, D.P., Richardson, A.L., Eklund, A.C., Wang, Z.C., Szallasi, Z., Li, Q., Juul, N., Leong, C.O., Calogrias, D., Buraimoh, A., Fatima, A., Gelman, R.S., Ryan, P.D., Tung, N.M., De Nicolo, A., Ganesan, S., Miron, A., Colin, C., Sgroi, D.C., Ellisen, L.W., Winer, E.P. and Garber, J.E. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. **J. Clin. Oncol.** 28 (2010) 1145-1153.

88. Rocco, J.W., Leong, C.O., Kuperwasser, N., DeYoung, M.P. and Ellisen, L.W. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. **Cancer Cell** 9 (2006) 45-56.
89. Thurfjell, N., Coates, P.J., Vojtesek, B., Benham-Motlagh, P., Eisold, M. and Nylander, K. Endogenous p63 acts as a survival factor for tumour cells of SCCHN origin. **Int. J. Mol. Med.** 16 (2005) 1065-1070.
90. Barbieri, C.E., Tang, L.J., Brown, K.A. and Pietenpol, J.A.. Loss of p63 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. **Cancer Res.** 66 (2006) 7589-7597.
91. Adorno, M., Cordenonsi, M., Montagner, M., Dupont, S., Wong, C., Hann, B., Solari, A., Bobisse, S., Rondina, M.B., Guzzardo, V., Parenti, A.R., Rosato, A., Bicciato, S., Balmain, A. and Piccolo, S. A mutant-p53/Smad complex opposes p63 to empower TGFbeta-induced metastasis. **Cell** 137 (2009) 87-98.
92. Carroll, D.K., Carroll, J.S., Leong, C.O., Cheng, F., Brown, M., Mills, A.A., Brugge, J.S. and Ellisen, L.W. p63 regulates an adhesion programme and cell survival in epithelial cells. **Nature Cell Biol.** 8 (2006) 551-561.
93. Su, X., Chakravarti, D., Cho, M.S., Liu, L., Gi, Y.J., Lin, Y.L., Leung, M.L., El-Naggar, A., Creighton, C.J., Suraokar, M.B., Wistuba, I. and Flores, E.R. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. **Nature** 467 (2010) 986-990.
94. Bamberger, C., Hafner, A., Schmale, H. and Werner, S. Expression of different p63 variants in healing skin wounds suggests a role of p63 in reepithelialization and muscle repair. **Wound Repair Regen.** 13 (2005) 41-50.
95. Thurfjell, N., Coates, P.J., Wahlin, Y.B., Arvidsson, E. and Nylander, K. Downregulation of TAp63 and unaffected levels of p63beta distinguishes oral wounds from SCCHN. **Cell Cycle** 5 (2006) 555-557.
96. Ma, D.K, Bonaguidi, M.A., Ming, G.L. and Song, H. Adult neural stem cells in the mammalian central nervous system. **Cell. Res.** 19 (2009) 672-682.
97. Gibelli, B., El-Fattah, A, Giugliano, G., Proh, M. and Grosso, E. Thyroid stem cells – danger or resource? **Acta Otorhinolaryngol. Ital.** 29 (2009) 290-295.
98. Wu, X., Wang, S., Chen, B. and An, X. Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy. **Cell Tissue Res.** 340 (2010) 549-567.
99. Snyder, J.C, Teisanu, R.M. and Stripp, B.R. Endogenous lung stem cells and contribution to disease. **J. Pathol.** 217 (2009) 254-264.
100. Little, M.H. and Bertram, J.F. Is there such a thing as a renal stem cell? **J. Am. Soc. Nephrol.** 20 (2009) 2112-2117.
101. Pincelli, C. and Marconi, A. Keratinocyte stem cells: friends and foes. **J. Cell. Physiol.** 225 (2010) 310-315.
102. Katsumoto, K., Shiraki, N., Miki, R. and Kume, S. Embryonic and adult stem cell systems in mammals: ontology and regulation. **Dev. Growth. Differ.** 52 (2010) 115-129.

103. Petersen, O.W. and Polyak, K. Stem cells in the human breast. Cold Spring Harb. **Perspect. Biol.** 2 (2010) a003160.
104. Ratajczak, M.Z., Zuba-Surma, E.K., Machalinski, B. and Kucia, M. Bone-marrow-derived stem cells – our key to longevity? **J. Appl. Genet.** 48 (2007) 307-319.
105. Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B. and Anversa, P. Adult cardiac stem cells are multipotent and support myocardial regeneration. **Cell** 114 (2003) 763-776.
106. Tumber, T., Guasch, G., Greco, V., Blanpain, C., Lowry, W.E., Rendl, M. and Fuchs, E. Defining the epithelial stem cell niche in skin. **Science** 303 (2004) 359-363.
107. Collins, C.A. and Partridge, T.A. Self-renewal of the adult skeletal muscle satellite cell. **Cell Cycle** 4 (2005) 1338-1341.
108. Herrera, M.B., Bruno, S., Buttiglieri, S., Tetta, C., Gatti, S., Deregibus, M.C., Bussolati, B. and Camussi, G. Isolation and characterization of a stem cell population from adult human liver. **Stem Cells** 24 (2006) 2840-2850.
109. Yang, A., Schweitzer, R., Sun, D.Q., Kaghad, M., Walker, N., Bronson, R.T., Tabin, C., Sharpe, A., Caput, D., Crum, C. and McKeon, F. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. **Nature** 398 (1999) 714-718.
110. Mills, A.A., Zheng, B.H., Wang, X.J., Vogel, H., Roop, D.R. and Bradley, A. p63 is a p53 homologue required for limb and epidermal morphogenesis. **Nature** 398 (1999) 708-713.
111. Pellegrini, G., Dellambra, E., Golisano, O., Martinelli, E., Fantozzi, I., Bondanza, S., Ponzin, D., McKeon, F. and De Luca, M. p63 identifies keratinocyte stem cells. **Proc. Natl. Acad. Sci. USA.** 98 (2001) 3156-3161.
112. Barbieri, C.E. and Pietenpol, J.A. p63 and epithelial biology. **Exp. Cell. Res.** 312 (2006) 695-706.
113. Dellavalle, R.P., Egbert, T.B., Marchbank, A., Su, L.J., Lee, L.A. and Walsh, P. CUSP/p63 expression in rat and human tissues. **J. Dermat. Sci.** 27 (2001) 82-87.
114. Rizzo, S., Attard, G. and Hudson, D.L. Prostate epithelial stem cells. **Cell. Prolif.** 38 (2005) 363-374.
115. Signoretti, S., Waltregny, D., Dilks, J., Isaac, B., Lin, D., Garraway, L., Yang, A., Montironi, R., McKeon, F. and Loda, M. p63 is a prostate basal cell marker and is required for prostate development. **Am. J. Pathol.** 157 (2000) 1769-1775.
116. Signoretti, S., Pires, M.M., Lindauer, M., Horner, J.W., Grisanzio, C., Dhar, S., Majumder, P., McKeon, F., Kantoff, P.W., Sellers, W.R., Loda, M. p63 regulates commitment to the prostate cell lineage. **Proc. Natl. Acad. Sci. USA** 102 (2005) 11355-11360.

117. Senoo, M., Pinto, F., Crum, C.P. and McKeon, F. p63 is essential for the proliferative potential of stem cells in stratified epithelia. **Cell** 129 (2007) 523-536.
118. Laurikkala, J., Mikkola, M.L., James, M., Tummers, M., Mills, A.A. and Thesleff, I. p63 regulates multiple signalling pathways required for ectodermal organogenesis and differentiation. **Development** 133 (2006) 1553-1563.
119. Mumm, J.S. and Kopan, R. Notch signaling: From the outside in. **Dev. Biol.** 228 (2000) 151-165.
120. Stylianou, S., Clarke, R.B. and Brennan, K. Aberrant activation of notch signaling in human breast cancer. **Cancer Res.** 66 (2006) 1517-1525.
121. Massi, D., Tarantini, F., Franchi, A., Paglierani, M., Di Serio, C., Pellerito, S., Leoncini, G., Cirino, G., Geppetti, P. and Santucci, M. Evidence for differential expression of Notch receptors and their ligands in melanocytic nevi and cutaneous malignant melanoma. **Mod. Pathol.** 19 (2006) 246-254.
122. Rose, S.L., Kunnimalaiyaan, M., Drenzek, J. and Seiler, N. Notch 1 signaling is active in ovarian cancer. **Gynecol. Oncol.** 117 (2010) 130-133.
123. Grudzien, P., Lo, S., Albain, K.S., Robinson, P., Rajan, P., Strack, P.R., Golde, T.E., Miele, L. and Foreman, K.E. Inhibition of Notch signaling reduces the stem-like population of breast cancer cells and prevents mammosphere formation. **Anticancer Res.** 30 (2010) 3853-3867.
124. Artavanis-Tsakonas, S., Rand, M.D. and Lake, R.J. Notch signaling: cell fate control and signal integration in development. **Science** 284 (1999) 770-776.
125. Lowell, S., Jones, P., Le Roux, I., Dunne, J. and Watt, F.M. Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. **Curr. Biol.** 10 (2000) 491-500.
126. Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J.C., Krishna, S., Metzger, D., Chambon, P., Miele, L., Aguet, M., Radtke, F. and Dotto, G.P. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. **EMBO J.** 20 (2001) 3427-3436.
127. Nickoloff, B.J., Qin, J.Z., Chaturvedi, V., Denning, M.F., Bonish, B. and Miele, L. Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma. **Cell Death Differ.** 9 (2002) 842-855.
128. Talora, C., Sgroi, D.C., Crum, C.P. and Dotto, G.P. Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. **Genes Dev.** 16 (2002) 2252-2263.
129. Nicolas, M., Wolfer, A., Raj, K., Kummer, J.A., Mill, P., van Noort, M., Hui, C.C., Clevers, H., Dotto, G.P. and Radtke, F. Notch1 functions as a tumor suppressor in mouse skin. **Nat. Genet.** 33 (2003) 416-421.
130. Okuyama, R., Ogawa, E., Nagoshi, H., Yabuki, M., Kurihara, A., Terui, T., Aiba, S., Obinata, M., Tagami, H. and Ikawa, S. p53 homologue, p51/p63,

- maintains the immaturity of keratinocyte stem cells by inhibiting Notch1 activity. **Oncogene** 26 (2007) 4478-4488.
131. Nguyen, B.C., Lefort, K., Mandinova, A., Antonini, D., Devgan, V., Della Gatta, G., Koster, M.I., Zhang, Z., Wang, J., Tommasi di Vignano, A., Kitajewski, J., Chiorino, G., Roop, D.R., Missero, C. and Dotto, G.P. Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. **Genes Dev.** 20 (2006) 1028-1042.
132. Yugawa, T., Narisawa-Saito, M., Yoshimatsu, Y., Haga, K., Ohno, S., Egawa, N., Fujita, M. and Kiyono, T. Δ Np63 α repression of the Notch1 gene supports the proliferative capacity of normal human keratinocytes and cervical cancer cells. **Cancer Res.** 70 (2010) 4034-4044.
133. Ma, J., Meng, Y., Kwiatkowski, D.J., Chen, X., Peng, H., Sun, Q., Zha, X., Wang, F., Wang, Y., Jing, Y., Zhang, S., Chen, R., Wang, L., Wu, E., Cai, G., Malinowska-Kolodziej, I., Liao, Q., Liu, Y., Zhao, Y., Sun, Q., Xu, K., Dai, J., Han, J., Wu, L., Zhao, R.C., Shen, H. and Zhang, H. Mammalian target of rapamycin regulates murine and human cell differentiation through STAT3/p63/Jagged/Notch cascade. **J. Clin. Invest.** 120 (2010) 103-114.
134. Yalcin-Ozuyisal, O., Fiche, M., Guitierrez, M., Wagner, K.U., Raffoul, W. and Brisken, C. Antagonistic roles of Notch and p63 in controlling mammary epithelial cell fates. **Cell Death Differ.** 17 (2010) 1600-1612.
135. Bienz, M. and Clevers, H. Linking colorectal cancer to Wnt signaling. **Cell** 103 (2000) 311-320.
136. Logan, C.Y. and Nusse, R. The Wnt signaling pathway in development and disease. **Annu. Rev. Cell. Dev. Biol.** 20 (2004) 781-810.
137. Kléber, M. and Sommer, L. Wnt signaling and the regulation of stem cell function. **Curr. Opin. Cell. Biol.** 16 (2004) 681-687.
138. Reya, T. and Clevers, H. Wnt signalling in stem cells and cancer. **Nature** 434 (2005) 843-850.
139. Gu, B., Watanabe, K. and Dai, X. Epithelial stem cells: an epigenetic and Wnt-centric perspective. **J. Cell. Biochem.** 110 (2010) 1279-1287.
140. Drewelus, I., Göpfert, C., Hippel, C., Dickmanns, A., Damianitsch, K., Pieler, T. and Döbelstein, M. p63 antagonizes Wnt-induced transcription. **Cell Cycle** 9 (2010) 580-587.
141. Iseki, S., Araga, A., Ohuchi, H., Nohno, T., Yoshioka, H., Hayashi, F. and Noji, S. Sonic hedgehog is expressed in epithelial cells during development of whisker, hair, and tooth. **Biochem. Biophys. Res. Commun.** 218 (1996) 688-693.
142. Ho, K.S. and Scott, M.P. Sonic hedgehog in the nervous system: functions, modifications and mechanisms. **Curr. Opin. Neurobiol.** 12 (2002) 57-63.
143. Freestone, S.H., Marker, P., Grace, O.C., Tomlinson, D.C., Cunha, G.R., Harnden, P. and Thomson, A.A. Sonic hedgehog regulates prostatic growth and epithelial differentiation. **Dev. Biol.** 264 (2003) 352-362.
144. Vezina, C.M. and Bushman, A.W. Hedgehog signaling in prostate growth and benign prostate hyperplasia. **Curr. Urol. Rep.** 8 (2007) 275-280.

145. Ramalho-Santos, M., Melton, D.A. and McMahon, A.P. Hedgehog signals regulate multiple aspects of gastrointestinal development. **Development** 127 (2000) 2763-2772.
146. Sicklick, J.K., Li, Y.X., Jayaraman, A., Kannangai, R., Qi, Y., Vivekanandan, P., Ludlow, J.W., Owzar, K., Chen, W., Torbenson, M.S. and Diehl, A.M. Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. **Carcinogenesis** 27 (2006) 748-757.
147. Yoshikawa, K., Shimada, M., Miyamoto, H., Higashijima, J., Miyatani, T., Nishioka, M., Kurita, N., Iwata, T. and Uehara, H. Sonic hedgehog relates to colorectal carcinogenesis. **J. Gastroenterol.** 44 (2009) 1113-1117.
148. Dormoy, V., Danilin, S., Lindner, V., Thomas, L., Rothhut, S., Coquard, C., Helwig, J.J., Jacqmin, D., Lang, H. and Massfelder, T. The sonic hedgehog signaling pathway is reactivated in human renal cell carcinoma and plays orchestral role in tumor growth. **Mol. Cancer** 8 (2009) 123.
149. Berman, D.M., Karhadkar, S.S., Hallahan, A.R., Pritchard, J.I., Eberhart, C.G., Watkins, D.N., Chen, J.K., Cooper, M.K., Taipale, J., Olson, J.M. and Beachy, P.A. Medulloblastoma growth inhibition by hedgehog pathway blockade. **Science** 297 (2002) 1559-1561.
150. Kubo, M., Nakamura, M., Tasaki, A., Yamanaka, N., Nakashima, H., Nomura, M., Kuroki, S. and Katano, M. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. **Cancer Res.** 64 (2004) 6071-6074.
151. Chen, X., Horiuchi, A., Kikuchi, N., Osada, R., Yoshida, J., Shiozawa, T. and Konishi, I. Hedgehog signal pathway is activated in ovarian carcinomas, correlating with cell proliferation: it's inhibition leads to growth suppression and apoptosis. **Cancer Sci.** 98 (2007) 68-76.
152. Sheng, T., Li, C., Zhang, X., Chi, S., He, N., Chen, K., McCormick, F., Gatalica, Z. and Xie, J. Activation of the hedgehog pathway in advanced prostate cancer. **Mol. Cancer** 3 (2004) 29.
153. Caserta, T.M., Kommagani, R., Yuan, Z.A., Robbins, D.J., Merce, r C.A. and Kadakia, M.P. p63 overexpression induces the expression of sonic hedgehog. **Mol. Cancer Res.** 4 (2006) 759-768.
154. Hatsell, S.J. and Cowin, P. Gli3-mediated repression of Hedgehog targets is required for normal mammary development. **Development** 133 (2006) 3661-3670.
155. Liu, S., Dontu, G., Mantle, I.D., Patel, S., Ahn, N.S., Jackson, K.W., Suri, P. and Wicha, M.S. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. **Cancer Res.** 66 (2006) 6063-6071.
156. Kubo, M., Nakamura, M., Tasaki, A., Yamanaka, N., Nakashima, H., Nomura, M., Kuroki, S. and Katano, M. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. **Cancer Res.** 64 (2004) 6071-6074.

157. Li, N., Singh, S., Cherukuri, P., Li, H., Yuan, Z., Ellisen, L.W., Wang, B., Robbins, D., DiRenzo, J. Reciprocal intraepithelial interactions between TP63 and hedgehog signaling regulate quiescence and activation of progenitor elaboration by mammary stem cells. **Stem Cells** 26 (2008) 1253-1264.
158. Boominathan, L. The guardians of the genome (p53, TA-p73, and TA-p63) are regulators of tumor suppressor miRNAs network. **Cancer Metastasis Rev.** 29 (2010) 613-639.
159. Davidson, M.R., Larsen, J.E., Yang, I.A., Hayward, N.K., Clarke, B.E., Duhig, E.E., Passmore, L.H., Bowman, R.V. and Fong, K.M. MicroRNA-218 is deleted and downregulated in lung squamous cell carcinoma. **PLoS One** 5 (2010) e12560.
160. Melo, S.A. and Esteller, M. Dysregulation of microRNAs in cancer: Playing with fire. **FEBS Lett.** (2010) Epub ahead of print.
161. Grelier, G., Voirin, N., Ay, A.S., Cox, D.G., Chabaud, S., Treilleux, I., Léon-Goddard, S., Rimokh, R., Mikaelian, I., Venoux, C., Puisieux, A., Lasset, C. and Moyret-Lalle, C. Prognostic value of Dicer expression in human breast cancer and association with the mesenchymal phenotype. **Br. J. Cancer.** 101 (2009) 673-683.
162. Wang, Y., Medvid, R., Melton, C., Jaenisch, R. and Blelloch, R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. **Nat. Genet.** 39 (2007) 380-385.
163. Cui, X.S., Shen, X.H. and Kim, N.H. Dicer1 expression in preimplantation mouse embryos: Involvement of Oct3/4 transcription at the blastocyst stage. **Biochem. Biophys. Res. Commun.** 352 (2007) 231-236.
164. Yi, R., Poy, M.N., Stoffel, M. and Fuchs, E. A skin microRNA promotes differentiation by repressing "stemness". **Nature** 452 (2008) 225-229.
165. Scheel, A.H., Beyer, U., Agami, R. and Döbelstein, M. Immunofluorescence-based screening identifies germ cell associated microRNA 302 as an antagonist to p63 expression. **Cell Cycle** 8 (2009) 1426-1432.
166. Lena, A.M., Shalom-Feuerstein, R., Rivetti di Val Cervo, P., Aberdam, D., Knight, R.A., Melino, G. and Candi, E. miR-203 represses "stemness" by repressing DeltaNp63. **Cell Death Differ.** 15 (2008) 1187-1195.
167. Papagiannakopoulos, T., Shapiro, A. and Kosik, K.S. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. **Cancer Res.** 68 (2008) 8164-8172.
168. Manni, I., Artuso, S., Careccia, S., Rizzo, M.G., Baserga, R., Piaggio, G. and Sacchi, A. The microRNA miR-92 increases proliferation of myeloid cells and by targeting p63 modulates the abundance of its isoforms. **FASEB J.** 23 (2009) 3957-3966.
169. Chan, J.A., Krichevsky, A.M. and Kosik, K.S. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. **Cancer Res.** 65 (2005) 6029-6033.

- 170.Si, M.L., Zhu, S., Wu, H., Lu, Z., Wu, F. and Mo, Y.Y. miR-21-mediated tumor growth. **Oncogene** 26 (2007) 2799-2803.
- 171.Meng, F., Henson, R., Lang, M., Wehbe, H., Maheshwari, S., Mendell, J.T., Jiang, J., Schmittgen, T.D. and Patel, T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. **Gastroenterology** 130 (2006) 2113-2129.
- 172.Craig, A.L., Holcakova, J., Finlan, L.E., Nekulova, M., Hrstka, R., Gueven, N., DiRenzo, J., Smith, G., Hupp, T.R. and Vojtesek, B. DeltaNp63 transcriptionally regulates ATM to control p53 Serine-15 phosphorylation. **Mol. Cancer** 9 (2010) 195.
- 173.Reya, T., Morrison, S.J., Clarke, M.F. and Weissman, I.L. Stem cells, cancer, and cancer stem cells. **Nature** 414 (2001) 105-111.
- 174.Tan, B.T., Park, C.Y., Ailles, L.E. and Weissman, I.L. The cancer stem cell hypothesis: a work in progress. **Lab. Invest.** 86 (2006) 1203-1207.
- 175.Schatton, T., Frank, N.Y. and Frank, M.H. Identification and targeting of cancer stem cells. **Bioessays** 31 (2009) 1038-1049.
- 176.Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J. and Clarke, M.F. Prospective identification of tumorigenic breast cancer cells. **Proc. Natl. Acad. Sci. USA** 100 (2003) 3983-3988.
- 177.Prince, M.E., Sivanandan, R., Kaczorowski, A., Wolf, G.T., Kaplan, M.J., Dalerba, P., Weissman, I.L., Clarke, M.F. and Ailles, L.E. Identification of a subpopulation of cells with cancer stem cells properties in head and neck squamous cell carcinoma. **Proc. Natl. Acad. Sci. USA** 104 (2007) 973-978.
- 178.Boldrup, L., Coates, P.J., Gu, X. and Nylander, K. DeltaNp63 isoforms regulate CD44 and keratins 4, 6, 14 and 19 in squamous cell carcinoma of head and neck. **J. Pathol.** 213 (2007) 384-391.
- 179.Du, Z., Li, J., Wang, L., Bian, C., Wang, Q., Liao, L., Dou, X., Bian, X. and Zhao, R.C. Overexpression of $\Delta Np63\alpha$ induces a stem cell phenotype in MCF7 breast carcinoma cell line through the Notch pathway. **Cancer Sci.** 101 (2010) 2417-2424.