Molecular focus in p63 and correlated human diseases

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ABSTRACT

The p63 gene, a member of the p53 gene family, is expressed into at least six protein isoforms.The transcription factor p63 is a homologue of the tumor suppressor p53. Unlike p53, which is dispensable for normal development, p63 is critical for the development of stratified epithelial tissues such as epidermis, breast, and prostate. p63, , is transcribed from two different promoters giving rise to two different proteins: p63, a member of the p53 family, is transcribed from two different promoters giving rise to two different proteins: TAp63 that contains the N-terminal transactivation domain and ΔN that lacks this domain. p63 encodes multiple protein isoforms with both transactivating and transcriptional repressor activities that can regulate a wide spectrum of target genes. p63 is also implicated in tumor formation and progression in stratified epithelia, with evidence for both tumor suppressive and oncogenic properties.

Keywords: p63; p53; isoforms; tumor suppressor; oncogen

p63 gene and protein structure

The human p63 gene resides on chromosome 3q27–29, and consists of 15 exons spread over about 220 kB, with introns up to 100 kB in length (Fig. 1C). p63 can be expressed from two different promoters, one immediately preceding the first exon and a second lying in the third intron. Transcription from the first and second promoters gives rise to TA- or ΔN- aminotermini of p63, respectively. Both TA- and ΔN-transcripts can be alternatively spiced at the carboxy-terminus, leading to α,β, and γ isoforms of TA- and ΔNp63. All p63 proteins encode a DNA-binding domain, which is approximately 60% identical at the amino acid level to the DNA-binding domain of p53, and an oligomerization domain with about 37% identity to that of p53 (Figs. 1A, B). TA isoforms possess an N-terminal acidic transactivation domain with low homology to the transactivation domain of p53 (about 22% identity), while ΔNp63 proteins lack this domain. The different C-termini of α, β, and γ isoforms also contribute to the diversity of p63 proteins; α, but not β and γ, isoforms contain a Sterile alpha-motif (SAM) domain that functions as a protein–protein interaction module in other proteins [6,7], (Fig. 1B). The complexity of p63 transcript and protein expression foretells functional complexity of this gene at the biochemical and biological levels.

Early experiments revealed that TAp63 isoforms could transactivate a reporter gene through a canonical p53 responsive DNA binding site, as well as induce cell death [2,3]. In contrast, ΔNp63 proteins can act in a dominantnegative manner toward p53-mediated transcriptional activation[2]. The DNA binding domains of p53 and p63 are highly homologous; all the amino acid residues in the p53 DNA binding domain that directly contact DNA or coordicoordinate a zinc ion necessary for DNA binding activity are 100% conserved in the p63 DNA binding domain. This implies not only sequence conservation, but also conservation of the structure of these protein domains. NMR studies of the p53 and p63 DNA binding domains have confirmed a highly similar global fold and essentially identical secondary structure elements for these protein domains [8]. Consistent with the sequence and structural homology of the p53 and p63 DNA binding domains, p63 proteins can bind to p53 consensus DNA binding sites in vitro and in vivo [9,10]. Other names for this gene include KET, p51, p40 and p73L [4].

p63 exhibit high amino acid identity with p53, especially among their transactivation (TA) domains, DNA-binding domain (DBD), and tetramerization (ISO) domain (fig. 1). Unlike *TP53, TP63* encode a number of isoforms (fig. 1). For *TP63,* two transcription initiation sites were initially described one that would give rise to proteins containing the TA domain (the TA isotypes) and another that would give rise to proteins lacking this domain (the DNisotypes).For *TP63,* additional transcripts were subsequently uncovered in human and rodents, resulting from both the use of at least four transcription initiation sites and extensive alternative splicing at the 5_ end of the gene. Additionally,extensive alternative splicing is seen at the 3_ end of the gene, resulting in three different C-termini for p63. For *TP63,* differential splicing of intron 8 creates additional variability in the final polypeptide sequences (either GTKRP or A), but the functional significance of this is not known. The extended 3_ coding sequences of the a isotypes of *TP63* encode a protein-protein–interaction motif that resembles the sterile-a-motif (SAM) domain which is not contained in p53. SAM domains are small globular protein-protein–interaction modules that are usually involved in homo- and heterooligomerization with other SAM domains. It has been demonstrated that the p63 SAM domains do not oligomerize with one another and the interacting proteins still need to be identified[11].

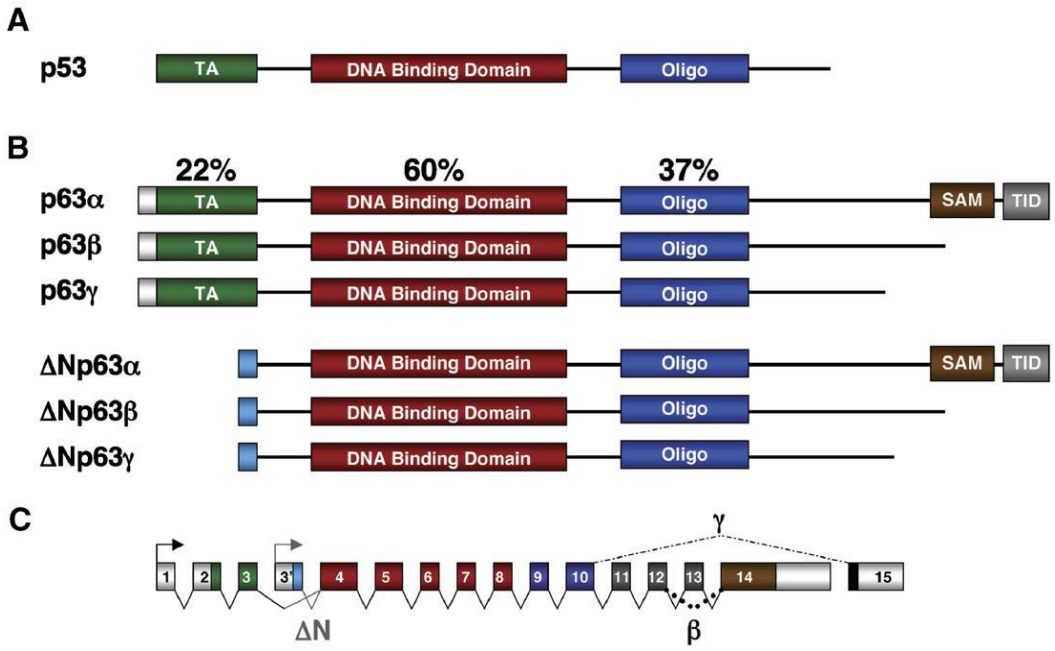

Fig. 1 – Functional domains of p53 and p63 proteins. (A) Functional domains of p53. p53 is composed of three primary domains: an N-terminal transactivation domain (TA), a central DNA binding domain, and a C-terminal oligomerization domain (Oligo). (B)Functional domains of p63 proteins. Percentages represent p53-identical residues found in p63. In addition, p63 has additional C-terminal domains called the sterile alpha motif (SAM) domain and transactivation inhibitory domain (TID). (C) Gene structure of p63. Both promoters and the #N and α, β, γ splicing events are shown.

Differential Properties of the p63 Isotypes

The *p*63 gene, is expressed into at least six protein isoforms which are divided into two groups, those containing the transcription activation domain (TA isoforms) and those that do not (N_isoforms). The TA isoforms are similar to p53 in that they are able to activate transcription of specific target genes and induce cell cycle arrest and apoptosis. Apoptosis is referred to as programmed cell death. The mechanisms controlling apoptosis remain largely obscure.

While there is considerable variation in the signals and requisite cellular metabolic events

necessary to induce apoptosis in diverse cell types, the morphological features associated with apoptosis are highly conserved [107,108]. Increase of cytosol calcium can affect the potential for mitochondrion membrane permeability and leaking cytochrom C and induce apoptosis[109,110]

The _N isoforms are unable to activate transcription, and act in adominant negative manner, inhibiting transcription activation by both p53 and TA isoformsThe functional significance of p63 in regulating cell proliferation in various stratified epithelial cells has previously been proposed.To investigate how these isoforms are used in ocular surface epithelia, the spatial distribution of p63 isoforms within human ocular surface epithelia.Regarding the C-terminal region, an α-isoform specific region was detected in all layers of the conjunctiva and limbus, as well as in the basal to intermediate layers of the cornea. β-isoform specific regionwas detected in the basal to intermediate layers of the limbus. γ-isoform specific region was detected in almost all layers of all epithelia.Among the six p63 isoforms, only ΔNp63a was detected in the basal to intermediate layers of the limbus and conjunctiva. These resultssuggest that ΔNp63a is the most dominant isoform within human ocular surface epithelia. This isoform may contribute, at least in part, to themaintenance of cell proliferative capacity within the ocular surface epith.

From the primary sequence, one would predict that only the p63 isotypes, which contain the acidic TA domain, have transactivation activity, whereas the ΔN isotypes, which lack this domain, do not have transactivation activity.Although this is generally true, there are still some exceptions to this rule. The largest p63 isotype, TAp63a , is unable to drive transcription on the optimized p53-responsive element PG13, in contrast to TA-p63b and TA-p63g. (fig. 1). This unexpected lack of activity is caused by an inhibitory effect that is contained within the a-specific C-terminal end. This inhibitory activity of the a tail also acts in *trans* toward TA-p63b/g transcriptional activation, indicating that the various p63 isotypes can have opposing properties. The repressive activity has been mapped to the region, downstream from the SAM domain, that has been denoted as the "transactivation inhibitory domain" (TID). Tentative evidence suggests the presence of other regions within p63 that either promote or repress transactivation activity. Interestingly, activation of transcription can be mediated by p63 domains other than the canonical TA domain[11]. In addition, there is accumulating evidence that TAp63 isoforms can be transcriptionally active at levels below the limit of detection by Western blot [14,36]. For this reason, the participation of TAp63 isoforms in the overall function of the p63 gene cannot be ruled out. However, we can unequivocally conclude that ΔNp63α plays a critical role in the biological function of the p63 gene. For this reason, the biochemical activity of the ΔNp63α protein merits further attention[1].

Expression patterns of p63 in adult tissues

p63 is expressed in a confined manner, with the highest expression found in the basal cells of various epithelial tissues and the ΔNp63α transcripts being the most abundant.

Expression patterns of p63 provide insight to its biological role.p63 is immunolocalized in the basal layers of stratified epithelial tissues. These include stratified squamous tissues,such as the epidermis, oral mucosa, and cervical epithelium;transitional epithelium, found in the mucosa of the urinary bladder; and complex glands, including the prostate and mammary, salivary, and lacrimal glands [2,40,41]. Generally, p63 protein expression is restricted to the basal layers of these epithelial structures, which lie directly on the basement membrane. This basal compartment of stratified epithelia is often considered to harbor cells of high proliferative capacity,which replenish the terminally differentiated populations in the more luminal strata [26]. p63 is also highly expressed in cancers derived from these tissues, including squamous cell carcinomas of the head and neck [40]. The lack of stratified epithelia in p63 −/− mice, combined with its normal expression in basal epithelia cells and squamous cancer cells, led to the hypothesis that p63 is required for the maintenance or differentiation of progenitor cell populations necessary for epithelial development[1].

Transcriptional regulation of p63 expression

The highly restricted expression patterns of p63 in normal and malignant tissues imply conditional and coordinated regulation of p63 expression. Despite increasing knowledge about the biological function of p63 in the tissues in which it is expressed, relatively little is known about the mechanisms governing transcription of the p63 gene. ΔNp63α plays a role in maintaining the viability and proliferative capacity of basal epithelial cells, therefore ΔNp63α expression may be controlled in part by upstream signals involved in the survival or proliferative capacity of these cells. One study has implicated EGFR signaling in regulation of ΔNp63α expression [47].p63 plays a critical role in embryonic development, and understanding its transcriptional regulation during this period can expose signaling pathways in which p63 is involved.ΔNp63α expression is directly induced by bone morphogenetic proteins (BMPs) during zebrafish development through binding sites for Smad4 and Smad5 in the ΔNp63 promoter [16].BMPs are growth factors which act as important determinants of cell fate and tissue lineage [51]. ΔNp63α transcript levels decline in both epidermal tissue and mammary cell lines after treatment with DNA damaging agents such as UV radiation, cisplatin, or adriamycin[28,52]. Interestingly, this may be mediated by the recruitment of ΔNp63α protein to a binding site in its own promoter following DNA damage, thereby repressing its own transcription [52]. UV-B-induced DNA damage decreases levels of DN-p63a (a naturally occurring dominant-negative form of the protein),before increasing levels of p53. Simultaneously, the levels of the transactivating TA-p63 isoforms increase. The down-regulation of dominant-negative DN-p63a, as well as the up-regulation that activates TA-p63 isoforms, may be a prerequisite for UV-induced apoptosis in skin This notion is supported by the recent observation that the transactivating TA-p63a isoforms are required for p53-dependent apoptosis induced by DNA damage. The role that this switch from inhibitory to activating p63 isoforms plays in normal skin development[11].

Regulation of p63 protein—stability and post-translational modifications

Like p53, ΔNp63α exists as a phosphoprotein [9], and phosphorylation of ΔNp63α increases following DNA damage or other cell stresses [43,54]. Coincident with this stress induced phosphorylation is an increase in the ubiqitination and proteosomal degradation of the ΔNp63α protein [43]. It has been hypothesized that the inverse regulation of p53 and ΔNp63α protein stability represent a mechanism allowing rapid modulation of coordinately regulated target genes destabilizing a repressor while stabilizing a transactivator through simultaneously executed similar mechanisms. In contrast to ΔNp63α, ectopically expressed TAp63 proteins can accumulate in response to genotoxic stress, but no evidence of endogenous TAp63 regulation by this mechanism has been reported [55,56]. p63 proteins can also be sumoylated, potentially affecting stability and transcriptional activity [57,58]. Interestingly, the stability of p63 proteins appears to be inversely correlated with their transactivation ability. TAp63γ, which is the most active transactivator of the p63 isoforms, is often undetectable by Western blot, even in amounts that have robust transcriptional activity [36]. TAp63α, with lower transactivation potential,is more easily detected, but deletion of the C-terminal TID from TAp63α increases its transcriptional activity and reduces protein expression levels [36]. it is possible that p63 stability are controlled through a similar mechanism of negative feedback. However, it is clear that Mdm2 itself is not a factor in p63 degradation [55,64–66]. Continued study of p63 post-translational modification and protein stability will provide insight to common and distinct mechanisms of regulation of the p53 family.All the aforementioned instances of regulation of ΔNp63α expression upregulation by activation of the pro-survival PI3K pathway, and downregulation by genotoxic stress and cell differentiation are consistent with the proposed function of p63 as a transcription factor that acts to maintain the viability and proliferative capacity of basal epithelial cells. However,these findings are also consistent with p63 being merely a marker for the viability and proliferative capacity of these cells.Additional information is necessary to confer functional importance namely, target genes modulated by p63[1].

DNA binding specificity of p63

The DNA binding domain of p63 retains significant homology to that of p53, and p63 proteins can bind to p53 consensus DNA binding sites in vitro and in vivo [9,10,70]. However, the divergent biological roles of these two genes imply that they regulate distinctsubsets of target genes. This paradox can be partially explained by the existence of a distinct p63 consensus DNA binding site, to which p63 proteins will bind preferentially.

Identification of p63 target genes

A new target gene Scotin induced byTAp63 during epithelial differentiation. This gene was previously isolated as a p53-inducible proapoptotic gene and the protein is located in the endoplasmic reticulum and in the nuclear membrane. Scotin expression is induced in response to endoplasmic reticulum (ER) stress in a p53 dependent or independent manner. We detected Scotin upregulation in primary keratinocyte cell lines committed to differentiate.
Scotin also is expressed in the supra basal layer of the epidermis in parallel with TAp63, but not ΔNp63 expression. We conclude that Scotin is a new p63 target gene induced during epithelial differentiation, a complex process that also involves ER stress induction[12].
Similar, but distinctive DNA-binding specificities implies distinct but overlapping subsets of target genes for p53 and p63 (Fig. 2). This idea is supported by the genes identified as targets of direct p63 regulation. p63 can regulate transcription of the well-characterized p53 target genes p21, 14-3-3σ, and GADD45α [5,9,35]. p21 is an inhibitor of cyclin-dependent kinases (cdks) that arrests cell cycle progression [71]; 14-3-3σ similarly inhibits cell proliferation by sequestering the proteins (cyclin B1 and cdc2) that initiate mitosis [72]. p53 induces expression of these genes following DNA damage, thereby preventing further cell proliferation [73,74]. p21- and 14-3-3σ- mediated growth arrest is important not only in the response to genotoxic stress, but also in cell cycle withdrawal characteristic of terminally differentiating cells [75–77]. ΔNp63α binds to p53-responsive elements in the promoters of both p21 and 14-3-3σ in vitro and in vivo [9]. Interestingly, ΔNp63α binds to one of the 14-3-3σ sites with higher affinity than p53; this site displays divergence from the perfect p53 consensus sequence, and supports the hypothesis that p63 can bind to certain DNA sequences preferentially compared to p53 [9].ΔNp63α represses transcription through these binding sites,and loss of ΔNp63α expression during keratinocyte differentiation corresponds with increased transcription of p21 and 14-3-3σ, and cell cycle withdrawal [9]. This implies a model of coordinate regulation of p21 and 14-3-3σ by ΔNp63α and p53, in which ΔNp63α represses transcription of these genes, and either loss of ΔNp63α-mediated repression during differentiation or transactivation by p53 following cell stress will result in upregulation of p21 and 14-3-3σ expression. Another example of a gene coordinately regulated by p53 and p63 is REDD1, a mediator of reactive oxygen species (ROS) and oxygen stress sensitivity. REDD1 is upregulated by p53 following cell stress; however, REDD1 expression colocalizes with p63 expression during development, and is virtually absent in p63 −/− mice [78].

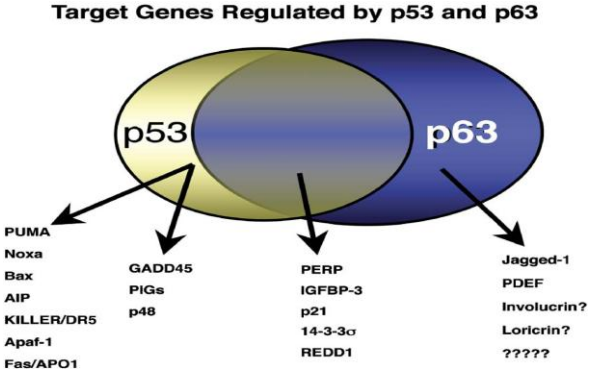

Figure 2. p53 and p63 regulate overlapping but distinct subsets of target genes. Diagram showing a proposed model for the relationship between p53 and p63 target genes. Genes reported to fall into each category of regulation are listed below[1].

Few genes have been identified as exclusive p63 target genes.The gene Jagged1 (JAG1) is directly regulated by p63 proteins but not p53 [81]. JAG1 encodes a ligand for Notch receptors;Notch signaling is critical for cell fate determination, and influences limb and craniofacial development, suggesting that p63 regulation of Notch signaling may play a role during embryogenesis [82,83]. Pigment epithelium derived factor (PEDF) is another gene recently identified as a target of p63, but not p53 [84]. Other genes that play a role in epidermal development and differentiation have been observed to be regulated by p63, such as the keratinocyte differentiation markers loricrin and involucrin [30]; however, whether these are direct target genes has not been established. In addition, a microarray screen has identified a large number of potentially p63-regulated genes using ectopically overexpressed p63 proteins in a non-epithelial cell line [85]. An approach to identify genes regulated by endogenous p63 in squamous cells will likely provide insight to transcriptional programs regulated by p63 under physiologically relevant conditions.

Target genes—p53 regulated, p63 regulated, or both?

The physiological role and functional significance of the target gene may be the most important consideration in defining it as a p53 or p63 target gene. For instance, PERP was originally identified as a p53 target gene [79]; however, the recent report that PERP is regulated by p63 and plays an important role in maintenance of epithelial integrity suggests that it should be functionally considered a p63 target gene [80]. It has also been shown that IGFBP-3 is a direct target gene of p63, and is negatively regulated by ΔNp63α in vivo [29]. IGFBP-3 was previously reported to be a p53 target gene; however, the relevance of IGFBP-3 in p53-mediated cell death is tenuous. These data suggest that regulation of IGFBP-3 by p63 may be more physiologically relevant[1].

Role of p63 in tumor suppressor

One study suggests that p63 +/− mice show an increased susceptibility to tumor formation, and that tumors forming in these mice often display loss of heterozygosity (LOH) for the remaining wild-type p63 allele [86]. Despite these data, a number of observations contradict the idea that p63 acts as a tumor suppressor in humans. p53 is the most commonly mutated gene in human cancer, supporting its role as a crucial tumor suppressor; in contrast, the p63 gene is very rarely mutated in human tumors or cancer cell lines [3,87]. In addition, LOH at chromosome 17p13, where p53 resides, is a common event during tumorigenesis that allows the elimination of a wild-type p53 allele [88–91]. No LOH occurs at the p63 locus in cancer; in fact, the 3q27–29 region containing the p63 gene is amplified in a number of human malignancies [92,93].

The most compelling evidence refuting a tumor suppressive role for p63 came with establishment of p63 −/− mice. p53 −/− mice are developmentally normal but highly susceptible to the rapid development of spontaneous tumors [94]. Mice deficient in other tumor suppressors, such as p16INK4A and p19ARF, similarly develop tumors at an early age [95]. In contrast, p63 −/− mice display gross developmental abnormalities. The most striking of these is a complete lack of all stratified squamous epithelia and their derivatives, including epidermis, mammary glands, prostate, and other tissues [11,12]. These data place the primary biological role of p63 outside the realm of tumor suppression governed by its more famous sibling, p53.

Role of p63 in apoptosis

Several studies have suggested that p63 is involved in apoptotic signaling, but its role this process remains controversial. Necrosis and apoptosis are two main separated

pathways for cell death. Apoptosis is a planned and genetically controlled cell death.

Expression of genes inducing apoptosis like BAX which are important for formation of membrane channels of mitochondrion and cytochrom C

leakage and in long periods, presence of active Protein, BAX is more prominent [111].
pathway loss due to over aggregation of glycogen in muscle cells and make apoptosis by phosphorylation of P65 and gene expression of NF.KB of muscle cell[112].Influenza virus by activating endogenous pathways of apoptosis through the expression of Excessive protein BAX and BCL inhibit the formation of channels and mitochondrial cytochrome c and output can be caused to induce apoptosis[114,113].

But role of cytokines are remarkable Search activity in the virus infected cells and commissioning process with the presence of inflammatory cytokines such as TNF-α can route FAS / FASL-induced apoptosis is caused [115-117].

The TA isoforms of p63 can bind to p53-consensus and induce p53-target genes.The TAp63_ isoform is the weakest transcription activator because of the presence of an inhibitory domain in its C-terminus[13].

TAp63 protein can induce apoptosis similar to p53, and both death receptor and mitochondrial apoptotic pathways have been implicated in this process [100]. In contrast, ΔNp63α appears to oppose apoptosis. Forced expression of ΔNp63α in mouse epidermis results in a reduction in UV-induced apoptosis [28], and disruption of ΔNp63α expression in squamous carcinoma cells increases sensitivity to apoptosis inducing agents [29]. In addition,ΔNp63α expression can be regulated by the PI3K pathway, a potent inhibitor of apoptosis [48]. Finally, ΔNp63α negatively regulates transcription of a pro-apoptotic gene, IGFBP-3 [29]. The role of other p63 isotypes in apoptosis is unclear.One study employing p63 −/− MEFs as a model system concluded that p63 was necessary for p53-mediated apoptosis [10]. In contrast, it was recently reported that p63 is completely dispensable for p53-dependent apoptosis in T cells [101]. The precise role of p63 proteins in apoptosis, the DNA-damage response, and modulation of p53 signaling will be elucidated with additional genetic and biochemical analyses. (figure 3)

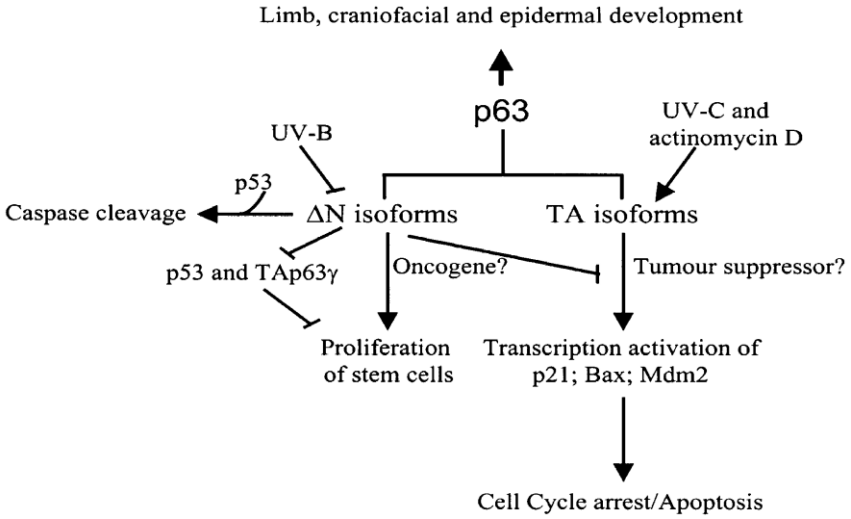

Fig. 3. The p63 isoforms can have opposing functions but a balance of these is required for normal development. The TA isoforms of p63, which may act as tumour suppressors, activate the transcription of genes ultimately leading to cell cycle arrest or apoptosis. The _N isoforms act in a dominant negative fashion, counteracting the effects of p53 and the TA isoforms, thus allowing for proliferation of stem cells. The regulation of these isoforms is critical for development of epithelial structures.

Role of p63 in human cancers

p63 is rarely mutated in human cancers. The majority of tumors maintain p63 expression, and in many cases p63 appears to be over expressed or the p63 locus is amplified, consistent with p63 performing a pro-proliferative or oncogenic role. A potential role for p63 in tumorigenesis is supported by the finding that p63 is a target of

genomic amplification and/or over expression in >80% of primary head and neck squamous cell carcinomas (HNSCC) as well as other squamous epithelial malignancies (Table 1). A genome-wide micro-array screen of non-small cell lung cancer revealed that the 3q26-29 locus encompassing p63 is frequently amplified in squamous cell carcinomas of the lung, suggesting that over expression of p63 facilitates tumorigenesis A study of 245
esophageal tumors demonstrated that both Tap63 and _Np63 isoforms are specifically upregulated at the transcript level in squamous cell carcinoma, and _Np63 was the predominant isoform expressed at the protein level \ Some tumor types have been reported to lose p63 expression, suggesting that p63 loss accelerates tumorigenesis. This is supported by in vitro data which reveal that disruption of p63 in squamous cell lines resulted in upregulation of genes associated with increased capacity for invasion and metastasis in tumors .(Table 1) gives an overview of p63 expression in different human tumor entities.

Table 1
p63 expression in human cancer

Organ/tissue	Isoform expressed	Comment	References
Esophagus: esophageal adenocarcinoma squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EA), Barrett's esophagus (BE)	ΔNp63	ΔNp63 expression in all squamous tissues	Glickman et al. (2001)
	Not determined	Protein expression in squamous cell carcinoma	Hara et al. (2004)
	Not determined	Nuclear expression increases with severity of neoplastic changes in BE	Hall et al. (2000)
	TAp63 and ΔNp63	Protein and mRNA expression of TAp63 and ΔNp63	Geddert et al. (2003)
	ΔNp63	ΔNp63 mRNA expression	Hu et al. (2002)
	TAp63 and Np63	TAp63 and ΔNp63 overexpressed in ESCC but not in EA	Cui et al. (2005)
Gastric cancer	TAp63 and ΔNp63	Tumor specific upregulation of TAp63 and ΔNp63	Tannapfel et al. (2001)
	TAp63	Tumor specific upregulation of TAp63γ	Huang and Xie (2002)
Pancreatic cancer	Not determined	Tumor specific upregulation	Ito et al. (2001)
	Not determined	Only in metastatic pancreatic adenocarcinoma	Hornick et al. (2005)
Bladder cancer	TAp63 and ΔNp63	Expression of ΔNp63 only in tumor tissue	Park et al. (2000), Urist et al. (2002)
	TAp63 and ΔNp63	Impaired TAp63 and ΔNp63 expression is associated with tumor grade, tumor stage and lymph node metastasis	Koga et al. (2003a)
Urothelial cancer	TAp63 and ΔNp63	Protein expression	Koga et al. (2003b)
Lung cancer	Not determined	Squamous cell carcinoma	Reis-Filho et al. (2003), Tonon et al. (2005)
	ΔNp63	Overexpression of ΔNp63 in tumor tissue	Valerie and Povirk (2003)
	TAp63	No p63 mutations	Tani et al. (1999)
	TAp63 and ΔNp63	Protein expression of TAp63 and ΔNp63	Hibi et al. (2000), Yamaguchi et al. (2000), Wang et al. (2002a), Massion et al. (2003)
Breast cancer	ΔNp63	Overexpression of ΔNp63	Barbareschi et al. (2001), Wang et al. (2002b), Ribeiro-Silva et al. (2003), Reis-Filho et al. (2003)
Ovarian cancer	ΔNp63	Protein expression	Reis-Filho et al. (2003)
Head and neck cancer	TAp63 and ΔNp63	Expression of ΔNp63 is correlated with clinical response to chemotherapy	Zangen et al. (2005)
	TAp63 and ΔNp63	Expression of TAp63 and ΔNp63 in Squamous cell carcinoma	Hibi et al. (2000), Yamaguchi et al. (2000), Crook et al. (2000), Choi et al. (2002)
Prostate cancer	ΔNp63	Expression of ΔNp63	Weinstein et al. (2002), Davis et al. (2002), Iczkowski et al. (2003)
Cervix cancer	Not determined ΔNp63	Squamous cell carcinoma protein expression	Nishi et al. (1999), Cviko et al. (2000), Wang et al. (2001), Quade et al. (2001), Cho et al. (2003), Lin et al. (2006)
Uterine cancer	Not determined	Protein expression	Koga et al. (2003b)

Role of p63 in Mammalian Embryonic Development

Immunohistochemical analyses of mouse embryos show high p63 levels in epithelial cells, especially in progenitor or stem-cell populations of epithelial tissues. The main isotype in these cells is the dominant-negative ΔN-p63a isotype, which likely acts in the maintenance of the proliferative capacity of such cells. As these cells start to differentiate, their ΔN-p63a levels gradually drop, and the levels of TA-p63 increase[15]. It thus appears that dominant-negative ΔN-p63a is crucial for the maintenance of the capacity of regenerative proliferation of epithelial stem cells. Indeed, application of retinoic acid, which prevents degradation of ΔN-p63a, effectively blocks the differentiation of skin epithelial stem cells in culture.In mouse embryos, *TP63* expression is first evident in nuclei of cells in the basal layer, which develop into the progenitor cells of the epidermis and related derivatives,such as hair and sweat glands. Basal

cells of the cervix,tongue, esophagus, mammary glands, prostate, and urothelium also show high levels of p63. Early *TP63* expression is further evident in ectodermal cells of the limb buds and tail bud, branchial arches, and the oral epithelium. In the developing limb bud, *TP63* expression is restricted to the apical ectodermal ridge (AER),a key determinant of limb-bud emergence and progression.Proper signaling along the antero-posterior axis between the AER and the underlying mesoderm is crucial for normal formation of the distal limb.

The sites of *TP63* expression are well in line with the phenotypic consequences of homozygous *TP63* inactivation in mice. These p63-deficient newborns exhibit striking limb defects. The forelimbs are severely truncated, and the hindlimbs are lacking altogether. The skin of the knockout animals is absent, and newborn animals die from dehydration shortly after birth. Other skin derivatives,such as hair shafts and follicles are not present.Finally, p63-deficient animals lack tooth primordia and eyelids. Both the maxilla and the mandible are truncated,and the secondary palate fails to close. Taken together, the defects in p63-deficient mice present as severe ectodermal dysplasia, abnormal limb development,and facial dysmorphism[11].

Upstream and Downstream from p63: Smad and Jagged

Although one may speculate that p63 is involved in epidermal differentiation through loricrin and involucrin. The first and only bona fide target genes for p63 are *Jagged1* (*JAG1*) and Jagged2 (*JAG2*), which encode ligands for Notch receptors.A cDNA microarray analysis showed an increased *JAG1* and *JAG2* expression in cell lines that were transfected with adenoviral vectors expressing TA-p63g. The physiological significance of this result was convincingly demonstrated by chromatin-immunoprecipitation experiments,which revealed binding of TA-p63g to promoter elements of *JAG1* in vivo. Also, co-culturing of Notch1 expressing Jurkat cells with p63-transfected cells led to an up-regulation of *HES-1*, a downstream target of Notch signaling. This indicates that p63 can trigger the Notch pathway in neighboring cells, possibly by induction of *JAG1* and *JAG2*. Although *JAG1* mutations cause Alagille syndrome in humans, no human disease has been linked to *JAG2* mutations. Interestingly, mice with homozygous inactivating *Jag2* mutations have syndactyly and defective craniofacial development, including cleft palate (CP) (Sidow et al. 1997; Jiang et al. 1998). Much work still needs to be done to elucidate other in vivo targets of p63 transactivation and to determine the downstream effects of this transactivation[11].

TP 63 and disease : EEC-like Family Syndromes

In 1999, linkage mapping of human EEC-like syndromes identified a locus on 3q27, coinciding with the localization of *TP63*. At the same time, these results established that germline mutations in p63 are not associated with a cancer-prone phenotype, as is the case for p53/Li-Fraumeni syndrome. Moreover, the implication of p63 in EEC syndrome paved the way to testing of the *TP63* gene in the EEC-like syndromes, and by that, provided insight into the molecular mechanisms underlying this group of disorders (table 2).A group of multiple-congenital-anomaly syndromes is characterized by EEC. The prototypic EEC syndrome has this triad of features. EEC syndrome frequently presents with other associated anomalies, such as lacrimal-tract anomalies, urogenital anomalies, anal atresia, and conductive hearing loss. EEC syndrome is relatively common, with 1200 cases having been reported in the literature, and is well known for having both variable expressivity and reduced penetrance. A comparison of interfamilial and intrafamilial variability in expressivity found significantly greater interfamilial variability, suggesting that more than one gene or allele might be involved.

Table 2

***TP63* Mutations in EEC Syndrome, LMS, AEC Syndrome, ADULT Syndrome, and Isolated SHFM**

Amino Acid Change	No. of Families (n = 78)	CpG Islet[a]	Disorder(s)	Reference(s)
Exon 3[b]:				
N6H	1	−	ADULT syndrome	Amiel et al. 2001
Exon 4:				
G76W	1	−	LMS	Authors' unpublished data
Exon 5:				
IVS4-2A→C[b]	1	−	SHFM	van Bokhoven et al. 2001
Y163C	1	−	EEC syndrome	Authors' unpublished data
Y192C	2	−	EE syndrome	Authors' unpublished data
K193E	1	−	SHFM	van Bokhoven et al. 2001
K194E	1	−	SHFM	Ianakiev et al. 2000
V202M	1	−	EE syndrome	Authors' unpublished data
Exon 6:				
R204W	6	+	EEC syndrome	Celli et al. 1999, van Bokhoven et al. 2001
R204Q	4	+	EEC syndrome	van Bokhoven et al. 2001, authors' unpublished data
R227Q	4	+	EEC syndrome	Authors' unpublished data
Exon 7:				
C269Y	1	−	EEC syndrome	van Bokhoven et al. 2001
S272N	1	−	EEC syndrome	Celli et al. 1999
C273Y	1	−	EEC syndrome	Authors' unpublished data
R279H	8	+	EEC syndrome	Celli et al. 1999, Ianakiev et al. 2000, van Bokhoven et al. 2001, authors' unpublished data
R279C	3	+	EEC syndrome	Kosaki et al. 2001, van Bokhoven et al. 2001
R279Q	1	−	EEC syndrome	van Bokhoven et al. 2001
R280C	5	+	EEC syndrome, SHFM	Ianakiev et al. 2000, van Bokhoven et al. 2001, authors' unpublished data
R280S	1	+	EEC syndrome	van Bokhoven et al. 2001
R280H	2	+	EEC syndrome, SHFM	van Bokhoven et al. 2001
R298Q	2	+	ADULT syndrome	Duijf et al. 2002, authors' unpublished data
Exon 8:				
R304W	4	+	EEC syndrome	Celli et al. 1999, Wessagowit et al. 2000, van Bokhoven et al. 2001, authors' unpublished data
R304Q	6	+	EEC syndrome	Ianakiev et al. 2000, van Bokhoven et al. 2001
C306R	1	−	EEC syndrome	Celli et al. 1999
C308S	1	−	EEC syndrome	van Bokhoven et al. 2001
P309S	1	−	EEC syndrome	van Bokhoven et al. 2001
D312H	1	−	EEC syndrome	van Bokhoven et al. 2001
Exon 11:				
IVS10-2A→G[b]	1	−	AEC syndrome	Barrow et al., in press
Exon 13:				
L518V	1	−	AEC syndrome	McGrath et al. 2001
L518F	1	−	AEC syndrome	McGrath et al. 2001
C526G	1	−	AEC syndrome	McGrath et al. 2001
C526W	1	−	AEC syndrome	McGrath et al. 2001
1689InsA	1	−	EEC syndrome	Celli et al. 1999
1693-1694DelTT	1	−	LMS	van Bokhoven et al. 2001
G534V	1	−	AEC syndrome	McGrath et al. 2001
T537P	1	−	AEC syndrome	McGrath et al. 2001
Q540L	1	−	AEC syndrome	McGrath et al. 2001
I541T	2	−	AEC syndrome	McGrath et al. 2001, authors' unpublished data
Exon 14:				
1859DelC	1	−	AEC syndrome	Authors' unpublished data
1860-1861DelAA	1	−	LMS	van Bokhoven et al. 2001
Q634X	1	−	SHFM	van Bokhoven et al. 2001
E639X	1	−	SHFM	Authors' unpublished data

[a] + = Mutation at a CpG site (n = 45); − = mutation not at a CpG site (n = 33).
[b] Intron mutation detected on analysis of the indicated exons.

Several autosomal dominant syndromes have been described that share features with EEC including lacrimo-auricular-dental-digital (LADD) syndrome (MIM149730) and LMS. Bamshad et al. (2000) proposed the combination of the aforementioned four syndromes as "LEAD syndrome" (named for limb, lacrimal, ectodermal, and apocrine dysplasia). Other dominant syndromes resemble the EEC syndrome in only one or two of the cardinal features; for example, AEC syndrome(also known as "Hay-Wells syndrome") and Rapp-Hodgkin syndrome (RHS [MIM 129400]) lack ectrodactyly,the ectrodactyly–cleft palate (ECP) syndrome(MIM

129830) lacks ectodermal dysplasia, ADULT syndrome and the ectrodactyly–ectodermal dysplasia (EE) syndrome (MIM 129810) lack cleft lip with or without cleft palate (CL/P), and isolated SHFM is characterized only by ectrodactyly[11]. We can see the highest rate of mutation in exson7 then exon6 and exon8 in table2.

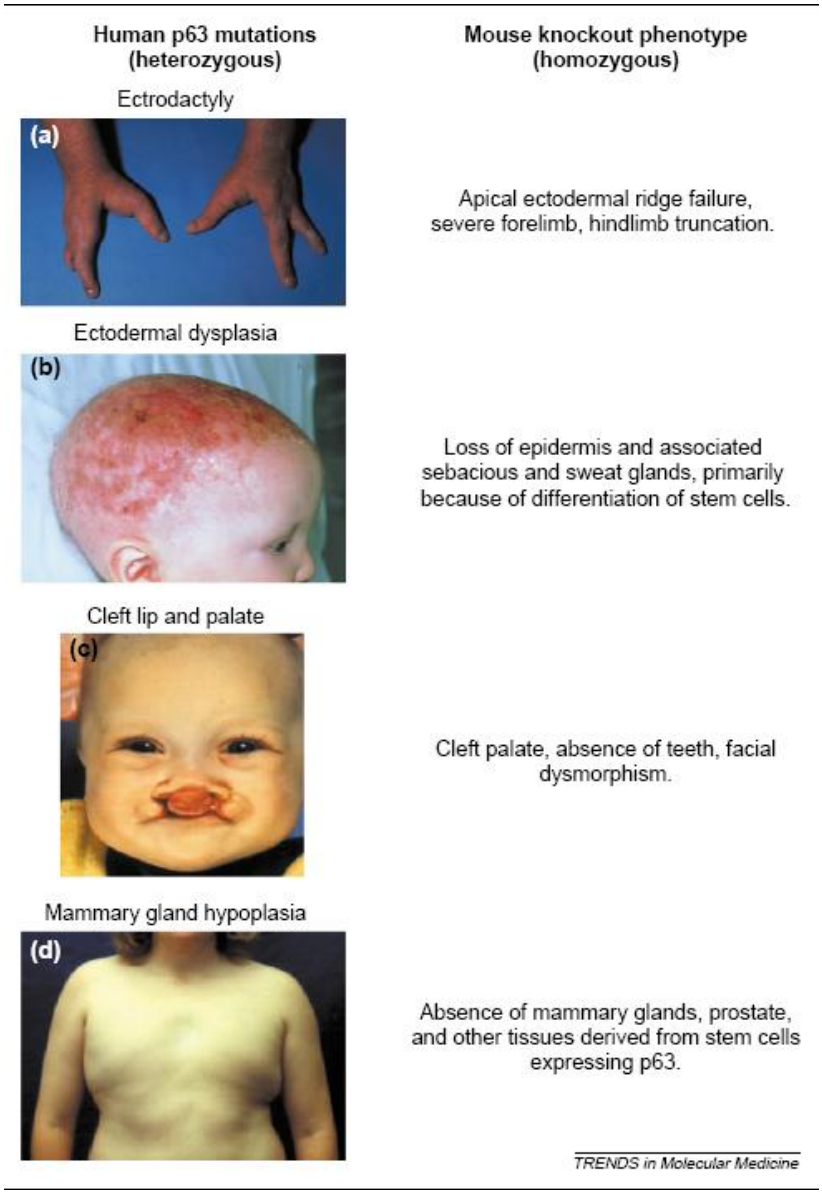

Fig. 4. Comparison of p63 phenotypes in humans and mice. Representative phenotypes of Human syndromes involving p63 mutations, including (a) ectrodactyly, (b) ectodermal dysplasia including absence of hair and eyebrows, and skin infections, (c) cleft lip and palate and lacrimal ductobstruction, and (d) mammary gland hypoplasia and absence of nipples

EEC Syndrome

To date, 20 different heterozygous p63 mutations in 53 families with EEC syndrome are known (reported by Celli et al. [1999], Ianakiev et al. [2000], Wessagowit et al.[2000], Kosaki et al. [2001], and van Bokhoven et al.[2001]. All except one of the mutations in families with EEC syndrome give rise to amino acid substitutions in the DBD that is common to all known p63 isoforms. The arginine codons 204, 227, 279, 280,

and 304 were mutated in several unrelated patients. These amino acids are crucially important for direct interactions with DNA target sequences, and their mutation is highly detrimental to DNA binding and transactivation activity. One explanation is the high mutability of the corresponding codons. Indeed, 46 of the 51 mutations in families with EEC syndrome are CrT transitions at CpG sites. Hence, the high mutability of 5-methylcytosine at CpG sites is a likely explanation for the high proportion of recurrent mutations in EEC syndrome. These data established that missense mutations in EEC syndrome disrupt DNA binding for all p63 isotypes. The effects on transactivation will differ, however,depending on the sum of the transactivating TAp63g and the dominant-negative ΔN-p63a activities, thereby making it difficult to predict the net result on transactivation in vivo. A single frameshift mutation found in a patient with EEC syndrome did not disrupt the DNA-binding capacity. Strikingly, this frameshift mutation, which affects the p63a isotypes only, conferred a gain of transactivation on the otherwise repressive ΔN-p63a isotype .

AEC (Hay-Wells) Syndrome

AEC syndrome, which is also known as "Hay-Wells syndrome,"has little or no limb involvement but instead includes ankyloblepharon, which is a partial or complete fusion of the eyelids that is very rare in other EEC-like syndromes (Hay and Wells 1976). Also, the ectodermal dysplasia is much more pronounced in AEC than in the other EEC-like syndromes. Severe infections of the scalp are common during the first years of life. Mutations in 12 unrelated patients with AEC have been detected, and 10 of these mutations are missense G76W) just upstream from the TA domain (P. Duijf, personal communication).

SHFM

SHFM is genetically heterogeneous, and three loci have previously been identified by linkage analysis and study of *SHFM1,* on 7q21-q22; *SHFM2* (MIM 313350), on Xq26; and *SHFM3* (MIM 600095), on 10q24. A subsequent analysis of a group of ~50 unrelated patients with SHFM revealed five mutations, suggesting that p63 mutations within the SAM domain of p63 . These mutations are predicted to disrupt protein-protein interactions,by either destroying the compact globular structure of the SAM domain or substituting amino acids that are crucial for such interactions (McGrath et al.2001). Tentative evidence indicates that the effects of the SAM-domain mutations varies for different isotypes and at different DNA target sites (L. Guerrini, personal communication).For the functional and developmental consequences of these mutations to be better understood, it will be necessary to identify the protein(s) interacting with the SAM domain[11].

ADULT Syndrome

ADULT syndrome differs from EEC syndrome by the absence of facial clefting in patients with the former (Propping and Zerres 1993). Instead, these patients show neurodermitic signs—namely, exfoliative dermatitis of the digits—and excessive freckling. Another missense mutation was reported in an isolated patient with features of ADULT syndrome. This mutation lies in exon 3_ and results in a substitution (N6H) that is specific to the DN-p63 isotypes[11].

LMS

Phenotypically, LMS is most similar to ADULT syndrome (Propping et al.2000). Three different mutations have been detected in patients with LMS. Two isolated patients with an LMS phenotype have, in exons 13 and 14, frameshift mutations that result in truncations of the p63a protein.Therefore, the abundant p63 product in epithelial cells would be missing the TID. The third mutation was identified in the large Dutch family with LMS. The mutation is in exon 4 and creates a substitution (mutations account for ~10% of these cases (van Bokhoven et al. 2001). Five of the seven p63 mutations seen in patients with SHFM are unique to this syndrome—namely, missense mutations K193E and K194E, nonsense mutations Q634X and E639X, and splice-site mutation IVS4-2ArC (which causes the insertion of a proline residue at position 233). The two aforementioned nonsense mutations create truncations of eight and three amino acids, respectively, in the Cterminal end of the a isotypes. This C-terminal domain contains

the repressive domain, and removal of the last eight amino acids partially abolishes this repression (V.Doetsch, personal communication). In addition, the lastfive amino acids, KEEGE, may form an endoplasmic retention signal, suggesting that protein routing may also be impaired. Two other mutations, both at the same codon, have been found in both SHFM and EEC syndrome—namely, R280C and R280H. This arginine,like the lysines at positions 193 and 194, is not in direct contact with the DNA, and mutation of these residues probably induces more-subtle effects on the DNA-binding capacity of p63[11].

Genotype-Phenotype Correlations: Molecular Dissection of the p63 Gene

The pattern of mutations in the five human disorders linked to p63 reveals a remarkable specificity of the molecular defects in this gene and clinical consequences.The clustering of mutations in the DBD, for EEC syndrome,and in the SAM domain, for AEC syndrome establishes a clear genotype-phenotype correlation. Furthermore,the mutations in ADULT syndrome, as well as most of the mutations in LMS and SHFM, are distinctive to these syndromes. Interestingly, within families, mutation of the arginine at position 280 always has the same phenotypic outcome namely, either SHFM or EEC syndrome supporting the notion that genetic modifiers or epigenetic factors have a modulatory effect.Evidence for genetic modifiers is found in mice with mutations in genes that are likely to be involved in p63 pathways. The limb phenotype of the *dactylaplasia* (*Dac*) mouse, a model for human *SHFM3*, not only requires mutation of the *dactylin* gene but also requires homozygosity for an as-yet-unknown modifier allele that has been denoted as "*mDac*" .Another fascinating example, in the *syndactylism* (*sm*) mouse, is caused by a disruption of the p63 target gene *Jag2*. The *sm* phenotype is strongly modified by genetic background, and several loci, acting as either enhancers or suppressors, have been mappe.One of these, the suppressor locus on mouse evolution of a tumor suppressor (p53) from a transcription factor that already functioned to handle decisions of cell fate (p63), and was specifically expressed in the relevant tissue. This chromosome 16,is syntenic to human chromosome 3q27-q29 and encompasses the *TP63* gene. *TP63* may be a modifier of the mutant *JAG2* phenotype, and, by analogy, *JAG2* may be a modifier of the mutant p63 phenotype. The hypothesis that there are specific modifier genes can be further pursued by molecular studies of large families with a single *TP63* mutation. Other candidate modifiers include (*a*) genes that are known to be mutated in human syndromes with features that overlap those of the EEC syndrome or (*b*) genes that are active in genetic programs that are governed by p63. For full comprehension of the normal and disrupted properties of the complex array of p63 isotypes, it will be necessary to identify those genes that act together with or in response to p63. It is to be expected that some of these will be found either to be modifiers of the spectrum of EEClike disorders or to underlie LADD syndrome or the 90% of cases of SHFM that lack *TP63* mutations[11].

Evolution of P63 gene

The p63 gene is extraordinarily conserved, even among distantly related species. For instance, p63 proteins shown 99% amino acid identity between human and mouse orthologs, and 93% amino acid identity between human and Xenopus laevis [2,105]. This implies an evolutionarily ancient function for p63. The N-terminal domain is the least conserved of the three domains among the family members (30% identity between p73 and p53, 22% identity between p63 and p53 and 30% identity between p63 and p73).Phylogenetic analysis of the p53 family members has suggested that the ancestor gene of the p53 family is most like p63, and that p53 is a more recent evolutionary adaptation [106]. This is an attractive hypothesis, because of the nature of the most prevalent genotoxic insult to which early organisms would have been exposed ultraviolet radiation and the tissue that bears the brunt of such exposure the skin. Thus, one can imagine a scenario by which UV radiation promoted the

evolutionary hypothesis is complicated by the fact that TAp63 isoforms have not been identified in zebrafish or Xenopus model systems at this time;

only ΔNp63 transcripts and proteins are detected. This leads to several possibilities:
1)TAp63 isoforms are evolutionarily ancient and physiological important players in the overall function of the p63 gene, but simply have not yet been identified in zebrafish and Xenopus.
2)TAp63 isoforms are dispensable for overall function of the p63 gene.
3)The most ancient p63 precursor gene did not possess a transactivation domain, and TAp63 isoforms are actually later evolutionary additions that appear in mammalian species, and permit the formation of a more complex and heavily stratified epidermis than that found in fish and amphibians.This last possibility is supported by the fact that in the p63 gene, the TA promoter and start site lies 120–160 kB upstream from the ΔN start site and DNA binding domain in both mice and humans. In contrast, the p53 transactivation domain and DNA binding domain lie within 4 kB of genomic sequence, suggesting that the addition of the TA domain to the p63 gene is a relatively recent event[1].

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:
IARC TP53 Mutation Database, http://www.iarc.fr/p53/ (for mutation frequencies in the *TP53* gene) Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for EEC syndrome [MIM 604292], LADD syndrome [MIM 149730],ADULT syndrome [MIM 103285], LMS [MIM 603543],AEC syndrome [MIM 106260], RHS [MIM 129400], ECP syndrome [MIM 129830], EE syndrome [MIM 129810],*SHFM1* [MIM 183600], *SHFM2* [MIM 313350], *SHFM3*[MIM 600095], and *SHFM4* [MIM 605289]).

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