Genes Involved in Stem Cell Fate Decisions and Commitment to Differentiation Play a Role in Skin Disease

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Multipotent stem cells residing in the bulge region of the hair follicle give rise to cells of different fates including those forming hair follicles, interfollicular epidermis, and associated glands. Stem cell fate determination is regulated by genes involved in both proliferation and differentiation, which are tightly regulated processes. Understanding the molecular mechanisms by which proliferation and differentiation are regulated will provide useful insight into treating human diseases caused by the deregulation of these processes. Two genes involved in regulating proliferation and differentiation are c-Myc and p63, both of which have been found to be deregulated/mutated in several human diseases. Accelerating proliferation leads to neoplastic human diseases and deregulated c-Myc has been implicated in a variety of cancers. Evidence indicates that c-Myc also diverts stem cells to an epidermal and sebaceous gland fate at the expense of the hair follicle fate. Therefore, deregulation of c-Myc has the potential to not only accelerate tumorigenesis, but also influence skin tumor phenotype. In addition, the inhibition of differentiation may also predispose to the development of skin cancer. Recent evidence suggests that the transcription factor p63, is not only responsible for the initiation of an epithelial stratification program during development, but also the maintenance of the proliferative potential of basal keratinocytes in mature epidermis. Mutations in the p63 gene have been shown to cause ectodermal dysplasias and deregulated expression of p63 has been observed in squamous cell carcinomas. In this review, we will discuss recent data implicating a role for both c-Myc and p63 in human skin diseases.

Key words: c-Myc/epidermal differentiation/p63/stem cells
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The epidermis is a self-renewing tissue that forms the outer layer of the skin through an intricate balance of cell proliferation and differentiation. Because the skin is readily accessible, it represents an attractive system to analyze the molecular mechanisms responsible for these processes. As a highly regulated organ, the skin maintains strict control of proliferation and differentiation. The balance between cell proliferation and differentiation results in the division of stem cells and the proper entry of daughter cells into differentiation, thus maintaining epidermal homeostasis. Alterations in either proliferation or differentiation have the potential to disrupt normal epidermal homeostasis and lead to disease. Recent studies focusing on c-Myc and p63, both of which are involved in regulating proliferation and differentiation, have provided new insight into the role of these genes in formation of the epidermis during development, the maintenance of stem cells in mature epidermis, and the commitment of multipotent stem cells to different cell fates. In addition, these studies have revealed that skin disorders arise when these genes are mutated or deregulated.

Abbreviation: SAM, sterile α motif

Epidermal Stem Cells

The self-renewing characteristic of the skin and its appendages is supported by stem cells residing within the epidermis and in the bulge region of hair follicles. Stem cells are unique from other cells because they have a high capacity for self-renewal and the ability to produce daughter cells (transit amplifying cells) that undergo terminal differentiation (Lajtha, 1979). In contrast, transit amplifying cells have a high potential to undergo differentiation and a low potential for self-renewal (Jones and Watt, 1993). Since cells in the epidermis continually differentiate to replenish cells sloughed from the external surface, the only cells capable of accumulating genetic mutations required for tumorigenesis are epidermal stem cells (Owens and Watt, 2003; Perez-Losada and Balmain, 2003).

Research over the past few years has expanded our knowledge about epidermal stem cells. Studies utilizing the unique characteristics of stem cells have led to the identification of a population of multipotent stem cells within the epidermis. Label retaining experiments have shown that cells residing in the bulge region of mouse pelage follicles are slow cycling and give rise to both the hair follicle and the epidermis (Taylor *et al*, 2000). This study established, for the first time, that these stem cells are bipotent. In addition, an elegant study using chimeric hair follicles created from

wild-type and Rosa26 mice, which were genetically engineered to express a reporter gene, β-galactosidase, showed that stem cells residing in the bulge region of the hair follicle are multipotent and give rise to hair follicles, sebaceous glands, and interfollicular epidermis (Oshima *et al*, 2001). Rosa26 cells that were initially present in the bulge region of the chimeric hair follicle were detected migrating downward along the hair follicle towards the hair bulb and upward into the epidermis 4 wk after transplantation. After 6 wk, the Rosa26 cells could be detected in the hair bulb, sebaceous glands, and interfollicular epidermis (Oshima *et al*, 2001). These studies provide very convincing evidence that a multipotent stem cell population resides in the bulge region of hair follicles.

To date, a unique epidermal stem cell marker has not been identified. But the use of a combination of markers and adhesive properties has allowed the isolation of enriched stem cell populations. Epidermal stem cells express higher levels of β1-integrin compared with transit amplifying cells, allowing the isolation of epidermal stem cells based on their adhesiveness (Jones and Watt, 1993). In addition, α6-integrin, a basal-specific integrin (Li *et al*, 1998), was used to further purify this population. A combination of these integrins with either CD71, a proposed negative selection marker (Tani *et al*, 2000) or CD34, a potential positive marker (Trempus *et al*, 2003), has also been used for the enrichment of epidermal stem cells. An additional approach using a combination of Hoechst and propidium iodide dye to sort cells has led to the isolation of three distinct populations of cells from the basal layer including stem cells, transient amplifying cells, and non-proliferative basal cells (Dunnwald *et al*, 2001). The ability to isolate a pure population of epidermal stem cells would be very beneficial for therapeutic applications for a variety of skin disorders.

Role of c-Myc in the Epidermis

Members of the MYC oncoprotein family, c-Myc, N-Myc, and L-Myc, play a role in the pathogenesis of many human neoplastic diseases (Nesbit *et al*, 1999). Throughout development each member is expressed in specific tissues (Zimmerman *et al*, 1986). The expression of c-Myc is high in rapidly proliferating cells and is downregulated during differentiation (Mugrauer *et al*, 1988; Hirning *et al*, 1991). N-myc is expressed at high levels in pre-B cells, kidney, forebrain, hindbrain, and intestine and continues to be expressed during differentiation (Mugrauer *et al*, 1988; Hirning *et al*, 1991). L-myc is expressed in the developing kidney, brain, and neural tube (Hatton *et al*, 1996). c-Myc knockout mice are lethal at E10.5 and it is thought that members can compensate to this point during development (Baudino *et al*, 2002). This review focuses on c-Myc since it is the predominant member expressed in the epidermis.

The oncoprotein c-Myc plays a role in proliferation, differentiation, and apoptosis. c-Myc is a transcription factor that heterodimerizes with members of the Max/Mad family. To activate transcription of target genes, c-Myc heterodimerizes with Max and binds to E box sequences in the promoters of target genes (Pelengaris and Khan, 2003). The transcription factor Mad can bind to both Max and c-Myc, which prevents transcriptional activation of target genes by competing out the association of c-Myc/Max complexes as well as binding E box sequences to block c-Myc/Max binding (Pelengaris and Khan, 2003). In addition, c-Myc has been shown to directly repress transcription of genes such as $p15^{INK4b}$ by associating with Max and Miz-1 (Staller *et al*, 2001). In the epidermis, c-Myc is expressed at high levels in the basal compartment and as cells differentiate c-Myc levels decline and Mad expression levels increase (Chin *et al*, 1995; Hurlin *et al*, 1995).

Recent studies have shown that c-Myc plays an important role in maintaining stem cells and regulating their commitment to different cell fates (Arnold and Watt, 2001; Waikel *et al*, 2001). There are a variety of mechanisms by which c-Myc is tightly regulated including: activation by the WNT signaling pathway, growth factors, and mitogens, as well as inhibition by the TGF-β signaling pathway (Pelengaris and Khan, 2003). Previous studies using transgenic mouse models have provided evidence that the WNT pathway regulates stem cell fate determination. Collectively, these studies have found that different levels of active β-catenin influences the specific fate adopted. An increase in β-catenin signaling leads to precocious hair formation (Zhou *et al*, 1995; Gat *et al*, 1998), whereas low levels of β-catenin signaling results in sebaceous-like cyst formation (van Genderen *et al*, 1994) and complete inhibition of the WNT pathway inhibits hair follicle formation (Andl *et al*, 2002). β-catenin levels, however, may not be the sole determinate of stem cell fate. c-Myc has been shown to induce differentiation of epidermal stem cells *in vitro* (Gandarillas and Watt, 1997), and recent *in vivo* studies have found that deregulation of c-Myc expression targeted to epidermal stem cells leads to increased sebaceous gland and epidermal differentiation at the expense of hair follicles (Fig 1) (Arnold and Watt, 2001; Waikel *et al*, 2001). Label retaining cell assays revealed that transgenic mice with deregulated c-Myc expression targeted to epidermal stem cells using a K14 promoter exhibited a depletion of stem cells compared with wild-type littermates (Waikel *et al*, 2001). These mice also

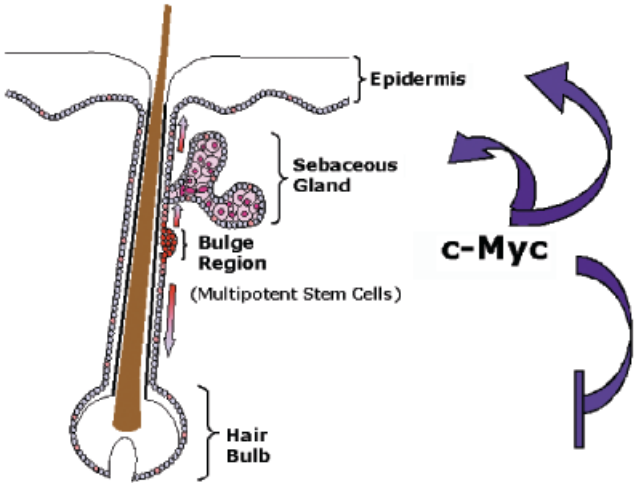

Figure 1
Influence of c-Myc on Stem Cell Fate. c-Myc diverts multipotent stem cells residing in the bulge region of the hair follicle to a sebaceous and/or interfollicular epidermis cell fate at the expense of a hair follicle fate (adapted from Honeycutt and Roop, 2003).

exhibited a decrease in integrin expression that accompanied a defect in wound healing. In addition, similar results were observed in an inducible MycER mouse model, which also exhibited terminal differentiation of keratinocytes into interfollicular epidermis and sebocytes at the expense of hair lineage differentiation (Arnold and Watt, 2001). Both studies indicate that bypassing the WNT pathway by deregulating c-Myc can also influence stem cell fate, suggesting that c-Myc may play a more pivotal role in stem cell fate determination than previously in realized.

The mechanism by which c-Myc influences stem cell fate is unknown; however, it is possible that c-Myc activates direct targets involved in stem cell fate determination. There have been numerous studies using gene expression profiling to determine potential downstream targets of c-Myc. Studies using an inducible transgenic mouse model have found targets of c-Myc to be involved in proliferation, cell cycle regulation, RNA regulation, protein synthesis and processing, cell adhesion, and regulation of the cytoskeleton (Frye *et al*, 2003). Among various downstream targets, this study found α6-integrin, a potential epidermal stem cell marker, to be decreased in expression 2-fold after activation of c-Myc expression. Other studies using *in vitro* gene expression profiling approaches also provide important insight into the potential downstream targets of c-Myc. For example, studies using an inducible human fibroblast line found an increase in c-Myc expression led to an increase in CD71 expression (Coller *et al*, 2000), which has been found to be a negative epidermal stem cell marker (Tani *et al*, 2000). This, in addition to the decrease in α6-integrin, correlates to evidence that c-Myc diverts stem cells from the stem cell compartment (Waikel *et al*, 2001). Additionally, a study that screened the human genome for direct targets of c-Myc found Smad7 to be a direct target of c-Myc (Fernandez *et al*, 2003). Smad7 is the inhibitory smad of the TGFβ pathway and overexpression of Smad7 targeted to the epidermal basal compartment has been shown to increase the number of sebaceous glands and induce epidermal hyperplasia (Wang XJ, personal communication), similar to the phenotype exhibited by K14.myc2 mice (Waikel *et al*, 2001). These results support the ability of c-Myc to divert stem cells from the stem cell compartment to a sebaceous fate at the expense of hair follicles through activating specific targets.

Role of c-Myc and Human Disease

Deregulated c-Myc has been found in several cancer types including breast (Nass and Dickson, 1997), colon (Kopnin, 1993), lung (Gazdar, 1994), and lymphomas (Cotter, 1993). The role c-Myc plays in cell proliferation is thought to be the key to its involvement in cancer. Studies analyzing c-Myc expression in non-proliferating cells of the suprabasal layer of the epidermis found c-Myc to induce proliferation while inhibiting terminal differentiation (Waikel *et al*, 1999). c-Myc is involved in the G1 to S phase transition of the cell cycle, which is the time when the DNA is repaired. Premature exit from G1 without proper DNA repair allows mutations to accumulate. Recent studies analyzing targets of c-Myc have found several genes involved in the G1 to S phase transition to be targets of c-Myc. Genes activated by c-Myc include cyclinD2 (Bouchard *et al*, 1999), CDK4 (Hermeking *et al*, 2000), and MCM7 (Fernandez *et al*, 2003), whereas c-Myc has been found to repress $p15^{INK4b}$ (Staller *et al*, 2001).

Deregulation of c-Myc alone may not be sufficient to induce tumorigenesis, since c-Myc does induce apoptosis (Pelengaris and Khan, 2003). But if c-Myc induced apoptosis is blocked, then, tumorigenesis could proceed. In fact, studies have looked at the effect of deregulated c-Myc in combination with overexpressing Bcl-x_L (Nass *et al*, 1996; Pelengaris *et al*, 2002), a member of the bcl-2 family. The anti-apoptotic family members include bcl-2, bcl-x_L, mcl-1, and bcl-w and the pro-apoptotic members include bax, bak, bad, bcl-x_S, bid, and hrk (Delehedde *et al*, 1999). This family regulates apoptosis by either promoting or inhibiting cell death. Human tumor studies have found Bcl-x_L to be highly expressed in human squamous cell carcinoma (SCC) (Delehedde *et al*, 1999). In a study using an inducible c-Myc pancreatic β cell-specific mouse line, it was found that induced expression of c-Myc induced apoptosis (Pelengaris *et al*, 2002). When this apoptosis was suppressed by co-expression of Bcl-x_L, c-Myc expression was able to induce tumor progression. Also, data from mammary gland tumors suggest cooperation between Bcl-x_L and c-Myc in transformation (Nass *et al*, 1996). Cell lines derived from mammary tumors, which arise in MMTV-myc transgenic mice, can be induced to undergo apoptosis by exogenous TGFβ and inhibited by exogenous epidermal growth factor (EGF). In a study to analyze apoptotic pathway genes in these cell lines in the presence or absence of the growth factors, it was found that the expression of Bcl-x_L increased with EGF and decreased with TGFβ. The change in Bcl-x_L expression was greater than the change in Bcl-2 expression (Nass *et al*, 1996). This data suggest that Bcl-x_L may be the predominant family member responsible for inhibiting c-Myc induced apoptosis. Although the cooperation between Bcl-x_L and c-Myc has not been analyzed in skin carcinogenesis, both have been found to play a role (Delehedde *et al*, 1999; Pelengaris *et al*, 1999).

Since the activation of oncogenes is important in tumorigenesis (Bishop, 1991), strategies based on oncogene inactivation are being investigated for cancer therapy (Jain *et al*, 2002). A concern raised with these therapies is that withdrawal of oncogene inactivation may result in tumor regrowth. Recent studies have analyzed the role of inactivation/activation of c-Myc in tumor regression and regrowth. The first study used an inducible mouse model in which c-Myc expression, targeted to the suprabasal layers by the involucrin promoter, could be activated by topical application of 4-hydroxytamoxifen (OHT) (Pelengaris *et al*, 1999). Activation of c-Myc in the suprabasal layer resulted in the proliferation of post-mitotic keratinocytes and prolonged activation induced the formation of preneoplastic lesions similar to human epithelial precancerous lesions. Inactivation of c-Myc in the preneoplastic lesions resulted in regression of lesions (Pelengaris *et al*, 1999). Additionally, studies using osteogenic sarcoma cells derived from a tetracycline-regulated transgenic mouse model with c-Myc targeted to lymphocytes have found that inactivating c-Myc leads to tumor regression (Jain *et al*, 2002). When c-Myc expression was reactivated, the cells underwent apoptosis as opposed to proliferation. This was further

analyzed *in vivo* by implanting osteogenic sarcomas subcutaneously into syngenic mice. Inactivation of c-Myc expression caused tumor regression and subsequent activation of c-Myc resulted in a marked increase of apoptosis in tumor cells (Jain *et al*, 2002).

In summary, c-Myc plays an important role in stem cell fate determination and carcinogenesis. It is tempting to speculate that the effect of c-Myc on stem cell fate determination could influence tumor phenotype. Nevertheless, c-Myc appears to be an important player in stem cell fate determination and the role of c-Myc in both tumor progression and regression indicates the potential use of c-Myc in therapeutic strategies for skin cancer treatment. For tumors with deregulated c-Myc expression, the acute short-term inhibition of c-Myc may lead to tumor-targeted apoptosis with the withdrawal of c-Myc inhibition. This would bypass the potential problems with systemically inhibiting c-Myc and therefore proliferation.

Function and Structure of p63

In addition to deregulated proliferation, alterations affecting proper cell differentiation have also been found to give rise to human disease. Recently p63, a member of the p53 gene family has been implicated in epidermal development and differentiation. The p53 gene family now consists of three genes: p53, p63, and p73. All three genes share sequence homology, although p63 and p73 are more similar to each other than to p53 (Saccone *et al*, 2002; Yang *et al*, 2002). Each p53 family member contains the three typical domains of a transcription factor: a transactivation domain, a DNA binding domain, and an oligomerization domain (Yang *et al*, 1998; Yang *et al*, 2000). In addition, each family member can bind to consensus p53 binding sites (Bian and Sun, 1997; Zeng *et al*, 1998; Sasaki *et al*, 2001; Fontemaggi *et al*, 2002). Furthermore, each family member, when overexpressed, can transactivate p53 target genes (Jost *et al*, 1997; Yang *et al*, 1998). It, however, remains to be determined if p63 and p73 regulate p53 target genes under physiological conditions. Despite these similarities between p53, p63, and p73, they differ in several important respects. Unlike p53, p63 and p73 do not represent classical tumor suppressor genes: mice heterozygous for either p63 or p73 are not predisposed to tumor development (Mills *et al*, 1999; Yang *et al*, 1999; Yang *et al*, 2000), p63 mutations are rarely found in human tumors (Osada *et al*, 1998; Ikawa *et al*, 1999; Nishi *et al*, 1999; Sunahara *et al*, 1999; Hibi *et al*, 2000), and germline mutations in p63 (as found in ectodermal dysplasias; see below) are not associated with a cancer-prone phenotype. Rather, p63 and p73 act as key regulators during development. p73 is required for the development of neuronal and pheromonal pathways and p63 for epithelial, limb, and craniofacial development (Mills *et al*, 1999; Yang *et al*, 1999; Yang *et al*, 2000). As expected, the phenotypes of both $p63^{-/-}$ and $p73^{-/-}$ mice can be linked to tissues that express high levels of p63 and p73 (Yang *et al*, 1998; Mills *et al*, 1999; Yang *et al*, 1999; Yang *et al*, 2000). Contrary to p63, the expression pattern of p73 and the phenotype of $p73^{-/-}$ mice do not suggest a role for p73 in skin development or skin cancer susceptibility.

p63 is expressed in at least six isoforms (Yang *et al*, 1998). The use of alternative promoters and transcription start sites gives rise to two classes of p63 transcripts, those encoding proteins with an amino terminal transactivation domain (TA isoforms) and those encoding proteins lacking this domain (ΔN isoforms). ΔNp63 isoforms were shown to be able to inhibit transactivation of a p53 reporter construct by TAp63 isoforms, suggesting that ΔNp63 isoforms have a dominant-negative function (Yang *et al*, 1998). ΔNp63 isoforms, however, were also shown to be able to transactivate target gene expression in cell lines (Dohn *et al*, 2001b; Wu *et al*, 2003) and primary keratincoytes (King *et al*, 2003). In addition to alternative promoter usage, alternative splicing in a part of the sequence that is not present in p53, gives rise to three different carboxy-termini designated α, β, and γ. The α C-terminus of p63 is the longest and is the only C-terminus that contains a SAM (sterile α motif) domain. SAM domains are evolutionary conserved domains that are found in proteins involved in the regulation of developmental processes and were shown to be able to bind to other SAM domains or to SH2 domains (Schultz *et al*, 1997). In addition, recent evidence has demonstrated that SAM domains can also bind to RNA or lipids (Aviv *et al*, 2003; Barrera *et al*, 2003; Green *et al*, 2003). Interestingly, although p63 and p73 do not form homo- or heterodimers through their SAM domains (Chi *et al*, 1999), the p73 SAM domain can interact with lipids, and a similar function was predicted for the p63 SAM domain (Barrera *et al*, 2003). The physiological relevance of these interactions, however, is not known. In addition to interaction with lipids, the SAM domain of p63 was shown to interact with apobec-1 binding protein (ABBP1), an RNA processing protein (Fomenkov *et al*, 2003). Upon binding to the p63 SAM domain, ABBP1 preferentially splices Fgfr2 into the epithelial-specific Fgfr2-IIIb (K-SAM) isoform. In the absence of this isoform, the epidermis exhibits a reduction in keratinocytes proliferation resulting in severe epidermal hypoplasia (Petiot *et al*, 2003). Therefore, the binding of the p63 SAM domain to ABBP1 may contribute to the role of p63 in epidermal development and differentiation. Although these are just two examples of interactions with the p63 SAM domain, it is likely that p63 participates in other interactions that mediate its function.

Role of p63 in the Epidermis

In the mature epidermis ΔNp63α is the major p63 isoform expressed and the highest expression levels are observed in the proliferating cells of the basal layer and hair follicles (Yang *et al*, 1998; Liefer *et al*, 2000). In the overlying differentiated layers of the epidermis, ΔNp63α expression is downregulated (Westfall *et al*, 2003). These expression data suggest a role for ΔNp63α expression in proliferation of basal keratinocytes. Consistent with this hypothesis, *in vitro* data using primary mouse keratinocytes demonstrated that ΔNp63α expression can block calcium-induced differentiation of primary keratinocytes, thereby maintaining cells in a proliferative state (King *et al*, 2003). In addition, studies in zebrafish embryos demonstrated that ΔNp63 isoforms are required for cell proliferation in the epidermis (Bakkers *et al*, 2002; Lee and Kimelman, 2002). In zebrafish embryos,

ΔNp63 is synthesized prior to epidermal proliferation; however, the nuclear translocation of ΔNp63 during zebrafish development correlates with the time that epidermal proliferation begins. Moreover, in the absence of ΔNp63 expression, epidermal cells in zebrafish embryos fail to proliferate (Lee and Kimelman, 2002). In addition, in the absence of ΔNp63 expression, zebrafish skin does not differentiate resulting in microbial infections and death. Consistent with a role for p63 in maintaining the proliferative potential of epidermal keratinocytes, the observed downregulation of p63 in differentiated layers of vertebrate epidermis was shown to be required for terminal differentiation to take place. In primary keratinocytes, ΔNp63α was shown to interact with the promoters of $p21^{WAF1/Cip1}$ and 14-3-3σ resulting in transcriptional repression (Westfall *et al*, 2003). Since $p21^{WAF1/Cip1}$ and 14-3-3σ are required for terminal differentiation of keratinocytes (Steinman *et al*, 1994; Missero *et al*, 1995; Dellambra *et al*, 2000), this repression may prevent basal keratinocytes from prematurely differentiating. During terminal differentiation, ΔNp63α expression is downregulated resulting in loss of binding of ΔNp63α to the $p21^{WAF1/Cip1}$ and 14-3-3σ promoters. This may result in the expression of $p21^{WAF1/Cip1}$ and 14-3-3σ thereby allowing for terminal differentiation to take place. Taken together, these studies suggest that p63 is required for the maintenance of the proliferative potential of basal keratinocytes in the mature epidermis and that p63 expression must be downregulated for terminal differentiation to take place.

In addition to its role in mature epidermis, p63 expression is required for development of the epidermis, as clearly demonstrated by the phenotype of $p63^{-/-}$ mice (Mills *et al*, 1999; Yang *et al*, 1999). $p63^{-/-}$ mice fail to form a stratified epidermis resulting in a lack of barrier formation causing dehydration and death within hours after birth. In addition to the failure to develop an epidermis, $p63^{-/-}$ do not develop epithelial appendages such as teeth, hair follicles, and mammary glands. This failure to develop appendages is presumably caused by a failure to participate in epithelial-mesenchymal signalling required for appendage development. In fact, several genes that are induced in the mesenchyme of the limb bud as a result of epithelial-mesenchymal signalling are absent from $p63^{-/-}$ limb buds (Mills *et al*, 1999; Yang *et al*, 1999). The single-layered surface epithelium of $p63^{-/-}$ mice does not express keratins K5 and K14. These keratins are the first differentiation markers expressed during normal epidermal development and are markers for epithelia that have committed to initiate an epithelial stratification program (Fig 2). Therefore, although it has been proposed that p63 is required for epithelial stem cell maintenance (Yang *et al*, 1999; Pellegrini *et al*, 2001), it is more plausible that p63 is required for the commitment of the originally single-layered surface ectoderm to an epithelial stratification program (Koster *et al*, 2002, 2004). In fact, we have recently demonstrated that ectopic expression of TAp63α in single-layered lung epithelia results in the development of squamous metaplastic lesions (Koster *et al*, 2004). Consistent with these data, it had previously been demonstrated that squamous metaplastic lesions that develop in the lung and uterus express p63, whereas the surrounding single-layered epithelia do not (Kurita and Cunha, 2001; Massion *et al*, 2003). In addition, we have demonstrated that deregulated expression of TAp63α in mature epidermis results in hyperproliferation and a delayed onset of terminal differentiation (Koster *et al*, 2004). Taken together, these data support a dual role for p63: initiating epithelial stratification during development and maintaining the proliferative potential of basal keratinocytes in mature epidermis (Fig 3). This hypothesis is further supported by the identification of p63 target genes that are involved in epidermal development and differentiation (Nishi *et al*, 2001; Sasaki *et al*, 2001; Dohn *et al*, 2001a; Fomenkov *et al*, 2003; Wu *et al*, 2003).

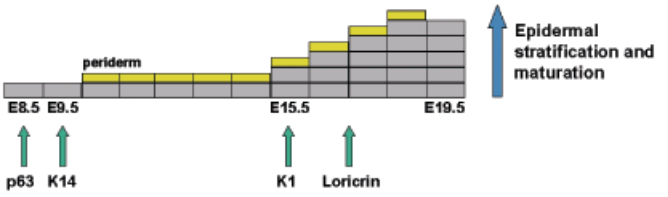

Figure 2
Schematic representing stages of epidermal development. Note that p63 is expressed at E8.5, prior to the onset of stratification (adapted from Koster and Roop, 2004).

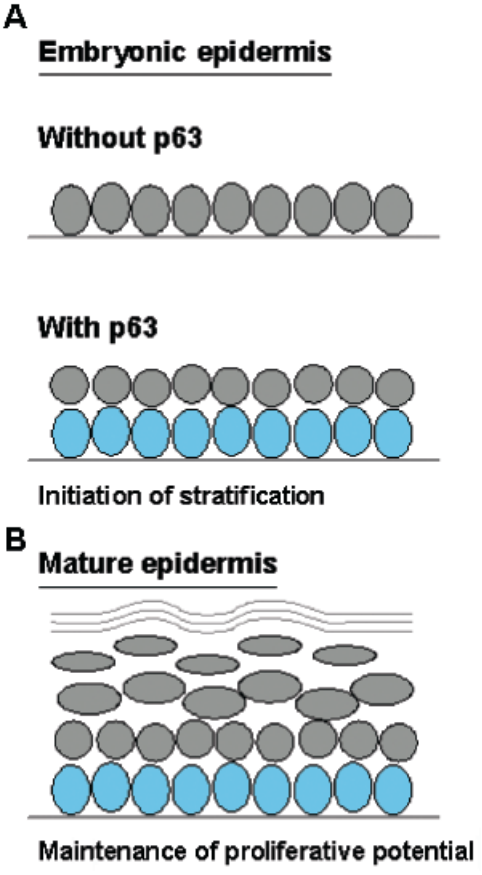

Figure 3
Proposed role of p63 in embryonic and mature epidermis. Epithelia that do not express p63 remain single-layered. Upon induction of p63 expression, epithelia commit to initiate a stratification program (*A*). In the mature epidermis, p63 is expressed in the basal layer, and its expression is downregulated in the differentiated layers. Expression of p63 in the basal layer may maintain the proliferative potential of keratinocytes (*B*) (adapted from Koster and Roop, 2004).

p63 and Human Disease

Mutations in p63 were shown to underlie a number of human ectodermal dysplasias, which are characterized by abnormalities of the limbs, hair, teeth, nails, sweat glands, and mammary glands (Brunner *et al*, 2002). Therefore, consistent with the phenotype of $p63^{-/-}$ mice, abnormalities in p63 gene function in humans appear to disrupt the differentiation process of epithelial tissues and their derivatives. All human ectodermal dysplasias caused by p63 mutations are inherited in an autosomal-dominant fashion. Since humans that have a heterozygous deletion of the p63 gene do not develop characteristics of ectodermal dysplasias, it has been suggested that the p63 mutations result in a dominant-negative effect or a gain-of-function. Interestingly, a genotype–phenotype correlation was shown to exist based on the clustering of p63 mutations in different ectodermal dysplasias. For example, patients with ectodermal dysplasia and cleft lip (EEC) harbor missense p63 mutations in the DNA binding domain (Celli *et al*, 1999). Patients with ankyloblepharon ectodermal dysplasia and clefting (AEC or Hay–Wells disease), however, have mutations in the SAM domain (McGrath *et al*, 2001). Interestingly it was found that mutations in the SAM domain abrogate the interaction of p63 with ABBP1, which may partially account for the defects in epithelial development and differentiation observed in these patients (Fomenkov *et al*, 2003). Since the SAM domain is only contained in p63α isoforms, this suggests that p63α isoforms are essential for development of ectodermally derived tissues.

Genes that are active during normal development are frequently found to be dysregulated during neoplastic transformation. A number of studies have investigated the role of p63 in neoplastic transformation and tumor progression. Although p63 does not function as a classical tumor suppressor gene (Osada *et al*, 1998; Ikawa *et al*, 1999; Nishi *et al*, 1999; Sunahara *et al*, 1999; Hibi *et al*, 2000), it was found that SCC from different organs express high levels of p63 (Crook *et al*, 2000; Hibi *et al*, 2000; Park *et al*, 2000; Yamaguchi *et al*, 2000; Choi *et al*, 2002; Di Como *et al*, 2002; Reis-Filho *et al*, 2002; Weber *et al*, 2002; Massion *et al*, 2003; Reis-Filho *et al*, 2003). The isoform that is most frequently overexpressed is ΔNp63α (Parsa *et al*, 1999; Crook *et al*, 2000; Hibi *et al*, 2000; Massion *et al*, 2003); however, overexpression of TAp63 isoforms has also been documented (Parsa *et al*, 1999; Nylander *et al*, 2000, 2002; Massion *et al*, 2003). In some cases, the overexpression of p63 may be caused by amplification of the genomic region which harbors p63 (Gebhart and Liehr, 2000; Hibi *et al*, 2000; Yamaguchi *et al*, 2000; Redon *et al*, 2001). We previously generated transgenic mice that express ΔNp63α in the epidermis (Liefer *et al*, 2000). These transgenic mice were more resistant to UV-B induced apoptosis than control littermates, suggesting that ΔNp63α has an oncogenic role. Based on *in vitro* evidence suggesting that ΔNp63 isoforms have a dominant-negative function towards TAp63 isoforms (Yang *et al*, 1998) and that TAp63 isoforms are capable of inducing apoptosis (Osada *et al*, 1998; Sasaki *et al*, 2001; Dohn *et al*, 2001a; Dohn *et al*, 2001b; Dietz *et al*, 2002; Flores *et al*, 2002; Okada *et al*, 2002), it has been proposed that TAp63 isoforms may have tumor suppressing abilities. But all of these *in vitro* studies have been performed in cell lines derived from tissues that normally do not express p63. Since ΔNp63α was shown to have cell type-specific functions (King *et al*, 2003), this may also be the case for TAp63 isoforms. Therefore, like ΔNp63 isoforms, TAp63 isoforms may also have an oncogenic function.

In summary, although the roles of the different p63 isoforms are still elusive, the current data suggest that p63 has a dual role. During development p63 is required for the initiation of an epithelial stratification program, whereas in the mature epidermis p63 is required for the maintenance of the proliferative potential of basal keratinocytes. Deregulation of p63 expression can result in ectodermal dysplasias and SCC. The molecular role of p63 in these disorders, however, remains to be determined and will be further elucidated by the identification of additional interacting proteins and downstream target genes.

c-Myc and p63 have complimentary roles in regulating epidermal homeostasis through the regulation of proliferation and differentiation, respectively. Deregulation of either of these processes leads to human disease. The molecular mechanisms by which these genes regulate epidermal homeostasis are currently being analyzed and future studies will uncover the missing links between these genes and the human diseases in which they are involved.

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References

Andl T, Reddy ST, Gaddapara T, Millar SE: WNT signals are required for the initiation of hair follicle development. Dev Cell 2:643–653, 2002

Arnold I, Watt FM: c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. Curr Biol 11:558–568, 2001

Aviv T, Lin Z, Lau S, Rendl LM, Sicheri F, Smibert CA: The RNA-binding SAM domain of Smaug defines a new family of post-transcriptional regulators. Nat Struct Biol 10:614–621, 2003

Bakkers J, Hild M, Kramer C, Furutani-Seiki M, Hammerschmidt M: Zebrafish DeltaNp63 is a direct target of Bmp signaling and encodes a transcriptional repressor blocking neural specification in the ventral ectoderm. Dev Cell 2:617–627, 2002

Barrera FN, Poveda JA, Gonzalez-Ros JM, Neira JL: Binding of the C-terminal SAM domain of human p73 to lipid membranes. J Biol Chem 278: 46878–46885, 2003

Baudino TA, McKay C, Pendeville-Samain H, *et al*: c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. Genes Dev 16:2530–2543, 2002

Bian J, Sun Y: p53CP, a putative p53 competing protein that specifically binds to the consensus p53 DNA binding sites: A third member of the p53 family? Proc Natl Acad Sci USA 94:14753–14758, 1997

Bishop JM: Molecular themes in oncogenesis. Cell 64:235–248, 1991

Bouchard C, Thieke K, Maier A, *et al*: Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. EMBO J 18:5321–5333, 1999

Brunner HG, Hamel BC, Bokhoven HH: P63 gene mutations and human developmental syndromes. Am J Med Genet 112:284–290, 2002

Celli J, Duijf P, Hamel BC, *et al*: Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 99:143–153, 1999

Chi SW, Ayed A, Arrowsmith CH: Solution structure of a conserved C-terminal domain of p73 with structural homology to the SAM domain. EMBO J 18:4438–4445, 1999

Chin L, Schreiber-Agus N, Pellicer I, *et al*: Contrasting roles for Myc and Mad proteins in cellular growth and differentiation. Proc Natl Acad Sci USA 92:8488–8492, 1995

Choi HR, Batsakis JG, Zhan F, Sturgis E, Luna MA, El Naggar AK: Differential expression of p53 gene family members p63 and p73 in head and neck squamous tumorigenesis. Hum Pathol 33:158–164, 2002

Coller HA, Grandori C, Tamayo P, Colbert T, Lander ES, Eisenman RN, Golub TR: Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. Proc Natl Acad Sci USA 97:3260–3265, 2000

Cotter FE: Molecular pathology of lymphomas. Cancer Surv 16:157–174, 1993

Crook T, Nicholls JM, Brooks L, O'Nions J, Allday MJ: High level expression of deltaN-p63: A mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma (NPC)? Oncogene 19:3439–3444, 2000

Delehedde M, Cho SH, Sarkiss M, Brisbay S, Davies M, El Naggar AK, McDonnell TJ: Altered expression of bcl-2 family member proteins in nonmelanoma skin cancer. Cancer 85:1514–1522, 1999

Dellambra E, Golisano O, Bondanza S, *et al*: Downregulation of 14-3-3sigma prevents clonal evolution and leads to immortalization of primary human keratinocytes. J Cell Biol 149:1117–1130, 2000

Di Como CJ, Urist MJ, Babayan I, *et al*: p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 8:494–501, 2002

Dietz S, Rother K, Bamberger C, Schmale H, Mossner J, Engeland K: Differential regulation of transcription and induction of programmed cell death by human p53-family members p63 and p73. FEBS Lett 525:93–99, 2002

Dohn M, Jiang J, Chen X: Receptor tyrosine kinase EphA2 is regulated by p53-family proteins and induces apoptosis. Oncogene 20:6503–6515, 2001a

Dohn M, Zhang S, Chen X: p63alpha and DeltaNp63alpha can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. Oncogene 20:3193–3205, 2001b

Dunnwald M, Tomanek-Chalkley A, Alexandrunas D, Fishbaugh J, Bickenbach JR: Isolating a pure population of epidermal stem cells for use in tissue engineering. Exp Dermatol 10:45–54, 2001

Fernandez PC, Frank SR, Wang L, *et al*: Genomic targets of the human c-Myc protein. Genes Dev 17:1115–1129, 2003

Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, Jacks T: p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 416:560–564, 2002

Fomenkov A, Huang YP, Topaloglu O, *et al*: P63 alpha mutations lead to aberrant splicing of keratinocyte growth factor receptor in the Hay–Wells syndrome. J Biol Chem 278:23906–23914, 2003

Fontemaggi G, Kela I, Amariglio N, *et al*: Identification of direct p73 target genes combining DNA microarray and chromatin immunoprecipitation analyses. J Biol Chem 277:43359–43368, 2002

Frye M, Gardner C, Li ER, Arnold I, Watt FM: Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. Development 130: 2793–2808, 2003

Gandarillas A, Watt FM: c-Myc promotes differentiation of human epidermal stem cells. Genes Dev 11:2869–2882, 1997

Gat U, DasGupta R, Degenstein L, Fuchs E: *De novo* hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95:605–614, 1998

Gazdar AF: The molecular and cellular basis of human lung cancer. Anticancer Res 14:261–267, 1994

Gebhart E, Liehr T: Patterns of genomic imbalances in human solid tumors (review). Int J Oncol 16:383–399, 2000

Green JB, Gardner CD, Wharton RP, Aggarwal AK: RNA recognition via the SAM domain of Smaug. Mol Cell 11:1537–1548, 2003

Hatton KS, Mahon K, Chin L, *et al*: Expression and activity of L-Myc in normal mouse development. Mol Cell Biol 16:1794–1804, 1996

Hermeking H, Rago C, Schuhmacher M, *et al*: Identification of CDK4 as a target of c-MYC. Proc Natl Acad Sci USA 97:2229–2234, 2000

Hibi K, Trink B, Patturajan M, *et al*: AIS is an oncogene amplified in squamous cell carcinoma. Proc Natl Acad Sci USA 97:5462–5467, 2000

Hirning U, Schmid P, Schulz WA, Rettenberger G, Hameister H: A comparative analysis of N-myc and c-myc expression and cellular proliferation in mouse organogenesis. Mech Dev 33:119–125, 1991

Honeycutt KA, Roop DR: c-Myc and epidermal stem cell fate determination. J Dermatol, 2003 (in press)

Hurlin PJ, Foley KP, Ayer DE, Eisenman RN, Hanahan D, Arbeit JM: Regulation of Myc and Mad during epidermal differentiation and HPV-associated tumorigenesis. Oncogene 11:2487–2501, 1995

Ikawa S, Nakagawara A, Ikawa Y: p53 family genes: Structural comparison, expression and mutation. Cell Death Differ 6:1154–1161, 1999

Jain M, Arvanitis C, Chu K, *et al*: Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science 297:102–104, 2002

Jones PH, Watt FM: Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. Cell 73:713–724, 1993

Jost CA, Marin MC, Kaelin WG Jr: p73 is a simian [correction of human] p53-related protein that can induce apoptosis. Nature 389:191–194, 1997

King KE, Ponnamperuma RM, Yamashita T, Tokino T, Lee LA, Young MF, Weinberg WC: deltaNp63alpha functions as both a positive and a negative transcriptional regulator and blocks *in vitro* differentiation of murine keratinocytes. Oncogene 22:3635–3644, 2003

Kopnin B: Genetic events responsible for colorectal tumorigenesis: Achievements and challenges. Tumori 79:235–243, 1993

Koster MI, Huntzinger KA, Roop DR: Epidermal differentiation: Transgenic/knockout mouse models reveal genes involved in stem cell fate decisions and commitment to differentiation. J Investig Dermatol Symp Proc 7: 41–45, 2002

Koster MI, Kim S, Mills AA, DeMayo FJ, Roop DR: p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev, 18:126–131, 2004

Koster MI, Roop DR: The role of p63 in development and differentiation of the epidermis. J Dermatol Sci, 34:3–9, 2004

Kurita T, Cunha GR: Roles of p63 in differentiation of Mullerian duct epithelial cells. Ann N Y Acad Sci 948:9–12, 2001

Lajtha LG: Stem cell concepts. Differentiation 14:23–34, 1979

Lee H, Kimelman D: A dominant-negative form of p63 is required for epidermal proliferation in zebrafish. Dev Cell 2:607–616, 2002

Li A, Simmons PJ, Kaur P: Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. Proc Natl Acad Sci USA 95:3902–3907, 1998

Liefer KM, Koster MI, Wang XJ, Yang A, McKeon F, Roop DR: Down-regulation of p63 is required for epidermal UV-B-induced apoptosis. Cancer Res 60:4016–4020, 2000

Massion PP, Taflan PM, Jamshedur Rahman SM, *et al*: Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res 63:7113–7121, 2003

McGrath JA, Duijf PH, Doetsch V, *et al*: Hay–Wells syndrome is caused by heterozygous missense mutations in the SAM domain of p63. Hum Mol Genet 10:221–229, 2001

Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A: p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 398:708–713, 1999

Missero C, Calautti E, Eckner R, Chin J, Tsai LH, Livingston DM, Dotto GP: Involvement of the cell-cycle inhibitor Cip1/WAF1 and the E1A-associated p300 protein in terminal differentiation. Proc Natl Acad Sci USA 92:5451–5455, 1995

Mugrauer G, Alt FW, Ekblom P: N-myc proto-oncogene expression during organogenesis in the developing mouse as revealed by *in situ* hybridization. J Cell Biol 107:1325–1335, 1988

Nass SJ, Dickson RB: Defining a role for c-Myc in breast tumorigenesis. Breast Cancer Res Treat 44:1–22, 1997

Nass SJ, Li M, Amundadottir LT, Furth PA, Dickson RB: Role for Bcl-xL in the regulation of apoptosis by EGF and TGF beta 1 in c-myc overexpressing mammary epithelial cells. Biochem Biophys Res Commun 227:248–256, 1996

Nesbit CE, Tersak JM, Prochownik EV: MYC oncogenes and human neoplastic disease. Oncogene 18:3004–3016, 1999

Nishi H, Isaka K, Sagawa Y, *et al*: Mutation and transcription analyses of the p63 gene in cervical carcinoma. Int J Oncol 15:1149–1153, 1999

Nishi H, Senoo M, Nishi KH, *et al*: p53 homologue p63 represses epidermal growth factor receptor expression. J Biol Chem 276:41717–41724, 2001

Nylander K, Coates PJ, Hall PA: Characterization of the expression pattern of p63 alpha and delta Np63 alpha in benign and malignant oral epithelial lesions. Int J Cancer 87:368–372, 2000

Nylander K, Vojtesek B, Nenutil R, *et al*: Differential expression of p63 isoforms in normal tissues and neoplastic cells. J Pathol 198:417–427, 2002

Okada Y, Osada M, Kurata S, *et al*: p53 gene family p51(p63)-encoded, secondary transactivator p51B(TAp63alpha) occurs without forming an immunoprecipitable complex with MDM2, but responds to genotoxic stress by accumulation. Exp Cell Res 276:194–200, 2002

Osada M, Ohba M, Kawahara C, *et al*: Cloning and functional analysis of human p51, which structurally and functionally resembles p53. Nat Med 4: 839–843, 1998

Oshima H, Rochat A, Kedzia C, Kobayashi K, Barrandon Y: Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 104: 233–245, 2001

Owens DM, Watt FM: Contribution of stem cells and differentiated cells to epidermal tumours. Nat Rev Cancer 3:444–451, 2003

Park BJ, Lee SJ, Kim JI, *et al*: Frequent alteration of p63 expression in human primary bladder carcinomas. Cancer Res 60:3370–3374, 2000

Parsa R, Yang A, McKeon F, Green H: Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. J Invest Dermatol 113:1099–1105, 1999

Pelengaris S, Khan M: The many faces of c-MYC. Arch Biochem Biophys 416:129–136, 2003

Pelengaris S, Khan M, Evan GI: Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell 109:321–334, 2002

Pelengaris S, Littlewood T, Khan M, Elia G, Evan G: Reversible activation of c-Myc in skin: Induction of a complex neoplastic phenotype by a single oncogenic lesion. Mol Cell 3:565–577, 1999

Pellegrini G, Dellambra E, Golisano O, *et al*: p63 identifies keratinocyte stem cells. Proc Natl Acad Sci USA 98:3156–3161, 2001

Perez-Losada J, Balmain A: Stem-cell hierarchy in skin cancer. Nat Rev Cancer 3:434–443, 2003

Petiot A, Conti FJ, Grose R, Revest JM, Hodivala-Dilke KM, Dickson C: A crucial role for Fgfr2-IIIb signalling in epidermal development and hair follicle patterning. Development 130:5493–5501, 2003

Redon R, Muller D, Caulee K, Wanherdrick K, Abecassis J, Du MS: A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. Cancer Res 61:4122–4129, 2001

Reis-Filho JS, Simpson PT, Martins A, Preto A, Gartner F, Schmitt FC: Distribution of p63, cytokeratins 5/6 and cytokeratin 14 in 51 normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray. Virchows Arch, 443:122–132, 2003

Reis-Filho JS, Torio B, Albergaria A, Schmitt FC: p63 expression in normal skin and usual cutaneous carcinomas. J Cutan Pathol 29:517–523, 2002

Saccone C, Barome PO, D'Erchia AM, D'Errico I, Pesole G, Sbisa E, Tullo A: Molecular strategies in Metazoan genomic evolution. Gene 300:195–201, 2002

Sasaki Y, Ishida S, Morimoto I, *et al*: The p53 family member genes are involved in the Notch signal pathway. J Biol Chem 277:719–724, 2001

Schultz J, Ponting CP, Hofmann K, Bork P: SAM as a protein interaction domain involved in developmental regulation. Protein Sci 6:249–253, 1997

Staller P, Peukert K, Kiermaier A, *et al*: Repression of p15INK4b expression by Myc through association with Miz-1. Nat Cell Biol 3:392–399, 2001

Steinman RA, Hoffman B, Iro A, Guillouf C, Liebermann DA, el Houseini ME: Induction of p21 (WAF-1/CIP1) during differentiation. Oncogene 9:3389–3396, 1994

Sunahara M, Shishikura T, Takahashi M, *et al*: Mutational analysis of p51A/TAp63gamma, a p53 homolog, in non-small cell lung cancer and breast cancer. Oncogene 18:3761–3765, 1999

Tani H, Morris RJ, Kaur P: Enrichment for murine keratinocyte stem cells based on cell surface phenotype. Proc Natl Acad Sci USA 97:10960–10965, 2000

Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM: Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102:451–461, 2000

Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, Reece JM, Tennant RW: Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. J Invest Dermatol 120:501–511, 2003

van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, Grosschedl R: Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. Genes Dev 8:2691–2703, 1994

Waikel RL, Kawachi Y, Waikel PA, Wang XJ, Roop DR: Deregulated expression of c-Myc depletes epidermal stem cells. Nat Genet 28:165–168, 2001

Waikel RL, Wang XJ, Roop DR: Targeted expression of c-Myc in the epidermis alters normal proliferation, differentiation and UV-B induced apoptosis. Oncogene 18:4870–4878, 1999

Weber A, Bellmann U, Bootz F, Wittekind C, Tannapfel A: Expression of p53 and its homologues in primary and recurrent squamous cell carcinomas of the head and neck. Int J Cancer 99:22–28, 2002

Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA: The Delta Np63 alpha phosphoprotein binds the p21 and 14-3-3 sigma promoters *in vivo* and has transcriptional repressor activity that is reduced by Hay–Wells syndrome-derived mutations. Mol Cell Biol 23:2264–2276, 2003

Wu G, Nomoto S, Hoque MO, *et al*: DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 63:2351–2357, 2003

Yamaguchi K, Wu L, Caballero OL, *et al*: Frequent gain of the p40/p51/p63 gene locus in primary head and neck squamous cell carcinoma. Int J Cancer 86:684–689, 2000

Yang A, Kaghad M, Caput D, McKeon F: On the shoulders of giants: p63, p73 and the rise of p53. Trends Genet 18:90–95, 2002

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

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

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

Zeng X, Levine AJ, Lu H: Non-p53 p53RE binding protein, a human transcription factor functionally analogous to P53. Proc Natl Acad Sci USA 95:6681–6686, 1998

Zhou P, Byrne C, Jacobs J, Fuchs E: Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. Genes Dev 9:700–713, 1995

Zimmerman KA, Yancopoulos GD, Collum RG, *et al*: Differential expression of myc family genes during murine development. Nature 319:780–783, 1986