and, besides MYC, stabilizes other growth- and tumour-promoting proteins, such as cyclin E, c-Jun and Notch[11]. Whether those FBW7 substrates are also stabilized by USP28 remains to be determined, although this seems to be the case at least for cyclin E[1]. Taken together, these considerations strongly suggest that USP28 may be endowed with oncogenic potential and, consistent with this hypothesis, Popov *et al.* report that it is overexpressed in various human cancers. However, USP28 is also involved in DNA-damage responses[12]. Oncogene- and in particular MYC-induced DNA-damage response is an important tumour-suppressor mechanism[13–16], warranting investigation of the role of USP28 in this process. In conclusion, USP28 may play key roles in both tumour-promoting and -suppressing pathways, the balance of which will need to be carefully understood before assessing its diagnostic and therapeutic potential.

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p63: revving up epithelial stem-cell potential

Cédric Blanpain and Elaine Fuchs

As legends go, when Alexander the Great crossed the Land of Darkness searching for the elixir of life, he encountered only desert. Thousands of years later, is it too optimistic to think that scientists have finally found the secret to a longer life? A recent study suggests that p63 may be key, at least for many epithelial stem cells.

As diverse as the coats in a fashion boutique, epithelia are the cellular cloaks of our body and its many organs. The epithelial cells that line our surfaces exist as single or multiple layers with flat, columnar or cuboidal appearance, depending on the tissue. Many epithelia are often exposed to physical trauma and hence, undergo relatively constant turnover to replace the damaged cells. To do so, epithelia set aside reservoirs of proliferative progenitor cells that self-renew and generate the differentiated cells that rejuvenate these tissues[1].

During development, a single (basal) layer of primitive epithelium initially covers the inner and outer surface of the embryo. As embryogenesis proceeds, epithelia that will be exposed to mechanical stress progressively acquire new layers of suprabasal cells that offer a better resistance against environmental cues. The skin epidermis has become the paradigm for exploring how a stratified epithelium develops in the embryo and how, in the adult, it maintains an inner layer of proliferating cells that gives rise to multiple layers of terminally differentiating cells that continuously reach and are shed from the body surface. This exquisite architecture allows the epidermis to generate a self-perpetuating barrier that keeps harmful microbes out and essential body fluids in[2].

A key question is how epithelial progenitor cells retain this self-renewing capacity, which is so critical for epidermal integrity. In the late 1990's, two independent groups interested in other members of the p53 family of protooncogenes made the surprising discovery that mice lacking p63 are severely compromised in their ability to generate the epidermis, as well as many other types of stratified epithelia[3,4]. Both groups reported that the mutant mice had very thin skin; however, in one case clumps of differentiated cells were detected in the epidermis[4], whereas in the other, uncommitted ectodermal

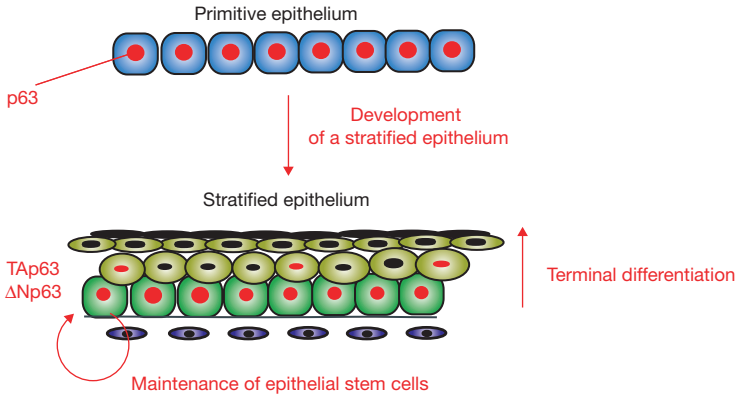

Figure 1 Postulated roles for p63 in the development of a stratified epithelium. Primitive embryonic epithelium exists as a single layer of cells. As development proceeds, epithelia that will be exposed to physical stress progressively stratify and acquire several layers of differentiated cells. p63 is expressed in primitive epithelia and is necessary for the development of these epithelia into stratified tissues. In addition, p63 has been implicated in the maintenance of epithelial stem cells, as well as in their terminal differentiation.

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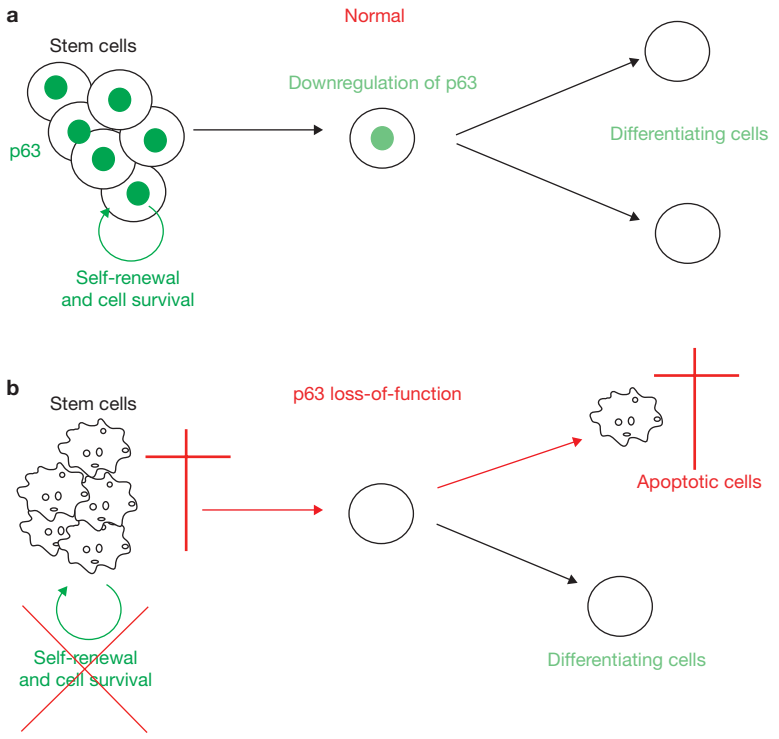

Figure 2 Potential mechanisms for p63 maintaining stem-cell populations. (**a**) In certain tissues, such as the skin epidermis, p63 may directly promote the proliferation of progenitor cells and their self-renewal. In other tissues, such as the thymus, p63 may maintain 'stemness' by stimulating cell survival. (**b**) In the absence of p63, stem cells and their progenies die by apoptosis (red dagger), and the crippled stem cells are not available to bolster cell proliferation and self-renewal. The prognosis for the tissue is poor, as it either progressively disintegrates (for example, epidermis) or shrivels (for example, thymus).

cells covered the body surface[3]. Whether strain-specific variations accounted for these differences was never fully clarified, but two divergent points of view emerged from these analyses: one group attributed the *p63*-null phenotype to an absence of lineage commitment and an early block in epithelial differentiation[3]; the other postulated that the phenotype was secondary to a defect in epithelial stem-cell renewal[4].

It thus remained uncertain whether the primary function of p63 was in control of differentiation or self-renewal, or both. Subsequent studies on p63 attempted to clarify this issue, but were further complicated with the discovery that p63 has two principal isoforms (ΔNp63 and TAp63), each of which seems to have distinct roles in epithelial development[5]. The skin of zebrafish is a single layer that expresses ΔNp63, a seemingly dominant-negative repressor of p53 target genes[6]. In mammals, the *p63* gene encodes an additional (larger) isoform, TAp63, which contains a putative amino-terminal transactivation domain[5].

Most researchers contend that, in mouse, ΔNp63 isoforms are expressed shortly after gastrulation when the skin is only a single layer of ectodermal cells, and that it persists thereafter in the basal layer of the stratified epithelium[7]. In contrast, TAp63 isoforms seem to be only weakly expressed in suprabasal cells[5,7]. When transgenic mice expressing either TAp63 and/or ΔNp63 were bred on the *p63*-null background, mice expressing ΔNp63, but not TAp63, partially rescued basal epidermal gene expression, whereas only mice coexpressing both isoforms presented a significant improvement in expression of terminal differentiation markers[8]. Supporting the view that TAp63 also has a role in epidermal differentiation, forced expression of TAp63 in lung induced transformation of a single-layered epithelium into a stratified keratinizing epithelium[9]. Thus, although not unequivocal, the cumulative data are consistent with the notion that ΔNp63 governs basal-epidermal gene expression, whereas ΔNp63, possibly together with TAp63, functions in an additional step to promote terminal differentiation (Fig. 1).

Taken together, these studies supported a role for p63 in differentiation and diverted attention from a possible role for p63 in self-renewal and long-term potential. In a new study, Senoo *et al.* have now taken advantage of an alternative model — development of the thymic epithelium — to take a fresh look at p63. The study provides compelling evidence that p63 does indeed play a major role in the maintenance of the proliferative potential of epithelial progenitors[10].

Thymic epithelium is a critical component of the microenvironment supporting T-lymphocyte development. It has a very different organization from epidermis, but like epidermis, expresses p63. The authors found that in mice lacking p63, the specification of primitive endoderm into thymic cells seemed to occur normally, but by birth, the epithelium was dramatically smaller in size[10]. In contrast to *p63*-null skin epidermis, which disintegrated during embryogenesis, the p63-deficient thymus, although hypoplastic, remained intact. For this reason, it offered a good model to study the function of p63 during epithelial morphogenesis.

To determine the cause of the thymic epithelial growth defect in p63-deficient embryos, the proliferative capacity of thymic epithelial cells was investigated in a three dimensional culture system. Under these conditions, the p63-deficient cells grew at a markedly reduced rate[10]. These findings provided a functional explanation for earlier and present correlations drawn between the level of p63 and the proliferative potential of corneal, thymic and epidermal epithelial cells[10,11].

The group next evaluated the importance of p63 for self-renewal potential by performing clonal analyses on cultured thymic epithelial and epidermal cells after short hairpin RNA (shRNA) knockdown of *p63* mRNA. Cells with reduced *p63* mRNA levels formed smaller colonies, displaying reduced proliferation rates and increased expression of terminal differentiation markers. Curiously, these results are in direct conflict with a recent study by Koster *et al.*, who conclude that p63 might trigger basal cells to switch from proliferation to terminal differentiation through induction of IκB kinase-α, which is thought to have an essential role in this process[12].

Based on the culture assays of Senoo *et al.*, p63 seems to be essential for proliferative potential. However, surprisingly, when the authors looked at the thymic epithelium *in vivo*, they found that absence of p63 did not affect epithelial-cell proliferation at all[10]. Why then was the thymus so small in the absence of p63? Intriguingly the authors observed that lack of p63 triggers apoptosis and clearance of the

proliferating cells of the thymic epithelium[10]. This phenomenon also occurs in mammary cell lines *in vitro*, where cells detach from their underlying substrates and execute apoptosis[13].

Overall, by concentrating primarily on the thymic epithelium, the study by Senoo *et al.* offers a new model (Fig. 2) for p63 as a gatekeeper for programmed cell death, and as a key factor for the maintenance of the proliferative potential of embryonic and adult epithelial stem cells. Calling up the stem-cell reserves becomes critical following tissue injury, when cell death is prevalent, and it might be particularly advantageous to protect the stem cells from surrounding death inducing factors that invade and repair the damaged tissue. The model of Senoo *et al.* beautifully explains how p63 might control the balance of stem-cell proliferation during regeneration of at least some epithelial tissues.

How might p63 prevent cell death? Several clues come from transcriptional profiling of cells following p63 gain- and loss-of-function studies[13,14]. Interestingly, p63 seems to directly regulate expression of extracellular matrix adhesion molecules, including basal integrins such as α6β4 (ref. 13) and desmosomal proteins (for example, PERP)[14], all of which are well-known determinants of epithelial integrity and proliferative maintenance[1]. Another possible p63 target is Fras1, an epidermal ECM protein that, when defective, results in severe blistering[15,16]. *Fras1* mRNA levels were reduced and basement membrane integrity was lost in the epidermis of recently generated *p63*-knockdown mice[12]. Strangely, however, the epidermis displayed no signs of enhanced apoptosis, but instead hyperproliferation.

The underlying reasons for the diametrically opposing results and conclusions of these recent studies remain mysterious. It would be interesting to determine whether the hyperproliferation and lack of apoptosis observed by Koster *et al.* in the epidermis of postnatal *p63*-knockdown mice also occur in the embryo, to exclude the possibility that the phenotype is not attributable to an interferon response and/or an ill-fated rescue attempt due to the loss of an intact barrier required for postnatal survival. Conversely, to dig further into the possible mechanisms underlying the *in vitro* data of Senoo *et al.*, it would be interesting to determine whether the defects in stem-cell self-renewal detected in cultured p63-deficient epithelial cells can be rescued by elevating α6β4, Fras1 or other putative adhesion and/or ECM targets of p63 identified by Koster *et al*. Finally, it may be relevant that spatial cues emanating from basal integrins assist in localizing the polarity complexes necessary to orient asymmetric cell divisions during epidermal stratification[17]. In the absence of p63, only symmetric divisions were observed, whereas in the absence of β1 integrin, proper spindle orientation was not maintained. Whether any of the key genes involved in this process are affected by p63 deficiency is, at present, unknown.

Although it will not resolve all of the controversy surrounding the functions of p63, Truong *et al.* suggest a possible reconciliation[18]. Using an *in vitro* model of human epidermal regeneration, these authors demonstrated that reducing *p63* mRNA levels also reduced cell proliferation. Most importantly, they discovered that when *p53* and *p63* small interfering RNAs (siRNAs) were added simultaneously, cell proliferation was normal, suggesting that p63 may regulate cell proliferation by inhibiting p53 function. If loss of p63 results in increased apoptosis, as suggested by Senoo *et al.*, then one way in which cell proliferation may be restored by *p53* shRNA is by disrupting p53-dependent apoptosis. Interestingly, *p53* siRNA did not rescue the block of terminal differentiation, suggesting that p63-deficiency impairs human epidermal differentiation independently of its role in cell proliferation, and possibly apoptosis.

In conclusion, cumulative data from studies performed on fish[6], mice[10] and humans[18] suggest that the mechanism by which p63 controls epidermal stem-cell proliferation has been conserved throughout vertebrate evolution. Although the impact of p63 on apoptosis and differentiation is still clouded by controversy, resolving these issues in the future will likely provide major and important insights into the role of p63 in cancers and cancer stem cells.

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