Association of p63 with Proliferative Potential in Normal and Neoplastic Human Keratinocytes

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p63, a recently identified member of the p53 gene family, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. We show that in normal human epidermis, in hair follicles, and in stratified epidermal cultures, p63 protein is principally restricted to cells with high proliferative potential and is absent from the cells that are undergoing terminal differentiation. In normal human epidermis and in hair follicles, basal cells with abundant p63 are interspersed with cells with little or no p63. Whenever p63 mRNA is present, it encodes mainly truncated, potentially dominant-negative isotypes. In squamous cell carcinomas, the number of cells containing p63 and their distribution depends on the degree of anaplasia. In highly differentiated tumors, p63 is confined to a ring of basal-like cells surrounding, but at a distance from, centers of terminal differentiation. In less differentiated tumors, most cells contain p63 and their distribution is chaotic with respect to centers of terminal differentiation. p63 appears to be a valuable diagnostic marker for anaplastic keratinocytes. *Key words: differentiation/epidermis/hair follicle/squamous cell carcinoma. J Invest Dermatol 113:1099–1105, 1999*

The p53 tumor-suppressor gene encodes a multifunctional DNA-binding protein important in cell cycle and cell death regulation, and is the most frequently altered gene in human cancers (Ko and Prives, 1996; Levine, 1997). The p53 protein contains three major functional domains: an N-terminal transactivation domain, a central, DNA-binding domain, and a C-terminal oligomerization domain. Regulation of cell growth and apoptosis by p53 occurs through its transactivation capability; mutations of p53 yielding either dominant-negative or novel gain-of-functions are thought to contribute to tumorigenesis (Levine, 1997).

The identification of a new protein, termed p73, having structural and functional similarities to p53, suggested that a family of p53-like proteins exists (Kaghad *et al*, 1997). Recently, a new gene termed variously KET, p40, p51, and p63, located on human chromosome 3q, has been shown to possess homology with p53 and p73 (Schmale and Bamberger, 1997; Osada *et al*, 1998; Trink *et al*, 1998; Yang *et al*, 1998). This gene, which we will refer to as p63, was detected in a variety of human and murine tissues (Osada *et al*, 1998; Trink *et al*, 1998; Yang *et al*, 1998) and encodes two categories of transcripts under the control of two alternative promoters (Yang *et al*, 1998). The first encodes proteins with an acidic N-terminal transactivation domain (TAp63) that, like p53 and p73, can activate transcription and induce apoptosis. The second encodes proteins lacking the N-terminal transactivation domain (Np63), and potentially acts in a dominant-negative manner to suppress transactivation by p53 and TAp63 (Yang *et al*, 1998). Cultured human keratinocytes predominantly express truncated, dominant-negative p63 isotypes (ΔNp63) (Yang *et al*, 1998). Investigation of 66 human primary tumors of various cell types and 102 tumor cell lines has revealed point mutations of p63 only in three tumors, all of epidermal origin (Osada *et al*, 1998). All three of these mutations were located in the DNA-binding domain (Osada *et al*, 1998).

We have now studied the expression of p63 in normal and neoplastic keratinocytes. p63 appears to be a marker for keratinocytes with proliferative capacity and may be useful for the prognostic study of neoplastic keratinocytes.

MATERIALS AND METHODS

Cell culture Human epidermal keratinocytes derived from foreskin of a normal newborn (strain YF29) were grown with supporting 3T3-J2 cells (Rheinwald and Green, 1975),using additives to the culture medium (Allen-Hoffmann and Rheinwald, 1984; Simon and Green, 1985), including 10% fetal calf serum (Hyclone, Logan, UT). Megacolonies of keratinocytes were grown on 150 mm dishes by inoculating 10–20 cells per dish and cultivating for 16–18 d (Tseng and Green, 1994). Well-isolated colonies were then chosen for experiments.

Centrifugal elutriation Keratinocytes were grown to confluence in 150 mm dishes, trypsinized, and collected by centrifugation. Centrifugal elutriation was performed as described previously (Tseng and Green, 1994). A total of seven fractions were collected as cell suspensions. A drop of each cell suspension was photographed in a hemacytometer for size measurement and cell counts. The remaining cells were lysed for RNA preparation.

Northern analysis, *in situ* hybridization and RT-PCR analysis Total RNA was isolated from cells, using RNeasy Total RNA kit (Qiagen, Santa Clarita, CA). Ten micrograms of total RNA from each cell fraction was loaded in each lane, separated on a 1% formaldehyde-agarose gel and transferred to nylon membranes (Amersham, Bucks, U.K.). Blots were prehybridized and hybridized at 68°C in Quickhyb hybridization solution (Stratagene, La Jolla, CA). The probes were labeled with (32P) dCTP by using a multiprime labeling system kit (Amersham). The RNA was first probed with a 420 bp fragment corresponding to a common region between TAp63 and Np63 cDNA (from nucleotides 765 to 1185 of TAp63 cDNA and from nucleotides 600
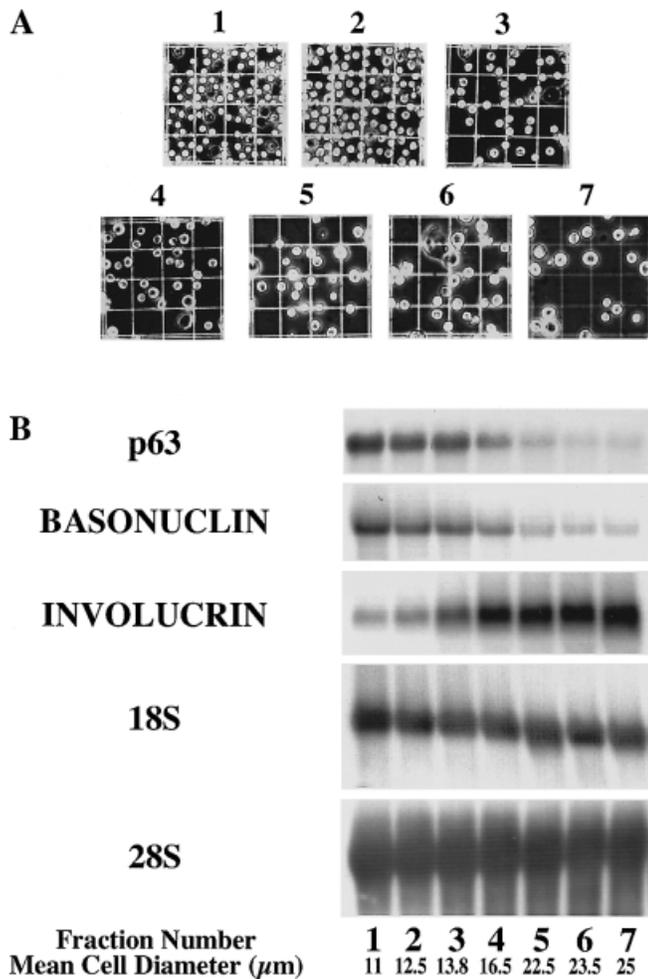
Figure 1. Change in abundance of p63 mRNA in cultured keratinocytes during their enlargement and terminal differentiation. Confluent cultures of keratinocytes were trypsinized and the cells were separated according to their size by centrifugal elutriation. (*A*) Size of the cells in seven elutriation fractions. (*B*) Northern analysis of total RNA prepared from elutriation fractions. The integrity of the RNA and the loading variations were verified by probing the blot with oligonucleotides corresponding to 18S and 28S ribosomal RNA. The small cells contain p63 and basonuclin mRNA, whereas the large cells contain involucrin mRNA.

to 1020 of $\triangle$Np63 cDNA) (Yang *et al*, 1998). The RNA was then dehybridized and reprobed sequentially, with cDNA encoding the human basonuclin and involucrin (Eckert and Green, 1986; Tseng and Green, 1994). 18S and 28S rRNA probed with specific oligonucleotides were used as controls for loading variation and RNA integrity.

In situ hybridization with digoxygenin-labeled cRNA probes corresponding to the p63 cDNA fragment (described above) were performed on frozen sections as described (Schaeren-Wiemers and Gerfin-Moser, 1993). The nuclei were counterstained with 1% methylene green (Fluka Chemical, Milwaukee, WI) for 5 min.

RT-PCR analyses were performed on RNA preparations, using primers specific for human p63 TA and human p63$\triangle$ N as described (Yang *et al*, 1998).

Immunohistochemistry   Cultured cells or frozen tissue sections were fixed as described previously (Tseng and Green, 1994). The mouse monoclonal antibody to p63 (Yang *et al*, 1998) and the rabbit antibody to basonuclin (Iuchi and Green, 1997) were detected with sheep antisera to mouse or rabbit IgG coupled to HRP (Amersham). The rabbit antibodies to human involucrin (Biomedical Technologies, Stoughton, MA) and to human Ki-67 (Dako, Carpinteria, CA) were detected with goat antiserum to rabbit IgG coupled to FITC (Boehringer-Mannheim Biochemicals, Indianapolis, IN). DNA was stained with Hoechst 33258 (Fluka Chemical) at 1 µg per ml for 2 min.
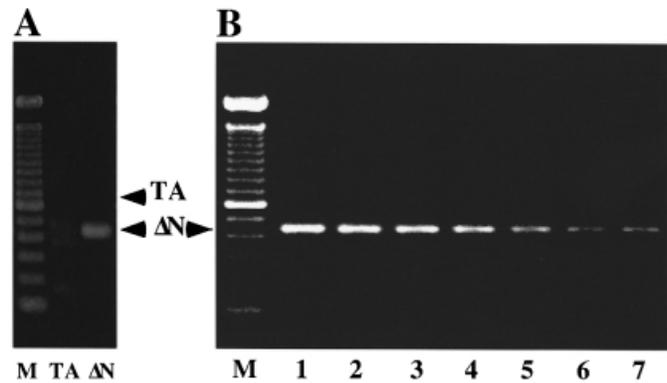

Figure 2. Analyses of p63 transcripts in cultured keratinocytes during their enlargement and terminal differentiation. (*A*) RT-PCR analyses of RNA from cultured keratinocytes before cell fractionation, using specific primers designed to amplify human TAp63 transcripts (*lane TA*) and $\triangle$Np63 transcripts (*lane $\triangle N$*). The TA lane shows little or no product corresponding to human TAp63 transcripts, whereas *lane $\triangle N$* shows a strong band corresponding to human $\triangle$Np63 transcripts. (*B*) Semi-quantitative RT-PCR analyses of total RNA prepared from elutriation fractions of cultured keratinocytes (as described in **Fig 1**), using primers designed to amplify human $\triangle$Np63 transcripts. The increase in cell size is associated with the disappearance of transcripts encoding truncated dominant-negative p63 isotypes.

RESULTS

p63 mRNA during differentiation of keratinocytes   In both the epidermis and the stratified epidermal cultures, the growth and differentiated properties of keratinocytes have been correlated with their size and location (Rowden, 1975; Sun and Green, 1976; Bergstresser *et al*, 1978). Proliferation is mostly confined to small cells in the basal layer. Keratinocytes that leave the basal layer withdraw from the cell cycle, undergo terminal differentiation, and enlarge as they move through suprabasal layers. In stratified epidermal cultures, enlargement and terminal differentiation of keratinocytes are accompanied by loss of both the ability to replicate their DNA (Sun and Green, 1976) and the ability to form colonies (Barrandon and Green, 1985; Tseng and Green, 1994). Differentiating cells also show a marked reduction or total loss of basonuclin expression (Tseng and Green, 1994) and begin to synthesize involucrin (Banks-Schlegel and Green, 1981; Watt and Green, 1981; Tseng and Green, 1994) and many other protein markers of terminal differentiation (Fuchs and Byrne, 1994).

We have examined the expression of p63 as the cells enlarge in stratified cultures of keratinocytes. Cells from confluent cultures were separated according to size by centrifugal elutriation. Seven fractions were collected. In each fraction, the average cell size was determined and the presence of p63, basonuclin, and involucrin mRNA was monitored by Northern analysis, using the coding regions of these genes as probes (Eckert and Green, 1986; Tseng and Green, 1994; Yang *et al*, 1998).

As shown in **Fig 1**, the expression of p63 mRNA is confined to the first four fractions, which contain small cells with a mean diameter of 11–16.5 µm. This population also contains basonuclin mRNA. Cells of this size range are known to include nearly all the colony-forming cells of the culture (Barrandon and Green, 1985; D'Anna *et al*, 1988; Tseng and Green, 1994). In the last three fractions, as the mean cell size increases above 16.5 µm in diameter, the abundance of p63 mRNA drops sharply, as does that of basonuclin mRNA. These cells accumulate involucrin mRNA, a characteristic of terminal differentiation. Cells of this size range do not synthesize DNA (Sun and Green, 1976) and are unable to form colonies (Barrandon and Green, 1985; D'Anna *et al*, 1988; Tseng and Green, 1994). These data clearly demonstrate that the p63 gene is expressed in the small colony-forming cells, and is absent from the larger cells that are undergoing terminal differentiation.
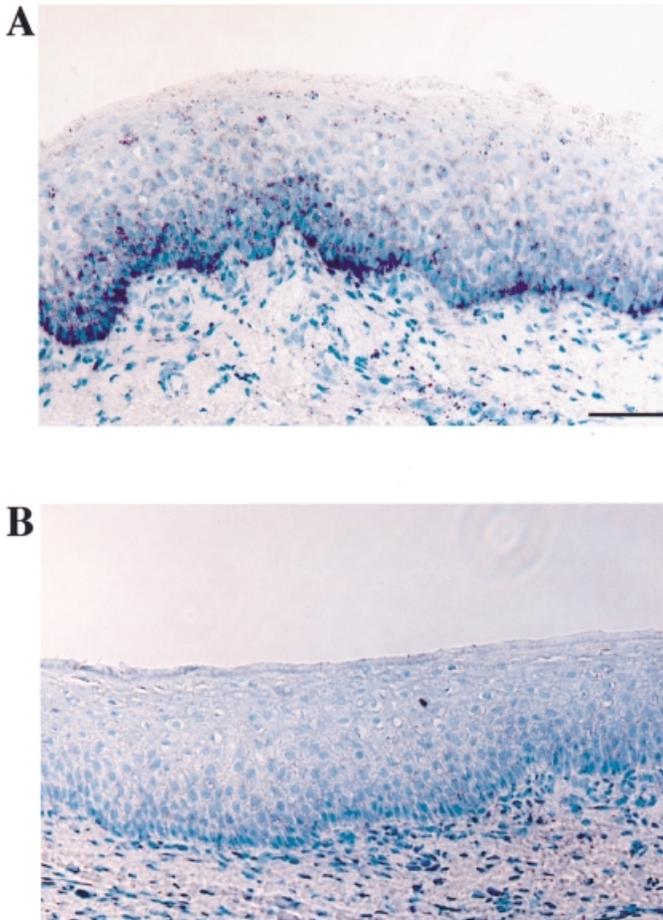
Figure 3. A representative *in situ* hybridization of p63 mRNA in normal human epidermis. (*A*) Frozen sections of human foreskin were hybridized with a digoxygenin-labeled antisense p63 cRNA corresponding to the p63 cDNA probe used in our Northern analysis. A strong signal was detected, predominantly in keratinocytes of the basal and lower spinous layers of the epidermis. The distribution of p63-containing cells was patchy. (*B*) Absence of hybridization when the corresponding sense cRNA was used as a probe. The nuclei were counterstained with methylene green to obtain a good contrast between the specific signal (purple) and the surrounding tissue. *Scale bar*: 50 μm.
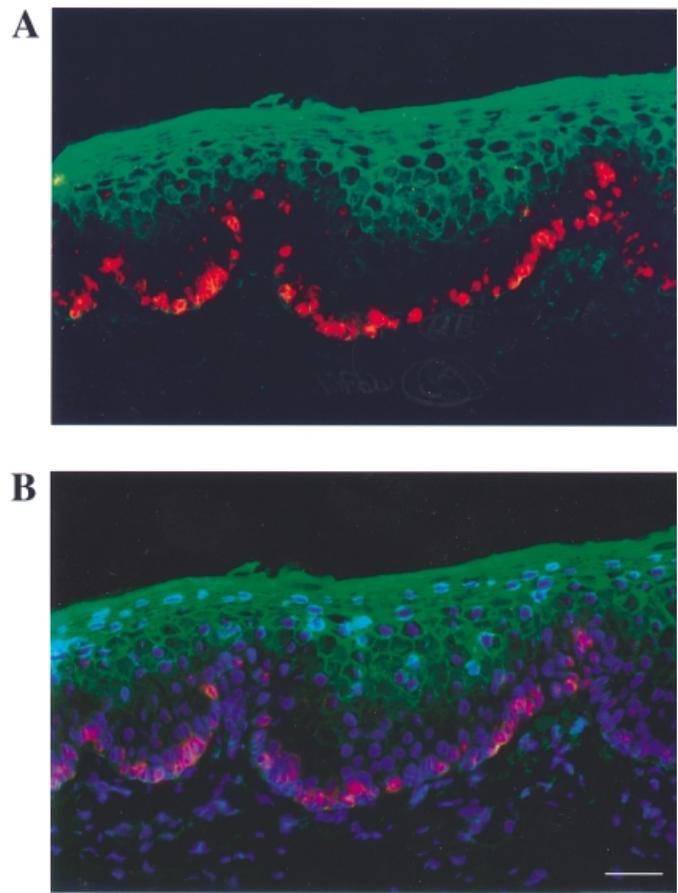
Figure 4. Distribution of p63 and involucrin in normal human epidermis. Frozen sections of human foreskin were fixed and stained for p63 (red) and involucrin (green) (*A*), and for DNA (blue) (*B*). Involucrin appears in the spinous layer beginning several layers above the first layer lacking p63 and is typically located in the peripheral cytoplasm, close to the cell membrane. p63 is located mainly in the nuclei of the basal layer and immediately suprabasal layers of the epidermis. As in the case of the mRNA, cells with the most abundant p63 protein appear to be clustered in patches. The green color staining in the dermis is non-specific. *Scale bar*: 50 μm.

Previous studies have shown that human keratinocytes express mainly truncated dominant-negative p63 isotypes (△Np63) rather than those with the N-terminal transactivation domain (TAp63) (**Fig 2***A*; Yang *et al*, 1998). Semi-quantitative RT-PCR reactions designed to amplify △Np63 transcripts in RNA preparations from fractionated cells showed that small keratinocytes express high levels of △Np63 transcripts (**Fig 2***B*). As the cell size increased, the abundance of △Np63 transcripts decreased. Comparison of **Fig 1** with **Fig 2***B* shows that the decrease in △Np63 transcripts detected by RT-PCR follows that of the p63 mRNA detected by Northern analysis. These data indicate that the differentiation of human keratinocytes is associated with the disappearance of transcripts encoding truncated dominant-negative p63 isotypes.

To determine the location of p63 mRNA within the epidermis, we performed *in situ* hybridization on frozen sections of human foreskin. A digoxygenin-labeled antisense p63 cRNA corresponding to the cDNA probe used in our Northern analysis revealed a strong signal in keratinocytes of the basal and lower spinous layers of the epidermis (**Fig 3***A*); however, the level of p63 mRNA in different cells of the basal layer appeared variable. The basal cells with the highest level of p63 mRNA were sometimes clustered in patches separated by basal cells containing little or no p63. No hybridization was detected when the sense cRNA was used as a probe (**Fig 3***B*).

P63 is localized in the nuclei of basal cells of epidermis and the outer root sheath of hair follicles   Frozen tissue sections of normal human foreskin were analyzed for the presence of p63 by immunofluorescence, using the 4A4 monoclonal antibody (Yang *et al*, 1998). Strong staining was confined to the nuclei of basal or immediately suprabasal keratinocytes (**Fig 4**); however, as suggested by the mRNA localization, the intensity of p63 protein staining in different cells of the basal layer appeared variable. The basal cells most brightly stained for p63 were clustered in patches, separated by basal cells less brightly stained or unstained. The protein was absent from the spinous layer, where terminal differentiation begins. In contrast to basonuclin, which is found in both nuclear and cytoplasmic compartments of the basal layer of human epidermis (Iuchi *et al*, 1999), p63 appeared strictly localized in the nucleus (**Fig 4**). Double staining for p63 and involucrin showed that involucrin appears in the spinous layer beginning several layers above the first layer lacking p63. Therefore, in normal human epidermis, all cells lose p63 considerably prior to the appearance of involucrin (**Fig 4***A*).

It has been proposed that hair follicles are an important reservoir of epidermal stem cells (Kobayashi *et al*, 1993; Lavker *et al*, 1993; Fuchs and Byrne, 1994; Rochat *et al*, 1994). We stained mature anagen hair follicles for p63 and involucrin. As in the case of basonuclin (Tseng and Green, 1994), the p63-containing cells are distributed in the basal layer of the outer root sheath, the part of the follicle continuous with the basal layer of the epidermis (**Fig 5**). In
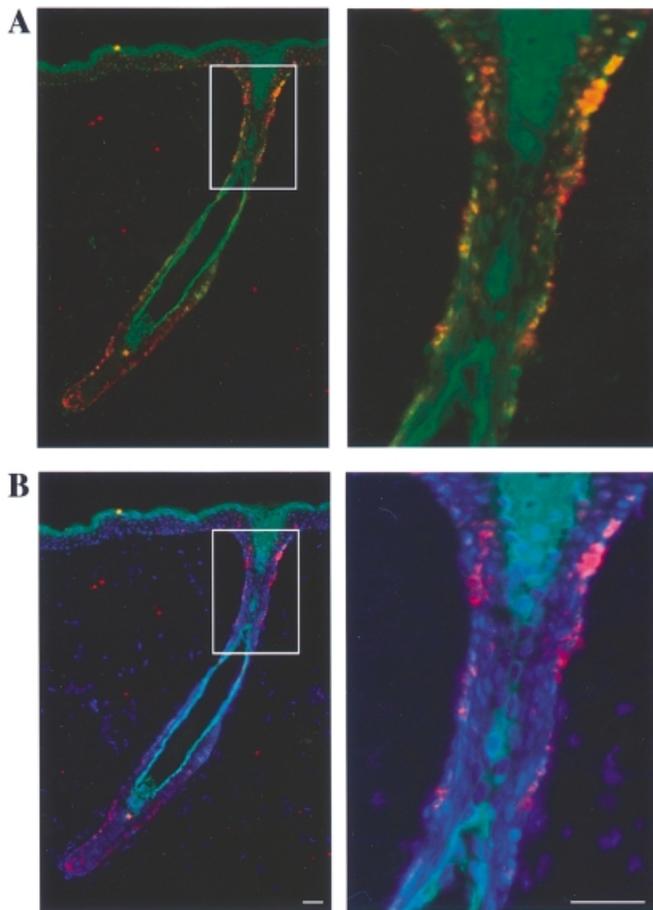
Figure 5. Comparison of distribution of p63 and involucrin in cells of the hair follicle. Frozen sections of human skin were fixed and stained for p63 (red) and involucrin (green) (*A*), and for DNA (blue) (*B*). The areas delineated by rectangles on the left panels are shown enlarged on the right panels. The p63-containing cells are distributed in the outer root sheath, which is continuous with the basal layer of epidermis. In the upper region of the follicle, cell clusters brightly stained for p63 could be seen. In the lower half of the follicle, p63 seems to be present in all layers of the outer root sheath. *Scale bar:* 50 μm.

the upper region of the follicle, cell clusters brightly stained for p63 could be seen (**Fig 5**). Cells of this region have been shown to contain very little basonuclin (Weiner and Green, 1998; Iuchi *et al*, 1999). In the lower half of the follicle, p63 seems to be present in all layers of the outer root sheath. In the region close to the bulb, the cells of the outer root sheath narrow into a single layer of cells retaining p63. In the bulb, p63-containing keratinocytes surround the surface of the follicular papilla (**Fig 5**). In contrast to basonuclin (Weiner and Green, 1998; Iuchi *et al*, 1999), no difference was observed in the distribution of p63 staining between newborn and adult epidermis, nor in staining intensity along the hair shaft (data not show). p63 is absent from the dermal cells within the follicular papilla or surrounding the follicle, and from the differentiated cells that form the medulla and inner root sheath, where involucrin is expressed.

p63 expression and the potential for cell proliferation   It has been shown that the growth of megacolonies depends on outward migration of the rapidly proliferating cells located in a rim close to the colony perimeter (Barrandon and Green, 1987). The multiplication rate internal to this rim is much lower. Although the small cells located in the center of the colonies are quiescent, they remain capable of growth (Barrandon and Green, 1987; Tseng and Green, 1994).

Ki-67 is a cell proliferation-associated human nuclear antigen found in all stages of the cell cycle (Gerdes *et al*, 1984). When located in the nuclei, Ki-67 has been reported as a specific marker of multiplying keratinocytes in human epidermis (Ando *et al*, 1989; Miyauchi *et al*, 1990; Heenen *et al*, 1998). Double staining for p63 and Ki-67 revealed that the rapidly proliferating cells located close to the megacolony perimeter contained both p63 and Ki-67 (**Fig 6A**). Ki-67 was concentrated in the nucleoli, a site from which p63 seemed to be excluded. In the central part of the colonies, virtually all cells in the basal layer of the stratified colony retain p63 but few cells contain Ki-67 (**Fig 6B**).

Similarly, in normal human epidermis, most cells in the basal layer of epidermis are not engaged in multiplication (Boezeman *et al*, 1987; Potten and Morris, 1988; Clausen and Potten, 1990). Double staining for p63 and Ki-67 revealed that only a small proportion of the cells containing p63 also contain Ki-67, but all the cells containing Ki-67 contained p63 (**Fig 7**). Thus, in stratified epidermal cultures and in normal human epidermis, p63 is expressed in the nuclei of cells that are either proliferating or possess the ability to proliferate.
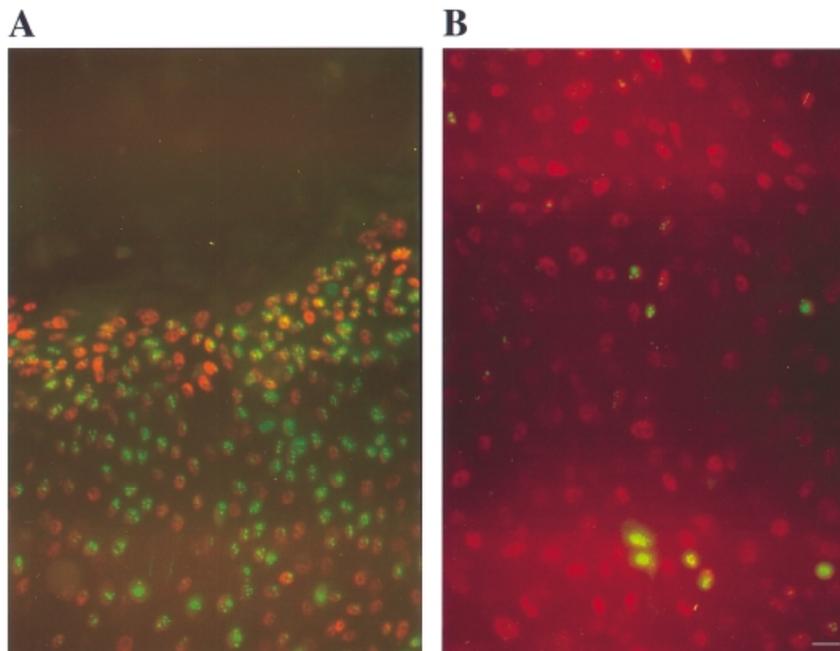

Figure 6. p63 in relation to Ki-67 in megacolonies. (*A*) Part of a 17 d megacolony located close to the perimeter, a region in which most cells of the basal layer contain Ki-67 (green) as well as p63 (red), consistent with a high rate of multiplication. Ki-67 is concentrated in nucleoli, sites from which p63 appear to be excluded. (*B*) Basal layer of the stratified central region of the same colony, in which most basal cells are quiescent. Only a small number of nuclei contain Ki-67, whereas virtually all cells in this region contain p63. The apparently fainter staining of p63 in this region is at least partly due to the stratification of the colony and the overlying suprabasal layers. *Scale bar:* 50 μm.
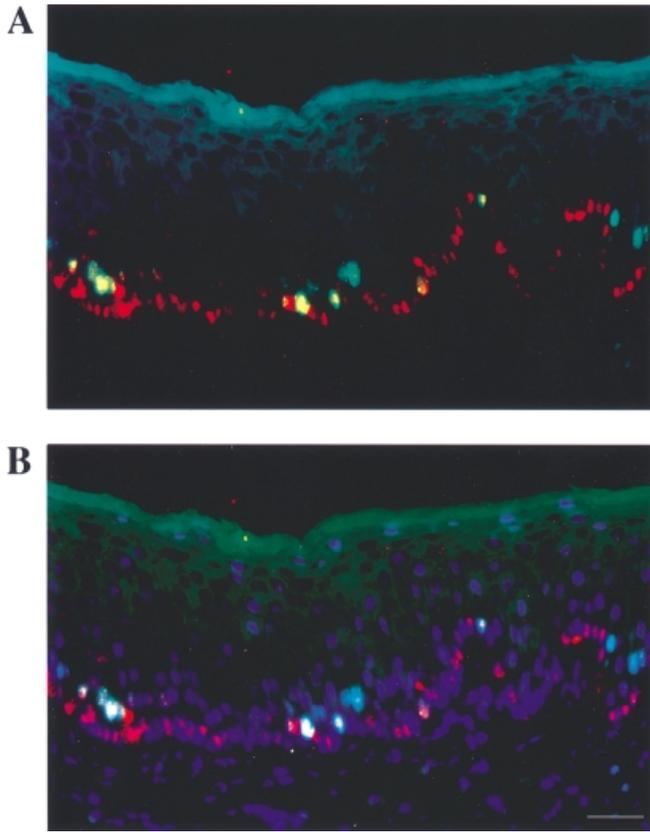
Figure 7. p63 in relation to Ki-67 in normal human epidermis. Frozen sections of human foreskin were fixed and stained for Ki-67 (green) and p63 (red) (*A*), and for DNA (blue) (*B*). Only a small proportion of cells expressing p63 contain also Ki-67. On the other hand, virtually all the cells containing Ki-67 contain p63. *Scale bar*: 50 μm.

Altered pattern of p63 expression in human squamous cell carcinoma Having shown the association of p63 with the ability of basal keratinocytes to multiply, we examined the expression of p63 in a squamous carcinoma cell line (SCC13) (Rheinwald and Beckett, 1980). RT-PCR analyses were performed on RNA preparations from these cells, using primers specific for each of the two different p63 N-termini, TA and △N (Yang *et al*, 1998). An RT-PCR reaction designed to amplify transcripts encoding the N-terminal transactivation domain showed little or no product. In contrast, a strong signal was detected in the RT-PCR reaction designed to amplify the truncated △Np63 transcript (data not shown). These data suggest that these cells, like normal keratinocytes, predominantly express truncated dominant-negative p63 isotypes (△Np63).

We also analyzed the expression of the p63 gene in squamous cell carcinomas derived from oral epithelium overlying the mandibular alveolus. Frozen sections from extensively, moderately, and poorly differentiated tumors were doubly stained for p63 and involucrin. In well-differentiated tumors, p63 was readily detected in the nuclei of cells arranged in a circle consisting of one or two layers of well-organized, basal-like cells. As in the normal epidermis, p63-containing cells were located at some distance from the terminally differentiated cells stained brightly for involucrin, and were generally absent from the zone immediately surrounding the involucrin-containing cells (**Fig 8*A*** compared to **Fig 4**). Less differentiated tumors showed a large increase in the number of cells containing p63 and those cells were irregularly distributed with respect to centers of terminal differentiation (**Fig 8*B*** and ***C***). Some of the cells containing p63 were located very close to involucrin-containing zones, instead of remaining at some distance, as in normal epidermis and in well-differentiated tumors. In poorly differentiated squamous tumors, there were very few cells containing involucrin; most of the cells stained very brightly for p63 (**Fig 8*C***), and the distribution of these p63-containing cells was chaotic with respect to centers of terminal differentiation.

DISCUSSION

In this report, we show that in cultured keratinocytes p63 mRNA is confined to the small cells known to be capable of DNA synthesis
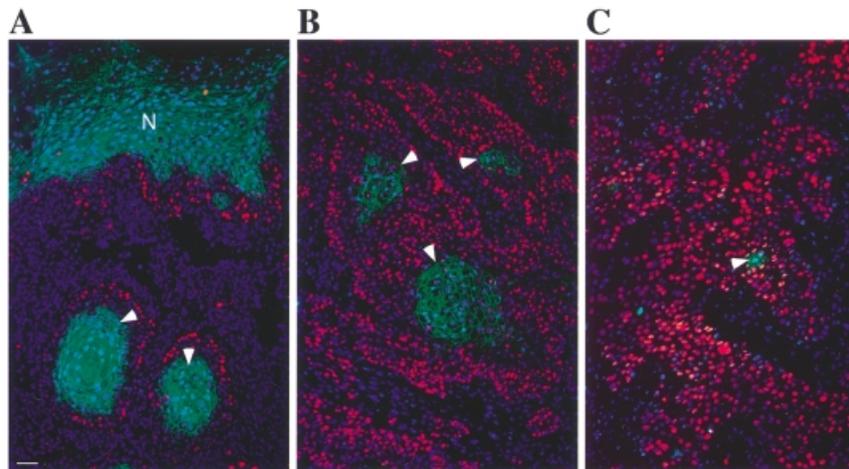
Figure 8. p63 in human squamous cell carcinoma. Frozen sections from well-differentiated (*A*), moderately differentiated (*B*), and poorly differentiated (*C*) tumors of mandibular alveolar epithelium were stained for p63 (red), involucrin (green), and DNA (blue). (*A*) Upper part of the photograph shows normal mandibular epithelium (N) with p63 confined to the basal layer at some distance from the nearest terminally differentiating involucrin-containing cells. The lower part of the photograph shows a well-differentiated tumor, in which p63-containing cells were distributed in one or two layers at some distance surrounding keratinizing nodules stained brightly for involucrin (*arrowheads*). As in the normal epithelium, a distance separates the nearest p63-containing cells from the involucrin-containing cells. (*B*) Moderately differentiated tumor showed many cells staining brightly for p63 and distributed more irregularly than in (*A*), The involucrin-containing regions were smaller and p63-containing cells were often located closer to involucrin-containing cells. (*C*) Poorly differentiated tumor. More cells contained p63, and their distribution was chaotic. Many of the nuclei seemed more brightly stained than in (*A*) or (*B*), suggesting an overexpression of p63. Similar results were obtained with frozen sections from well-differentiated, moderately differentiated and poorly differentiated tumors of buccal epithelium (data not shown). All tumors were obtained through the kindness of Dr. Fiona Watt of the Imperial Cancer Research Institute, London. The degree of differentiation of these tumors was determined by pathologic examination of the tumors at that Institute. *Scale bar*: 50 μm.

and colony formation, and absent from the larger cells that are undergoing terminal differentiation. Our data indicate that the irreversible growth arrest and differentiation of human keratinocytes are associated with the disappearance of transcripts encoding truncated dominant-negative p63 isotypes. In the epidermis, the mRNA for p63 is concentrated in the basal and lower spinous layers, in agreement with the previously reported result for the rat homologue KET mRNA (Schmale and Bamberger, 1997); however, the amount of p63 mRNA and protein in different cells of the basal layer appeared variable. Basal cells with abundant p63 mRNA or protein were clustered in patches, separated by basal cells with little or no p63 mRNA or protein. Heterogeneity of cells within the basal layer with respect to proliferative capacity and expression of protein markers is well known (Clausen and Potten, 1990; Jones et al, 1995). Whether the heterogeneity in p63 staining of the basal layer means that p63 is more highly expressed in epithelial stem cells deserved investigation. Interestingly, another marker of epidermal basal cells, basonuclin, is more uniformly present in cells of the basal cell layer than in p63 (Tseng and Green, 1994; Weiner and Green, 1998), supporting the notion that p63 distribution is differentially expressed in basal cell populations. Whereas more directed studies will be required to determine whether p63 is a reliable marker for epidermal stem cells, a functional link between p63 expression and the maintenance of the epidermal stem cell population is evident from the p63–/– mouse (Mills et al, 1999; Yang et al, 1999).

In its distribution, p63 is in many respect similar to that of basonuclin (Tseng and Green, 1994), a zinc finger protein (Tseng and Green, 1992); however, there are two important differences: (i) in the case of p63, strictly nuclear localization has been demonstrated in this report and in Yang et al (1998). In contrast, basonuclin, although often nuclear in its location, is sometimes cytoplasmic (Yang et al, 1997; Mahoney et al, 1998; Weiner and Green, 1998; Iuchi et al, 1999). The basis for cytoplasmic localization of basonuclin in keratinocytes has been demonstrated to reside in phosphorylated serine residues located very close to the nuclear localization signal sequence, particularly serine 541 (Iuchi and Green, 1997). There is no evidence on whether p63 possesses such a mechanism affecting its localization. (ii) In the hair follicles, p63 is strongly stained in the nuclei of the outer root sheath along its entire length, whereas nuclear staining for basonuclin is confined to the region below the sebaceous gland (Tseng and Green, 1994; Weiner and Green, 1998).

In squamous cell carcinomas, we found that the number of cells containing p63 and their distribution depends on the degree of anaplasia of the tumor. In highly differentiated tumors, p63 is confined to a ring of basal–like cells surrounding, but at a distance from, centers of terminal differentiation. In less differentiated tumors, the number of cells containing p63 increases and their distribution becomes chaotic with respect to centers of terminal differentiation; in poorly differentiated tumors cell cells express p63 and there is virtually no relation between the distribution of cells containing p63 and the small zones of terminal differentiation. Similar experiments were carried out for basonuclin, but variability in its staining made it impossible to correlate its expression with the degree of anaplasia of the tumor (our unpublished data). Whereas our analysis was limited to only two examples of each well differentiated, moderately differentiated, and poorly differentiated tumors, the consistency of the findings suggest that p63 may be a valuable diagnostic and prognostic marker of anaplasia of keratinocyte tumors. Investigations of a much larger panel of tumors will be necessary to ascertain the clinical importance of p63 as a marker in this disease.

The presence of p63 in the basal cells of the epidermis, in the cells of the outer root sheath of the hair follicle, and in the small colony-forming cells of stratified epidermal cultures suggests that this protein may have a function in maintaining the proliferative potential of keratinocytes and prevention of terminal differentiation. This concept is further supported by the much greater abundance of p63-containing cells in poorly and moderately differentiated squamous cell carcinomas than in well-differentiated ones. Finally, the strongest evidence for a stem-cell function of p63 is provided by the recent demonstration that the disruption of the p63 gene in mice results in embryonic failure to sustain keratinocyte multiplication, thus culminating in the total absence of all stratified squamous epithelia and their derivatives (Mills et al, 1999; Yang et al, 1999).

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