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Expression of *p63* in primary cutaneous adnexal neoplasms and adenocarcinoma metastatic to the skin

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***p63*, a recently identified homologue of the *p53* gene, has been reported to be essential in the development of epithelia and is mainly expressed by basal and myoepithelial cells. The purpose of this study was to investigate the pattern of *p63* expression in cutaneous adnexal neoplasms and to assess its possible value in the differential diagnosis of primary cutaneous neoplasms *vs* adenocarcinomas metastatic to the skin. Immunohistochemical analysis for *p63* was performed on formalin-fixed, paraffin-embedded archival tissue from 20 benign adnexal tumors, 10 malignant adnexal tumors and 14 adenocarcinomas metastatic to the skin. The expression of *p63* was evaluated in epidermal cells, skin appendages and metastatic tumor cells. *p63* was consistently expressed in the basal and suprabasal cells of epidermis and cutaneous appendages, including the basal/myoepithelial cells of sweat glands. Out of 20 benign adnexal tumors, 13 (65%) showed strong (score 3) *p63* expression; the remaining seven (35%) cases had score 2. All primary cutaneous carcinomas, including adenocarcinomas, expressed *p63*. In contrast, none of the metastatic adenocarcinomas to the skin was positive for *p63* ($P < 0.001$). Based on our findings, analysis of *p63* expression may help in the differential diagnosis of primary *vs* metastatic cutaneous adenocarcinomas.**
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The *p63* gene, a recently described member of the *p53* gene family, is located on the chromosome 3q27–29.[1] It contains two separate promoters and expresses at least six major transcripts that lead to two fundamentally different classes of proteins.[1–4] Three of the *p63* isoforms (TA*p63*) encode proteins with roles similar to *p53* (ie transactivation and induction of apoptosis), whereas the other three isoforms (ΔN*p63*) lack the acidic amino (N)-terminal transactivation domain and exert inhibitory effects on *p53* activity. It has been recently shown that *p63* is highly expressed in the basal cells of human epithelial tissues.[5,6] Notably, the analysis of RNA from keratinocytes indicated that the major *p63* transcripts in these cells encoded ΔN*p63* isotypes.[1] Therefore, in these cells the expression of ΔN*p63* might block the apoptosis-inducing activity of *p53* and thus could help maintain the proliferative capacity of basal/progenitor cells.[5–8]

It has been postulated that the *p63* plays an essential role in epithelial development, fact demonstrated by the defects or agenesis of squamous epithelia, mammary, lacrimal, salivary glands and craniofacial structures in the *p63*-deficient mice.[7–9] In addition, *p63* is also expressed in many normal human tissues including prostate basal cells, uterine cervix or urogenital tract.[10–13] Furthermore, *p63* is a selective nuclear marker of the myoepithelial cells in breast and has an increasingly diagnostic value in differentiating between *in situ* and invasive carcinomas.[10,11,14,15] Moreover, it has been shown that primary or metastatic carcinomas derived from glandular epithelia, including adenocarcinoma of the breast or prostate, consistently fail to express *p63*.[10,12,14]

The expression of *p63* in normal human epidermis, cutaneous appendages and skin carcinomas has been recently assessed.[16,17] In these studies, *p63* was detected in the epidermal and adnexal basal/myoepithelial cells and it has been suggested that *p63* might be used as a diagnostic marker for epidermal or adnexal tumors in the skin.[16–18]

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Cutaneous metastases may be the initial manifestation of some neoplasms, including adenocarcinomas. The distinction between such metastases and primary cutaneous neoplasms, especially adnexal tumors, on histologic grounds alone, may be difficult. The purpose of this study was to investigate the pattern of *p63* expression in cutaneous adnexal neoplasms, including adenocarcinomas and to assess its possible value in the differential diagnosis of primary cutaneous neoplasms *vs* adenocarcinomas metastatic to the skin.

Materials and methods

Patients

The pathology database at the University of Texas MD Anderson Cancer Center was retrospectively reviewed to identify cases of primary adnexal carcinomas and metastatic adenocarcinomas to the skin between January 1997 and July 2003. There were retrieved 20 benign adnexal tumors (six poromas, three syringomas, nine trichoepitheliomas, one spiradenoma, one papillary eccrine adenoma), 10 malignant adnexal tumors (four microcystic adnexal carcinomas, two eccrine adenocarcinomas, one hidradenocarcinoma, two tricholemmal carcinomas and one mucinous sweat gland adenocarcinoma), and 14 metastatic adenocarcinomas to the skin (12 from breast, two from the gastrointestinal tract). Clinical information was obtained from the medical records. The pathology reports and the hematoxylin and eosin (H&E)-stained glass slides were reviewed in all cases.

Immunohistochemical Staining for *p63*

Immunohistochemical analysis for *p63* was performed on formalin-fixed, paraffin-embedded archival tissue using the streptavidin–biotin-peroxidase technique. For all cases, a 4 μm histologic section was deparaffinized and rehydrated in graded alcohols and distilled water. After blocking the endogenous peroxidase (3% hydrogen peroxide for 5 min), the heat-induced antigen retrieval was performed in a water bath, using citrate buffer at pH 6.0 for 20 min. Biotin-conjugated secondary antibody was applied for 20 min, and streptavidin-biotin-peroxidase complex (Strept-AB complex, dilution 1:200, DAKO Corp., Carpinteria, CA, USA) was added for 20 min. A mouse antihuman monoclonal antibody that reacts with all *p63* isoforms (clone 4A4, dilution 1:200; Santa Cruz, Biotechnology Inc.; Santa Cruz, CA, USA) was used. Positive and negative controls were included in each slide run.

The distribution of the immunoreactivity in the neoplastic cells was analyzed by quantifying nuclear staining, following previous methodology.[19] A consensus between the investigators was obtained to score the nuclear staining of *p63* as follows: 0 for less than 5% positive nuclei; 1 for 5–25% positive nuclei, 2 for 26–75% positive nuclei and 3 for over 75% positive nuclei. Positivity of cells was defined regardless of staining intensity. By convention, we considered that greater than 25% positive cells represented the cutoff between negativity and positivity of the *p63* immunostaining. Two investigators (VGP and DI) have independently reviewed and scored slides by estimating the percentage of cells exhibiting characteristic nuclear staining. Interobserver variation was addressed by averaging the individual values. The distribution of *p63* expression in the basal and suprabasal cells of normal epidermis, hair follicles, sweat glands and sebaceous glands was evaluated in normal skin adjacent to the neoplasms. It was also assessed the pattern of *p63* expression in endothelial cells, dermal mesenchymal cells, pilar erector muscles, nerve bundles, adipocytes or inflammatory infiltrates. At least 10 high-power fields were chosen randomly, and 100 cells were counted in each field.

Statistical Analysis

The statistical association between the distribution of *p63* expression in benign or malignant primary cutaneous neoplasms or metastatic adenocarcinomas to the skin was analyzed using χ^2 and Fisher's exact tests, with $P<0.05$ considered to be statistically significant.

Results

Expression of *p63* in Normal Epidermis and Skin Appendages

p63 was consistently expressed in the basal and suprabasal cells of normal epidermis; the spinous, granular and corneum layers cells were negative for *p63*. The outer root sheath cells and the hair bulb of the hair follicle exhibited positivity for *p63* whereas the inner root sheath cells and the hair matrix were negative for *p63*. A variable *p63* nuclear staining was identified in the pilar erector muscles. The germinative cells of the secretory portion of the sebaceous glands, as well the duct cells, strongly expressed *p63*. No immunoreactivity was seen in the mature sebocytes. The myoepithelial cells of the eccrine coils and the basal cells of the eccrine glands ducts were positive for *p63*. The myoepithelial cells of the secretory component of the apocrine glands and the outer layer cells of the apocrine ducts did also express *p63*. No differences in the labeling pattern were observed between the epidermis and adnexal structures of the skin. *p63* immunostaining was not evident in the nuclei of the endothelial cells, dermal mesenchymal cells, nerves, adipocytes or inflammatory infiltrates.

Expression of *p63* in Benign Adnexal Tumors

The analysis of *p63* expression in trichoepitheliomas (Figure 1a) revealed that *p63* was expressed in over 75% of the basaloid cells (nuclear score 3) in six out of nine (67%) cases, including desmoplastic trichoepitheliomas. The other three cases were also positive for *p63* and had a nuclear score 2. Notably, the latter had areas of *p63* negativity alternating with areas of strongly *p63* labeling (almost 100% of the nuclei).

The pattern of expression of *p63* in six eccrine poromas (Figure 1b) included in our study revealed that the cords of proliferating basaloid cells were strongly positive (nuclear score 3) in three cases and had a nuclear score 2 in the remaining three cases. Only the luminal cells in the tumor nests did not expressed *p63*.

The myoepithelial cells of the ductal structures of the syringomas (Figure 1c) were positive for *p63*; two cases had nuclear score 3 and one case, score 2. The inner layer of cuboidal epithelium did not express *p63*.

Strong *p63* positivity was identified in the outer cells of the basaloid nodules in the eccrine spiradenoma (Figure 1d), whereas the inner cells had a *p63* labeling of less than 25% of the nuclei. The papillary eccrine adenoma case had a similar pattern of *p63* immunostaining with strong positivity (nuclear score 3) of the outer cells.

Expression of *p63* in Malignant Adnexal Tumors

Both cases of eccrine adenocarcinoma (Figure 2a) were strongly positive (nuclear score 3) for *p63*, as well as the hidradenocarcinoma (Figure 2b). The basaloid islands of the mucinous eccrine adenocarcinoma included in our study expressed *p63* in the less than 25% of cells (nuclear score 1).

All four cases of microcystic adnexal carcinoma expressed *p63* in more than 75% of cells (Figure 2c). No differences in the labeling pattern were observed between the superficial and the deeper nests or cords of neoplastic cells. *p63* staining highlighted

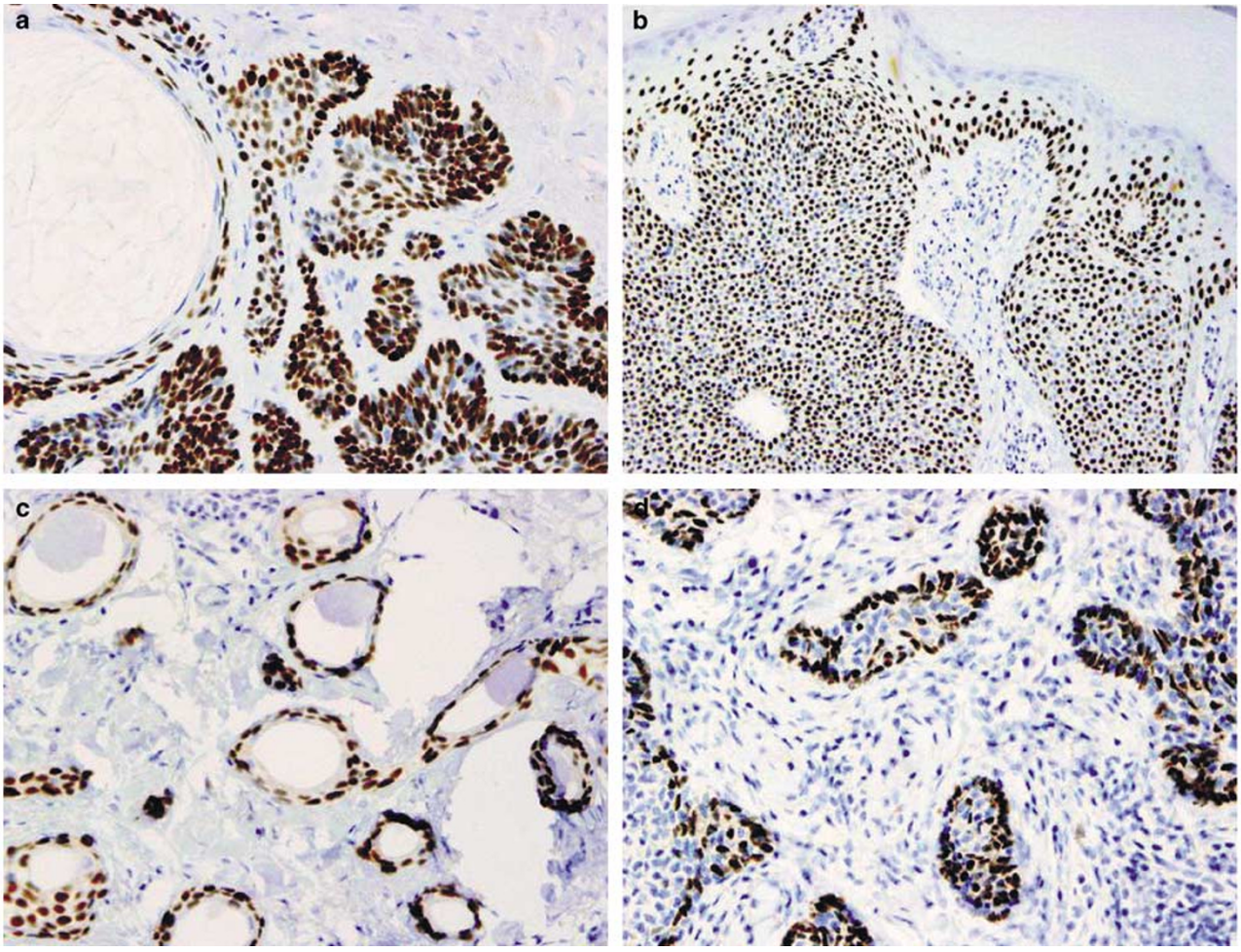

Figure 1 Immunohistochemical staining for *p63* reveals strong and diffuse nuclear reactivity in the basaloid cells of trichoepitheliomas (**a**) and eccrine poromas (**b**). The myoepithelial cells of the ductal structures of the syringomas expressed *p63*; the inner layer of cuboidal epithelium did not (**c**). Also, strong *p63* positivity was identified in the outer cells of the basaloid nodules of the eccrine spiradenomas (**d**).

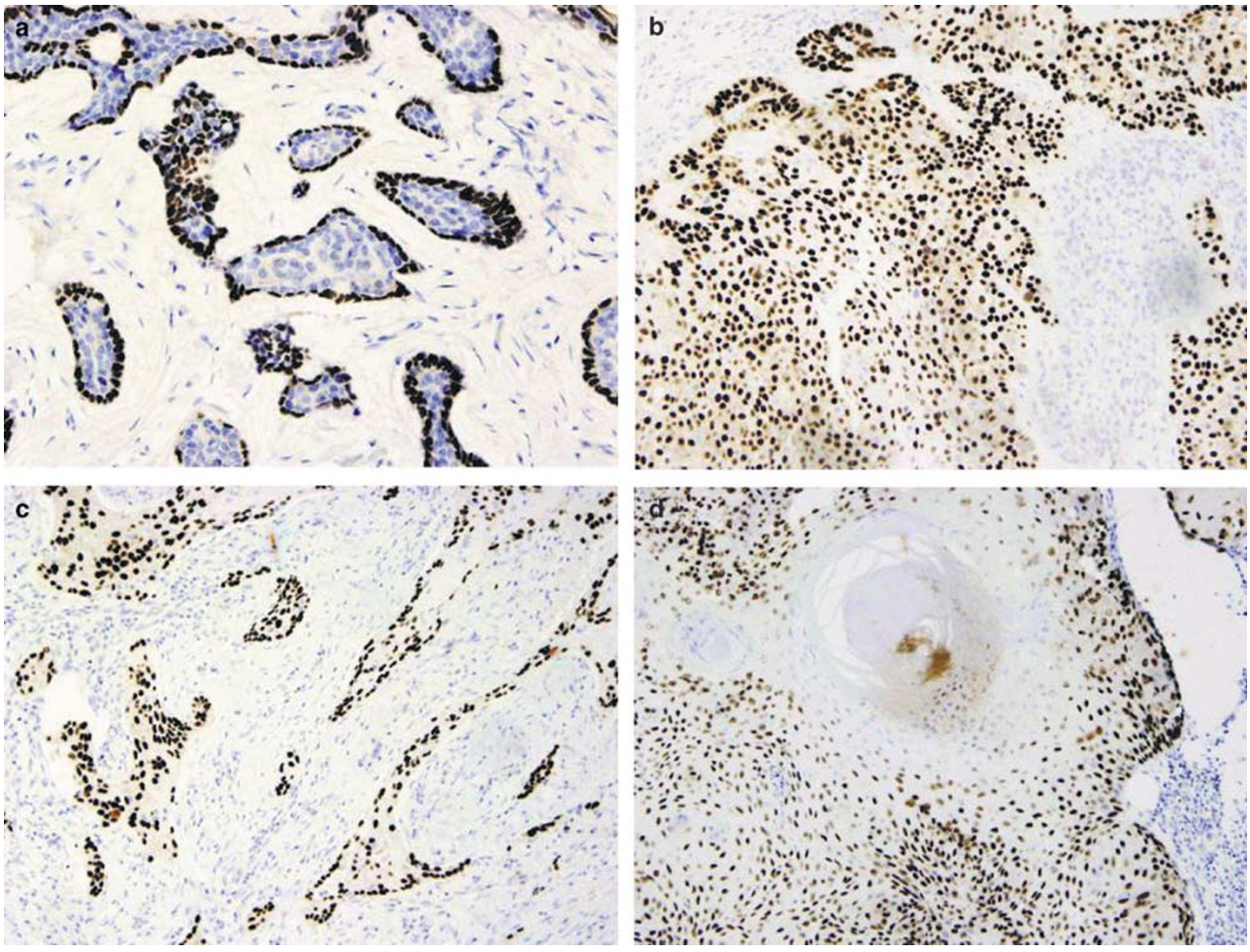

Figure 2 The eccrine adenocarcinomas (**a**) and the hidradenocarcinoma (**b**) were strongly positive for *p63*. The microcystic adnexal carcinomas (**c**) also expressed *p63*, as well as the infiltrating lobules of tricholemmal carcinoma (**d**).

the perineural invasion in one case, since the nerve bundles did not express *p63*.

The infiltrating lobules of tricholemmal carcinoma (Figure 2d) were also positive for *p63* (nuclear score 3).

No cytoplasmic labeling with *p63* was observed in any of the primary adnexal carcinomas included in the study.

Expression of *p63* in Metastatic Adenocarcinomas to the Skin

A total of 14 metastatic adenocarcinomas to the skin (12 from breast, two from the gastrointestinal tract) were included in our study and they were all negative for *p63* (Figure 3). The majority of the metastatic breast carcinomas did not express *p63* in any of the cells. In three (25%) of the cases, focal cytoplasmic *p63* positivity was identified. The skeletal muscle that was included in some of the studied slides was strongly positive for *p63* and the muscle striations were evident.

In the carcinomas metastatic from the gastrointestinal tract (one colonic adenocarcinoma, one mucinous adenocarcinoma with appendiceal origin), *p63* was expressed in less than 5% of the neoplastic cells. The apical cells revealed mild cytoplasmic staining.

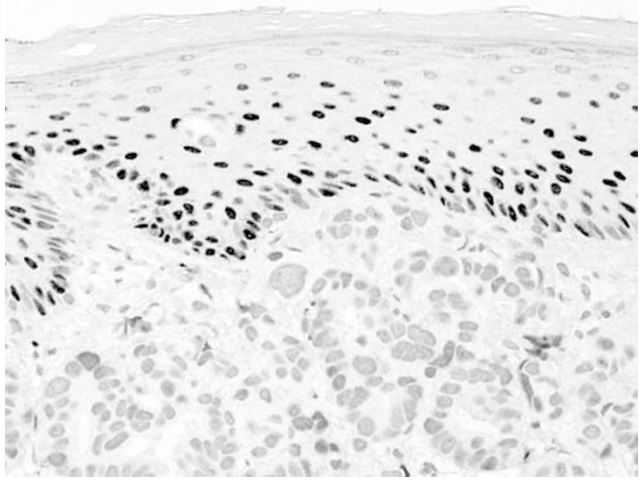

Figure 3 Similar to this case of breast metastatic carcinoma to the skin, none of the metastatic adenocarcinomas expressed *p63*.

The differences in *p63* immunolabeling between the primary cutaneous adnexal tumors and metastatic adenocarcinomas to the skin were statistically significant ($P<0.001$).

Discussion

Analysis of *p63* expression in normal tissue and neoplastic conditions has been recently the subject of several basic research studies and also gained a special attention in the applicative pathology literature. It has been reported that ΔN*p63* isoforms are consistently expressed in the nuclei of the basal cells of the multilayered epithelia, including skin, cervical and vaginal mucosa, urothelium, respiratory tract, as well as by the myoepithelial cells of the breast and sweat glands.[1–4,10–15] Analysis of *p63* expression has been recently incorporated by some in the routine breast pathology diagnosis provided that *p63* is a reliable myoepithelial cell marker.[14,15] Similarly, analysis of *p63* expression is used to identify prostatic basal cells in challenging cases.[12]

Reis-Filho *et al*[11] studied the distribution of *p63* in 400 neoplastic conditions, the largest case studies to date, and have shown that the vast majority of the adenocarcinomas from breast, prostate, lung, colon and ovary did not expressed *p63*. Kaufmann *et al*[20] had similar results in their case series of 141 adenocarcinomas, where only 20 cases had variable and mild *p63* positivity.

The expression of *p63* in normal human epidermis, cutaneous appendages and skin carcinomas has been recently assessed.[16–18] *p63* seems to play a major role in ectodermal development, in the maintenance of the basal cell population of stratified epithelia, and also in the terminal differentiation of epithelia.[6–8] *In vitro* studies, using transformed human keratinocytes, have shown that *p63* is a nuclear transcription factor that triggers keratinocyte differentiation and is downregulated in terminally differentiated cells.[4,5,16] Moreover, *p63* might block the apoptosis-inducing activity of *p53* and thus could help to maintain the proliferative capacity of basal/progenitor cells.[5–8]

However, there is only limited knowledge regarding the pattern of *p63* expression in cutaneous adnexal neoplasms, including adenocarcinomas. The potential use of this marker as diagnostic tool in distinguishing between primary *vs* metastatic cutaneous carcinomas is not fully investigated.

Our study confirmed and expanded the results of previously published studies, namely constant *p63* expression in the basal and suprabasal cells of normal epidermis and cutaneous appendages, including basal/myoepithelial cells of the eccrine and apocrine sweat glands.

In the hair follicle-derived tumors, the cell components originating from the outer root sheath cells or matrix cells expressed *p63*. All trichoepitheliomas exhibited *p63* positivity. The small cords and islands of basaloid cells of desmoplastic trichoepitheliomas were strongly highlighted by *p63* labeling. The benign tumors derived from the sweat glands of either eccrine or apocrine origin (syringomas, eccrine poromas and benign tubular adenoma) also exhibited *p63*. The tricholemmal carcinomas, derived from outer root sheath cells, preserved *p63* expression. In the microcystic adnexal carcinomas, the islands and strands of basaloid cells expressed *p63*, in contrast with the keratinous cysts. The eccrine adenocarcinomas did also exhibit strong *p63* positivity. Only in the case of mucinous adenocarcinoma *p63* was expressed in less than 25% of the cells.

None of the metastatic carcinomas exhibited *p63* positivity ($P<0.001$) and this raises the hypothesis that this marker expression might be used for distinguishing metastatic adenocarcinomas to the skin from primary cutaneous adnexal neoplasms.

In summary, *p63* is consistently expressed in a wide variety of epidermal appendages thus suggesting the participation of basal/myoepithelial cells in the ontogenesis of these tumors. The *p63* expression is a sensitive and specific marker for benign or malignant adnexal tumors. Moreover, the analysis of *p63* expression may help in the differential diagnosis of primary adnexal carcinomas, including adenocarcinomas *vs* metastatic cutaneous adenocarcinomas.

References

1 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 1988;2:305–316.
2 Yang A, McLeon F. *p63* and *p73*: *p53* mimics, menaces and more. Nat Rev Mol Cel Biol 2000;3:199–207.
3 Yang A, Kaghad M, Caput D, *et al.* On the shoulders of giants: *p63*, *p73* and the rise of *p53*. Trends Genet 2002;18:90–95.
4 Levrero M, De Laurenzi V, Constanzo A, *et al.* The *p53/p63/p73* family of transcription factors: overlapping and distinct functions. J Cell Sci 2000;113:1661–1670.
5 Pellegrini G, Dellambra E, Golisano O, *et al. p63* identifies keratinocyte stem cells. Proc Natl Acad Sci USA 2001;98:3156–3161.
6 Mills AA, Zheng B, Wang XJ, *et al. p63* is a *p53* homologue required for limb and epidermal morphogenesis. Nature 1999;398:708–713.
7 Yang A, Schweitzer R, Sun D. *p63* is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999;398:714–718.
8 Parsa R, Yang A, McLeon F, *et al.* Association of *p63* in proliferative potential in normal and neoplastic human keratinocytes. J Invest Dermatol 1999;113:1099–1105.
9 Celli J, Duijf P, Hamel BC. Heterozygous germ cell mutations in the *p53* homolog *p63* are the cause of ECC syndrome. Cell 1999;99:143–147.
10 Di Como CJ, Urist MJ, Babayan I, *et al. p63* expression profiles in normal and tumor tissues. Clin Cancer Res 2002;8:494–501.

11 Reis-Filho JS, Simpson PT, Martins A, *et al.* Distribution of *p63*, cytokeratins 4/6 and cytokeratin 14 and 51 in normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray. Virch Arch 2003;443:122–132.
12 Signoretti S, Waltregny D, Dilks J. *p63* is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000;157:1769–1773.
13 Glickman JN, Yang A, Shahsafei A, *et al.* Expression of *p53*-related protein *p63* in the gastrointestinal tract and in esophageal metaplastic and neoplastic disorders. Hum Pathol 2001;32:1157–1165.
14 Barbareschi M, Pecciarini L, Cangi MG. *p63*, a *p53* homologue, is a selective nuclear marker of myoepithelial cells of human breast. Am J Surg Pathol 2002;25:1054–1062.
15 Reis-Filho JS, Schmitt FC. Taking advantage of basic research: *p63* is a reliable myoepithelial and stem cell marker. Adv Anat Pathol 2002;9:280–289.
16 Reis-Filho JS, Torio B, Albergaria A, *et al. p63* expression in normal skin and usual cutaneous carcinomas. J Cutan Pathol 2002;29:517–523.
17 Tsujita-Kyutoku M, Kuichi K, Danbara N, *et al. p63* expression in normal human epidermis and epidermal appendages and their tumors. J Cutan Pathol 2003;30:11–17.
18 Qureshi HS, Ormsby AH, Lee MW, *et al.* The diagnostic utility of *p63*, CK5/6, CK7, and CK20 in distinguishing primary cutaneous adnexal neoplasms from metastatic carcinomas. J Cutan Pathol 2004;31:145–152.
19 Ivan D, Diwan AH, Esteva FJ, *et al.* Expression of cell cycle inhibitor $p27^{Kip1}$ and its inactivator *Jab1* in melanocytic lesions. Mod Pathol 2004;17:811–818.
20 Kaufmann O, Fietze E, Mengs J, *et al.* Value of *p63* and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001;116:823–830.