J Cutan Pathol 2002: 29: 517–523
Blackwell Munksgaard . Printed in Denmark

Journal of
Cutaneous Pathology
ISSN 0303-6987

p63 expression in normal skin and usual cutaneous carcinomas

Background: p63 is a p53 homologue that is mapped to chromosome 3q27. This gene encodes six different isoforms, which have either transactivating or dominant negative effects on p53-reporter genes. It has been described that in contrast to p53, p63 seems not to be associated with tumor predisposition, as neither p63 knockout mouse models nor germline p63 mutations are related to an increased risk of tumorigenesis. It has been demonstrated that p63 is a reliable keratinocyte stem cell marker and that it is involved in the maintenance of the stem cell population. Scant data on p63 expression in normal skin, basal cell carcinomas (BCCs), keratoacanthomas and squamous cell carcinomas (SCCs) have been reported. We herein evaluated p63 expression in 16 BCCs, one keratoacanthoma and 13 SCCs.

Methods: Immunohistochemistry according to the streptavidin-biotin-peroxidase technique, using the antibody 4A4 raised against all p63 isoforms, was performed. p63 expression was evaluated in epidermal cells and skin appendages. Semi-quantitative evaluation (–, +, ++, +++) of p63 expression in BCCs, keratoacanthoma and SCCs was carried out. Only nuclear expression was considered as specific.

Results: p63 was expressed in the nuclei of epidermal basal and suprabasal cells, in the cells of the germinative hair matrix and the external root sheath of hair follicles, in the basal cells of the sebaceous gland and in the myoepithelial/basal cells of the sweat glands. All terminally differentiated cells were negative for p63. All BCCs showed ++ to +++ immunoreactivity. At variance, keratoacanthomas and grade I and II SCCs showed variable p63 reactivity in a basal layer-like distribution, whereas undifferentiated cells of grade III SCCs showed ++ to +++ positivity. A grade IV spindle SCC showed + immunoreactivity. The SCCs *in situ* showed remarkable expression of p63 in all cell layers. Terminally differentiated squamous cells were either negative or showed only focal immunoreactivity in the carcinomas.

Conclusions: p63 is consistently expressed in the basal cells of epidermis and cutaneous appendages, including the basal/myoepithelial cells of sweat glands. Based on our findings, the balance of probabilities favors that p63 might play a role in the pattern of differentiation and in the oncogenesis of usual carcinomas of the skin.

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

Jorge S. Reis-Filho[1,2], Beatriz Torio[3], André Albergaria[1] and Fernando C. Schmitt[1,4]

[1]IPATIMUP, Institute of Molecular Pathology and Immunology, University of Porto, Porto, [2]School of Health Sciences, University of Minho, Braga, [3]Department of Pathology, Hospital Fernando da Fonseca, Amadora-Sintra, Lisbon, and [4]Porto Medical Faculty, University of Porto, Porto, Portugal

R. Roberto Frias, S/N, 4200 Porto, Portugal
Tel: +35 1225570700
Fax: +35 1225570799
e-mail: jreis@ipatimup.pt

Accepted 15 April, 2002

p63 is a recently characterized p53 homologue whose gene is located on 3q27 and encodes six different isoforms: three with transactivating (TA) properties (TA-p63α, TA-p63β, and TA-p63γ), which are capable of transactivating p53 target genes and also inducing apoptosis, and three truncated dominant negative isoforms that lack the N-terminal domain (ΔN) necessary for activation of p53-reporter genes (ΔN-p63α, ΔN -p63β and ΔN -p63γ).[1–4]

Based on p63-knockout mouse models[2,3] and *in vitro* studies,[5,6] p63 seems to play a major role in ectodermal development, in the maintenance of a basal cell population of stratified epithelia, and also in the terminal differentiation of stratified epithelia.[2,3,5,6] Yang et al.[2] and Mills et al.[3] elegantly described that p63-/-mice have severe cranio-facial defects, defective epidermal differentiation, as well as lack of hair follicles, teeth, prostate, sebaceous, sweat, mammary, lachrymal or salivary glands.[1–3]

Consistent p63 expression has been demonstrated in normal epidermal and basal cells of hair follicles.[1–7] According to Pellegrini et al.[7] p63 is a reliable keratinocyte stem cell marker[4,7] and may be used to identify these cells in the skin.[7] Several lines of evidence point toward a major role of p63 in the regulation of expression markers associated with keratinocyte differentiation in stratified squamous epithelia, such as keratins 5 and 14, loricrin, filaggrin, involucrin, and transglutaminase 1.[1–3,5,6] Indeed, it seems that p63 may induce expression of differential markers and that shortly after, p63 levels are progressively reduced.[5,6] These findings are in accordance with previous observations describing a progressive reduction of p63 levels from basal to suprabasal epidermal cells.[1–6]

Regardless of the huge amount of data concerning p63 expression in several types of human neoplasms using tissue microarray technology,[8] only scant studies of p63 expression in normal skin and usual primary skin carcinomas[5,8] have hitherto been performed on routinely processed formalin-fixed paraffin-embedded tissue sections. We herein describe p63 expression in normal epidermis, cutaneous appendages, *in situ* squamous cell carcinoma (isSCC), invasive squamous cell carcinomas (ISCC), and basal cell carcinomas (BCC). As ΔN-p63 isoforms may act as 'oncogenes' (may constitute an alternative mechanism to overcome p53-driven cell cycle arrest and apoptosis)[1–4] we also evaluated whether or not p63 overexpression could be related to some of the morphologic traits frequently associated with the aggressiveness of usual cutaneous neoplasms, including growth pattern, degree of desmoplasia, presence of vascular/lymphatic invasion and perineural infiltration.

Materials and methods

Cases selection

Thirteen consecutive cases of squamous cell carcinomas, with and without an associated *in situ* component, and 17 consecutive cases of basal cell carcinomas were retrospectively retrieved from the files of the Department of Pathology, Hospital Fernando da Fonseca, Lisbon, Portugal. The clinical pathologic data was collected from the pathology reports. All cases were independently reviewed by two of the authors (JSRF, BT) and the diagnoses were reconfirmed in all but two cases (one squamous cell carcinoma was reclassified as a keratoacanthoma and one 'metatypical' carcinoma was reclassified as a squamous cell carcinoma). Squamous cell carcinomas (SCC) were graded according to Broders' criteria[9] and basal cell carcinomas were classified according to Rippey's classification.[10] All cases were also classified according to the presence of an *in situ* component, growth pattern (infiltrative vs. expansile), degree of desmoplasia, presence of vascular/lymphatic invasion, and perineural infiltration. Mitotic counting was reported as the number of mitotic figures per 10 consecutive high power magnification fields (HPF) in the most proliferative areas.

Immunohistochemical analysis

For all cases, a 4-μm histologic section was cut and mounted on a silane-coated slide. Immunohistochemistry using the streptavidin-biotin-peroxidase technique with a monoclonal antibody raised against p63 (clone 4A4, dilution 1:150, Neomarkers, Freemont, CA, USA) was performed as described elsewhere.[11] Heat-induced antigen retrieval using the Dako Antigen Retrieval Solution (Dako, Glostrup, Denmark) was previously performed in a wet bath for 20 min in all cases. Positive and negative controls were included in each slide run. Only nuclear p63 expression was accepted as specific.

The distribution of p63 expression in the normal epidermis, sweat glands, sebaceous glands, hair follicles, dermal mesenchymal cells, endothelial cells, pericytes, erector muscles, nerve bundles, and adipocytes was evaluated in normal skin adjacent to the neoplasms. A semi-quantitative assessment of p63 expression was performed for *in situ* squamous cell carcinomas, invasive squamous cell carcinomas, and basal cell carcinomas, according to the following criteria: –, negative nuclear staining of neoplastic cells; +, focal (< 5%) positivity of neoplastic cells; ++, moderate (5–50%) positivity of neoplastic cells; and +++, diffuse (> 50%) positivity of neoplastic cells. Owing to the remarkable differential p63 expression in cells showing basal cell-like/undifferentiated morphology and terminally differentiation morphology in SCC and BCC, p63 expression was separately semiquantified in each cell type.

Statistical analysis

Statistical analysis was performed using Statview software (4.0, SAS Institute Inc., Cary, NC, USA). Statistical differences between p63 expression and histologic type, histologic grade of SCCs, BCC types, presence of vascular/lymphatic invasion, and the presence of perineural infiltration were calculated using the Chi-square test. Analysis of variance (ANOVA) with Yates' correction was used to compare p63 expression and mean values for age and mitotic counting. A level of $p < 0.05$ was considered significant.

Results

Clinical and pathologic data

The age of the patients ranged from 32 to 82 years (median age 62 years for patients with SCC and 73.5 for patients with BCC). All patients were white. All tumors affected sun-exposed skin. Table 1 summarizes the clinical pathologic data.

All squamous cell carcinomas showed variable keratinization; according to Broders' criteria[9] three were classified as grade I, five as grade II, four as grade III, and one as grade IV. The latter was a bona fide example of spindle cell SCC,[9] showing intersecting fascicles of variably pleomorphic spindle cells. In seven cases, an associated *in situ* component was depicted. Eleven cases showed infiltrative borders and two exhibited a remarkably expansile growth pattern. Eleven cases showed moderate desmoplasia and two showed a prominent desmoplastic reaction. Vascular/lymphatic invasion was observed in five cases and perineural invasion was observed in three cases. Mitotic figures ranged from 13 to 181/10HPF, with a mean of 43.3/10HPF and a median of 30/10HPF.

The case initially diagnosed as a grade I SCC and reclassified as a keratoacanthoma showed the typical morphologic features that characterize keratoacanthomas. Briefly, it was a symmetric lesion with deeply situated bulbous lobules of squamous cells showing variably atypical nuclei and a rather remarkable eosinophilic glassy cytoplasm. There was an abrupt transition between the lesion and the adjacent epidermis which lacked atypical cells suggestive of isSCC. Admixed with the squamous cell lobules, there were scattered microabscesses.[9] Surrounding the lesion, a mixed inflammatory infiltrate predominantly composed of lymphocytes and eosinophils was depicted.

Table 1. Summary of the pathologic clinical data of the cases

Biopsy	Age	Diagnosis	Histologic type/grade	Borders	Desmoplasia	Perineural invasion	Vascular invasion	Mitotic
1	77	BCC	Metatypical	Infiltrative	Intense	Present	Absent	24
2	66	BCC	Metatypical	Infiltrative	Intense	Absent	Absent	34
3	75	BCC	Metatypical	Infiltrative	Intense	Absent	Present	56
4	82	BCC	Nodular	Expansile	Low	Absent	Absent	18
5	80	BCC	Mixed	Infiltrative	Low	Absent	Absent	29
6	59	BCC	Mixed	Infiltrative	Moderate	Absent	Absent	23
7	62	BCC	Mixed	Infiltrative	Moderate	Absent	Present	31
8	72	BCC	Nodular	Expansile	Low	Absent	Absent	42
9	70	BCC	Nodular	Expansile	Moderate	Absent	Absent	10
10	75	BCC	Nodular	Expansile	Low	Absent	Absent	19
11	80	BCC	Micronodular	Expansile	Low	Absent	Absent	4
12	63	BCC	Nodular	Expansile	Low	Absent	Absent	2
13	75	BCC	Nodular	Expansile	Low	Absent	Absent	46
14	64	BCC	Nodular	Expansile	Low	Absent	Absent	16
15	64	BCC	Nodular	Expansile	Low	Absent	Absent	8
16	75	BCC	Nodular	Expansile	Low	Absent	Absent	ND*
17	76	Keratoacanthoma		Expansile	Low	Absent	Absent	ND
18	75	SCC	GI	Expansile	Moderate	Absent	Absent	26
19	67	SCC	GI	Infiltrative	Moderate	Absent	Absent	30
20	54	SCC	GI	Infiltrative	Intense	Absent	Absent	45
21	57	SCC	GII	Infiltrative	Moderate	Absent	Present	54
22	66	SCC	GII	Infiltrative	Moderate	Present	Present	13
23	42	SCC	GII	Infiltrative	Moderate	Present	Absent	26
24	32	SCC	GII	Infiltrative	Intense	Absent	Absent	33
25	74	SCC	GII	Infiltrative	Moderate	Absent	Absent	13
26	55	SCC	GIII	Expansile	Moderate	Absent	Absent	33
27	71	SCC	GIII	Infiltrative	Moderate	Absent	Absent	19
28	59	SCC	GIII	Infiltrative	Moderate	Absent	Present	181
29	62	SCC	GIII	Infiltrative	Moderate	Absent	Present	74
30	67	SCC	GIV/SCC	Infiltrative	Moderate	Present	Present	16

BCC, basal cell carcinoma; GI, grade I; GII, grade II; GIII, grade III; GIV, grade IV; HPF, high power fields; ND, not available; SCC, squamous cell carcinoma.
*The biopsy did not have enough tissue to allow the evaluation of 10 high power magnification fields.

Concerning BCCs, the histologic subtypes[10] are specified in Table 1. Ten cases were of the nodular histologic type (including four with an adenoid pattern and four pigmented BCCs) and one showed a micronodular pattern. In three cases there was a complex admixture of two types of cells: 1) cells with darkly stained nuclei and scant cytoplasm, indistinguishable from those observed in bona fide BCCs, 2) and neoplastic cells showing a more vesicular and atypical nuclei, with prominent nucleoli, and more abundant eosinophilic cytoplasm. In all of these cases, neoplastic cells were arranged in infiltrative cords with ill-defined peripheral palisades, and were immersed in a highly desmoplastic stroma, showing obvious stromal-epithelial separation artifacts. In addition, the features of squamous differentiation were unevenly observed, but squamous pearls were not found.[12] We preferred to classify these cases as 'metatypical carcinomas', but they could also be classified as basal cell carcinomas with incomplete squamous differentiation, infiltrative borders and prominent desmoplasia.[10] Ten cases showed expansile borders and six showed an infiltrative growth pattern. Ten cases showed low desmoplastic stromal reactions; and moderate and intense desmoplasia were observed in three cases each. Vascular/lymphatic invasion was observed in two cases and perineural infiltration in a further case. Mitotic figures ranged from 2 to 56/10HPF, with a mean of 24.1/10HPF and a median of 23.0/10HPF.

P63 expression

Normal skin

In normal skin, p63 was consistently expressed in the nuclei of the epidermal basal cells(Fig. 1A), cells of the germinative hair matrix and the external root sheath of the hair follicles (Fig. 1B). No p63 staining was observed in the cells of the Henle's layer, Huxley's layer, cuticle, or in the perifollicular connective tissue sheath. The nuclei of the sebaceous gland basal cells were strongly stained by p63 (Fig. 1C) and an uneven nuclear staining of the apocrine and eccrine sweat glands' basal/myoepithelial cells was depicted (Fig. 1D). No dermal mesenchymal cell, endothelial cell, pericyte, smooth muscle cell, neural cell, or adipocyte showed any immunoreactivity for p63.

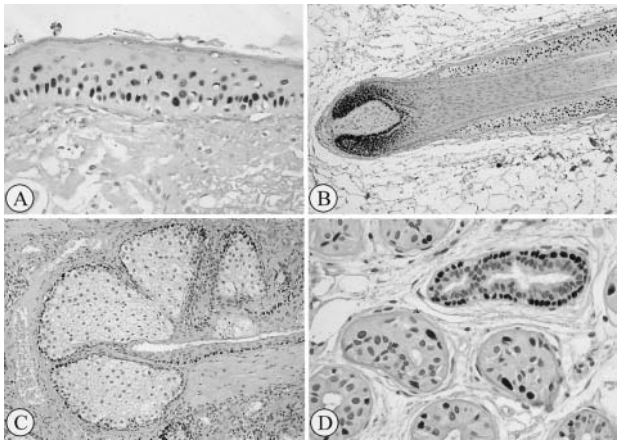

Fig. 1 p63 expression in normal skin. *(A)* Basal and suprabasal epidermal cells with nuclei decorated by p63. *(B)* Hair follicle (anagen growth phase): strong p63 expression in the nuclei of hair matrix cells and cells of the external root sheath. *(C)* p63 expression in basal cells of the sebaceous glands. *(D)* Sweat glands with p63-positive cells in a basal cell distribution. (Streptavidin-biotin-peroxidase/DAB). Original magnifications: A, 200×; B, 100×; C, 100×; D, 200×.

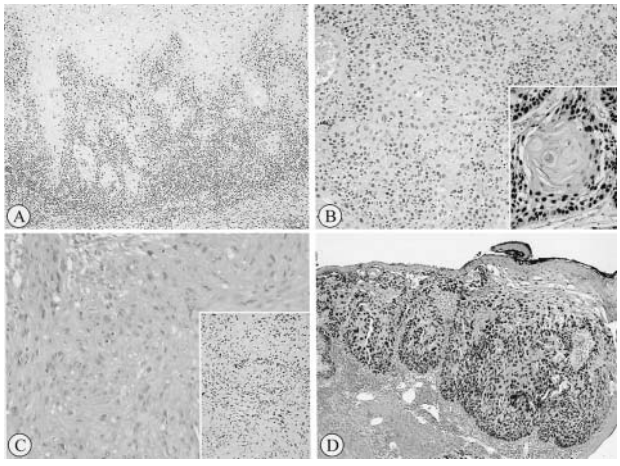

Fig. 2 p63 expression in keratoacanthoma and squamous cell carcinoma (SCC). *(A)* p63 expression in a keratoacanthoma showing a basal cell layer-like pattern. *(B)* Grade III SCC with strong p63 immunoreactivity in undifferentiated cells and lack of p63 staining in cells showing terminal squamous differentiation. Inset: p63 expression in a squamous pearl. *(C)* Spindle cell SCC. Inset: focal but strong immunoreactivity for p63 in the nuclei of neoplastic cells. *(D)* SCC *in situ* adjacent to invasive GIII SCC. Note the p63 overexpression. (A, B, B inset, C inset and D: streptavidin-biotin-peroxidase/DAB; C: H&E). Original magnifications: A, 40×; B, 100×? B (inset), 400×; C (inset), 200×; D, 100×.

Squamous cell carcinomas and keratoacanthoma

As previously reported,[5,8,13,14] p63 expression in squamous cell carcinomas showed a peculiar distribution. In grade I lesions and in the keratoacanthoma, p63 stained the nuclei of cells arranged in a basal-like pattern(Fig. 2A); the terminally differentiated, keratinized squamous cells lacked any p63 immunoexpression. In grade II and grade III lesions, the nuclei of undifferentiated squamous cells were strongly decorated by p63 (Fig. 2B); conversely, a gradual reduction of p63 was observed toward the more differentiated cells. Only two cases showed focal immunoreactivity for p63 in the differentiated squam-

Table 2. p63 expression in usual cutaneous carcinomas

Cases	p63 in basal/undifferentiated cells				p63 expression in differentiated squamous cells			
	–	+	++	+++	–	+	++	+++
BCC	0	0	4	12	16	0	0	0
Solid	0	0	2	3	5	0	0	0
Micronodular	0	0	0	1	1	0	0	0
Mixed	0	0	2	5	7	0	0	0
Metatypical	0	0	0	3	1	2	0	0
Keratoacanthoma	0	1	0	0	0	0	0	0
SCC	0	5	3	5	11	1	1	0
GI	0	2	1	0	3	0	0	0
GII	0	2	1	2	3	1	1	0
GIII	0	0	1	3	4	0	0	0
GIV	0	1	0	0	1	0	0	0

–, Negative nuclear staining; +, focal positivity (<5% of neoplastic cells); ++, moderate positivity (5–50% of neoplastic cells); +++, diffuse immunoreactivity (>50% of neoplastic cells).
BCC, basal cell carcinoma; GI, grade I; GII, grade II; GIII, grade III; GIV, grade IV; SCC, squamous cell carcinoma.

ous cells. In the spindle cell carcinoma, moderate/focal immunoreactivity was seen (Fig. 2C).

Table 2 summarizes the semiquantitative distribution of p63 immunoreactive cells in SCCs. Briefly, in the grade I tumors, two cases showed + and one case showed ++ reactivity for p63; in the grade II tumors, two cases showed +++, two cases showed + and one case showed + immunoreactivity for p63. The grade III SCCs were diffusely positive for p63 in all but one case, which showed ++ positivity. The grade IV spindle cell carcinoma showed + (focal) immunoreactivity.

In the *in situ* component adjacent to the invasive SCCs, p63 showed a rather peculiar distribution. While in the normal epidermis, p63 was restricted to basal and occasional suprabasal cells, in isSCC, p63 decorated almost all cell layers (Fig. 2D). Interestingly, no other areas showed p63 expression in superficial (granular and prickle) epidermal layers.

Basal cell carcinomas

All basal cell carcinomas with infiltrative borders showed diffuse (+++) positivity for p63(Fig. 3A) independently of the other variables. Six and four BCC cases with expansile growth patterns showed +++ (Fig. 3B) and ++ immunoreactivity for p63, respectively, regardless of their histologic subtype and degree of desmoplastic stromal reaction. Interestingly, in eight cases scattered terminally differentiated squamous cells were observed. In two out of these cases, focal nuclear immunoreactivity for p63 was also observed.

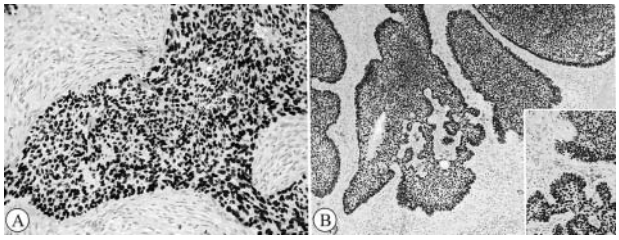

Fig. 3 p63 expression in basal cell carcinomas (BCCs). *(A)* Infiltrative BCC with strong p63 expression. *(B)* BCC with expansile borders showing marked p63 immunoexpression. Inset: note that all cells, including those in a palisade arrangement, are decorated by p63. (Streptavidin-biotin-peroxidase/DAB). Original magnifications: A, 100×; B, 100×; (B) inset, 400×.

Statistical analysis

There was a statistically significant more diffuse expression of p63 in the BCCs as compared to the SCCs ($p = 0.025$). No other statistically significant association between p63 and other pathologic parameters, including age, growth pattern, desmoplasia, vascular/lymphatic invasion, perineural infiltration and mitotic counting was observed.

Discussion

p63 is a p53 homologue that seems to play distinctive roles in the physiology of skin. First, knockout mouse models[2,3] have demonstrated that p63 is fundamental for the development of skin and cutaneous appendages.[2,3] Histologic analysis of these mice revealed that their skin lacked the structured stratification normally observed;[2,3] p63-/-late-stage embryos and newborns only retain scattered patches of disorganized epithelial cells along the epidermal surface.[1–3] Interestingly, in these mice, epidermal basal cells fail to express the markers of squamous differentiation, such as keratin 5 and 14, loricrin, filaggrin, involucrin, and transglutaminase 1.[1–3,6] Based on these findings, p63 seems to be fundamental for the differentiation and specialization of epithelial cells forming stratified epithelia. In addition, Parsa et al.[5] and De Laurenzi et al.[6] elegantly demonstrated that p63 expression is gradually

reduced from the basal cells to the terminally differentiated keratinocytes.[5–7] These findings are in accordance with what was observed in our study: strong p63 expression in basal cells and occasional suprabasal cells and lack of p63 staining in superficial epidermal layers.

Another role played by p63 involves the maintenance of a basal/stem cell population in stratified epithelia.[1–4,7,15,16] The p63 gene encodes at least six proteins: three with transactivating (TA) and three with dominant negative (ΔN) effects on p53 reporter genes.[1–4] As p53 plays a major role in cell cycle arrest by inducing the cyclin-dependent kinase inhibitor p21 and the pro-apoptotic factor bax, ΔN-p63 isoforms might constitute an alternative mechanism to overcome p53-mediated cell cycle arrest and apoptosis in basal cells.[1–4,7,15,16] Interestingly, it has been shown that in normal skin and SCCs, there is preferential expression of ΔN-p63 isoforms.[5,6,14] Moreover, Nylander and colleagues[14] demonstrated that there is an inverse correlation between expressions of ΔN-p63α and p53 in the normal and hyperplastic squamous epitheliums of the oral mucosa.[14] This is in accordance with functional genetic studies,[1–3] which demonstrate that lack of p63 leads to a failure in the maintenance of a stem cell population in stratified epithelia, as well as an absence of structures derived from the epidermal stem cells, such as hair follicles, mammary, salivary and lachrymal glands. In our study, we also observed preferential p63 expression in epidermal basal cells, basal (germinative hair matrix and external root sheath) cells of hair follicles, sebaceous glands and in sweat glands.

p53 is the most frequently altered tumor suppressor gene in human neoplasms.[17] Several lines of evidence support the concept that imbalances in the p53 pathway are fundamental to tumor development.[17] Whereas p53 mutations are highly prevalent in SCCs, they are not usually observed in BCCs.[18] As expression of ΔNp63 isoforms seems to be necessary for maintaining a basal cell population, owing to their ability to overcome p53-driven cell cycle arrest and apoptosis[1–4] we attempted to evaluate p63 expression in BCCs. As expected, p63 was strongly and diffusely expressed in BCCs, as these neoplastic cells maintain their basal cell morphology. In addition, as the ΔNp63 isoforms are those preferentially expressed in epidermal basal cells[5,7] our findings give further support to the putative role of p63 as an efficient alternative mechanism to overcome p53-driven tumor suppressing properties in human epidermal basal cells.

In contrast to the BCCs, the SCCs showed variable expression of p63. As in normal epidermis, the terminally differentiated cells observed in the squamous pearls lacked p63 expression; conversely, the immature/anaplastic cells of the SCCs showed strong p63 expression. It should be noted that in the present study, p63 expression failed to correlate with the SCCs degree of differentiation. We may advance two explanations for this finding: 1) the small number of SCC herein evaluated was insufficient to demonstrate a statistically significant association between these two parameters; or, 2) based on a previously reported genetic analysis of SCCs,[19–21] one of the most frequently amplified loci in SCCs of the skin is 3q27–28, which is the locus of p63.[19–21] Interestingly, 3q amplification is the earliest event in SCC tumorigenesis and seems not to be associated with tumor grade.[21] Moreover, according to studies by Hibi et al.[19] and Yamaguchi et al.,[20] the p63 gene is frequently amplified in squamous cell carcinomas of the head and neck and lung.[19,20] Furthermore, p63 mRNA and protein expression has been described in SCCs of the cervix, lung, head and neck and skin.[5,8,13,14,19,21] Based on these findings, the balance of probabilities favors that p63 overexpression may play a role in the oncogenesis of SCCs, but it is probably not associated with their degree of differentiation and with other features associated with aggressive biological behavior.

A putative application of p63 in routine dermatopathology practice makes distinct isSCC from its mimics. In the present study, all seven cases of isSCC associated with invasive SCC showed diffuse expression of p63 in all cell layers. At variance, p63 expression was restricted to the basal and suprabasal layers in the normal epidermis and reactive epidermis adjacent to the foci of the ulceration. In contrast to our findings, Noszczyk & Majewski[22] reported that p63 is also expressed in the basal and prickle layers of the epidermis during wound healing.[22] However, systematic studies evaluating p63 expression in putative preneoplastic epidermal lesions, such as actinic keratosis, Bowen's disease and bowenoid lesions, as well as in their mimics, must be carried out to definitively clarify the putative role of p63 expression in the differential diagnosis of these lesions.

In conclusion, we herein evaluated expression of p63 in normal skin, including cutaneous appendages, as well as in usual skin carcinomas. p63 is consistently expressed in epidermal and adnexal basal cells. Moreover, our findings warrant that further studies evaluating differential expression of p63 isoforms in BCCs and SCC must be carried out in order to clarify the role of the different p63 isoforms in the oncogenesis and differentiation of cutaneous carcinomas.

Acknowledgements

This study was partially supported by PhD grants from the Portuguese Science and Technology Foundation (FCT) (reference SFRH/BD/5386/2001) to JSRF and from Programa Operacional Ciência, Tecnologia e Inovação (POCTI) do Quadro Comunitário de Apoio (QCA) II.

The authors are grateful to Mrs Athely Pinto Guedes for typing the manuscript and for its final review.

References

1. Levrero M, De Laurenzi V, Costanzo A, et al. The p53/p63/p73 family of transcription factors: overlapping and distinct functions. J Cell Sci 2000; 113: 1661.
2. Yang A, Schweitzer R, Sun D, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398: 714.
3. Mills AA, Zheng B, Wang XJ, et al. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398: 708.
4. 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.
5. Parsa R, Yang A, McKeon F, Green H. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. J Invest Dermatol 1999; 113: 1099.
6. De Laurenzi V, Rossi A, Terrinoni A, et al. p63 and p73 transactivate differentiation gene promoters in human keratinocytes. Biochem Biophys Res Commun 2000; 273: 342.
7. Pellegrini G, Dellambra E, Golisano O, et al. p63 identifies keratinocyte stem cells. Proc Natl Acad Sci U S A 2001; 98: 3156.
8. Di Como CJ, Urist MJ, Babayan I, et al. p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002; 8: 494.
9. Broders AC. Squamous cell cancer of the lip: a study of five hundred and thirty-seven cases. JAMA 1920; 74: 656.
10. Rippey JJ. Why classify basal cell carcinomas? Histopathology 1998; 32: 393.
11. Reis-Filho JS, Milanezi F, Silva P, Schmitt FC. Maspin expression in myoepithelial tumors of the breast. Pathol Res Pract 2001; 197: 817.
12. Barrett TL, Smith KJ, Hodge JJ, et al. Immunohistochemical nuclear staining for p53, PCNA, and Ki-67 in different histologic variants of basal cell carcinoma. J Am Acad Dermatol 1997; 37: 430.
13. Kaufmann O, Fietze E, Mengs J, Dietel M. 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.
14. 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 2000; 87: 368.
15. Barbareschi M, Pecciarini L, Cangi MG, et al. p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. Am J Surg Pathol 2001; 25: 1054.
16. Signoretti S, Waltregny D, Dilks J, et al. p63 is a prostate basal cell marker and is required for prostate development. Am J Pathol 2000; 157: 1769.
17. Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. J Oral Pathol Med 2000; 29: 413.
18. Cho S, Hahm JH, Hong YS. Analysis of p53 and BAX mutations, loss of heterozygosity, p53 and BCL2 expression and apoptosis in basal cell carcinoma in Korean patients. Br J Dermatol 2001; 144: 841.
19. Hibi K, Trink B, Patturajan M, Westra WH, Caballero OL. Hill DE, Ratovitski EA, Jen J, Sidransky D. AIS is an oncogene amplified in squamous cell carcinoma. Proc Natl Acad Sci U S A 2000; 97: 5462.
20. 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 2000; 86: 684.
21. Bockmuhl U, Schwendel A, Dietel M, Petersen I. Distinct patterns of chromosomal alterations in high- and low-grade head and neck squamous cell carcinomas. Cancer Res 1996; 56: 5325.
22. Noszczyk BH, Majewski ST. p63 expression during normal cutaneous wound healing in humans. Plast Reconstr Surg 2001; 108: 1242.