Journal of Interdisciplinary Histopathology
www.scopmed.org
DOI: 10.5455/jihp.20160823075838

Original Research

Immunohistochemical expression of P53 protein in cutaneous basal cell carcinoma: A clinicopathological study of 66 cases

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Received: June 10, 2016
Accepted: August 23, 2016
Published: August 26, 2016

ABSTRACT

Objective: Nuclear expression of p53 protein is associated with a biological behavior in a variety of human malignancies. In cutaneous basal cell carcinoma (BCC), however, many studies have provided conflicting results in this regard. We aimed to determine whether there is relationship between p53 expression and different histologic subtypes of BCC, and whether it may indicate tumor aggressiveness. **Materials and Methods:** Biopsy samples from 66 cutaneous BCCs from 57 patients were collected. P53 expression was demonstrated by immunohistochemical staining using the anti-p53 antibody. Among them, 52 cases were also evaluated for Ki-67 antigen. **Results:** Immunoreactivity of p53 protein varied in the range of 0 to 100% of total tumor tissue (mean value 46.0%). The expression exceeding 5% of cancer tissue (positive staining) was found in 54 BCCs (81.8%). Within this group, there were 25 cases (37.9%) with low and 29 cases (43.9%) with high expression. In superficial, superficial-nodular, nodular, nodular-infiltrative and infiltrative BCCs, p53 protein positivity was found in 100% (8/8), 80% (8/10), 70.4% (19/27), 88.2% (15/17) and 100% (4/4), respectively. We did not reveal a significant correlation between the extent of p53 protein expression and BCC subtypes except for nodular BCC, in which a number of negative cases (8/27, 29.6%) were just above the threshold of statistical significance ($P = 0.04$). After merging cancers into non-aggressive and aggressive growth phenotype, no association with expression of p53 protein was found. There was no relationship between p53 protein expression and topographical sites after they have been gathered into sun-exposed and sun-protected locations. We did not observe any association between expression of p53 protein and Ki-67 antigen. **Conclusion:** In cutaneous BCC, the expression of p53 protein does not seem to reflect a biological behavior and tumor aggressiveness. Therefore, in a routine dermatopathological practice, immunohistochemical assessment of p53 protein may not serve as a reliable prognosticator of this malignancy.

KEY WORDS: Basal cell carcinoma, p53 protein, immunohistochemistry

INTRODUCTION

The p53 gene, also called as "guardian of the genome," belongs to the most important tumor suppressor genes, mutations of which are one of the most frequent oncogenic alterations in the majority of human neoplasms [1-3]. It encodes a multifunctional phosphoprotein that plays a significant role in the cell proliferation regulation by holding the cell cycle at the G1/S point and induction of apoptosis [1,2]. Under normal conditions, the inactive form of p53 protein (wild-type [wt]-p53) occurs at low levels in the cytoplasm of the cells. Because it is unstable and easily degradable with a short half-life, it is usually below the immunohistochemical detection level in paraffin-embedded tissue material [1,4,5]. However, in response to DNA damage, p53 protein starts accumulating in the nuclei, resulting in either cell cycle arrest and subsequent DNA reparation, or starting apoptotic mechanisms [1,4]. P53 gene mutations usually cause an overproduction of aberrant (mutated) p53 protein, which is in contrast to wt-p53 much more stable, making it possible to visualize immunohistochemically [1]. Thus, nuclear expression of p53 protein with an immunohistochemical method is generally accepted as an indirect indicator of the p53 gene mutation [2,5].

Basal cell carcinoma (BCC) of the skin is the most common malignancy in the human population. Molecular pathogenesis of this neoplasia is very complex and still not fully understood [6,7]. Given the key role of p53 gene mutations in BCC carcinogenesis [6,7], this gene has been expected a promising candidate for prediction of tumor biological behavior. However, although many studies have been published to date dealing with this issue [1-5,8-17], they have provided inconsistent results. In particular, marked discrepancies exist about whether its immunohistochemical expression differs between BCCs at

distinct anatomic sites or different histomorphology. This topic is attractive since there are suggestions that the pathophysiology of BCC arising on different (sun-exposed vs. sun-protected) parts of the body and developing into different histological subtypes may differ [18]. Herein, we focused on the immunohistochemical analysis of p53 protein expression in BCC to elucidate these controversies and to better clarify whether it could be a predictor of tumor aggressiveness.

MATERIALS AND METHODS

Patients and Tumor Specimens

Biopsy samples from 66 chosen cases of primary cutaneous BCCs from various topographic locations were enrolled into this study. They were obtained from 57 patients, who have been treated in the clinical departments of the Faculty Hospital in Zilina (Slovakia), and all biopsy samples were histopathologically investigated at the Department of Pathology in Faculty Hospital in Zilina. For the purpose of the study, we selected a set of representative samples of five BCC subtypes: 8 cases of superficial, 10 cases of mixed superficial-nodular, 27 cases of nodular, 17 cases of mixed nodular-infiltrative, and 4 cases of infiltrative subtypes. Based on histomorphology, they were, subsequently, categorized into two separate subgroups. The former comprised BCCs with non-aggressive (indolent) growth phenotype (superficial, superficial-nodular, nodular), the later included BCCs with (at least focal) aggressive (infiltrative) growth phenotype (nodular-infiltrative, infiltrative). Clinical features of the patients such as gender, age, and lesion site were collected from their files.

Immunohistochemistry

Biopsy samples were routinely processed and immunohistochemically stained for protein p53, as well as Ki-67 antigen, according to manufacturer's instructions. Shortly, representative 4-μm tissue sections applied on silanized slides were baked for 2 h in an oven at 56°C. Then, the sections were deparaffinized in xylene, rehydrated in series of descending ethanol concentrations and treated with microwaves in a 0.01 M citrate buffer (pH 6.0) for 15 min. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min, followed by incubation with tris-buffered saline solution (pH 7.6). Subsequently, specific monoclonal mouse anti-human antibody against p53 protein (clone DO-7, DAKO, dilution 1: 50) and Ki-67 antigen (clone MM1, LEICA, dilution 1: 200) were used for staining. After incubation at ambient temperature, postprimary antibody was applied and an immunoreaction was visualized by means of the DAB (3,3'-diaminobenzidine) detection chromogen solution. Slides were counterstained with Weigert's hematoxylin, dehydrated, mounted, and finally evaluated in the light microscope.

Data Interpretation and Statistical Analysis

After including a total percentage of immunolabeled tumor cells, based on the study published by Mateoiu *et al*. [1], we adopted following simplified semiquantitative grading system: (a) Negative p53 expression (≤5% positive tumor cells), (b) low p53 expression (6-50% positive tumor cells), and (c) high p53 expression (51% positive tumor cells). The topographic sites affected were merged into chronically sun-exposed (head and neck) and sun-protected (trunk and extremities) regions, according to the previous studies [8,16]. Data were collected in a databank, using SPSS statistics software. For the statistical analysis, Chi-square test was employed and $P < 0.05$ was considered significant.

RESULTS

Clinical Data

The clinicopathological characteristics and immunohistochemical findings in our set of BCCs investigated are shown in Tables 1 and 2. The specimens comprised 29 men (50.9%) and 28 women (49.1%) aged 41-90 years (mean age 70.5 years). The mean age of men and women were 68.0 and 73.5 years, respectively. The anatomical sites affected were as follows: Face (27 cases), extrafacial regions of the head (eleven cases), neck (two cases), trunk (19 cases), upper extremities (five cases), and lower extremities (two cases). Women had tumors located much more frequently on the head and neck (22/31, 71%) compared with men (18/35, 51.4%). The vast majority of BCCs arising on the trunk and extremities were of the non-aggressive subtypes (23/26, 88.5%), whereas those on the head and neck were more commonly of the aggressive subtypes (18/40, 45%).

Table 1: An overview of clinicopathological parameters in relation to the extent of p53 protein expression

Clinicopathological parameter (%)	Negative expression	Low expression	High expression
Number of cases	12 (18.2)	25 (37.9)	29 (43.9)
Mean age of patients	70.7 years	68.5 years	72.2 years
Gender			
Corresponding to male	4 (33.3)	14 (56)	17 (58.2)
Corresponding to female	8 (66.7)	11 (44)	12 (41.8)
Tumor location			
Head and neck	10 (83.3)	14 (56)	16 (55.1)
Trunk and extremities	2 (16.7)	11 (44)	13 (44.9)
BCC growth phenotype			
Non-aggressive	10 (83.3)	18 (72.0)	17 (58.6)
Aggressive	2 (16.7)	7 (28.0)	12 (41.4)
Mean Ki-67 index	18.5	23.3	24.6

Table 2: A summary of p53 protein expression data in different BCC subtypes

BCC subtype	N	Mean value (%)	Negative expression (%)	Low expression (%)	High expression (%)
Superficial	8	57.5	0	4 (50)	4 (50)
Superficial-nodular	10	44.5	2 (20)	4 (40)	4 (40)
Nodular	27	34.6	8 (29.6)	10 (37)	9 (33.8)
Nodular-infiltrative	17	57.9	2 (11.8)	5 (29.4)	10 (58.8)
Infiltrative	4	54.0	0	2 (50)	2 (50)

BCC: Basal cell carcinoma

Immunohistochemical Data

In our series, a nuclear immunoreactivity of p53 protein varied in the range of 0-100% of total tumor tissue (mean value 46.0%, standard deviation [SD] 36.8). The expression exceeding 5% of total cancer tissue (positive staining) was found in 54 BCCs (81.8%). Within this group, there were 25 cases (37.9%) with low and 29 cases (43.9%) with high expression. In superficial, superficial-nodular, nodular, nodular-infiltrative and infiltrative BCCs, p53 protein positivity was found in 100% (8/8), 80% (8/10), 70.4% (19/27), 88.2% (15/17) and 100% (4/4), respectively [Figures 1-3]. All five subtypes exhibited a wide expression range varying between 15% and 100% in superficial, between 0% and 100% in superficial-nodular, nodular and nodular-infiltrative, and between 6% and 100% in infiltrative subtype. The lowest average value was observed in nodular BCC (34.6%), whereas the highest mean values were confirmed in nodular-infiltrative and superficial BCCs (57.9% and 57.5%, respectively). In mixed superficial-nodular and nodular-infiltrative BCC cases, a percentage of p53-positive tumor tissue in both structural components seemed to be about the same.

We did not reveal a significant correlation between the extent of p53 protein expression and individual BCC subtypes except for nodular BCC, in which a number of negative cases (8/27, 29.6%) were just above the threshold of statistical significance ($P = 0.04$). After merging all cancers into non-aggressive (indolent) and aggressive (at least focally infiltrative) growth phenotype, there was no association between expression of p53 protein and both given tumor subgroups. However, there was a tendency of rising proportion of BCCs with aggressive growth features with increasing extent of p53-positive tumor tissue in regards to three semiquantitative categories evaluated. Similarly, there was no relationship between p53 protein expression and topographical sites, after they have been merged into chronically sun-exposed (head and neck) and sun-protected (trunk and extremities) locations, although p53-negative BCCs occurred most frequently (83.3%) on the head and neck regions ($P = 0.07$). Among 52 cases, in which a Ki-67 antigen was immunohistochemically analyzed, proliferative Ki-67 index varied from 1% to 90% (mean value 23.0%, SD 16.9). We observed no significant association between the expression of p53 protein and Ki-67 antigen.

DISCUSSION

P53 gene mutations occur in the early stages of cutaneous carcinogenesis [2,8,9] and perhaps are responsible for the initiation of both aggressive and non-aggressive histological BCC subtypes [9]. Despite undisputable role of this gene in the pathogenesis of cutaneous BCC, many controversies about the importance of the p53 protein detection in tumor tissue at the biopsy examination have persisted. Some authors [2-5,10] demonstrated immunohistochemical positivity of p53 protein in the vast majority (80-100%) of BCCs studied, whereas another group of the authors [9,11,12] found it in less than half of all (42.9-48.7%) cases. Even a percentage range of p53-positive cancer cells in the individual BCCs is striking and varies from 0% to 100% [2,3,10,13] with a mean values of 30.1-67.7% [2,3,13]. These discrepancies could be attributed

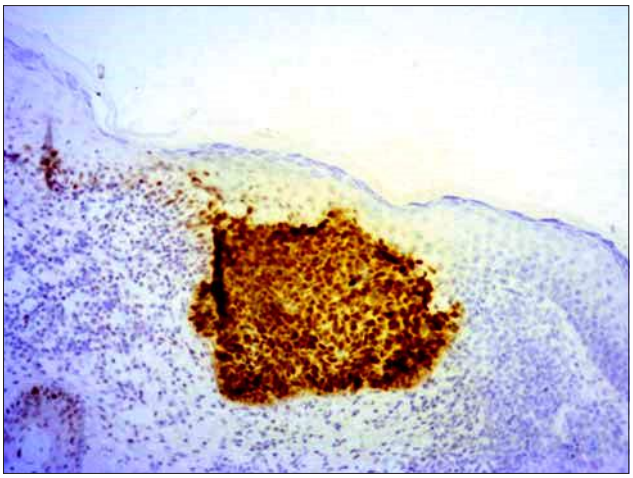

Figure 1: Diffuse high expression of p53 protein in superficial basal cell carcinoma (clone DO-7, DAKO, original magnification ×120)

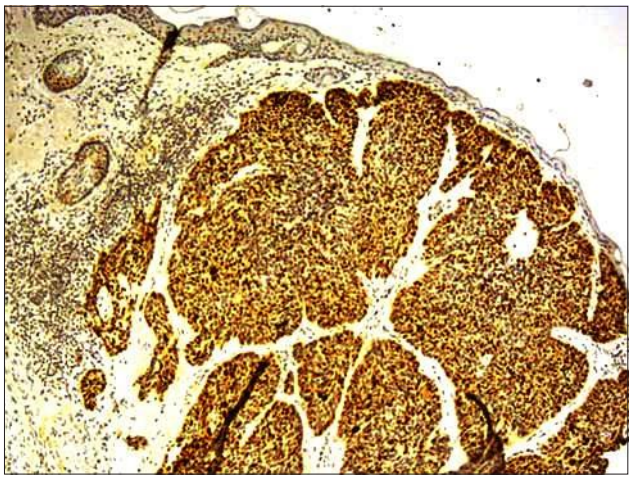

Figure 2: Diffuse high expression of p53 protein in nodular basal cell carcinoma (clone DO-7, DAKO, original magnification ×120)

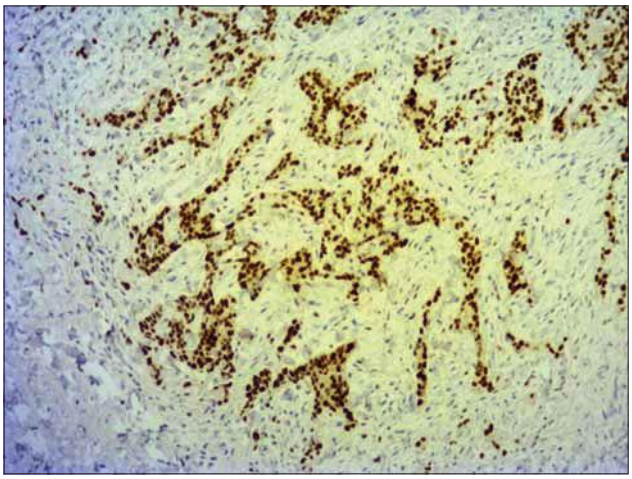

Figure 3: Diffuse high expression of p53 protein in infiltrative basal cell carcinoma (clone DO-7, DAKO, original magnification ×120)

to differences in technique, sample size, cases selection, and multifactorial pathogenesis of this malignancy. In our current study, we have found p53 protein expression in 81.8% cases investigated, based on which we state, it is a common histopathological finding. Although it is well-known that an excessive exposure of solar ultraviolet (UV) radiation is the key etiological factor of cutaneous BCCs and the main cause of p53 gene alterations, many authors [8,10,11,16,17] did not confirm any significant differences in immunohistochemical expression of p53 protein between BCCs localized on the parts of the body permanently exposed to sunlight (head) and on the sun-protected regions, such as we did not. One reason for these conflicting observations could be that the sunlight is not the only causative factor of p53 gene damage [7,9], as well as another UV-induced p53-independent molecular mechanisms are included in BCC carcinogenesis [7]. In addition, p53 gene mutations are very heterogeneous and deletions of the genome may result in the complete loss of protein p53 production. Since antibodies against p53 protein will not detect cases in which the mutations result in the complete failure to produce this protein, even p53-negative BCCs at immunohistochemistry may actually possess a p53 gene mutation [1]. So far, it has been questionable, as to whether cutaneous BCCs with more aggressive clinicomorphological phenotype exhibit different expression of p53 protein compared to indolent variants, and thus, a prognostic significance of this marker remains unclear. Several papers [1,10,11,14,15] have shown that immunohistochemical nuclear p53-positivity was higher in aggressive variants. Based on these observations, this marker might be of predictive value for the biological behavior of cutaneous BCCs. Contrary to this theory, many other researchers [5,16,8,9,12] revealed no statistically significant differences in p53 expression between aggressive and non-aggressive BCC variants. Our experience rather supports conclusions of the latter group of the authors.

We have also assessed a relationship between the expression of p53 protein and Ki-67 antigen, which is the most reliable marker of proliferating cells. Ki-67 antigen is visible in the nuclei during active phases of cell cycle (G1, S, G2 and mitosis), being absent in "resting" (G0) phase [1,2,16]. Immunohistochemical assessment of nuclear Ki-67 expression in tumor cells (Ki-67 index) allows a quantitative measure of their growth fraction. As mentioned above, p53 protein possesses a role in regulating transcription of genes that suppress cell proliferation, especially those affecting passage from the late G1 to S phase of the cell cycle. In other words, this protein has a biological function as a G1 checkpoint controller. Consistent with this role, it has been previously found that there was a significant association between p53 positivity and the number of Ki-67-positive cells in actinic keratosis [19] and cutaneous squamous cell carcinoma [20]. In some reports, similar finding has also been shown in BCC of the skin. For example, Koseoglu *et al*. [14] found that p53-positive cases had a higher mean value for Ki-67 index and they concluded, the expression of both these markers seemed to be related. Stratigos *et al*. [13] and Batinac *et al*. [3] demonstrated that p53 protein expression correlated positively with cell proliferation measured by Ki-67 index. On the other hand, Esmaeili *et al*. [16] have not revealed such results and also in the current study; we have failed to confirm such association.

CONCLUSION

Nuclear expression of p53 protein does not seem to reflect a biological behavior of cutaneous BCC, as well as it is not directly related to its histomorphology. BCC aggressiveness may rather be attributable to other genetic changes or events that occur during carcinogenesis. Therefore, in a routine dermatopathological practice, immunohistochemical assessment of p53 protein may not serve as a reliable prognosticator of this malignancy. Inconsistent results presented in several studies may be at least in part due to the complex biology of this skin neoplasia.

REFERENCES

1. Mateoiu C, Pirici A, Bogdan F. Immunohistochemical nuclear staining for p53, PCNA, Ki-67 and bcl-2 in different histologic variants of basal cell carcinoma. Rom J Morphol Embryol 2011;52 1 Suppl:315-9.
2. Khodaeiani E, Fakhrjou A, Amirnia M, Babaei-Nezhad S, Taghvamanesh F, Razzagh-Karimi E, *et al*. Immunohistochemical evaluation of p53 and Ki67 expression in skin epithelial tumors. Indian J Dermatol 2013;58:181-7.
3. Batinac T, Zamolo G, Jonjic N, Gruber F, Petrovecki M. p53 protein expression and cell proliferation in non-neoplastic and neoplastic proliferative skin diseases. Tumori 2004;90:120-7.
4. Adamkov M, Halasova E, Rajcani J, Bencat M, Vybohova D, Rybarova S, *et al*. Relation between expression pattern of p53 and survivin in cutaneous basal cell carcinomas. Med Sci Monit 2011;17:BR74-80.
5. Karagece Yalçin U, Seçkin S. The expression of p53 and COX-2 in basal cell carcinoma, squamous cell carcinoma and actinic keratosis cases. Turk Patoloji Derg 2012;28:119-27.
6. Tilli CM, Van Steensel MA, Krekels GA, Neumann HA, Ramaekers FC. Molecular aetiology and pathogenesis of basal cell carcinoma. Br J Dermatol 2005;152:1108-24.
7. Lacour JP. Carcinogenesis of basal cell carcinomas: Genetics and molecular mechanisms. Br J Dermatol 2002;146 Suppl 61:17-9.
8. Monsef A, Ansar A, Behnoud S, Monsef F, Esmaeili R. Frequency of p53 immunohistochemical expression in all histopathological types of basal cell carcinoma and its correlation with clinicopathological features. Life Sci J 2012;9:106-11.
9. Bolshakov S, Walker CM, Strom SS, Selvan MS, Clayman GL, El-Naggar A, *et al*. p53 mutations in human aggressive and nonaggressive basal and squamous cell carcinomas. Clin Cancer Res 2003;9:228-34.
10. Ansarin H, Daliri M, Soltani-Arabshahi R. Expression of p53 in aggressive and non-aggressive histologic variants of basal cell carcinoma. Eur J Dermatol 2006;16:543-7.
11. Auepemkiate S, Boonyaphiphat P, Thongsuksai P. p53 expression related to the aggressive infiltrative histopathological feature of basal cell carcinoma. Histopathology 2002;40:568-73.
12. Demirkan NC, Colakoglu N, Düzcan E. Value of p53 protein in biological behavior of basal cell carcinoma and in normal epithelia adjacent to carcinomas. Pathol Oncol Res 2000;6:272-4.
13. Stratigos AJ, Kapranos N, Petrakou E, Anastasiadou A, Pagouni A, Christofidou E, *et al*. Immunophenotypic analysis of the p53 gene in non-melanoma skin cancer and correlation with apoptosis and cell proliferation. J Eur Acad Dermatol Venereol 2005;19:180-6.
14. Koseoglu RD, Sezer E, Eyibilen A, Aladag I, Etikan I. Expressions of p53, cyclinD1 and histopathological features in basal cell carcinomas. J Cutan Pathol 2009;36:958-65.
15. Chuprov IN. Immunomorphological features of cutaneous basal-cell carcinoma. Vopr Onkol 2008;54:715-9.
16. Esmaeili R, Khorasani MS, Monsef A. Immunohistochemical expression of p53 and Ki-67 in different histopathological variants of basal cell carcinoma. Middle East J Cancer 2015;6: 29-34.
17. Khalesi M, Waterhouse M, Whiteman DC, Johns R, Rosendahl C, Hackett T, *et al*. Comparison of PTCH1, COX-2, p53, and Ki-67 protein expression in basal cell carcinomas of nodular and superficial

subtypes arising on the head and trunk. Int J Dermatol, 2016;28. DOI: 10.1111/ijd.13276.
18. Khalesi M, Whiteman DC, Rosendahl C, Johns R, Hackett T, Cameron A, *et al.* Basal cell carcinomas on sun-protected versus sun-exposed body sites: A comparison of phenotypic and environmental risk factors. Photodermatol Photoimmunol Photomed 2015;31:202-11.
19. Shimizu T, Oga A, Murakami T, Muto M. Overexpression of p53 protein associated with proliferative activity and histological degree of malignancy in solar keratosis. Dermatology 1999;199:113-8.
20. Shimizu T, Muto M, Murakami T, Furumoto H, Mogami S, Asagami C. Overexpression of p53 protein associated with proliferative activity as evaluated by Ki-67 immunostaining in well-differentiated squamous cell carcinoma of the skin. Dermatology 1997;195:224-7.

Source of Support: Nil, Conflict of Interest: None declared.