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Immunohistochemical Expression of P53 and Ki-67 in Different Histopathological Variants of Basal Cell Carcinoma

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Abstract

Background: Basal cell carcinoma is the most common form of skin cancer in men. Increased marker expressions of apoptosis and cell proliferation have been found in the majority of basal cell carcinomas. Our purpose was to determine whether any correlation between immunohistochemical expressions of P53 and Ki-67 in different histopathological variants of basal cell carcinoma and patient's age, gender and tumor localization existed.

Methods: We evaluated basal cell skin specimens obtained from 100 previously diagnosed cases of basal cell carcinoma at the Dermatology Clinic of Farshchian Hospital, Hamadan, Western Iran. At first the specimens were fixed by formalin and stained by hematoxylin-eosin. Histopathological types of the tumor were determined. Immunohistochemical expressions of Ki-67and P53 were examined. Clinical features of the patients such as age, gender, and lesion site were collected from their files.

Results: The specimens were obtained from 62 (62%) men and 38 (38%) women with an age range of 22-107 years. Of basal cell carcinoma specimens, 76% expressed P53 and 60% expressed Ki-67. There was strong staining intensity of P53 protein in 70% and Ki-67 antigen in 30% of specimens. Strong staining intensity of Ki-67 in patients of lower ages was significantly correlated. There was no statistically significant correlation between these markers and other variables ($P>0.05$).

Conclusion: This study highlighted the value of P53 and Ki-67 markers in basal cell carcinoma. We did not observe any significant difference between the histopathological types based on the P53 and Ki-67 expressions and their staining intensities.

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Introduction

Basal cell carcinoma (BCC) is one of the most common cancers in humans. Multiple factors contribute to its development, including genetics and environment. Although the role of the genetic factor is clear in cancer predisposition, there are a wide variety of other components. Some agents have been suggested as causes of BCC and include ionizing radiation, exposure to chemical carcinogens and presumably infection with papillomaviruses. The most causative agent is UV radiation which possibly explains why BCC occurs in hairy skin and particularly on the face.[1-4] Metastasis and mortality from BCC is not common as these tumors tend to grow slowly and may become locally invasive.

Most studies in recent years have focused on recognition of markers that have proven predictive value in determining the risk of progression and recurrence. P53 is a cell cycle control protein that provides the conditions such as cell arrest and apoptosis of cancerous cells. P53 leads to cessation of their proliferation by protection in a hypophosphorylate status of retinoblastoma protein.[5] Another cellular marker, Ki-67, is appreciably associated with cell proliferation. This marker is present in all active cell cycle phases including G1, S, G2 and mitosis.[6] Nevertheless, the normal function of the P53 and Ki-67 genes can be changed by mutations. Conflicting results about expressions of these markers, their relationship, and histopathological types of BCC are not clear. This study aims to investigate both the correlation between immunohistochemical expressions of P53 and Ki-67 in different histopathological variants of BCC and expressions of these markers to clinicopathological features such as age, gender and anatomical sites of the lesions.

Materials and Methods

Cases studied

Archived specimens from 100 cases diagnosed with BCC were collected from 2008 to 2009 at the Dermatology Clinic of Farshchian Hospital in Hamadan, Western Iran. Clinical findings such as age, gender and tumor localization were obtained from the Department's archive records and report copies. Specimens were re-evaluated independently by two expert pathologists who agreed on all specimens involved in the study. Histopathological types of BCC were determined and histopathological classification of the lesions was performed according to the criteria proposed by Bolognia.[7]

Immunohistochemistry

In order to detect P53 protein expression in BCC specimens, we performed immunohistochemical staining as follows.[8] Initially we chose the best represented BCC paraffin blocks. These blocks were deparaffinized and dehydrated. For antigen retrieval, we boiled the sections in 0.01 M sodium citrate buffer (pH 6.0) for 5 min in a microwave oven. In order to block the activity of endogenous peroxidase, the slides were treated with 0.5% H_2O_2 for 10 min after which the sections were incubated with primary and secondary mouse monoclonal antibody P53 protein (Novocastra, Newcastle, UK, DO-7), mouse monoclonal antibody Ki-67 protein (Novocastra, Newcastle, UK, DO-7), and avidin biotin complex-horseradish peroxidase (ABC-HPR). Immunoreactivity was illustrated by 3, 3 - diaminobenzidine tetrahydrochloride (DAB) and the slides were counterstained with hematoxylin and eosin. We used a microscope to examine the immunostained sections at a magnification of 400x and counted the numbers of positive cells per standardized unit area. Both P53 (breast carcinoma) and Ki-67 (tonsil sample) strongly positive control slides were used as references in each run of the staining procedure.

Interpretation

We recorded the percentage of positively stained cells. P53 expression was scored as 0: no staining; 1+: staining in 1%-25% of the cells; 2+: staining in 26%-50% of the cells; 3+: staining in 51%-75% of the cells; and 4+: staining in 76%-100% of the cells. Staining intensity was evaluated as 0: no staining, 1+: weak staining and 2+: strong staining. For evaluation of Ki-67 expression we

Table 4. Correlation between age, chest wall separation and toxicity for Group B.

Complication	Age (*P*-value)	Chest Wall Separation (*P*-value)
Skin toxicity	0.20	<0.0001
Edema	0.69	<0.0001
Pain	0.46	<0.0001
Pigmentation	0.53	<0.0001
Fibrosis	0.31	=0.029

examined the average number of stained nuclei at 400x magnification per standardized unit area. We scored the level of staining as follows: 0 positive cells: negative; 1-3 positive cells: low; 4-6 positive cells: moderate; and more than 7 positive cells: high. Ki-67 expression intensity was graded as follows: 1+ represented weak labeling whereas 2+ was considered strong labeling.

Statistical analysis

Data analysis was performed using SPSS software (version 17). Descriptive statistics was used to calculate the number, percentage and mean±standard deviation (SD). Analytical statistics was used to determine the difference in proportion by applying by the t-, chi-square or Fisher's exact tests where appropriate. We considered *P*-values of <0.05 to be statistically significant.

Results

Clinical and pathological data

We analyzed 100 BCC specimens in this study. These specimens comprised of biopsies from 62 (62%) men and 38 (38%) women with an age range of 22-107 years (mean±SD: 63.97±14.36). Tumor localizations were obtained and most lesions were located on areas that had exposure to the sun. The majority of samples were from the face (40%) and nose (27%) followed by the scalp (15%), ears (7%), trunk (7%), neck (2%) and extremities (2%)which corresponded to 91% from sun exposed areas and 9% from non-sun exposed areas. Histopathological types were classified in one of the groups defined by 10-fold and summarized in Table 1. We divided specimens into two groups according to pathological behavior (recurrentor aggressive): high risk (morphoeic, micronodular, superficial and metatypical) and low risk (other). Nodular (19%) and micronodular (16%) were the most common types. Among histopathological types, 44% of specimens were considered high risk and 56% were low risk. There was no significant correlation between high and low risk group tumors and sun-exposure (*P*=0.34). Among the 44 cases in the high risk group, 40 cases had sun-exposure and out of 56 from the low risk group, 51 had sun-exposure. The distribution of histopathological types based on site lesions is summarized in Table 2.

Immunohistochemistry data

Table 3 shows results of immunohistochemical expressions of P53 and Ki-67. The P53 protein was expressed in 76% of specimens and the Ki-67 antigen expressed in 60% of specimens. We observed no significant association between expression and intensity of these markers with both low and high risk groups according to the chi-square test. The *P*-value of P53 expression and staining intensity based on the high and low risk groups was 0.66 and 0.44, respectively. For Ki-67 these correlations were 0.11 (high risk) and 0.57 (low risk). The frequencies of P53 and Ki-67 expressions based on the high and low risk groups are shown in Figures 1 and 2. We observed no significant relation between P53 expression (*P*=1.00) and staining intensity (*P*=0.66) to sun-exposed tumor. There was also no significant expression of Ki-67 (*P*=1.00) and staining intensity (*P*=0.42) to sun-exposed tumor. An evaluation of the association between the immunohistochemical findings of these markers and mean age of cases only revealed a statistically significant difference between staining intensity of Ki-67 and mean age (*P*=0.036).

Discussion

Basal cell carcinoma, the most common form of skin cancer, is a multi-factorial disease that usually occurs in people over the age of 40.[9] This cancer begins in the top layer of the skin and has different histopathological types with various clinical behaviors. Oncogenic potential may increase by mutation of the P53 and Ki-67 genes; several studies have reported overexpression of these markers in cancers.[8,10,11] Therefore, according to the importance of BCC and the lack of similar studies in Iran, we examined the immunohistochemical expressions and intensities of P53 and Ki-67 as tumor markers and their correlations with age, gender and anatomical site of the tumor in all histopathological types of BCC.

This study was conducted in Hamadan, Western Iran on 100 archived specimens collected from BCC patients who referred to the Dermatology Clinic of Farshchian Hospital. The men comprised 62% of the sample size and specimens belonged to patients between the ages of 22-107 years. These findings indicated a higher frequency of BCC risk in men and older individuals. Several studies reported that incidence of BCC in men is more than women[12-14] however some studies are not agreed with this result.[15] The higher incidence of BCC in men could be due to increased exposure of men to the sun for job reasons. The effect of gender was not significant enough be introduced as risk factor for BCC. Typically BCC has been shown to occur after the fourth decade of life[9] and its incidence increases with increasing age which was confirmed by Raasch et al.[16]

In the present study the face and nose were the most common sites for lesions. These sites are exposed to the sun more than other anatomical sites. In our literature search the role of UV radiation and the risk of sites exposed to sunlight have been confirmed.[15,17-19]

In this study P53 expressed in 76 (76%) and Ki-67 expressed in 60 (60%) of BCC cases. P53 expressed in 41 (54%) specimens with 4+ labeling and high expression for Ki-67 was observed in 31 (51.7%) specimens. Staining intensity of P53 was strong in 70% of specimens whereas Ki-67 showed strong staining intensity in 30% of specimens that expressed these markers. Our results confirmed those of previous studies which showed overexpression of these markers in the studied tumors.[10,11,20,21]

This study showed no correlation between expression and intensity of these tumor markers with histopathological types of BCC based on the high and low risk groups. Examinations of these correlations were performed in a few studies with conflicting results. Several studies supported our results[22-24] however a number of other studies did not support these results.[25-27] These contradictory results could be attributed to differences in technique, sample size, case selection and multi-factorial pathogenesis of BCC. Two important factors surveyed were the association of the possibility of P53 and Ki-67 expressions and their intensity with age and sun exposure. Previous studies that evaluated the correlation immunohistochemical findings with exposure to the sun supported the results of the current study.[26-28] However other studies showed a strong linear correlation.[29,30] One reason for these conflicting results could be that the P53 gene was not affected solely by UV. Other factors such as smoking, ionizing radiation, arsenic and coal tar might be effective factors. The age of patients that expressed P53 was higher than specimens with no P53 expression. Also this association there was about P53 intensity but in none of them (P53 expression and intensity), there were no significant difference.

Ki-67 antigen expression occurred more in younger patients but this difference was not statistically significant. However the strong staining intensity of Ki-67 in younger patients was significantly higher. Based on our key words research we did not observe similar studies with these evaluations. Limitations of the study should be noted. This study was performed on only 100 BCC samples. Therefore it should be repeated on a large number of various skin tumor specimens in order to compare different age ranges and achieve more accurate results. The immunohisto-

chemical technique we have used to evaluate P53 expression may not reveal the actual molecular event. Therefore additional more molecular analysis is required. Other contributing factors involved in the pathogenesis of BCC can be useful.

The study highlights the value of both the P53 and Ki-67 markers in BCC. The results have not demonstrated a significant correlation between immunohistochemical findings and clinicopathological characteristics. In addition, we have not observed a significant difference between the high and low risk groups based on the P53 and Ki-67 expressions and their staining intensities.

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Conflict of interest statement

No conflict of interest is declared.

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