1P53 over expression in skin lesions: An immunohistochemical study

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Abstract

Background and objective: Immunohistochemistry is the application of immunologic principles and techniques to demonstrate molecules in cells and tissues. Gene p53 is a tumor-suppressor gene. The product of this gene is a nuclear protein thought to be involved in the control of the cell cycle, apoptosis, and the maintenance of genomic stability. Gene p53 is the most frequently mutated tumor suppressor gene found in human cancer. The aim of this study was to investigate p53 protein over expression in skin cancer and compare it with benign skin lesion.

Methods: A retrospective study was conducted on paraffin block from skin biopsy of 36 patients with various skin lesions; eight benign cases and 28 malignant cases. The sample of patients was collected from the Pathology Laboratory in Rizgary Teaching Hospital in Erbil, Kurdistan region, Iraq from December 2011 to December 2012. The age of the patients ranged from 34 to 80 years. The p53 protein over expression was investigated by immunohistochemical staining. Sample sections were stained and scored.

Results: Nineteen out of 28 (76.8%) skin cancer showed over expression of p53 gene compared with benign skin lesion and there was a statistically significant difference. There was statistical significant difference in relation to the age group of patients with various skin cancers which was higher in patient above 40 years.

Conclusion: Increased expression of p53 a nuclear protein can be detected in human skin cancer compared with benign skin lesion and it may play an important role in pathogenesis of many types of skin cancers.

Keywords: Immunohistochemistry, Tumor Suppressor gene (p53) protein and skin cancers.

Introduction

Mutation of the p53 gene represents the most common genetic alteration in human tumors. The accumulation of the protein can also occur as a result of epigenetic changes, and therefore it is not an obligatory indicator of a gene mutation. P53 protein has an intracellular half-life of only 20 minutes. It is present at very low concentration below the limit of Immunohistochemical detection in most untransformed cells and non neoplastic tissue. The altered protein product of the mutant gene has a much extended half-life and can be detected with immunohistochemical techniques. Epidemiological data strongly implicate sunlight as the principal environmental cause of skin cancer. Sunlight acts as tumorigenic mutagen, it also acts as tumor promoter by favoring the clonal expansion of p53-mutated cells. These combined actions of sunlight result in normal individuals carrying a substantial burden of keratinocytes predisposed to cancer. The role of sunlight is supported by the finding of sunlight-induced mutations in the p53 tumor suppressor gene in actinic keratosis. The precancerous lesion for squamous cell carcinoma of the skin and other precancerous conditions is squamous-cell carcinoma in situ. It may progress to invasive disease if not treated completely. The thickness of the epidermis and skin color are known to...
attenuate the amount of UV light that reaches the basal layer of the epidermis and these factors may modify the distribution of p53 expression in the upper and basal layers of the epidermis.8,9 The aim of the study is to investigate p53 protein over expression in malignant skin lesions and compare it with the benign skin lesions and to evaluate the frequency rate of p53 expression measured in biopsies from various skin cancers.

**Methods**

This is a retrospective study conducted on paraffin block from skin biopsy of 36 patients with various skin lesions including 8 benign lesions and 28 malignant cases. The sample of patients was collected from the Pathology Laboratory in Rizgary Teaching Hospital in Erbil, Kurdistan region, Iraq for the period from December 2011 to December 2012. The age of the patients ranged from 34 to 80 years. Ethical approval was obtained from the Ethical Committee of Hawler Medical College. Immunohistochemical staining method was carried out on these samples using the DO-7 monoclonal antibody (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) at a 1:50 dilution using microwave antigen retrieval. All slides were counterstained with hematoxylin and examined at 400 magnification. For each case, a negative control section was examined to monitor staining quality based on the level of nonspecific background staining. The negative control section was prepared identical as the test section except that no primary antibody was placed over the slide. All other procedures were the same. The distribution of p53 immunoreactivity in epidermal cells in which the nucleus was stained brownish-red, including keratinocytes and melanocytes, were interpreted as p53-positive cells. The examination and interview were done by three pathologists. P53 immunoreactivity were assessed by the proportion score (percentage) of p53-positive cells in the whole epidermis. The results were quantified by expressing the number of positive keratinocytes as a percentage of 1000 cells counted in each section.11-13 Tumor cells with staining <10% were regarded as negative and >10% were considered positive.3 Tumor staining pattern was divided into 5 categories (nil, <10%, 10-20%, 21-50%, >50%). The proportional score of positively staining cells in each tumor was recorded as (10-20% = +), (21-50% = ++) and (>50% = ++++) but was classified as either p53-positive or p53-negative for analysis. **Statistical Analysis:**

Statistical parameters as mean, standard deviation, t-test, z-test and chi-square test were used in the study. P value ≤0.05 was regarded as significant, values <0.01 were regarded as highly significant.

**Results**

There was statistical significant value in relation to age group examined in patients with various skin cancers which was higher in patient aged more than 40 years versus less than 40 years as shown in Table 1.

<table>
<thead>
<tr>
<th>Age group (Year)</th>
<th>Benign</th>
<th>B.C.C</th>
<th>S.C.C</th>
<th>Melanoma</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;40</td>
<td>2</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>16.6</td>
</tr>
</tbody>
</table>
Regarding gender, there was no statistical significant difference in different type of skin cancer (Table 2). The p53 immunoreactivity of the skin lesions of the total 36 collected sample from patients with various skin lesions is shown in Table 3. The IHC expression of p53 in malignant cases was positive in only 19 of total 28 cases (76.8%). The samples with most biopsies showed medium to intense p53-positive cells (Table 4); positive staining constituted 68.4% while negative 32.4%.

**Discussion**

Recently p53 mutation has been implicated in the pathogenesis of many skin cancer. Most of these mutations are acquired, not inherited. Assessment of p53 status in human cells can be performed by immunohistochemical detection of nuclear p53 accumulation giving a rapid preliminary indication of p53 status in tumors. Most skin cancers in the present study occur in old age group above 40 year. Age is considered to be a determinant of the neoplastic process. Other studies agree that skin cancers typically arise in patients aged 50–70. Differences in the estimation of p53 cut off value level in different studies account for some of the variability as some studies consider benign skin lesion as being positive. In the current study there was scattered discrete immunoreactivity of p53 staining in keratinicytes which are considered as negative (the cut off value in present study >10%). In the current study there was statistical significant difference in p53 expression in patients with skin cancer compared with benign skin lesion. One investigator found that higher p53 immunoreactivity in the malignant cases. The observed staining pattern in our study were scattered in basal layer of epidermis in benign skin, but in BCC, SCC, cutaneous melanoma there was intense diffuse staining throughout the lesion. P53 staining intensity among malignant cases, shows statistically significant association most biopsies showing medium to intense

<table>
<thead>
<tr>
<th>Gender</th>
<th>Benign</th>
<th>B.C.C</th>
<th>SCC</th>
<th>Melanoma</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>0.103</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Gender factor in relation to various skin lesion.

<table>
<thead>
<tr>
<th>Pathological Diagnosis</th>
<th>Positive p53 No.</th>
<th>Negative p53 No.</th>
<th>Total cases No.</th>
<th>Statistic Mean±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td>1.3572±0.09132</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>1.0335±0.27631</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>1.3521±0.24709</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>1.2560±0.27877</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>17</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 3:** Number of positive and negative p53 immunoreactivity cases.

<table>
<thead>
<tr>
<th>P53 scoring</th>
<th>10-20%=++</th>
<th>21-50=+++</th>
<th>&gt;50=++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>3(15.8%)</td>
<td>3(15.8%)</td>
<td>13(68.4%)</td>
</tr>
</tbody>
</table>

*P value less than 0.05 is significant*
p53-positive cells, positive staining constituted 68.4%. In the present study p53 over expression was detected in 70% of BCC, 70% of SCC and 83% in M.M. while variable results was found in other studies that 90% of squamous cell carcinoma and approximately 50% of basal cell carcinomas, and 20% of melanomas.5-7 This variability is due to small sample size in this study. The prevalence of abnormal p53 expression in series of invasive melanomas from Queensland was 83%. Estimates of p53 expression vary widely, due to in part the differing characteristics of the antibodies used for detection and the criteria used for determining positive staining.17 In addition, individuals exhibit heterogeneity in their ability to repair DNA damage induced by UV radiation. This heterogeneity will affect the relationship between sun exposure and p53 mutation.20

**Conclusion**

Increased expression of p53 protein can be detected in human skin cancer compared with benign lesion and mutation of p53 may form an important part of pathogenesis of many type of skin cancer.

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**Figure 1:** Basal cell carcinoma of skin IHC staining for p53 magnified X400.

**Figure 2:** Squamous cell carcinoma of skin IHC staining for p53 magnified X400.

**Figure 3:** Malignant melanoma of skin IHC staining for p53 magnified X400.

**Figure 4:** Intradermal nevus stained by IHC for p53 negative magnified X400.
Conflicts of interest
The authors report no conflicts of interest.

References