Expression of p53 in Arsenic-Related and Sporadic Basal Cell Carcinoma

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Background: The TP53 gene has been shown to have an important role in the genesis of sporadic, presumably mainly sunlight-related, basal cell carcinoma (BCC). However, its role in arsenic-related BCCs is not clear, although the trivalent form of arsenic has been long recognized as a cause of BCC. Arsenic treatment has been shown to cause hypermethylation of the TP53 gene in lung carcinoma cell lines, but it is not known if this occurs in vivo in arsenic-related BCCs.

Objective: To compare the immunohistochemical expression of the p53 protein in arsenic-related and sporadic BCCs to determine if the expression pattern is consistent with gene silencing.

Setting: A research institute and hospital in Australia.

Cases: One hundred seventeen white patients with 121 sporadic BCCs and 21 white patients with 92 arsenic-related BCCs.

Main Outcome Measures: The expression and the intensity of p53 were scored semiquantitatively. Statistical analysis was performed using the χ² test.

Results: Arsenic-related BCCs express p53 less often and at a lower intensity than sporadic BCCs (P = .001; 2-tailed test). The BCCs from sun-exposed sites, whether arsenic related or sporadic, more frequently showed overexpression of p53 than those from less-exposed areas (P = .004; 2-tailed test). The more aggressive subtypes of BCC show a higher level of expression of p53 than the less aggressive forms (P = .04; 2-tailed χ² test).

Conclusions: These results are consistent with the hypothesis that the TP53 gene is down-regulated by methylation in arsenic-related BCC, particularly those from less-exposed sites. However, an alternative possibility is that mutations in TP53 that stabilize the protein are less common in arsenic-related BCCs. Further analysis will be necessary to distinguish between these hypotheses.


Basal cell carcinomas (BCCs) are the most common cancer in humans. Genetic and environmental factors contribute to their development. Although the genetic component is clear in the cancer predisposition syndrome (nevoid basal cell carcinoma syndrome), other genetic traits, such as skin and hair color and the tendency to freckle, also influence risk indirectly. The major causative factor is UV radiation (UVR), which probably explains the incidence of BCC in Queensland, Australia, is the highest reported for any cancer. However, the risk for BCC is less strongly associated with sun exposure than that for squamous cell carcinoma. Other agents, such as ionizing radiation, exposure to chemical carcinogens, and possibly infection with human papillomaviruses have also been implicated in the etiology of BCC. Exposure to the trivalent form of inorganic arsenic has also been long recognized as a cause of BCC, mainly through ingestion of arsenic-containing herbal medicine or contaminated drinking water.

The TP53 tumor suppressor gene, which is frequently mutated in human cancers, appears to be an early target in UVR-associated skin carcinogenesis and has been shown to play an important role in the development of BCC. Mutations have been found in BCCs from sun-exposed and less-exposed sites, although the types of mutations in these sites differ, with transitions more common in BCCs from sun-exposed sites and transversions in those from less-exposed sites. Various studies have reported that 40% to 90% of BCCs show at least focal immunopositivity for p53. Expression of the p53 protein has also been detected in normal epidermis that has suffered usual sun exposure and in the normal epidermis adjacent to BCCs, which in 1 case also carried a missense mutation in TP53. These data suggest that UVR can result in mutations of TP53, which stabilize the protein and are therefore detected by increased immunoreactivity. It is not known whether BCCs arising largely as a conse-
IMMUNOHISTOCHEMICAL ANALYSIS

Paraffin sections (4 μm) were affixed to adhesive slides (Menzel Superfrost Plus; Menzelgläser, Braunschweig, Germany) and air dried. The sections were deparaffinized in xylene and rehydrated through descending graded alcohol to Trisbuffered saline solution (TBS), pH 7.4. Antigen retrieval was performed by boiling the sections in citrate buffer (0.01 mol/L, pH 6.0) in a microwave. Endogenous peroxidase activity was blocked by 1.0% hydrogen peroxide (H₂O₂) and 0.1% sodium azide in TBS. Nonspecific antibody binding was inhibited by use of 4% skim milk powder in TBS and 10% normal goat serum (Zymed Laboratories, San Francisco, Calif). The sections were then incubated overnight at room temperature with a mouse monoclonal anti–p53 antibody (DO17; Dako, Carpinteria, Calif) diluted 1:100 in TBS followed by prediluted biotinylated goat anti–mouse immunoglobulin (Zymed Laboratories) for 30 minutes and then prediluted streptavidin-horseradish peroxidase (Zymed Laboratories). Following each incubation step, the sections were thoroughly washed in 3 changes of TBS, the first containing 0.5% Triton X-100 detergent (Ajax Chemicals, Auburn, Australia). Immunoreactive sites were visualized using 3,3’-diaminobenzidine (SigmaAldrich Corporation, St Louis, Mo) with H₂O₂ as substrate. The sections were counterstained with Mayer hematoxylin, dehydrated in ascending alcohols, cleared in xylene, and permanently mounted using DePeX (BDH Gurr, Poole, England). As negative control samples, serial sections were stained as described above, but incubated with TBS alone in lieu of primary antibody.

DATA EVALUATION

Slides were coded and scored by one of us (W.B.) who was unaware of the origin and site of the tumor. The criteria for scoring the stained sections were as follows: 1 plus sign indicated 0% to 25% of the whole tumor mass stained; 2 plus signs, 26% to 50%; 3 plus signs, 51% to 75%; and 4 plus signs, 76% to 100%. The sections were also scored (0–4) for the intensity of p53 staining, where 4 indicated the greatest intensity. The χ² test was used to compare the difference between different groups of tumors, using a significance level of P < .05 and 2-tailed tests. The Mantel-Haenszel test was used for trend analysis.

RESULTS

The sporadic and arsenic-related BCCs showed a heterogeneous pattern of p53 nuclear staining within individual tumor masses. There were regions of the BCC in which the nuclei were strongly stained, whereas other regions in the same tumor had only focal, but reproducible, staining. Positive p53 staining was prominent at the periphery of some nests, especially in the solid histological subtype.

Positive nuclear staining of p53 (>23% positive cells) was significantly more common in sporadic BCCs (64/121 [52.9%]) than in arsenic-related BCCs (28/92 [30.4%]) (P = .001) (Table 1). Furthermore, the intensity of p53 staining was much greater in sporadic BCCs compared with arsenic-related tumors, with 50 (41.3%) of 121 sporadic tumors having intensities of 3 or 4, compared with only 15 (16.3%) of 92 arsenic-related BCCs (P < .001) (Figure).
Overall, BCCs from sun-exposed sites, whether arsenic-related or sporadic, more frequently showed p53 immunopositivity (defined by staining in $\geq 25\%$ of cells) than tumors from less sun-exposed areas ($P = .004$; 2-tailed test). However, the difference between sporadic and arsenic-related BCCs in their p53 immunopositivity was maintained when the comparison was limited to tumors from sun-exposed sites ($P = .03$), or from less-exposed sites, although the latter comparison was not statistically significant ($P = .22$). Among the arsenic-related BCCs, p53 immunopositivity was more common in tumors from sun-exposed sites (15/40 [37.5%]) compared with those from less-exposed sites (13/50 [26.0%]), but this difference was not significant ($P = .24$). The lowest frequency of p53 immunopositivity was found in the arsenic-related BCCs from less-exposed sites (26.0%), and the highest was in sporadic tumors from sun-exposed sites (57.8%).

Immunopositivity of p53 was assessed with respect to age of the patient at the time of excision. There was no evidence that immunopositivity was less common in the BCCs removed at an earlier age, which might be expected if TP53 mutations are less common in BCCs occurring at a young age. Indeed, to the contrary, there was a tendency in the sporadic BCCs for more staining ($P = .06$, test for trend), which may imply a higher frequency of mutations in BCCs removed at a younger age. The BCCs were categorized as aggressive, which included infiltrative and basosquamous types, or nonaggressive, which included superficial, solid, and cystic types (Table 2). The aggressive forms showed a higher number of p53-positive cells than the nonaggressive forms ($P = .04$).

### Table 1. Immunoreactivity of p53 in Arsenic-Related and Sporadic BCCs*

<table>
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<th>Sporadic BCCs‡</th>
<th>Arsenic-Related BCCs§</th>
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<tr>
<td>p53 Expression†</td>
<td>All</td>
<td>Less-Exposed Areas</td>
</tr>
<tr>
<td>+</td>
<td>57 (47.1)</td>
<td>17 (60.7)</td>
</tr>
<tr>
<td>++ to ++++</td>
<td>64 (52.9)</td>
<td>11 (39.3)</td>
</tr>
<tr>
<td>All</td>
<td>121 (100.0)</td>
<td>28 (100.0)</td>
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*BCC indicates basal cell carcinoma. Data are given as number (percentage) of patients. For arsenic-related vs sporadic BCCs, $P = .001$; BCCs from sun-exposed vs less-exposed areas, $P = .004$; arsenic-related BCCs from less-exposed vs sun-exposed sites, $P = .24$; and arsenic-related BCCs vs sporadic BCCs from less-exposed sites, $P = .22$.

†One plus sign indicates no more than 25% of the whole tumor mass stained; 2 plus signs, 26% to 50%; 3 plus signs, 51% to 75%; and 4 plus signs, 76% to 100%.

‡Three of 121 tumors came from unknown sites.

§Two of 92 tumors came from unknown sites.

### Table 2. p53 Immunopositivity in Different Histological Subtypes of BCC*

<table>
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<tr>
<th>p53 Expression†</th>
<th>Aggressive BCCs</th>
<th>Nonaggressive BCCs</th>
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<tr>
<td>+</td>
<td>15 (41.7)</td>
<td>105 (59.7)</td>
</tr>
<tr>
<td>++ to ++++</td>
<td>21 (58.3)</td>
<td>71 (40.3)</td>
</tr>
</tbody>
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*BCC indicates basal cell carcinoma; aggressive BCCs, infiltrative or basosquamous subtypes; and nonaggressive BCCs, superficial, solid, or cystic subtypes. Data are given as number (percentage) of patients. $P = .04$, $\chi^2$ 2-tailed.

†The symbols are explained in the second footnote to Table 1.

Immunopositivity of p53 is highly correlated with mutations in TP53 that stabilize the protein, but that also may occur if the protein is overexpressed in response to a recent genotoxic assault. Various studies have reported that 40% to 90% of BCCs have at least focal p53 immunopositivity. We have found in our study that arsenic-related BCCs show significantly less p53 immunopositivity (30.4%) than sporadic BCCs (52.9%) ($P = .001$). This may be explained partly by the fact that we also found that BCCs from...
less sun-exposed sites had lower levels of p53 immunopositivity than tumors from sun-exposed sites. These results suggest some differences in the genesis of BCCs from sun-exposed and protected sites, and also suggest that arsenic-related BCCs may arise somewhat differently from those occurring largely as a result of UVR exposure. Our results are in contrast to those of Chang et al., who reported p53 immunopositivity in all 10 BCCs examined from an area of endemic arsenic exposure from contaminated drinking water. However, it is difficult to compare our results directly with those of Chang et al, because of differences in the definition of immunopositivity. Furthermore, the timing and duration of exposure probably differ between both studies, as well as the form of arsenic taken.

However, there are data to suggest that BCCs with an earlier age of onset are less likely to have TP53 mutations, and so it was important to determine if our results may simply reflect a difference in the ages in the patients with arsenic-related and sporadic BCC. D’Errico et al. suggest that this might be related to a p53-independent mechanism occurring in the BCCs of early onset, which were associated with acute sun exposure in childhood, as opposed to the more chronic sun exposure associated with BCCs of a later age of onset (>40 years). The mean age of the patients with sporadic BCCs in our study was 69 years, as opposed to 52 years for the patients with arsenic-related cases. However, there was no evidence that p53 immunopositivity was related to age, and the only 2 sporadic BCCs excised at ages less than 40 years had very high levels of p53 immunopositivity. It is therefore unlikely that the difference in p53 immunopositivity between the sporadic and arsenic-related BCCs was related to the age difference.

In sporadic BCC, most TP53 mutations are those associated with UV-B radiation, and the TP53 gene appears to be an early target in UVR-associated skin carcinogenesis. Therefore, the low-level of p53 expression in arsenic-related BCC may indicate that p53 is less often an early target than in sporadic BCC, or that the spectrum of TP53 mutations is different in arsenic-related BCCs and less likely to stabilize the protein. Alternatively, the arsenic-related BCCs may have promoter mutations that down-regulate the gene or other epigenetic insults that account for the reduced rates of p53 immunopositivity. The latter possibility is particularly intriguing in the light of the recent report showing that arsenic alters cytosine methylation patterns in the promoter of p53, and that this is associated with decreased p53 expression.

Our study also shows that p53 immunopositivity is more common in aggressive forms of BCC (P < .04). This is consistent with the report of Barrett et al., who found that p53 expression was highest in the aggressive subtypes and correlated with the expression of PCNA, a marker of proliferation. Similarly, overexpression of p53 was found by De Rosa et al. to be correlated with a more aggressive clinicopathological behavior of BCCs.

Further investigation into the molecular events relating to p53 expression are needed in these tumors, together with further samples from other arsenic-exposed individuals, to determine why the rates of p53 immunopositivity are so different in sporadic and arsenic-related BCCs. It would also be of interest to examine the expression of other genes involved in the genesis of BCC, such as the patched gene (PTCH), which is frequently mutated in BCC and acts as a gatekeeper, but no PTCH antibodies are available to perform such an examination.

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REFERENCES