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 \( \chi^2 \) 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 \( \chi^2 \) 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.

MATERIAL AND METHODS

TUMOR SAMPLES

One hundred twenty-one BCCs from 117 patients were identified using a SNOMED (Systemized Nomenclature Of human and veterinary MEDicine) search from the archives at the Royal Brisbane Hospital, Brisbane, Queensland. These BCCs were assumed to be related largely to UVR exposure, given the high rates of BCCs in these subtropical latitudes among patients of mainly Anglo-Celtic origin, and the very small likelihood that these patients had been exposed to arsenic. However, sun exposure history of these cases could not be determined because the patients could not be contacted, and nor could they be questioned about their arsenic exposure. The patients included 76 men and 41 women, with a mean age at the time of excision of 69 years (range, 41-95 years). Ninety (74.4%) of these tumors came from sites that are regarded as receiving considerable sun exposure in the subtropical climate of Brisbane, eg, face, arms, and legs. Ninety-two arsenic-related BCCs from 21 patients (12 men and 9 women) were identified from the archives of private pathology laboratories in Queensland. These patients were self-referred to this research project because of a history of arsenic ingestion, ie, they used a local medication, Bel’s Asthma Mixture, in their childhood or adolescence. The medication was an antihistaminic preparation that was distributed in Queensland during the 1940s through the 1960s. It is reported to have contained 1% arsenic trioxide (T. McGuire, MD, Queensland Medication Helpline, Queensland Institute of Medical Research, Brisbane, Australia; available by calling 61 7 3636 8111), although this cannot be confirmed, as the medication is no longer available. However, all patients displayed multiple cutaneous signs of chronic arsenicism, such as palmar-plantar keratoses, arsenical keratoses, Bowen disease, squamous cell carcinomas, and BCCs, and in many cases hundreds of lesions had been removed. The mean age at tumor(s) excision was 52 years (range, 39-76 years). Of the 92 BCCs from these 21 patients, 50 (54.3%) came from less sun-exposed sites such as the trunk and scalp.

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), pH7.4. Antigen retrieval was performed by boiling the sections in citrate buffer (0.01 mol/L, pH6.0) 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 (DO7; 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 (Sigma-Aldrich Corporation, St Louis, Mo) with H2O2 as substrate. The sections were counterstained with Mayer hematoxylin, rinsed in ascending alcohols, dehydrated 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).

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Overall, BCCs from sun-exposed sites, whether arsenic-related or sporadic, more frequently showed p53 immunopositivity (defined by staining in 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>
<thead>
<tr>
<th>p53 Expression†</th>
<th>Sporadic BCCs‡</th>
<th>Arsenic-Related BCCs§</th>
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<tbody>
<tr>
<td></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>
</tr>
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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>
<thead>
<tr>
<th>p53 Expression†</th>
<th>Aggressive BCCs</th>
<th>Nonaggressive BCCs</th>
</tr>
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<tbody>
<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>
</table>

*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, x² 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.26 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 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 younger 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,24,25 and the TP53 gene appears to be an early target in UVR-associated skin carcinogenesis. Therefore, the low-level of p53 immunopositivity occurs 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.17

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.40 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