Human patched (PTCH) mRNA Is Overexpressed Consistently in Tumor Cells of Both Familial and Sporadic Basal Cell Carcinoma

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Abstract

Recently, a human homologue of the Drosophila patched gene, PTCH, was identified as a putative tumor suppressor mutated in both hereditary and sporadic basal cell carcinomas. Because PTCH controls its own transcription, inactivating mutations in PTCH may lead to overexpression of mutant PTCH mRNA due to loss of autoregulation. The present study is aimed at evaluating whether deregulation of PTCH mRNA expression is a general feature of BCCs of varying histological growth pattern and malignant potential.

Irrespective of histological subtype, PTCH mRNA was overexpressed consistently as determined by in situ hybridization in all of the sporadic (n = 16) and hereditary (n = 20) tumors examined. PTCH expression was found in all of the tumor cells but appeared stronger in the peripheral palisading cells. PTCH mRNA was not detected in adjacent nontumor epidermal cells or in other parts of the epidermis. In the majority of tumors (20 of 36), nuclear immunostaining for p53 was found in scattered cells, whereas seven tumors completely lacked p53 immunoreactivity.

Our finding of an up-regulation of PTCH mRNA levels in all of the BCCs analyzed indicates that deregulation of the PTCH signaling pathway constitutes an early rate-limiting event in BCC development.

Introduction

BCC is the most common human cancer in the Western world, showing a continuing increase in incidence (1). The tumors grow slowly and hardly ever metastasize or cause death. Nevertheless, BCC can cause considerable morbidity through local invasion and tissue destruction. UV radiation is believed to be an important etiological factor based mainly on epidemiological studies (2). BCCs usually arise in elderly light-skinned people as sporadic tumors, but are also the major feature of the NBCCS, a multisystem autosomal dominant disorder characterized primarily by cancer susceptibility and embryonic abnormalities (3).

Recently, mutations in the human homologue of Drosophila patched (PTCH) were identified as the underlying genetic event in NBCCS (4, 5). Coupled with data demonstrating frequent LOH of the genomic area containing PTCH in sporadic BCCs (6–8) and the subsequent finding of PTCH mutations in both hereditary and sporadic BCCs, these results suggest strongly that PTCH is a tumor suppressor of critical importance in BCC development (9–11). The PTCH gene encodes a transmembrane protein serving as receptor for the secreted factor sonic hedgehog and its homologues (12, 13). Studies of both Drosophila and mammalian systems demonstrate that PTCH down-regulates its own transcription, and inactivating mutations of the PTCH gene are therefore expected to lead to overexpression of the PTCH mRNA due to loss of negative feedback (14). In support of this hypothesis, we showed recently that PTCH mutations in single samples of sporadic BCCs were associated with increased expression of mutant PTCH mRNA (9).

Among other major genetic changes occurring in BCCs are mutations of the p53 tumor suppressor gene. The frequency of mutation is estimated to about 50% (15–17), although reports vary rather widely. Interestingly, p53 mutations have also been found in epidermal cells of normal appearance adjacent to BCCs and SCCs (18–20). Such cells were first detected as “patches” of cells showing distinct immunostaining with anti-p53 antibodies, indicating the presence of stabilized p53 protein.

The aim of the present study was to evaluate whether deregulation of the PTCH mRNA expression pattern is a general finding in hereditary and sporadic BCCs of varying histological subtypes and malignant potential and to compare this feature with aberrations in the p53 protein as assessed by increased immunoreactivity.

Materials and Methods

Tumor Samples. A total of 36 formalin-fixed paraffin-embedded BCCs from 22 patients (15 males and 7 females) was obtained from the Department of Dermatology, Karolinska Hospital (Stockholm, Sweden). The mean age of the patients at diagnosis was 54.5 years (range, 31–86). The majority (25 of 36) of the tumors were located on sun-exposed areas. Of these, 14 tumors were located on the face. All of the histological subtypes of BCC were included: superficial (n = 6), nodular (n = 23), and infiltrative (n = 7). Twenty tumors were from NBCCS patients, 3 were from two young patients with multiple BCCs (not classified as NBCCS), and 13 were sporadic BCCs. In addition, samples from two superficial malignant melanomas, one SCC of the skin, one intradermal nevus from a NBCCS patient, and one breast carcinoma were included. The histological diagnoses were confirmed by an experienced dermatopathologist.

In Situ Hybridization. Preparation of PTCH-RNA probes and in situ hybridization were performed as described previously (9, 21). Briefly, a human patched cDNA fragment (bases 190–628) was cloned into PGEM5, appropriately linearized and in vitro transcribed to obtain antisense and sense probes (9). Sections were treated with proteinase K (Sigma Chemical Co.) and washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. Subsequently, sections were hybridized overnight with 2.5 × 10⁶ cpm labeled antisense or sense probe (9). Sections were treated with proteinase K (Sigma Chemical Co.) and washed in 0.1 M triethanolamine buffer containing 0.25% acetic anhydride. Subsequently, sections were hybridized overnight with 2.5 × 10⁶ cpm labeled antisense or sense probe at 55°C. Autoradiography was for 14 days. After development of the photographic emulsion, slides were stained with H&E. The strength of the PTCH mRNA signal was ranked on a semiquantitative scale from + to ++.

Immunohistochemistry. Dewaxed and rehydrated sections were treated with 0.3% hydrogen peroxide solution for 30 min to exhaust endogenous peroxidase activity. After microwave treatment (22), the slides were cooled in tap water and preincubated in 1% BSA in PBS for 20 mm followed by incubation with a monoclonal antibody (DO-7) against human p53 protein. After rinsing in PBS, the slides were incubated with a biotinylated rabbit antimouse antibody (DAKO/A/S, Glostrup, Denmark), diluted 1:100, for 60 min. After rinsing in PBS, the slides were incubated with a biotinylated rabbit antimouse antibody (DAKO), diluted 1:100, for 30 min. The immunoreaction was visualized with...
Fig. 1. *PTCH* mRNA is expressed in BCC tumor cells. A, dark-field photomicrograph of a sporadic nodular BCC tumor hybridized with $^{35}$S-labeled antisense RNA for *PTCH*. Abundant autoradiographic signal for *PTCH* mRNA is seen only in tumor cells. B, section of the same tumor hybridized with the sense probe had no signal. Bar, 150 μm. C and D, paired bright- and dark-field photomicrographs of a tumor nodule under higher magnification. The expression of *PTCH* mRNA appears most pronounced in the palisading periphery of the tumor nests. Bar, 25 μm.

avidin-biotin complex (DAKO) with 0.004% hydrogen peroxidase as substrate and diaminobenzidine as a chromogen. Counterstaining was performed with Mayer's hematoxylin. The proportion of immunoreactive cells exhibiting strong nuclear staining was estimated, and the overall p53 immunoreactivity was evaluated as − (no positive cells), + (single scattered cells), and ++ (most tumor cells positive).

Fig. 2. *PTCH* mRNA is overexpressed in superficial BCC but not in the adjacent epidermis. A and B, paired bright- and dark-field photomicrographs of a superficial sporadic BCC tumor. Autoradiographic signal for *PTCH* mRNA is evident only in tumor cells (tu). Bar, 100 μm. C, tumor nest under high power demonstrates specific signal in tumor cells, whereas no signal is seen in the adjacent histologically normal epidermis (ep). Bar, 10 μm.
Results

Expression of PTCH mRNA. To investigate whether increased expression of PTCH mRNA is common in BCCs, we analyzed 36 BCCs of different histological types by in situ hybridization. Notably, a positive signal for PTCH mRNA was observed in all of the BCCs examined, including tumors from non-sun-exposed sites (Figs. 1–3 and Table 1). A strong signal, graded ++ (Table 1), was seen in 2 of 7 infiltrative BCCs, 8 of 23 nodular BCCs, and 0 of 6 superficial ones. Of familial tumors, 6 of 20 displayed strong signals compared to 4 of 16 sporadic BCCs. The three nodular BCCs derived from young patients with multiple BCCs, not diagnosed with NBCCS, all showed low to moderately positive signals for PTCH mRNA.

The positive signal was confined consistently to the tumor cells, and no expression was detected in adjacent normal epidermal or dermal cells (Fig. 2). All of the parts and all of the cells of the tumor nests appeared positive. However, the strongest signal was often seen in the peripheral palisading cells, with weaker expression in the smaller, more differentiated cells in the center of the nests (Fig. 1, C and D).

In contrast, there was no detectable signal for PTCH mRNA in any of the other samples examined: malignant melanoma, benign intradermal nevus, SCC of the skin, or breast carcinoma (not shown).

Expression of p53 Protein. Aberrant expression of the p53 protein, in many cases associated with mutations in the p53 gene, is often seen in BCCs and SCCs (16, 17, 19). To compare such aberrant p53 protein expression with PTCH mRNA expression, we analyzed the same set of tumors for p53 immunoreactivity (Fig. 3; Table 1). Positive nuclear immunoreactivity, in at least some tumor cells, was
detected in 80% (29 of 36) of the BCCs. In the majority of tumors, 27 of 36, either no or only scattered p53-positive cells were seen in a dispersed pattern. All of the negative tumors (n = 7) were derived from NBCCS patients and included three tumors from non-sun-exposed sites. Only one tumor showed positive p53 immunoreactivity in all of the tumor cells. These results indicate that between 25 and 80% of the BCCs may contain cells with alterations in the p53 gene. In several samples, we observed p53 immunoreactivity in histologically normal epidermal cells adjacent to the tumor, in agreement with earlier studies (17, 18, 20; data not shown).

Notably, one 31-year-old male NBCCS patient presented with multiple large and aggressive BCCs, which later led to his death (Fig. 3). Interestingly, one of these tumors from the abdomen (tumor 10 in Table 1), showed abundant PTCH mRNA expression involving all of the tumor cells but completely lacked p53 immunoreactivity (Fig. 3).

Discussion

Tumor development is believed to be a multistage process involving the accumulation of various genetic alterations in the tumor cell. It has been proposed that in most cases one of these genetic alterations is a critical determinant (gatekeeper) of the onset and/or growth rate of the tumor (23). BCCs occur frequently, but thus far no precursor lesion has been identified, indicating that a key genetic event sufficient for acquisition of the tumor cell phenotype may be required. Germ-line mutations of one allele of the PTCH gene found in NBCCS patients predispose to development of BCC (4, 5). An important role in the development of sporadic BCC is also strongly suggested by frequent LOH (60—70%) in the corresponding genomic region, 9q22.3, as well as by demonstration of direct PTCH mutations (9, 10).

Our present results, using overexpression of PTCH mRNA in BCCs as a read-out, lead us to propose that deregulation of the signal transduction pathway controlled by the PTCH protein serves as an early rate-limiting step in BCC development. Arguments in favor of this hypothesis are: (a) overexpression of PTCH mRNA involving all of the tumor cells was observed in all 36 tumors examined, and (b) PTCH mRNA expression was not detected in normal epidermal cells from either sporadic BCC or NBCCS patients. That the increased PTCH mRNA does not merely reflect the presence of actively proliferating keratinocytes is supported by our recent finding that sporadic skin SCCs do not express elevated PTCH mRNA levels, nor do they contain mutations in the PTCH gene.4 The specificity of PTCH mRNA overexpression in BCC is indicated further by the lack of detectable PTCH mRNA signals in two malignant melanomas and one breast carcinoma. However, as demonstrated previously, PTCH mRNA can be detected in normal epidermis using the sensitive reverse transcription-PCR technique (4). An important question is the degree of correlation between the consistent overexpression of PTCH mRNA and the presence of mutations in the PTCH gene. Based on the reasons given above, we favor the interpretation that increased PTCH mRNA levels reflect a clonal genetic change resulting in loss of autoregulation. PTCH mutations have been reported in about one-third of sporadic BCCs (9). This is likely to be an underestimation due to nonoptimal detection methods, as also suggested by the presence of LOH in 60—70% of BCCs (6—8). However, loss of PTCH autoregulation can result not only from homozygous inactivation of the PTCH gene; mutations in other genes participating in the PTCH signaling pathway may have the same effect. Thus, the deregulation of PTCH

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mRNA in all of the BCCs examined may be the net result of mutations in several genes involved in the same signal transduction pathway.

In the present study, positive nuclear immunostaining for p53 was observed in 80% of the tumors. However, most of the positive tumors showed only relatively few scattered positive cells. Although there is no absolute correlation between p53 immunoreactivity and the presence of p53 mutations for BCCs, the fraction of tumors (about 50%) reported as having p53 mutations and displaying positive immunostaining is similar (17, 24). The demonstration of clonal groups of normal-appearing epidermal cells immunoreactive with p53 in normal epidermis as well as adjacent to BCCs (17, 18, 20) suggests that such groups of cells may be precursors of tumor formation. However, it has recently been shown that p53 mutations in adjacent p53-immunoreactive cells differ from p53 mutations present in tumor cells, which seriously questions this hypothesis (17). Our present data also demonstrate that BCCs can arise and even be highly aggressive without any sign of p53 immunoreactivity. To resolve the relative importance of p53 and PTCH mutations in BCC development, mutational analysis of both genes in the same tumors will be important in future studies.

In summary, our results show that PTCH mRNA is overexpressed consistently in all of the BCC tumor cells, suggesting that deregulation of the PTCH signaling pathway is an early and rate-limiting event in BCC development. Positive p53 immunostaining is observed frequently in BCCs, but BCCs can arise and be highly aggressive without displaying any p53-positive cells.

Acknowledgments

We thank Elisabeth Henriksson for technical assistance with immunohistochemistry.

References


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*Cancer Res* 1997;57:2336-2340.