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Short Communication

Overexpression of p53 Protein in Basal Cell Carcinomas of Human Skin

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Basal cell carcinoma (BCC) of the skin is the most common human cancer, but its molecular-genetic pathogenesis is unclear. In many other types of cancer, mutations of the tumor-suppressor gene p53 occur frequently and may lead to overexpression of a long-lived mutant form of p53 protein. In this study, overexpression of p53 protein was detected immunohistochemically in 30 (83%) of 36 specimens of BCC of the head and neck. The same regions of tumor typically were reactive both with a monoclonal antibody (PAb240) specific for the mutant protein and with one (PAb1801) directed against an epitope common to both wild-type and mutant p53 protein. Keratinocytes of chronically sun-exposed epidermis adjacent to BCCs also focally overexpressed p53 protein in the majority of cases, whereas those of sun-protected buttock skin did not. Mutation of p53 may form an important part of the pathogenetic sequence in a majority of cases of BCC. (Am J Pathol 1992, 141:25–29)

Basal cell carcinoma (BCC) of the skin is the most common type of human cancer; the estimated incidence of BCC in the United States is more than 500,000 cases yearly, representing more than one-third of all cancers.[1] BCC occurs most frequently on the head, neck, and other chronically sun-exposed regions of fair-skinned persons in the fourth decade of life and later. BCC is a low-grade tumor with a low potential for metastasis[2] but with a significant risk of local invasion, destruction, and recurrence.[3] Thus, although the per-case mortality from BCC is low,[4] the cumulative morbidity is high. Chronic solar exposure, a source of mutagenic ultraviolet radiation (UVR), is the most important etiologic factor identified in most cases of BCC.[5] The subsequent molecular-genetic steps in the pathogenesis of BCC, however, are poorly understood.

An interesting candidate molecule for investigation of this process is p53, a 53 kd nuclear phosphoprotein encoded by the *p53* gene,[6] located on chromosome 17, band p13.[7,8] The wild-type (wt) p53 protein downregulates cellular proliferation by interrupting progression through the cell cycle.[9] Transfection of the wt *p53* gene into cells mitigates transformation mediated by cotransfected oncogenes,[10–12] indicating that it is a tumor-suppressor gene.[13,14] Documented structural abnormalities of the *p53* gene include deletions, inactivations, truncations, and point mutations. The mutant *p53* gene can act as a dominant-negative oncogene, with the mutant gene-product interfering with the normal, growth-suppressive function of the wt p53 protein.[15,16] Transgenic mice bearing a point mutation of the *p53* gene have an increased incidence of cancer.[17] Mutations of the *p53* gene also occur in a wide variety of human tumor types,[18] and are in fact the most commonly detected genetic abnormality in cancer.[19] We hypothesized that the *p53* gene and its product might be involved in the pathogenesis of human BCC as well.

To test this hypothesis, we have examined human BCCs immunohistochemically with two anti-p53-protein monoclonal antibodies (MAbs). One of these (PAb240) has high specificity for mutant forms of p53 protein, while the other (PAb1801) reacts with both wt and mutant p53 protein. Because wt p53 protein has an intracellular half-life of only 20 minutes,[20] it is present at a very low concentration (below the limits of immunohistochemical detection) in most untransformed cells and non-neoplastic

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tissues.[21,22] Point mutation of the *p53* gene, on the other hand, typically results in overexpression of a stable, long-lived form of p53 protein[23] that can be detected by sensitive immunohistochemical methods. A portion of this work has been reported previously in abstract form.[24]

Materials and Methods

Tissue Specimens

Forty-one consecutively accessioned, frozen specimens of human skin were studied after excision for the definitive treatment of previously biopsied BCC located on chronically sun-exposed regions of the head and neck. The patients were 38 adult Caucasoid men (n = 16) and women (n = 22) with an age range of 34 to 88 years (mean = 61 years; age not known in 4 patients). All patients gave informed consent for study of their specimens. To assess the potential role of chronic UVR exposure on p53 protein expression of human skin, 14 frozen biopsy sections of nonlesional buttock skin taken from other patients (5 male and 9 female, age range = 12–86 years, mean = 46 years) were also examined. These buttock skin biopsy specimens had been submitted for immunofluorescence study, and all were negative for deposition of IgG, IgM, IgA, the third component of complement, and fibrin.

Antibodies

Two murine IgG_1-class MAbs (Oncogene Science, Inc., Manhasset, NY) were used for immunohistochemical localization of p53 protein. PAb1801[25] recognizes an epitope near the N-terminus of human p53 protein, at a region distant from the most common mutations[26] and therefore reacts with both mutant and wt protein. PAb240,[27] on the other hand, does not usually react with native, wt p53 protein, but instead recognizes a conformational epitope common to many mutant forms of p53 protein of human and other species; PAb240 can also react with denatured wt p53 protein in certain circumstances.

Immunohistochemistry

In the following protocol, sections were rinsed twice with phosphate buffered saline (PBS) after each change of solution, up to the dehydration steps; all incubations were done at 25°C unless otherwise specified, and all stated concentrations are final. Sections 6 μm thick were cut from frozen specimens of human skin, fixed for 10 minutes in acetone, postfixed for 10 minutes in 0.3% p-formaldehyde in PBS, and reacted for 10 minutes with aqueous 0.3% H_2O_2 to quench endogenous peroxidase activity. Nonspecific binding sites were blocked by exposure of sections to 5% normal equine serum in diluting buffer (DB) composed of PBS supplemented with 2% fetal bovine serum and 0.1% NaN_3. Sections were concurrently exposed to primary antibodies, namely either normal murine serum at 0.1% in DB as a negative control, PAb1801 at 0.04% in DB, or PAb240 at 0.1% in DB. After 18 hours incubation at 4°C in a humidified chamber, sections were sequentially treated for 30 minutes each with biotinylated, equine anti-murine-IgG at 0.5% in PBS and with 2% avidin-biotin-peroxidase complex in PBS (Vectastain Elite kit, Vector Laboratories, Burlingame, CA), reacted for 2–4 minutes with aqueous 0.05% diaminobenzidine plus 0.75% H_2O_2, dehydrated in 95% ethanol, counterstained with eosin-Y in 95% ethanol, dehydrated in 100% ethanol, cleared in Americlear (Stephens Scientific, Denville, NJ), mounted in S/P Accumount 60 (Baxter, McGaw Park, IL), coverslipped, and examined by light microscopy. Cells were counted as positive if they exhibited strong, nuclear staining. The proportion of immunoreactive cells was ranked on a semiquantitative scale from 0 to 4+, as specified in Table 1.

Results

Of the 41 specimens excised for treatment of BCC, 36 (88%) contained residual BCC in the sections examined, and 39 (95%) contained sufficient epidermis for evaluation. Positive nuclear immunoreactivity (1+ or greater) was demonstrated with PAb1801 in 30 (83%) of the 36 BCCs, and with PAb240 in 21 (58%) (Table 1). In serial sections, the same regions of BCC typically exhibited similar topographic patterns of positive immunoreactivity with both MAbs (Figure 1A,B). Qualitatively (i.e., with regard to simple positivity or negativity), the immunohisto-

Table 1. *Immunoreactivity of 36 Basal Cell Carcinomas with the Anti-p53-protein Monoclonal Antibodies PAb1801 and PAb240*

Extent of immunoreactivity*	PAb1801 Number (%) of cases	PAb240 Number (%) of cases
0	6 (16.7)	15 (41.7)
1+	21 (58.3)	15 (41.7)
2+	3 (8.3)	3 (8.3)
3+	0 (0)	2 (5.6)
4+	6 (16.7)	1 (2.8)

* Immunoreactivity is ranked on a semiquantitative scale. 0 ≈ less than 1% of tumor parenchymal cells exhibiting strong, nuclear reactivity; 1+ ≈ 1–25%; 2+ ≈ 26–50%; 3+ ≈ 51–75%; 4+ ≈ 76–100%.

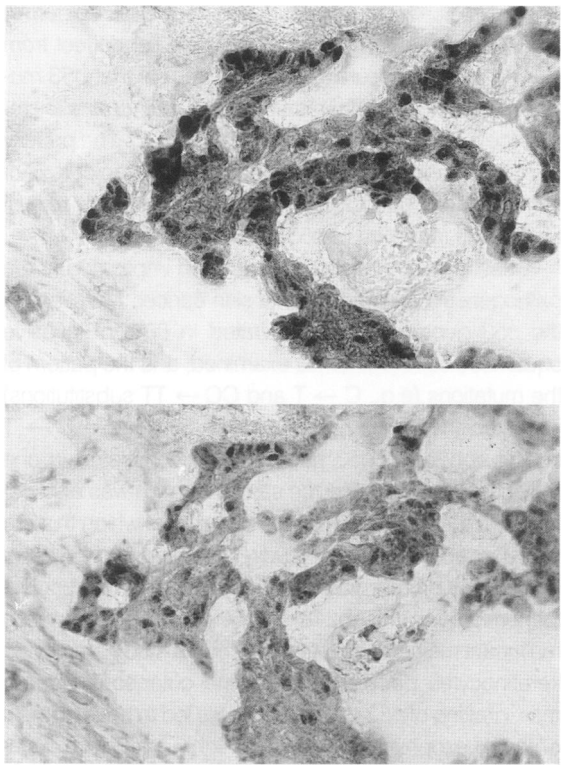

Figure 1. *Parenchymal-cell nuclei of an infiltrating BCC are immunoreactive with the antibody PAb1801, which recognizes both wild-type and mutant p53* **(top)**. *In an adjacent section of the same specimen, the BCC is immunoreactive with the antibody PAb240, which specifically recognizes mutant forms of p53 protein* **(bottom)**. *Note colocalization of immunoreactivity with both MAbs in similar regions of tumor. (Diaminobenzidine and eosin; ×480)*

chemical results obtained with PAb240 and PAb1801 were concordant in 69% of BCCs studied. The extent of immunoreactivity with either MAb was not related to the age or sex of the patient or to the histologic type of the BCC.

Of the 39 BCC excision specimens that contained sufficient epidermis for evaluation, 37 (95%) exhibited focally positive immunoreactivity of the nuclei of epidermal keratinocytes with PAb1801. The pattern of reactivity in some specimens was restricted to scattered, isolated keratinocytes located predominantly in the basal layer, and in others involved discrete clusters of keratinocytes, sometimes sparing the intervening appendages in the pattern of an actinic keratosis. Immunoreactivity of epidermal keratinocytes with PAb240 was positive in 16 (42%) of 38 specimens tested. Serial sections typically demonstrated colocalized immunoreactivity of particular epidermal regions with both MAbs (Figure 2A,B).

Of the 14 specimens of sun-protected buttock skin, 13 (92%) were negative for epidermal p53 immunoreactivity with PAb1801 and PAb240 (with each MAb, a single, positively stained keratinocyte was present in 1 specimen). The prevalence of epidermal p53 immunoreactivity was significantly decreased in nonlesional buttock skin compared with chronically sun-exposed, perilesional skin from the tumor specimens ($P < 0.01$ and $P < 0.05$ for PAb1801 and PAb240, respectively, by the chi-square test).

Discussion

The molecular pathogenesis of human cutaneous BCC is incompletely understood. Aneuploidy occurs in a few cases of BCC [28]; cytogenetic abnormalities were found in 8 of 33 cases.[29] Mutational activation of the K-*ras* and H-*ras* proto-oncogenes was shown in 4 of 30 cases of BCC.[30] In another study, the pattern of expression of the c-*fos*, c-*myc*, H-*ras*, and N-*ras* proto-oncogenes in one BCC was similar to that of normal skin.[31] Activated oncogenes were not detected in recipient fibroblasts transfected with DNA from BCCs.[32,33] Thus to date, no consistent molecular-genetic alterations that might explain the development of most BCCs have been demonstrated.

In the present study, a majority of the BCCs was shown to overexpress p53 protein. The positive immunoreactivity of BCCs with PAb240, a MAb with high speci-

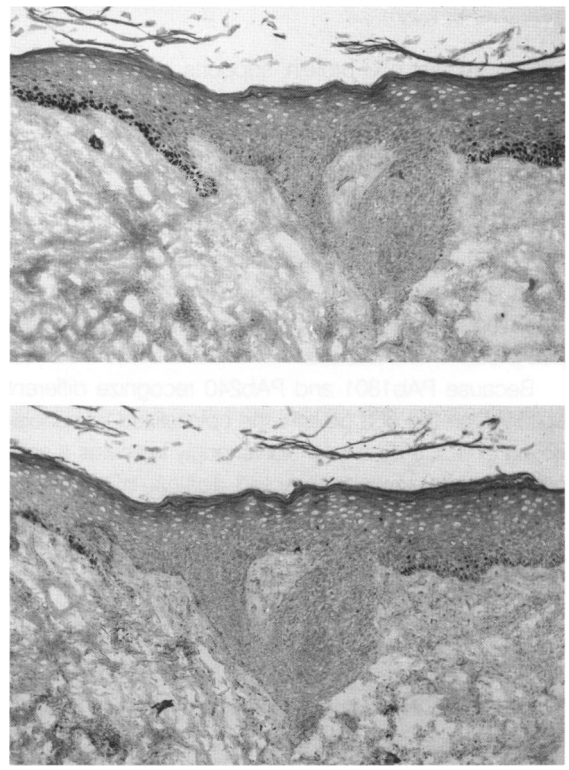

Figure 2. *Nuclei of epidermal keratinocytes located <1 cm from BCC are focally immunoreactive with PAb1801* **(top)** *and PAb240* **(bottom)**, *in a colocalized pattern as demonstrated in these adjacent sections. Note absent immunoreactivity of intervening keratinocytes of the follicular epithelium. (Diaminobenzidine and eosin; ×120)*

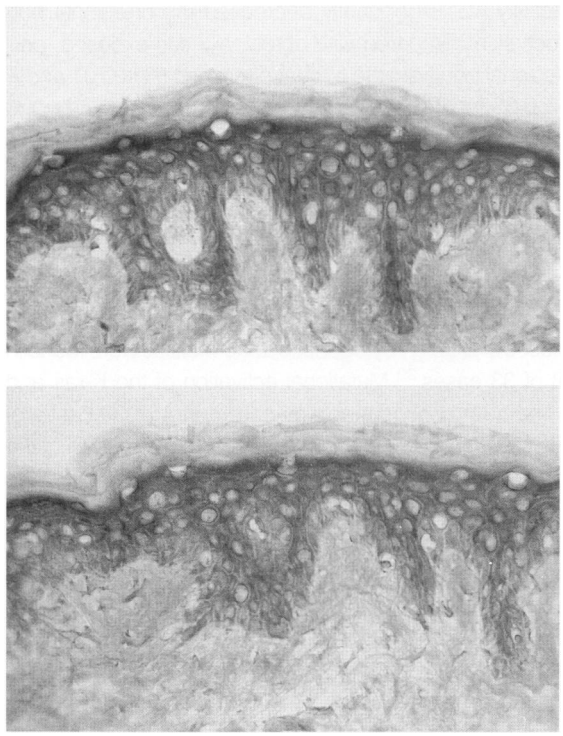

Figure 3. *Nuclei of epidermal keratinocytes from chronically sun-protected, nonlesional buttock skin are nonreactive with PAb1801* **(top)** *and PAb240* **(bottom).** *(Diamonibenzidine and hematoxylin and eosin; ×480)*

ficity for mutant p53 protein, supports the interpretation that the *p*53 gene is frequently mutated in BCC. Positive nuclear immunoreactivity was observed more often with PAb1801 than with PAb240, both in BCCs and in perilesional epidermis. This finding may reflect overexpression in some specimens of a form of mutant p53 protein reactive with PAb1801 but not with PAb240; alternatively, some specimens may express an increased amount of stabilized, wt p53 protein, perhaps complexed to mutant p53 protein in the nucleus.[34]

Because PAb1801 and PAb240 recognize different epitopes on the p53 protein, the colocalized immunolabeling of BCCs by both MAbs strongly indicates a specific immunoreactivity directed against p53 protein, rather than fortuitous crossreactivity to an irrelevant antigen. The true prevalence of p53 immunoreactivity with both MAbs may in fact be even higher than indicated in Table 1; for accurate quantification, only strong, discrete, nuclear staining was counted as positive. A perinuclear or diffuse, cytoplasmic pattern of immunoreactivity with both MAbs, with or without nuclear labeling, was present in some specimens but was not counted. Such cytoplasmic reactivity may be biologically significant; however, mutant (unlike wt) p53 protein does localize in the cytoplasm under certain circumstances,[35] e.g., when complexed with the heat-shock stress protein hsp70.[36]

The presence of focally immunoreactive keratinocytes in the chronically sun-exposed, non-neoplastic epidermis adjacent to BCCs suggests a possible field effect from chronic UVR exposure,[29] and also implies that *p*53 mutation may occur rather early in the pathogenetic sequence eventuating in the formation of BCC. The paucity of p53-immunoreactive keratinocytes in the epidermis of sun-protected buttock skin further supports the role of UVR in the induction of p53 overexpression.

Recently *p*53 mutations have been implicated in the pathogenesis of other types of skin cancer. Mutations in the *p*53 gene were demonstrated in 58% of invasive squamous cell carcinomas examined, and the pattern of the mutations (e.g., C → T and CC → TT substitutions) indicated their induction by UVR.[37] Similarly, a C → T substitution in the *p*53 gene was demonstrated in one melanoma cell line, and the same mutation was also detected in the original tumor specimen from which this line was derived.[38] In another study, increased expression of mutant p53 protein was reportedly detected immunohistochemically with PAb240 in 85% of human cutaneous malignant melanomas examined.[39] In a study employing keratinocytes derived from murine cutaneous papillomas, grafting of p53-transfected cells led to development of large, exophytic tumors with highly dysplastic histologic features.[40] Taken together, these findings suggest that *p*53 mutations may be important in the pathogenesis of many types of cutaneous tumors, particularly those related to chronic UVR exposure.[37]

In summary, we have shown overexpression of p53 protein in a majority of human cutaneous BCCs examined. This expression was confirmed by the use of two different anti-p53 MAbs, one of which is highly specific for mutant p53 protein. Perilesional, chronically sun-exposed epidermal keratinocytes also focally overexpressed p53 protein, but epidermis of buttock skin chronically protected from UVR did not. These findings indicate that mutation of the tumor suppressor gene *p*53, as a result of chronic UVR exposure, is likely to play an important role in the pathogenesis of BCC of human skin.

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