Expression of p53 in the evolution of squamous cell carcinoma: Correlation with the histology of the lesion

Aviv Barzilai, MD, MSc,[a,b,c] Anna Lyakhovitsky, MD,[a,b] Henri Trau, MD,[a,b] Mina Fogel, PhD,[c,d] and Monika Huszar, MD[c,e]
Tel-Hashomer and Rehovot, Israel

Background: The evolution of squamous cell carcinoma (SCC) on sun-exposed areas is a multistep process triggered by ultraviolet radiation (UVR), in which precursor lesions exist. However, the exact classification of the various lesions in this process, mainly solar keratosis (SK), is still disputed, and its pathogenesis requires further clarification.

Objective: To further elucidate the evolution of SCC on sun-damaged skin by correlating the levels of p53 protein expression, a parameter that reflects UVR damage to cells, and the morphology of the lesions that develop on sun-exposed areas.

Methods: Biopsy specimens from normal skin (n = 4), normal skin with various degrees of solar elastosis (SE) (n = 16), various degrees of SK (n = 17) and SCCs from sun-exposed (n = 12) and sun-protected (n = 7) areas were stained with anti-p53 antibodies. A semiquantitative evaluation of the degree of staining was performed and correlated with the histological features.

Results: Nuclear staining in keratinocytes was observed already in normal skin with mild SE and was increased gradually to its highest level of expression in advanced SK. It was also expressed in SCCs, but to a lesser degree. Statistical analysis revealed association between the morphology of the lesion and the level of p53 expression ($P < .01$); it also showed that in general the level of p53 is correlated with the histology of the lesion ($P < .001$). Furthermore, with regard to p53 expression, two groups of lesions exist: one showing a low level of expression of p53 that includes normal skin, skin with various degrees of SE and SCC from sun-protected areas, and a second group showing a high level of expression that includes SK and SCC occurring on sun-damaged skin.

Limitation: This is an immunohistochemical study of relatively few cases and in which the antibody detects all types of p53 protein.

Conclusions: This study furnishes further evidence that the development of SCC on sun-damaged skin is a gradual process not only morphologically but also on the molecular level. The process starts already in normal-appearing epidermis with SE. In that respect, SK should be regarded as a part of the continuum in the development of SCC, analogous to the situation in other epithelia. The molecular events involved in the development of SCC on sun-exposed areas may be different from those involving the development of SCC on sun-protected areas. (J Am Acad Dermatol 2007;57:669-76.)

The development of cutaneous squamous cell carcinoma (SCC) is strongly associated with sun exposure.[1] Furthermore, both from the clinical and histological aspects, precursors or low-degree lesions exist: solar or actinic keratosis (SK) are referred by some as a premalignant lesion and by

From the Department of Dermatology, Sheba Medical Center, Tel-Hashomer[a]; Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv[b]; Department of Pathology, Kaplan Medical Center, Rehovot[c]; the Faculty of Health Sciences, HaNegev University, Beer Sheba[d]; and the Faculty of Medicine, The Hebrew University, Jerusalem.[e]

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Reprint requests: Aviv Barzilai, MD, MSc, Department of Dermatology, The Chaim Sheba Medical Center, 52621 Tel-Hashomer, Israel. E-mail: avivb@post.tau.ac.il.

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Abbreviations used:

BD: Bowen's disease
SCC: squamous cell carcinoma
SE: solar elastosis
SK: solar (actinic) keratosis
UVR: ultraviolet radiation

others as being one type of SCC.[1,2] Sun-damaged skin also has typical clinical and histological characteristics, the latter being mainly solar elastosis (SE).

P53 is a tumor suppressor gene, the mutation of which has been involved in the genesis of various cancers, including skin cancer.[3,4] It has been shown that mutant p53 accumulates in the cell nucleus, probably due to increased half-life of the protein.[2,5] Thus, overexpression of p53 in solar keratosis, Bowen's disease (BD), and cutaneous SCC has been reported in many studies.[6-22] Furthermore, expansion of p53 in the epidermis correlates with sun exposure and sun damage, even in the absence of premalignant changes.[23-26]

In the present study we assessed the expression of p53 in normal skin, in skin with chronic sun damage, in various degrees of SK and in SCCs and correlated it with the morphology of the lesions.

MATERIAL AND METHODS

Cases studied

Cases showing normal skin, solar elastosis only, SK, and cutaneous SCC were randomly retrieved from the archives of the Institute of Pathology at the Kaplan Medical Center. The original hematoxylin-eosin slides were reviewed. Cases were selected as detailed below.

The first group examined included 16 specimens of skin with SE. These were graded according to their morphologic appearance into categories 1, 2, and 3: (1) slight increase in the size of elastic fibers; (2) aggregates of coarse, twisted bands of elastic fibers in the upper dermis; (3) amorphous basophilic deposits extending to the upper reticular dermis.

The second group included 17 cases of SK. To semiquantify the morphologic changes, these were classified into two groups: (1) atypical keratinocytes situated mostly in the lower half of the epidermis and (2) atypical keratinocytes present in more than half of the thickness of the epidermis. Thus this latter group also included lesions that would be designated as SCC in situ.

The third group consisted of 19 cases of invasive SCC of the skin with varying degrees of differentiation. Twelve cases from chronically sun-exposed skin sites and 7 cases from non-sun-exposed skin sites were included. The tumors were graded as well differentiated, moderately differentiated, and poorly differentiated according to the extent of keratinization and nuclear pleomorphism.

Four specimens of normal skin (margins of excision of benign skin lesions) taken from patients matched in age with those of the study group, served as controls.

Tissues

Sections, 4 μm in thickness, from the paraffin-embedded formalin fixed blocks were used for immunohistochemical studies.

Immunohistochemistry

Deparaffinized and rehydrated tissue sections were microwaved in 10 mmol/L citrate buffer (pH 6) for 10 minutes in a 1300-W microwave oven. After endogenous peroxidase blocking and incubation in normal goat serum (Vector Laboratories, Burlingame, Calif) for 20 minutes, sections were incubated at 4°C overnight with monoclonal anti p53 antibodies (clone BP53.12, Isotype: IgG2a, kappa, Zymed Laboratories, Inc, So San Francisco, Calif), followed by staining with the avidin-biotin-peroxidase method[27] (Vectastain Elite, Vector Laboratories, Burlingame, Calif). The reaction product were visualized with amino-ethylcarbazole (Zymed Laboratories, Inc), counterstained with Mayer's hematoxylin and mounted. Negative controls underwent a similar procedure, with exclusion of the primary antibody.

Evaluation of staining

Two independent observers (A. B, A. L.), who had no knowledge of initial diagnosis, scored the immunostained slides using a standard light microscope. In cases where scores differed, a third observer (M. H.) scored the sections and the majority decision was adopted.

Positivity of the staining was indicated by nuclear staining. Two parameters were assessed: the intensity of the nuclear staining and the percentage of immunopositive cells.

The intensity of the staining in the majority of stained cells was classified into 4 grades: (1) no staining—no expression of p53; (2) faint bronze nuclear staining—low level of expression; (3) bronze nuclear staining—moderate level of expression; (4) dark bronze to brown nuclear staining—high level of expression. In every evaluation, a comparison with a reference slide for grades 2-4 was performed.

All positively nuclear-stained keratinocytes in 5 randomly chosen, high-power fields (400×) were counted. The percentage of positively nuclear stained keratinocytes from the total count of