Smooth Muscle-associated Antigen in Experimental Cutaneous Squamous Cell Carcinoma, Keratoacanthoma, and Papilloma

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SUMMARY

Cryostat sections of 19 squamous cell carcinomas, 13 keratoacanthomas, and 12 papillomas, induced by 7,12-dimethylbenz(a)anthracene in the skin of rabbits and rats were examined by indirect immunofluorescence with human serum containing antibody to smooth muscle. Linear or granular staining of the cell outlines of the basal squamous cell layers was seen most extensively in the carcinomas, less in keratoacanthomas, and least in papillomas. In addition, squamous cell carcinomas showed this pattern of staining at advancing tumor margins and in invasive cords and tumor cell nests in the dermis. Four keratoacanthomas also showed prominent staining of the basement membrane area. The specificity of the staining reaction was established by its prevention on neutralization absorptions of the serum with extracts or homogenates of smooth muscle. The epidermal cells of normal rabbit and rat skin gave negative staining reactions. The presence of smooth muscle-associated antigen probably corresponds to cellular microfilaments.

INTRODUCTION

SMA has been found in the sera of some patients with malignant melanoma, ovarian carcinoma, lymphoma, leukemia, hepatocellular carcinoma, and cervical carcinoma. Sera containing SMA have also been used to demonstrate the presence of smooth muscle-like antigen in some tumor cell lines, i.e., Epstein-Barr virus-transformed lymphoblastoid cells and HeLa cells. Ultrastructurally, actin-like microfilaments have been demonstrated in HeLa cells, Ehrlich ascites tumor cells, and epidermal cancer cells, and it is probable that SMA sera react with these microfilamentous structures. This study was initiated to determine the presence of smooth muscle-like antigen in chemically induced experimental skin tumors of different invasive potential, squamous cell carcinoma, keratoacanthoma, and squamous papilloma.

MATERIALS AND METHODS

Animals and Tumor Induction. Skin tumors were induced in albino rabbits and DA Agouti rats by painting the skin with 1% 7,12-dimethylbenz(a)anthracene for 5 months as previously described (4, 10); the skin lesions were excised and bisected 2 to 8 weeks after their 1st appearance, usually about 9 to 18 weeks. One half was fixed in 10% phosphate-buffered formalin, and paraffin-embedded sections stained with hematoxylin and eosin were examined for histological diagnosis. The other half was snap-frozen in isopentane-liquid nitrogen at −70° for immunofluorescence tests.

Forty-four skin tumors were examined for their reactivity with SMA sera. These comprised 32 rabbit (15 squamous cell carcinomas, 8 papillomas, and 9 keratoacanthomas) and 12 rat lesions comprising 4 each of squamous cell carcinomas, papillomas, and keratoacanthomas. The histological diagnostic criteria for the various cutaneous lesions were as previously described (10). As controls, comparable areas of normal skin from albino rabbits and DA Agouti rats were similarly examined.

SMA Sera. Seven SMA sera were examined for their reactivity with representative examples of the above cutaneous lesions. All showed a similar pattern of staining but only 1 of these sera, from a patient with active chronic hepatitis (5, 6, 18), was used in the main test series. This serum had a SMA titer of 1:256 when assessed by indirect immunofluorescence on rat and rabbit stomach; it also stained rat renal glomeruli and rabbit liver parenchymal cells in a polygonal pattern (2, 3, 5, 6, 18).

Immunohistology. Standard “sandwich” immunofluorescence tests were performed as described by Naimn (12), using 6-μm cryostat sections stained with SMA serum. Parallel control sections were treated with phosphate-buffered saline (0.145 M NaCl, 0.01 M sodium phosphate, pH 7.1) or normal human serum. All sera were used at a dilution of 1:8. The conjugate for immunofluorescent tracing of any bound immunoglobulin was a fluorescein isothiocyanate-labeled goat anti-human γ-globulin with a fluorescein:protein molar ratio of 4.0 and a protein content of 0.8 g/100 ml. Before use, it was absorbed with homogenates of rat liver, kidney, and gastrointestinal tract and smooth muscle of pig stomach, so that by itself it gave no staining reaction on test sections of the skin or tumors.

After immunofluorescent staining, the microscopic preparations were examined by dark-ground UV fluorescent microscopy using a condenser fitted with a toric lens beneath and a colorless barrier filter.

Specificity of the tests was established by failure to obtain staining with normal control serum or by SMA serum neutralized by absorption with extracts or homogenates of smooth muscle from pig stomach (1).

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2 The abbreviation used is: SMA, smooth muscle antibody.

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RESULTS

Epidermal squamous cells of normal rabbit or rat skin showed no staining with SMA serum; fluorescence staining was seen only in the walls of blood vessels, muscle around hair follicles, and the erector pili muscle (Fig. 1).

The 12 papillomas gave a predominantly negative-staining reaction with SMA serum (Fig. 2). Only occasional single cells or focal groups of squamous cells showed a finely granular or linear fluorescence of part or the whole of the cell outline (Fig. 3). These cells were mostly located in the basal layers of the epidermis. Staining was absent in the more differentiated and mature squamous cells and cells undergoing keratinization.

The 13 keratoacanthomas gave a variable staining reaction with SMA serum, although 3 basic patterns were observed. The 1st, seen in 3 lesions, was much the same as in the papillomas, i.e., they were largely negative except for occasional cells in the basal layers showing spotty or linear staining of the cell outline. The 2nd pattern, seen in 6 lesions, was predominantly a fine linear staining of the cell outlines in most squamous cells of the basal region of the tumor (Fig. 4); staining was again weaker or absent in the more superficial squamous cells. The last pattern, observed in 4 cases, was a uniform band of staining of the epidermal basement membrane area; associated with this was staining of the cell outline of some adjacent epidermal cells (Fig. 5).

In 19 squamous cell carcinomas examined, 16 showed linear or granular staining of the cell outlines of the basal layers at the advancing tumor edge; in general, the cell outline staining was coarser than in the keratoacanthomas and papillomas. Much weaker staining was seen in cells above these layers, and staining was mostly absent in the more mature prickle cells (Fig. 6). Where invasive cords or groups of cells were found in the dermis, these showed strong reactivity with SMA serum (Fig. 7). In addition to the cell outline staining, some cells showed granular cytoplasmic fluorescence (Fig. 8). The staining of the advancing tumor edge or invasive cords was commonly associated with streaky, noncellular staining in the adjacent dermis (Fig. 8). In 3 other lesions the staining was confined to the outlines of occasional cells in the basal layers, but here the dermal, noncellular staining was especially marked.

In 3 representative rabbit lesions, 1 each of papilloma, keratoacanthoma, and squamous cell carcinoma, titrations with the SMA serum were carried out and these gave a titer of 1:256 for each lesion.

DISCUSSION

The results demonstrate that cutaneous epidermal tumors induced by 7,12-dimethylbenz(a)anthracene contain varying amounts of smooth muscle-associated antigen. This was most extensive in squamous cell carcinoma and less in keratoacanthoma; the antigen was present only in focal areas and occasional cells in squamous cell papillomas. The more mature and differentiated prickle cells contain little or no antigen. Gabbiani et al. (3) have observed a similar pattern of staining in healing skin wounds, using anti-smooth muscle sera. They demonstrated positive epidermal cell staining in the center of wounds, while the periphery, where epithelialization was complete, was negative. They suggested that the staining corresponds to a microfilamentous network in growing epidermal cells, which disappears when the cells establish mature junctional complexes with their neighbors.

Recently, microfilaments have also been observed in mouse epidermal cancer cells induced by 3,4-benzpyrene (9). These microfilaments were closely associated with the cell membrane and were abundant in the cells at the leading edge of invasive carcinomas; they were disrupted by cytochalasin B. We suggest that the epidermal cell staining observed in the present study corresponds to these microfilamentous structures.

The presence of smooth muscle-associated antigen is not a feature of cancer per se, inasmuch as it is also present in benign papillomas, keratoacanthomas, and healing wounds (3). However, the apparent quantitative increase of this antigen in squamous cell carcinomas and, to a lesser extent, in keratoacanthomas compared to papillomas; its localization to the advancing tumor edge; and its invariable presence in invasive cords and tumor cell nests suggest that it may be associated with local tumor invasiveness. The variable pattern of cell outline staining in keratoacanthomas may reflect the local growth activity of individual lesions.

The emergence of smooth muscle-associated antigen in cutaneous tumors stands in contrast to deletion of pemphigus and bullous pemphigoid antigens from the intercellular-cell membrane and basement membrane regions of the invading margins of rabbit and rat squamous cell carcinomas (10). It has previously been suggested that deletion of these antigens may also contribute to local tumor invasiveness (10, 11).

An alternative but not mutually exclusive explanation for the presence of smooth muscle-associated antigen in epidermal tumors is that the antigen reflects the varying capacity of benign and malignant tumor cells to undergo cell division. The presence of contractile rings composed of microfilaments in dividing cells (14) and the absence of the antigen in more differentiated cells lend support to this view.

An interesting observation in this study is the staining of the basement membrane area in some keratoacanthomas by smooth muscle antiserum. This parallels our previous observation that the basement membrane area of keratoacanthomas may be thickened when assessed by bullous pemphigoid sera, which specifically stain this area (10). The apparent condensation of the smooth muscle antigen at the tumor edge in some keratoacanthomas might limit its cellular extension (cf. Ref. 10). Its presence as a thickened band at the tumor margin also raises the intriguing possibility that it may be a secretory product of tumor cells. The dermal, streaky, acellular staining in squamous cell carcinomas might be explained on this basis.

It has yet to be demonstrated that smooth muscle-associated antigen is a general feature of neoplastic cells. However, the release of such antigenic structures following cell death or secretion could account for the production of SMA in some patients with cancer (8, 13, 15, 17).
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Smooth Muscle Antigen in Skin Tumors

Fig. 1. Immunofluorescent staining of erector pili muscle of normal rabbit skin by SMA. × 125.
Fig. 2. Absence of immunofluorescent staining of rabbit papilloma with SMA. × 315.
Fig. 3. Immunofluorescent staining of the cell outline of a focal nest of epidermal cells of rabbit papilloma by SMA. × 200.
Fig. 4. Linear immunofluorescent staining of the cell outline of the basal layers of epidermal cells of rabbit keratoacanthoma by SMA. × 200.
Smooth Muscle Antigen in Skin Tumors

Fig. 5. Uniform immunofluorescent staining of basement membrane area of rabbit keratoacanthoma by SMA. Some adjacent cells show positive staining of the cell outline. × 125.

Fig. 6. Immunofluorescent staining of the cell outline of epidermal cells of the advancing tumor edge of rabbit squamous cell carcinoma by SMA. × 315.

Fig. 7. Immunofluorescent staining of the cell outline of an invasive cord of rabbit squamous cell carcinoma by SMA. In part the staining is granular. × 315.

Fig. 8. Immunofluorescent staining of rabbit squamous cell carcinoma by SMA showing streaky, noncellular dermal staining and granular staining of epidermal cell outlines and cytoplasm. × 315.
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