Involucrin in Squamous and Basal Cell Carcinomas of the Skin: An Immunohistochemical Study

Jonathan W. Said, M.D., Aaron F. Sassoon, B.S., I. Peter Shintaku, Ph.D., and Susan Banks-Schlegel, Ph.D.

Involucrin is a precursor of the cross-linked envelope protein of human stratum corneum, and its appearance in the upper layers of the epidermis is a function of the normal differentiation of the keratinocyte. Cases of basal cell and squamous cell carcinoma were evaluated for the presence of involucrin using immunoperoxidase techniques on paraffin sections. Basal cell carcinomas were negative for involucrin with staining restricted to squamous horn cysts, while squamous cell carcinomas stained strongly, particularly in large keratinized cells. Cases of squamous cell carcinoma in situ (Bowen’s disease) revealed increased staining for involucrin with staining of dyskeratotic cells at all levels in the epithelium. Abnormal patterns of staining were also noted in non-neoplastic epidermis adjacent to carcinomas. Immunohistochemical staining for involucrin identifying abnormal or premature keratinization is a sensitive marker for dyskeratosis in squamous epithelia and may have applications in the histopathologic evaluation of skin specimens.

During the late stages of differentiation, human epidermal keratinocytes develop an insoluble protein envelope that contributes to the protection of the skin against the environment [1-4]. Involucrin, one of the soluble precursor proteins of this envelope isolated from cultured human epidermal keratinocytes [5], is found in the cytoplasm of the outer squamous cell layers of the epidermis and in the envelope of the stratum corneum [5,6]. A 36K soluble precursor named keratolinin has recently been isolated from the bovine snout and human epidermis, and does not cross-react with involucrin [7]. The cross-linking of these soluble envelope protein precursors to form insoluble polymers is catalyzed by transglutaminase in a calcium-dependent reaction [7,8]. Tissue culture studies have shown that squamous cell carcinoma lines produce involucrin but reveal aberrations in terminal differentiation and formation of corified envelopes [9]. Presence of involucrin in skin neoplasms may therefore be expected to serve as a marker for squamous differentiation, and alterations in involucrin synthesis might be anticipated in dysplastic or preneoplastic squamous epithelium. In this study immunoperoxidase techniques are used to localize involucrin in histologic sections of basal cell and squamous cell carcinomas in order to assess patterns of distribution in these neoplasms and the surrounding epidermis.

MATERIALS AND METHODS

Cases of skin tumors (24 squamous cell and 21 basal cell carcinomas) were retrieved from the files of the surgical pathology division of Cedars-Sinai Medical Center. In selected cases, sections were reviewed independently by a dermatopathologist not otherwise involved with the study (Dr. Theodore H. Kwan, Beth Israel Hospital, Boston, Massachusetts). Cases of basal cell carcinoma were subclassified according to conventional histologic criteria [10] into solid type (7 cases), superficial multifocal (3 cases), adenoid (3 cases), sclerosing (4 cases), keratotic (3 cases), and cystic (1 case). Cases of squamous cell carcinoma comprised 14 cases of squamous cell carcinoma in situ (Bowen’s disease) and 10 cases of infiltrating squamous cell carcinoma. Thirteen cases revealed evidence of sun damage in the surrounding epidermis with solar elastosis and/or solar keratosis.

Details of preparation and characterization of the anti-involucrin antiserum (kindly supplied by Dr. Howard Green) have been previously reported [5]. Briefly, a soluble envelope precursor protein was purified from cultures of human epidermal keratinocytes and antiserum was prepared in rabbits by injection of this purified protein dissolved in complete Freund’s adjuvant. Formalin-fixed paraffin-embedded blocks were cut and 4-μm sections were mounted on glue-coated slides for immunoperoxidase staining as previously described [11]. Parallel sections were evaluated with and without trypsin digestion (0.12 mg/ml trypsin at 37°C for 30 min) with similar results. Following hydration of paraffin sections, they were incubated for 15 min in a solution of methanolic peroxide to consume endogenous peroxidase activity. Sections were rinsed with phosphate-buffered saline (PBS) at pH 7.4 and incubated with swine serum to reduce background staining. The sections were then incubated sequentially with antibodies to involucrin produced in rabbits and diluted 1:1000, swine antirabbit serum protein, and horseradish peroxidase-rabbit-antihorseradish peroxidase soluble complexes (Dako Corporation, Santa Barbara, California). Antibody localization was effected by incubation with a solution of 6 mg 3,3’-diamino benzidine in 10 ml PBS to which 0.1 ml of hydrogen peroxide was added just before use. Sections were counterstained with methyl green and mounted in Permount. Immunofluorescence microscopy was performed on unfixed cryostat sections incubated sequentially with normal swine serum, primary antiserum, and fluorescein-conjugated swine antirabbit immunoglobulins. Sections were examined with a Zeiss microscope equipped with an epifluorescent condensor, primary (440-490 nm) and secondary (530 nm) filters, and a dichroic reflector (610 nm).

Similar to previous reports [5,12] when frozen sections of normal epidermis were stained with antiserum to involucrin, the envelope precursor was seen throughout the cell cytoplasm, but was also concentrated at the cell periphery of the outer spinous and granular cells, and appeared diffuse in the cells of the stratum corneum (data not shown). In formaldehyde-fixed sections, the outer spinous and granular cells still stained well but staining of the stratum corneum was suppressed, presumably because the formaldehyde fixation prevented penetration of the antiserum (Fig 1) [5,6,13]. While formaldehyde fixation also reduced the staining of the peripheral uncross-linked involucrin, it appeared to have little effect on the cytoplasmic staining (Fig 1), as previously noted [5,6]. Negative controls consisted of parallel sections with elimination of the primary antiserum and substitution with preimmune serum or anti-involucrin serum which had been adsorbed with purified envelopes. These controls were included for each case and revealed no staining.
RESULTS

Squamous Cell Carcinomas

Twenty-four cases of squamous cell carcinoma were evaluated (14 cases of invasive squamous cell carcinoma and 10 cases of carcinoma in situ). In 11 cases, normal skin was present on the sections away from the tumors and showed the normal pattern of localization of involucrin to the upper layers of the epidermis similar to results illustrated in Fig 1. Thirteen cases of infiltrating carcinoma showed diffuse cytoplasmic staining both in the intraepidermal and infiltrating component of the tumors regardless of extent of infiltration (Fig 2a). In unfixed frozen sections, staining was predominantly located at the cell periphery (Fig 2b). Staining was of greatest intensity in the center of squamous nests, particularly in larger keratinized cells (Fig 3). Staining was unaffected by minor degrees of inflammation, although in 2 cases staining was focally decreased in areas of extensive ulceration and necrosis. One case showed a variation in staining for involucrin in that the majority of the tumor was negative, with highly keratinized foci staining strongly.

Ten cases of squamous cell carcinoma in situ of the epidermis (Bowen's disease) were evaluated, and all showed an abnormal pattern of staining for involucrin. In 8 cases a diffuse pattern of staining for involucrin was observed in all cell layers of the neoplastic epidermis down to the basal layer. In 3 tumors, staining was focal and was restricted to nests of dyskeratotic cells at all levels within the neoplastic epidermis (Fig 4).

The epidermis immediately adjacent to but not involved by carcinoma was also evaluated in all 24 cases. In 2 cases we found a normal pattern of localization to the upper cell layers. However, in the remaining cases staining was abnormal, extending to the basal layer in 18 cases and two-thirds of the way to the base of the epidermis in 4 cases (Fig 5). These abnormal patterns of increased staining for involucrin were present in dyskeratotic and hyperplastic epidermis, but also in cases where the epidermis adjacent to the tumors appeared histologically normal.

Basal Cell Carcinomas

Twenty-one cases of basal cell carcinoma were evaluated. The tumors were negative for involucrin in all cases with the exception of squamous horn cysts which stained strongly for involucrin in 7 cases (Fig 6). In 4 cases individual keratinized cells also stained strongly. Unlike cases of squamous cell carcinoma, the skin adjacent to the basal cell carcinomas showed a normal pattern of staining. The skin immediately overlying the basal cell carcinomas, however, showed an abnormal pattern of staining in 17 cases with stain extending to the base in 15 cases and two-thirds of the thickness of the epidermis in 2 cases (Fig 7). These changes were particularly evident at sites of attachment between the basal cell carcinomas and overlying epidermis.
DISCUSSION

Involucrin is one of the soluble protein precursors of the cross-linked envelope found in normal stratum corneum [4-6]. Cross-linking of soluble precursor proteins including involucrin and keratinin [7,8] into insoluble polymers is catalyzed by epidermal transglutaminase in a calcium-dependent reaction. The appearance of mRNA for involucrin and synthesis of the protein is an orderly function of normal terminal maturation, which correlates with the size of keratinocytes and their level within the epidermis [5,6,14,15]. Alterations in involucrin synthesis have been demonstrated in squamous carcinoma cell lines [9] and with changes in the cell microenvironment [16,17].

In this study strong staining for involucrin was noted in squamous cell carcinomas of the skin. Staining for involucrin was most intense in the larger neoplastic cells, paralleling findings in cultured human epidermal keratinocytes which have demonstrated that accumulation of mRNA for involucrin and synthesis of the protein correlates with cell size [15]. Rheinwald and Beckett [9] demonstrated reduced cornified envelope formation in squamous cell carcinoma cell lines in culture, and suggested that this alteration might induce malignant transformation by evading mechanisms for limiting cell growth. Our results indicate that in human epidermal neoplasms inappropriate or premature terminal differentiation may occur, and that presence of involucrin in these lesions correlates with differentiation of neoplastic keratinocytes and presence of large keratins [18].

The presence of involucrin in squamous cell carcinomas of the skin is in accordance with the presence of numerous cells in these neoplasms which are strongly labeled with antisera to high-molecular-weight keratins [18,19]. Altered patterns of staining for involucrin in Bowen’s disease and squamous cell carcinoma in situ, with loss of polarity and increased staining of cells at all levels of the epidermis, suggest that the orderly sequence of terminal differentiation is defective in these epithelia. Similar intense staining for involucrin and high-molecular-weight keratins occurs in cases of squamous cell carcinoma in situ and Bowen’s disease, corresponding to the presence of numerous dyskeratotic cells.

The presence of increased staining for involucrin in squamous cell carcinoma of the skin differs from that described in cervical intraepithelial neoplasms, which demonstrate lack of staining for involucrin in comparison with normal epithelium [17]. These differences may be explained by biologic differences in these squamous epithelia. Squamous cell carcinoma of the cervix is predominantly characterized by undifferentiated malignant cells of basal type without surface maturation or keratinization [20], unlike the numerous dyskeratotic cells with prominent tonofilament bundles present in squamous cell carcinomas of the skin [10]. In our laboratory, although small and large cell anaplastic carcinomas of the cervix are negative for involucrin, large cell keratinizing squamous cell carcinomas in situ and infiltrating squamous cell carcinomas with keratinization stain strongly for involucrin (unpublished observations).

Basal cell carcinomas were strikingly different from squamous cell carcinomas in their patterns of staining for involucrin. These tumors were uniformly negative except for individual keratinized cells and squamous horn cysts. This result is expected since normal basal cells lack involucrin.

Of interest were the abnormal patterns of increased staining for involucrin in the lower spinous cell layers of the epidermis adjacent to squamous cell carcinomas and overlying basal cell carcinomas. These changes apparently represent staining of keratinocytes with faulty or premature keratinization, either as a reaction to the tumors or as a feature of the dysplastic or preneoplastic state. Deep epidermal staining for involucrin
Figure 6. Basal cell carcinoma showing absence of staining for involucrin except for squamous horn cysts (arrowheads). The overlying epidermis shows a normal pattern of staining for involucrin (methyl green counterstain, × 150).

Figure 7. Epidermis overlying basal cell carcinoma shows abnormal staining for involucrin (black) which extends to the junction with the underlying tumor. The basal cell carcinoma is unstained except for squamous horn cyst (arrowhead) (methyl green counterstain, × 225).

Involving all differentiated cell layers of the epidermis is not specific for neoplasia, and is also encountered in various benign epidermal hyperplasias including verruca and solar keratosis (unpublished observations).

In summary, this study demonstrates staining for involucrin in situ and infiltrating squamous cell carcinomas but not in basal cell carcinomas of the skin. Increased staining was also noted in dyskeratotic squamous epithelium and in epidermis adjacent to carcinomas. Immunohistochemical staining for involucrin represents a sensitive marker for abnormal or premature squamous maturation and an adjunct to the histopathologic evaluation of skin lesions.

The authors are grateful for the word processing skills of Mavis Thompson and Meyer Bekhore, and the photographic assistance of Kai Chien and Robert Heuser. We thank Dr. Theodore H. Kwan, Beth Israel Hospital, Boston, Massachusetts for review of the histology in selected cases.

REFERENCES