Immunohistochemical Localization of Basic Fibroblast Growth Factor in Skin Diseases

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The basic fibroblast growth factor (bFGF) is an angiogenic factor and also a mitogen for epidermal keratinocytes. In order to investigate the role of bFGF in human skin we examined the distribution of bFGF immunoreactivity in normal and diseased human skin. Antigen expression was demonstrated by direct immunofluorescence staining of cryostat sections with a polyclonal anti-bFGF antibody. In normal human skin, bFGF-like immunoreactivities were observed in the basal cells, while in the case of psoriasis, positive immunoreactivities were observed in the basal cells and several supra-basal layers at rete ridges. Seborrheic keratosis and basal cell epithelioma showed diffuse immunoreactivities in the basaloid cells of the tumor. Concurrently, benign nevus cell nevus, capillary hemangioma, squamous cell carcinoma and malignant melanoma displayed negative immunoreactivities. These results suggest that bFGF is important for basal or basaloid cell proliferation. ***Key words: Human skin; bFGF; Immunohistochemistry.***

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Fibroblast growth factors (FGF) are a family of polypeptides which have a high affinity for heparin (1). Basic FGF (bFGF) is a major member of the FGFs. It acts as an angiogeneic factor(2) and also as a mitogen for various types of mesoderm- and ectoderm-derived cells (3, 4). Although bFGF is easily purified from a variety of tissues including placenta, prostate, thymus, kidney, corpus luteum, adrenal gland, retina, brain, and pituitary gland (1–3), little is known about its physiologic role.

Psoriasis and skin tumors were chosen for the detection of bFGF since they have accelerated keratinocyte proliferation. We report here the distribution of bFGF in normal human skin and several skin diseases showing benign or malignant hyperproliferation.

MATERIAL AND METHODS

Antibodies

Polyclonal antibodies were raised against recombinant human bFGF (Synergen Inc., Boulder, CO) in rabbits, and IgG fractions were prepared by protein A sepharose column chromatography as previously described (5).

The antibody did not show any cross-reactivity with acidic FGF by enzyme-linked immunosorbent assay (ELISA) or with hst/K-FGF. The flow-through fractions of the antibodies were prepared by repeatedly applying anti-bFGF IgG fractions onto bFGF-coupled agarose beads. They did not demonstrate any cross-reactivity with bFGF by ELISA and were utilized in the control studies to show the specificity of staining. Polyclonal antibodies and their flow-through fractions were generously donated by Dr. D. B. Rifkin (NYU Medical Center, NY). Fluorescein-conjugated goat anti-rabbit IgG was purchased from CAPPEL Research Products (Durham, USA).

Samples

Normal human skin (10 cases) was obtained from plastic surgery. Skin biopsy specimens were obtained from patients with psoriasis vulgaris (involved skin of 6 untreated cases), seborrheic keratosis (5 cases), basal cell epithelioma (4 cases), benign nevus (5 cases), hemangioma (granuloma pyogenicum, 4 cases), squamous cell carcinoma (6 cases) and malignant melanoma (5 cases).

Immunohistology

Sample specimens were embedded in tissue compound (Miles Inc., USA), snap–frozen in liquid nitrogen and then stored at -80°C. Four μm cryostat sections were air-dried, fixed in cold acetone; and preincubated in phosphate-buffered saline containing 2% normal goat serum and 1% bovine serum albumin (2%NGS-1%BSA-PBS) for 15 min at room temperature. The sections were then incubated overnight at 4°C with anti-bFGF antibodies diluted in 2%NGS-1%BSA-PBS (1 μg/ml). After having been washed in PBS, the sections were incubated with FITC-conjugated goat anti-rabbit IgG for 1 h at 37°C. The sections were then washed in PBS and mounted in glycerin-PBS. The same sections were later stained with hematoxylin-eosin to confirm the histological structures of the tissues.

RESULTS

A summary of our results is given in Table I. In normal human skin, we observed bFGF-like immunoreactivities in the basal cells, including staining of the hair bulbs and sweat gland ducts (Fig. 1). However, the upper part of the spinous layer and the granular layer was not stained with this antibody though nonspecific binding was observed in the horny layer in some regions. The cellular distribution of bFGF in keratinocytes could not be clearly resolved, although it appeared to be cytoplasmic. No bFGF immunoreactivity was seen in the ex-

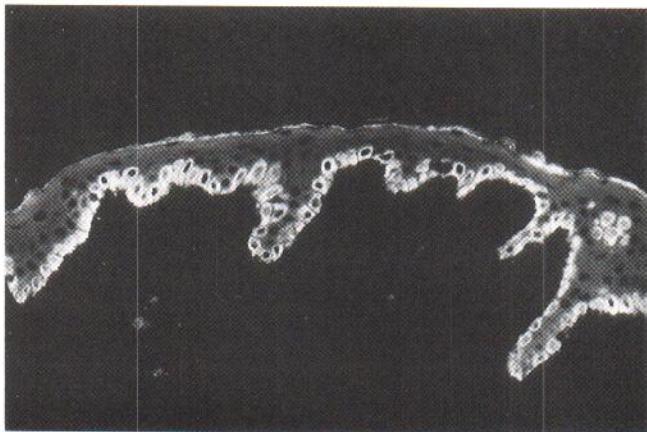

Fig. 1. Immunofluorescent staining of normal skin by bFGF antibody. Positive stainings were observed in the basal cells, and at two or three layers of keratinocytes from the basal cells in some regions (× 200).

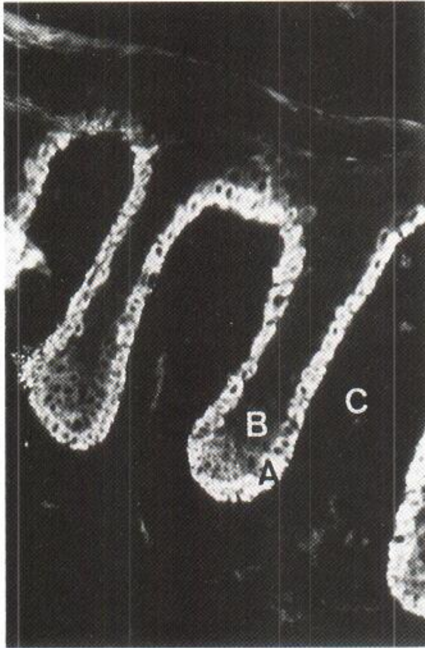

Fig. 2. Immunofluorescent staining sections of psoriasis. Positive stainings were observed at the basal cell layer (*A*) and several supra-basal layers of keratinocytes (*B*) at rete ridges. *C*: Papillary dermis (× 400).

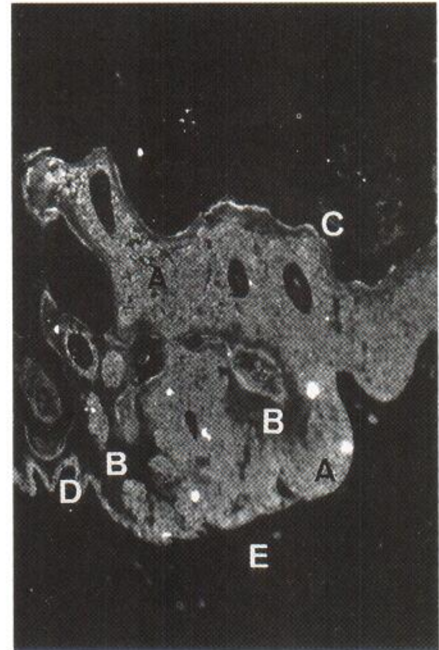

Fig. 3. Immunofluorescent staining sections of acanthotic type seborrheic keratosis. *A*: basaloid cells, *B*: squamous layer, *C*: corneal layer, *D*: basal layer, of adjacent normal skin, *E*: dermis. Positive stainings were observed in the basaloid cells (× 100).

tracellular spaces. The specificity of these immunoreactivities for bFGF was confirmed using anti-bFGF IgG chromatography. Dermal elements, including capillary endothelial cells, fibroblasts and matrices, did not demonstrate any immunoreactivity.

Psoriatic skin showed positive immunoreactivities in the basal cells (Fig. 2A) and a faint staining in the supra-basal layers of keratinocytes at rete ridges (Fig. 2B). Neither infiltrated cells nor capillary endothelial cells in the upper dermis were stained (Fig. 2C). These findings were consistent in all the tested sections taken from untreated psoriatic patients.

Subsequently, we examined the localization of bFGF in skin tumors. Seborrheic keratosis of the acanthotic type, a benign epithelial tumor, showed diffuse immunoreactivities in basaloid cells (Fig. 3A), although differentiated keratinocytes and horny layers (Fig. 3B, C) were not stained. Adjacent skin had positive immunoreactivity only in the basal cells (Fig. 3D). In the section of basal cell epithelioma (Fig. 4), strong immunofluorescence was observed in all the basaloid tumor cells, although adjacent matrices did not show any immunoreactivity. Differentiated squamous cell carcinoma and capillary hemangioma did not show immunofluorescence (results not shown). Normal melanocytes in epidermis, nevus cells and malignant melanoma did not show immunoreactivities (results not shown).

Table I. *Distribution of bFGF in normal and diseased skin*

Tissues cells	No. of biopsies	bFGF positive cases	bFGF positive cells
Normal skin	10	10	Basal cells
Psoriasis	6	6	Basal and supra-basal cells
Seborrheic keratosis	5	5	Basaloid cells
Basal cell epithelioma	4	4	Basaloid cells
Squamous cell carcinoma	6	6	No staining
Capillary hemangioma	4	0	No staining
Nevus cell nevus	5	0	No staining
Malignant melanoma	5	0	No staining

DISCUSSION

A variety of growth factors may be responsible for the regulation of cell proliferation by forming a network. Basic FGF is known as one of the major growth factors for various types of cells, including keratinocytes (3,4, 6). Many biochemical and immunohistochemical studies have revealed autocrine regu-

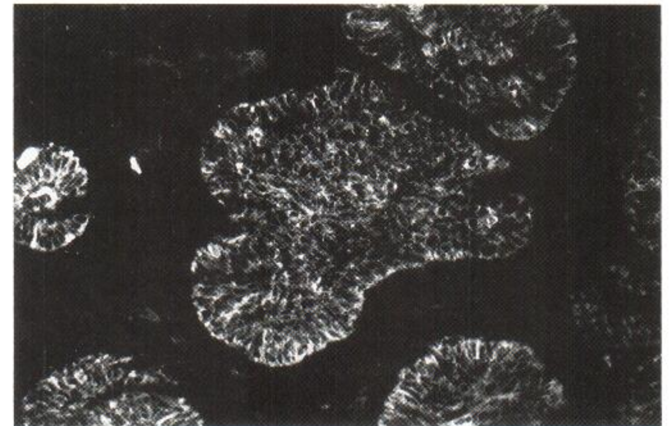

Fig. 4. Immunofluorescent staining sections of basal cell epithelioma. Positive stainings were observed in all the basaloid cells (× 400).

lation of cell growth by bFGF. However, most immunohistochemical studies on the localization of bFGF have been confined to embryonal organs, brain tumors and angiogenic tumors (5, 7–10). In this study, we have elucidated the localization of bFGF in normal human skin and several skin diseases using specific antibodies. This study provides evidence that only basal and basaloid cells have bFGF-like immunoreactivity in human skin. These cells may serve as storage sites of bFGF for differentiating keratinocytes and melanocytes. In this context it is interesting to note that the psoriatic lesion, which is characterized by accelerated proliferation and turnover of keratinocytes, contained higher levels of bFGF at the supra-basal layer of keratinocytes than their normal counterparts. The results from three selected epidermal neoplasmas, seborrheic keratosis, basal cell epithelioma and squamous cell carcinoma also confirmed that basaloid cells had bFGF-like immunoreactivity independent of their malignancy.

In reviewing previous studies on the localization of bFGF in various organs (5, 7–15), Sorg et al. demonstrated positive immunoreactivity in normal basal keratinocytes using independently raised polyclonal antibodies against intact human bFGF (16–18). Our results support their findings, although no antibody could detect bFGF-like immunoreactivity in endothelial cells. Nevertheless, in some studies, such immunoreactivity was detected using monoclonal antibodies against bFGF (8, 13–15). It is apparent from our study that basal and basaloid cells have endogenous bFGF. However, it remains to be shown whether endogenous bFGF works as an autocrine growth stimulant under physiologically normal conditions and in some skin diseases.

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