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Basic Fibroblast Growth Factor: A Missing Link between Collagen VII, Increased Collagenase, and Squamous Cell Carcinoma in Recessive Dystrophic Epidermolysis Bullosa

Jack L. Arbiser,[1,2] Jo-David Fine,[3] Dedee Murrell,[4] Amy Paller,[5] Susan Connors,[2] Karen Keough,[2] Elizabeth Marsh,[6] and Judah Folkman[2]

Departments of [1]Dermatology, Harvard Medical School, and [2]Surgery, Childrens Hospital and Harvard Medical School, Boston, Massachusetts, U.S.A.
[3]Department of Dermatology, University of North Carolina, Chapel Hill, National Epidermolysis Bullosa Registry, Chapel Hill, North Carolina, U.S.A.
[4]Department of Dermatology and St. George Hospital, University of New South Wales, Sydney, New South Wales, Australia
[5]Departments of Pediatrics and Dermatology, Northwestern University, Chicago, Illinois, U.S.A.
[6]Department of Dermatology, Cornell University Medical Center, New York, New York, U.S.A.

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Abstract

Background: Patients with recessive dystrophic epidermolysis bullosa (RDEB) have deficiencies of collagen type VII and have elevated levels of fibroblast collagenase, and a greatly increased risk of cutaneous squamous cell carcinoma. Patients with other genetic blistering disorders do not have elevated collagenase or an increased risk of squamous cell carcinoma, despite chronic wounding. The connection between collagen type VII deficiency, increased collagenase, and squamous cell carcinoma is not understood.

Materials and Methods: Urine from 81 patients with RDEB (39 patients), junctional epidermolysis bullosa (JEB; 12 patients), and epidermolysis bullosa simplex (EBS; 30 patients), as well as unaffected family members of RDEB patients (33 patients), was tested for the presence of basic fibroblast growth factor (bFGF) using a sensitive radioimmunoassay. These patients included many who were enrolled in the Epidermolysis Bullosa Registry and others who were referred by their physicians.

Results: Fifty-one percent of patients with RDEB had elevated levels (>5000 pg/g) of urinary bFGF. In contrast, none of the patients with JEB had elevated levels of bFGF. Twenty-one percent of clinically unaffected family members had elevated levels of bFGF, and 13% of patients with EBS had elevated levels of bFGF. The frequency of elevated bFGF values among all groups was statistically significant ($p = 0.002$), and the levels of bFGF in RDEB patients were significantly elevated compared with those of other groups ($p < 0.05$).

Conclusions: We have found that patients with RDEB have elevated levels of bFGF, which may contribute to increased fibroblast collagenase and the development of squamous cell carcinoma. These results suggest a novel treatment for RDEB, namely, angiogenesis inhibitors, which may antagonize the effects of bFGF in this disorder. There are currently no other means of treatment for this disorder, which has a high morbidity and mortality rate.

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is usually a severe and often fatal genetic disease characterized by mechanically fragile skin and subepidermal blistering (1–4). Patients with RDEB have elevated levels of fibroblast-derived collagenase and a high incidence of squamous cell carcinoma, neither of which is observed in the two other major epidermolysis bullosa subtypes, junctional epidermolysis bullosa (JEB) and epidermolysis bullosa simplex (EBS) (5,6). Clinically, RDEB is characterized by a high degree of morbidity from scarring, chronic erosions, chronic severe pruritus, fusion of fingers, esophageal stenosis, recurrent infection, and squamous cell carcinoma (1–4). Patients with all major subtypes of RDEB have greatly diminished levels of collagen type VII. This is a component of anchoring fibrils, which are structures that attach the epidermis to the dermis (7–10). We found that a patient with severe RDEB and bilateral above-the-elbow amputations for recurrent squamous cell carcinoma had elevated levels of urinary basic fibroblast growth factor (bFGF). To assess the role of bFGF in this genetic bullous disease, urine samples from patients with RDEB, JEB, EBS, and unaffected family members were identically screened.

Materials and Methods

Urine samples from patients with RDEB ($n = 39$) and their parents and unaffected siblings ($n = 33$) were analyzed for the presence of bFGF. As potential controls, urine from patients with JEB ($n = 12$) and EBS ($n = 30$) was similarly analyzed. Samples were refrigerated at 4°C during transit and stored at −80°C prior to bFGF radioimmunoassay (11).

A 20-ml aliquot of thawed urine was centrifuged at 2300 rpm for 8 min at 4°C. The supernatant was filtered using a filter with a pore size of 1.2 μm. The sample was then analyzed using a radioimmunoassay for bFGF according to the manufacturer's instructions (Human bFGF, Quantikine HS, R+D Systems, Minneapolis, MN).

We used the Kruskal-Wallis test to determine overall differences in the levels of bFGF among EB groups, and Duncan's Multiple Range Test on ranked bFGF values to identify specific differences.

Immunohistochemical Staining

Sections from a bullae from a patient with RDEB were deparaffinized and stained with a rabbit polyclonal antibody against von Willebrand factor (Dako, Carpentaria, CA), at a dilution of 1/100. A secondary mouse anti-rabbit IgG antibody conjugated to diaminobenzidine was incubated at a concentration of 1/50, and the slide was stained according to the manufacturer's directions.

Results

Over 50% of patients with RDEB were found to have levels of urinary bFGF greater than 5000 pg/gram of urine. Normal values of urinary bFGF are less than 5000 pg/g (11). In contrast, none of the patients with JEB had urinary bFGF values greater than 5000 pg/g of urine. Seven out of thirty-three unaffected family members of RDEB had urinary bFGF above 5000 pg/g (21%), and 4 out of 30 patients with epidermolysis bullosa simplex had urinary bFGF levels above 5000 pg/g (13%). The Kruskall-Wallis test showed that there was statistical significance between groups, with one group being significantly different from all other groups ($p = 0.002$). The Duncan's Multiple Range test showed that bFGF values in RDEB significantly differed from those of the other three groups ($p < 0.05$) (Fig. 1, Table 1). In contrast, values from non-RDEB sources were not significantly different from each other. No correlation was seen between the extent of body surface involvement and the presence of squamous cell carcinoma in RDEB patients.

Discussion

Epidermolysis bullosa comprises a heterogenous group of genetic disorders of the skin characterized by blister formation within different layers of the skin. RDEB has been linked to defects in collagen type VII, and it is usually characterized by severe cutaneous and extracutaneous disease activity and a highly increased risk of cutaneous squamous cell carcinoma (1–6). Whereas both RDEB and JEB can be associated with severe

Address correspondence and reprint requests to: Dr. Jack L. Arbiser, Enders 1025 Childrens Hospital, 300 Longwood Ave., Boston, MA 02115, U.S.A. Phone: (617) 355-6369 ext 1444; Fax: (617) 355-7043.

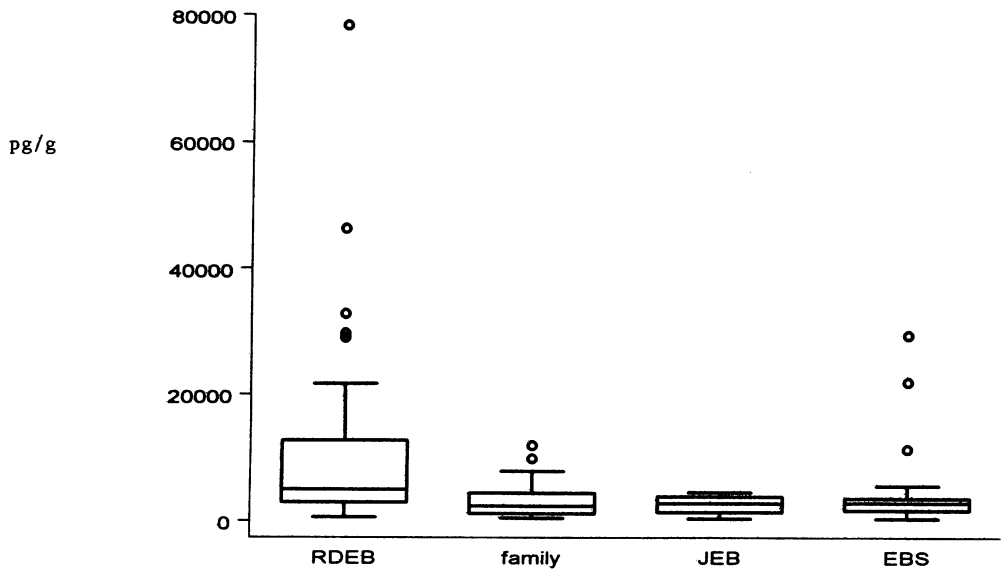

FIG. 1. Comparison of urinary bFGF values between patients with recessive dystrophic epidermolysis bullosa (RDEB), unaffected family members of patients with RDEB (family), junctional epidermolysis bullosa (JEB), and epidermolysis bullosa simplex (EBS). The top dots on the bar graph represent extreme values, the upper side of the rectangle represents the upper quartile values, the bar inside the rectangle represents the median value, and the lower side of the box represents the lower quartile. The error bars above and below each rectangle represent 1.5 × the interquartile range drawn towards the nearest data point.

morbidity and mortality, only RDEB is associated with elevated levels of collagenase and an extremely high incidence of cutaneous squamous cell carcinoma. This study provides a potential explanation for these findings.

Prior to the discovery of collagen VII defects in RDEB, these patients had been shown to have elevated collagenase activity both in dermal extracts and in supernatants from fibroblast cultures (12,13). Based on this finding, phenytoin, a weak inhibitor of collagenase, was tested for its ability to ameliorate the symptoms (14–16). Initial studies appeared to demonstrate a beneficial effect, although a double-blinded, crossover, placebo-controlled larger study failed to confirm any significant overall benefit (17). In addition, no mutations in collagenase genes were discovered in RDEB. While the association between alteration in anchoring fibrils, blister formation, and type VII collagen mutations is apparent, the connection between these mutations and the presence of elevated collagenase and squamous cell carcinomas is not readily apparent.

Table 1. Range of urinary bFGF values

	bFGF Values (pg/g)		
Diagnosis	Median	Range	N (Number of patients)
RDEB	5021	609–78297	39
Family	2396	422–11916	33
JEB	2785	351–4604	12
EBS	2830	311–29407	30

We have discovered that patients with RDEB have elevations of basic fibroblast growth factor in their urine. Examination of the dermis in a patient with RDEB and elevated bFGF reveals numerous microvessels beneath a typical bulla (Fig. 2). The subepidermal location of bullae at the sites where heparan sulfate proteoglycans bind bFGF may contribute to release of bFGF.

bFGF has several activities that may contribute to the pathogenesis of RDEB. First, it is a stimulant of collagenase synthesis, which may account for the elevated collagenase and perpetuation of blistering observed in these patients (12). bFGF is also a potent angiogenic factor, which may enhance the growth of squamous cell carcinoma (18,19). Finally, bFGF is a keratinocyte mitogen, and chronic proliferative stimuli to

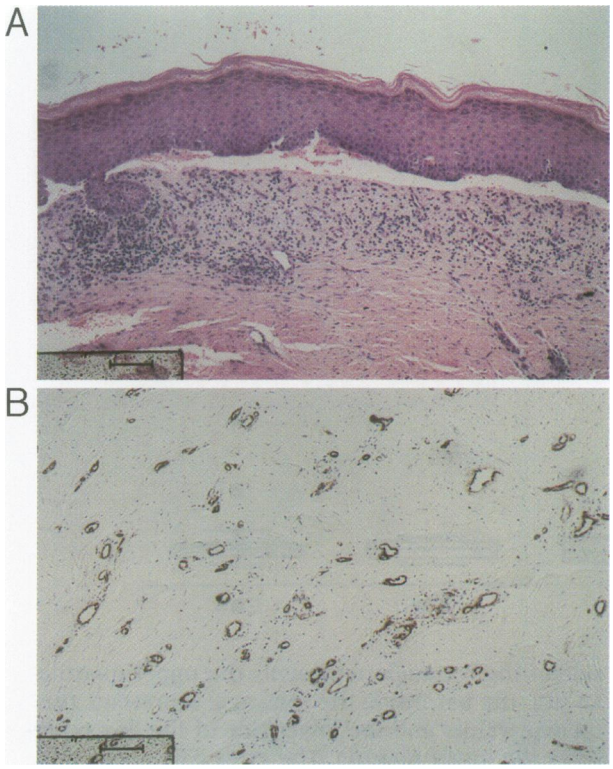

FIG. 2. Histological and immunohistochemical staining of involved tissue in a patient with RDEB. (A) Hematoxylin and eosin stain of a bulla from a patient with RDEB (×100); (B) von Willebrand staining of the dermis from the same section. The black-stained lumens represent microvessels (×100). The bars represent 50 μm.

keratinocytes may predispose to squamous cell carcinoma (20). Overexpression of collagenase in the skin of transgenic mice causes an elevated incidence of cutaneous squamous cell carcinoma (21). Thus elevation of bFGF and collagenase may contribute to cutaneous carcinogenesis.

We did not observe a strict correlation between the presence of squamous cell carcinoma and bFGF values. The measurements of bFGF in this study were at a single time point. Continued observation of this cohort over a period of years is needed to determine whether bFGF levels correlate with the eventual development of cutaneous squamous cell carcinoma.

We did not find urinary bFGF elevated in all RDEB patients. We postulate at least two possible explanations. First, bFGF may be present in high levels at skin lesions, and once local binding is saturated, it may enter the systemic circulation. Some of our patients may not have had fully saturated tissue binding sites, resulting in undetectable levels within their urine. Secondly, it is possible that different mutations in collagen type VII, which cause variable levels of basement membrane disruption, may result in differing rates of release of bFGF. We feel that the release of bFGF is not strictly a function of wounding, however, as patients with JEB have equally severe cutaneous wounding but lack elevated bFGF levels.

These results suggest strategies that may be useful in alleviating the morbidity of RDEB. Inhibition of bFGF in tumor cells by α interferon has been suggested as a potential mechanism for the anti-tumor activity of α interferon, and this activity may be of benefit in RDEB (22). Also, inhibitors of bFGF activity, such as tyrosine kinase inhibitors, may antagonize bFGF-mediated collagenase expression (23). Finally, potent and specific inhibitors of collagenase may be useful in the prevention of bullae formation and scarring (24).

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