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Urinary Basic Fibroblast Growth Factor

A Biochemical Marker for Preosseous Fibroproliferative Lesions in Patients With Fibrodysplasia Ossificans Progressiva

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Angiogenesis is a prominent histopathologic feature of preosseous fibroproliferative lesions in patients who have fibrodysplasia ossificans progressiva. Basic fibroblast growth factor is an extremely potent in vivo stimulator of angiogenesis, and has been implicated in the growth of solid tumors. An enzyme linked immunosorbant assay for basic fibroblast growth factor was performed on urine samples from patients who had active (n = 28) and inactive (n = 39) fibrodysplasia ossificans progressiva, and compared with urine samples from normal age and gender matched control subjects (n = 54). Median basic fibroblast growth factor levels were 2705 pg/g of creatinine in the normal control group, 5058 pg/g of creatinine in patients with inactive fibrodysplasia ossificans progressiva (no significant difference), and 8793 pg/g of creatinine in patients with active fibrodysplasia ossificans progressiva. Female subjects, both normal and with fibrodysplasia ossificans progressiva, had higher levels of urinary basic fibroblast growth factor than did male subjects. There was no correlation of urinary basic fibroblast growth factor levels with age or severity of preexisting disability. These data document an elevation of urinary basic fibroblast growth factor during acute flareups of fibrodysplasia ossificans progressiva and provide a biochemical basis for considering antiangiogenic therapy for inhibiting endochondral osteogenesis in this disorder.

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List of Abbreviations Used

aFGF	acidic fibroblast growth factor
AP 1	associated protein 1
bFGF	basic fibroblast growth factor
BMP	bone morphogenetic protein
IGF I	insulinlike growth factor I
IL 8	interleukin 8
TGF α	transforming growth factor alpha
TGF β	transforming growth factor beta
TNF α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

Embryonic growth and development, fracture healing and wound repair, and tumor growth and regression are all dependent on the control of angiogenesis. Angiogenesis is also an absolute requirement for the formation and development of the skeleton.[7,9,10,12,13,17,32] The angiogenesis that accompanies skeletogenesis often is described as a late event that follows calcification of cartilage in endochondral ossification.[31] Although the absolute requirement for angiogenesis has been well documented in the late stages of endochondral osteogenesis, little attention has been given to the requirement for angiogenesis during the early stages of osteogenesis when angiogenic events accompany the formation of the mesenchymal anlage of the skeleton.[9,16] This early stage of skeletal embryogenesis corresponds to the highly vascularized preosseous fibroproliferative lesion in patients who have fibrodysplasia ossificans progressiva, a rare genetic disorder of progressive postnatal ectopic osteogenesis (Fig 1).[22,23,33]

Basic fibroblast growth factor is an extremely potent heparin binding angiogenic endothelial cell mitogen involved in the control of numerous biologic processes that are critical for development and survival of the organism.[1,2,14,15,19,20,25,27,28,30,34,37,38,40] Basic fibroblast growth factor has been detected in the serum and urine of tumor bearing animals and in the serum and urine of patients who have a wide spectrum of benign and malignant tumors.[28] In the tumorigenesis pathway from mild fibromatosis to aggressive fibromatosis, there seems to be a discrete molecular switch that correlates with the cellular export of bFGF.[21] The preosseous lesions of fibrodysplasia ossificans progressiva are histologically indistinguishable from the fibroproliferative lesions of aggressive fibromastosis.[22] The authors therefore examined whether bFGF also was implicated in the preosseous lesions of fibrodysplasia ossificans progressiva. In this article, the authors provide evidence that urinary bFGF levels are elevated in patients who have fibrodysplasia ossificans progressiva during times of acute flareups of the disease process.

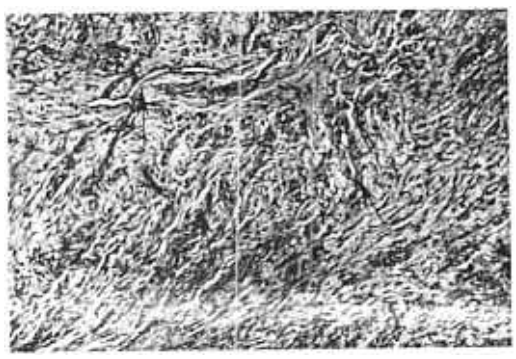

Fig 1. A medium power photomicrograph of an early fibroproliferative lesion in a patient with fibrodysplasia ossificans progressiva. There are numerous small vessels (arrows) supported by a characteristic fibroproliferative stroma. No bone or cartilage is present. (Stain, hematoxylin and eosin; original magnification, ×250).

MATERIALS AND METHODS

Patients and Controls

Sixty-seven patients with fibrodysplasia ossificans progressiva were seen in the off site fibrodysplasia ossificans progressiva clinic of the University of Pennsylvania Medical Center between October 1995 and March 1997. There were 40 females and 27 males ranging in age from 3 years to 48 years. The diagnosis of fibrodysplasia ossificans progressiva was confirmed in all patients on the basis of congenital malformation of the great toes, and progressive postnatal heterotopic ossification in characteristic temporal and anatomic patterns. Fifty-four normal individuals (25 females; 29 males) ranging in age from 3 to 70 years served as control subjects.

Patients were evaluated and categorized as having active flareups of fibrodysplasia ossificans progressiva if they were experiencing symptoms and signs of acute soft tissue swelling secondary to the presence of a soft tissue tumor. Patients were categorized as having inactive disease if they had not experienced any symptoms or signs of an acute flareup of fibrodysplasia ossificans progressiva during the 3 months before evaluation.

Basic Fibroblast Growth Factor Assay

Spot urine specimens were obtained from all patients and controls. Specimens were refrigerated at 4°C, and sent on ice by overnight mail to the Children's Hospital of Boston where they were analyzed blindly by immunoassay for human basic fibroblast growth factor according to the manufacturer's guidelines (Quantikine HS, R and D Systems, Minneapolis, MN). Results were normalized for urinary creatinine.

Statistical Evaluation

Logarithms of the bFGF levels were analyzed in accordance with standard procedure for data vary over a wide range. Median bFGF levels are reported as descriptive statistics for normal controls, for patients with inactive fibrodysplasia ossificans progressiva, and for patients with active fibrodysplasia ossificans progressiva. The significance of the group differences was assessed using an analysis of variance (ANOVA) of the log bFGF levels. Paired group differences were determined using the Welch two sample procedure; all significance levels are two tailed.[36]

RESULTS

Subjects were classified into three groups: patients with active fibrodysplasia ossificans progressiva, patients with inactive fibrodysplasia ossificans progressiva, and normal controls. Median bFGF levels were 2705 pg/g of creatinine in the normal control group (n = 54), 5058 pg/g of creatinine in patients with inactive fibrodysplasia ossificans progressiva (n = 39), and 8793 pg/g of creatinine in patients with active flareups of fibrodysplasia ossificans progressiva (n = 28).

An ANOVA of the logarithms of the bFGF levels revealed a significant difference between patients with active fibrodysplasia ossificans progressiva and all other subjects (Table 1). The difference in urinary bFGF levels between the active group and the normal control group was highly significant ($p = 7 \times 10^{-6}$). The difference in urinary bFGF levels between patients with active and inactive disease was also significant ($p = 0.0023$). The difference in urinary bFGF levels between patients with inactive disease and normal controls was not significant ($p = 0.21$). Normal female subjects and those with fibrodysplasia ossificans progressiva (n = 65), had higher levels of urinary bFGF than did male subjects (n = 56; $p = 0.0198$). There was no apparent correlation of urinary bFGF levels with age or level of preexisting disability.

DISCUSSION

The major finding of this study was that urinary bFGF levels were elevated in patients who had fibrodysplasia ossificans progressiva at a time that corresponded to the formation of an early fibroproliferative lesion. In contrast, elevations of urinary bFGF were not detected during times of disease quiescence in this cross sectional study. These

TABLE 1. Urinary Median Basic Fibroblast Growth Factor (bFGF) Levels

Group	Number of Subjects	Urinary bFGF Levels	Significance	
Control	54	2705		
Inactive fibrodysplasia ossificans progressiva	39	5058	NS	
Active fibrodysplasia ossificans progressiva	28	8739	$p = 0.0023$	$p = 7 \times 10^{-6}$

NS = not significant.
Values are pg/g creatinine.

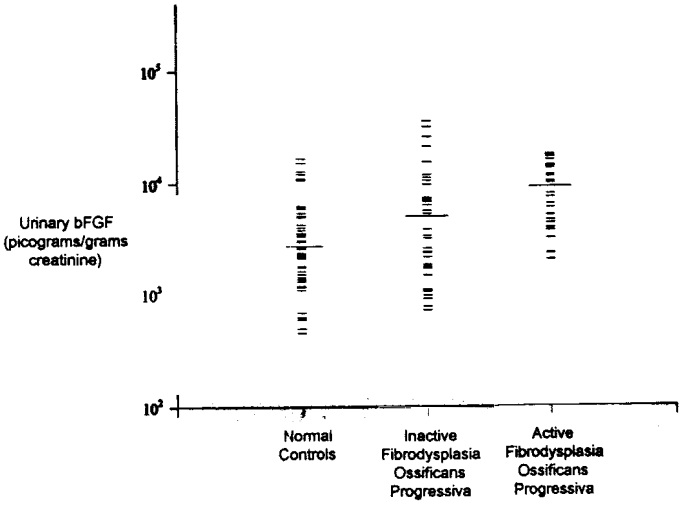

Fig 2. Logarithmic scale of bFGF levels in normal controls and in patients with fibrodysplasia ossificans progressiva. Transverse bar in each column denotes median bFGF level.

data suggest, therefore, that urinary bFGF may be a biochemical marker for preosseous fibroproliferative tumors in patients who have fibrodysplasia ossificans progressiva.

Despite these interesting findings, some precautions are necessary. First, although median levels of urinary bFGF were significantly different between those patients with and without active fibroproliferative lesions, there was a wide range of values within each group and much overlap between groups (Fig 2). Such findings may limit the clinical use of individual bFGF levels in assessing disease activity. Second, although the sample size was small and cross sectional, it represented approximately 1/3 of all patients currently identified with fibrodysplasia ossificans progressiva worldwide. In all, the authors think that the findings of this study are representative of the disease activity that may be seen at any time in the fibrodysplasia ossificans progressiva community. However, further long term longitudinal studies are necessary to establish the general applicability of these findings to the molecular pathophysiology of fibrodysplasia ossificans progressiva, and to the potential use in monitoring disease activity in individual patients.

To date, numerous angiogenic factors have been identified and include aFGF, bFGF, IL 8, angiogenin, TGF α, TGF β, TNF α, prostagandins, and VEGF.[7,9,10,12,13,17,32] The cells producing bFGF during flareups of fibrodysplasia ossificans progressiva could be plausibly lesional fibroblasts or lesional endothelial cells but the source of the secreted bFGF is unknown at present. Inflammatory cells such as monocytes also are known to be robust producers of bFGF and may be involved in the disease process.[6]

Basic fibroblast growth factor, BMP, and TGF β are thought to be involved in the early pathophysiology of fibrodysplasia ossificans progressiva.[6,23,33] These factors plausibly contribute to a cascade of molecular events that inhibit myogenesis and promote osteogenesis, leading to the transdifferentiation of muscle to bone (Fig 3).[3,6,8,11,24,26,39,42] Although the exact relationship between BMP 4 and bFGF signaling remains unknown, recent studies have shown that BMP 4 and bFGF signal through a Ras/Raf/AP 1 pathway, even though their signals are transduced by totally different families of transmembrane receptors.[41]

Despite the attention that has been focused on bFGF as an angiogenic factor, it cannot fully explain the profound edema that accompanies the preosseous lesions of fibrodysplasia ossificans progressiva. Vascular endothelial growth factor is also a potent angiogenic factor that has unique, profound, and immedi-

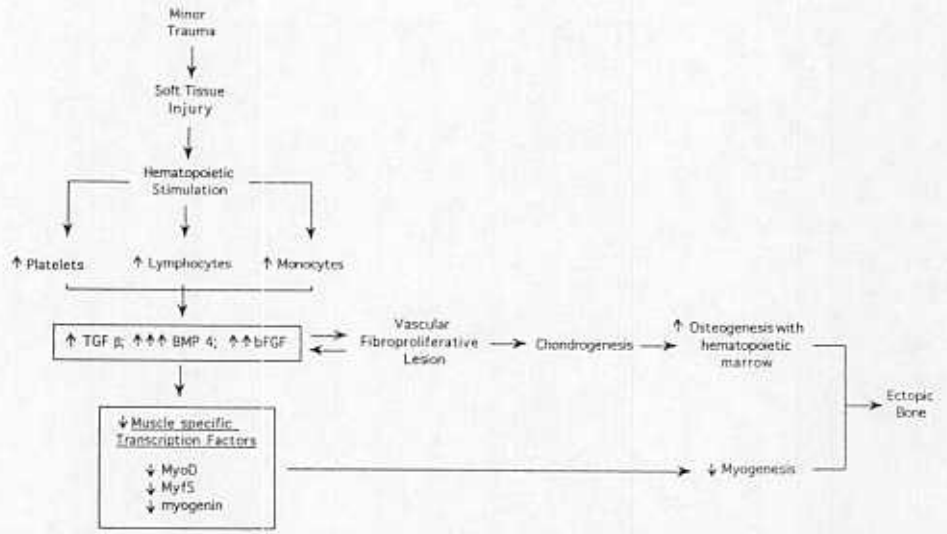

Fig 3. Hypothetic schema for pathophysiology of ectopic osteogenesis in patients with fibrodysplasia ossificans progressiva. Activated platelets and monocytes express TGF β and bFGF, respectively. Lymphoblastoid cells in patients with fibrodysplasia ossificans progressiva overexpress BMP 4. All three secreted factors have distinct effects on promoting osteogenesis and inhibiting myogenesis.

ate effects on vascular permeability. Vascular endothelial growth factor is induced by hypoxia, parathyroid hormone, IGF I, and most potently 1, 25 dihydroxyvitamin D in osteoblastlike cells.[4,18,35] The authors did not measure vascular endothelial growth factor levels in this study, and presently there are no data on the role of vascular endothelial growth factor in early lesions of fibrodysplasia ossificans progressiva.

This study raises many provocative questions about the role of angiogenesis and angiogenic peptides in the pathogenesis of fibrodysplasia ossificans progressiva:

(1) How does angiogenesis become activated during the evolution of a fibrodysplasia ossificans progressiva lesion; (2) what cells produce and excrete bFGF during an acute flareup of fibrodysplasia ossificans progressiva; (3) what is the relationship between bone morphogenetic protein expression and basic fibroblast growth factor expression in the early fibroproliferative lesions of fibrodysplasia ossificans progressiva; (4) what are the exogenous and endogenous stimuli that trigger the formation and regression of a fibrodysplasia ossificans progressiva lesion, and what effect do those stimuli have on the molecular pathophysiology of the angiogenic lesions; and (5) are endogenous inhibitors of angiogenesis responsible for changing the balance of the angiogenic switch that leads to the spontaneous regression of the fibrodysplasia ossificans progressiva lesions? If so, what are those factors and how can they be mobilized for therapeutic advantage?

Answers to these questions will prove critical to understanding the pathogenesis of preosseous fibroproliferative lesions in fibrodysplasia ossificans progressiva and in designing therapies that will be effective in controlling renegade osteogenesis in this condition. Antiangiogenic factors such as thalidomide, gamma interferon, angiostatin, and squalamine affect various parts of the angiogenic pathway and may be valuable tools in dissecting the molecular events that lead to angiogenesis in this condition.[5,29] (nonpublished data, Sills AK, Tyler B, Caterra J, Brem H: Squalamine blocks endothelial activation by common brain tumor mitogens. Proceedings of the Society for Neuro-oncology 1996.) The

data presented in the study suggest that a therapeutic approach based on inhibiting the biologic activity of angiogenic peptides may be a rational approach to consider in treating the early fibroproliferative lesions of fibrodysplasia ossificans progressiva.

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