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Immunohistochemical detection of acidic fibroblast growth factor in bladder transitional cell carcinoma*

V. Ravery[1], J. Jouanneau[3], S. Gil Diez[1], C. C. Abbou[1], J. P. Caruelle[2], D. Barritault[2], and D. K. Chopin[1]

[1]Department of Urology, Henri Mondor Hospital, Creteil, France
[2]Laboratory of Biotechnology, University of Paris XII, Creteil, France
[3]URA, CNRS 1337, Paris, France

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Summary. Acidic fibroblast growth factor (aFGF) is a regulatory peptide which, on account of its structural homologies with the products of oncogenes, is involved in cell proliferation, differentiation, and motility. We previously reported the presence of aFGF in the urine of patients with transitional cell carcinoma (TCC). aFGF can also induce the motility of a rat-derived bladder carcinoma cell line (NBT_{II}). This immunohistochemical study used polyclonal rabbit antibodies against acidic and basic FGF and peroxidase detection. Native NBT_{II} nude mice xenografts and aFGF transfected NBT_{II} (NFS14) nude mice xenografts were used as tissue controls for antibody specificity. The samples included 4 normal urothelia and 12 TCC. In addition, cytospins of 4 different tumoral cell lines of human bladder and normal bladder cells were stained. The results showed strong immunostaining in all tumoral urothelium samples using anti-aFGF and a very low amount of staining or none at all in healthy tissues. A primary analysis suggested that the strongest reaction was obtained in high-grade tumors ($3+$ *vs* $+$ for lower-grade tumors). Using bFGF antibody, strong immunohistochemical staining was detected on basal membranes and stromal vessels and none in urothelium. These data confirm aFGF expression in the epithelial cell compartment of bladder cancer and the likely involvement of this regulatory peptide in the biology of TCC.

Key words: Immunohistochemistry – Bladder cancer – Growth factors – Acidic fibroblast growth factor – Epithelial cell compartment

Growth factors form a group of low-molecular-weight peptides. They are involved in a wide range of actions, such as proliferation, inhibition, motility, and differentiation [12], and can induce a cellular response through binding to specific receptors [4, 10]. They have structural and functional homologies and regulatory relationships with products of oncogenes [15, 21], further supporting the autocrine growth hypothesis of cancer cells [19, 20, 27] with a loss of control of critical steps in the process of cell division. Previous studies [6, 11] have shown that among these regulatory peptides, the fibroblast growth factor (FGF) family appears to be an important signal for cell mitogenesis and differentiation.

Previously, acidic fibroblast growth factor (aFGF)-like activity was detected in the urine of patients with transitional cell carcinoma (TCC) [5] and, using an original enzyme immunoassay [3], we were able to detect aFGF immunoreactivity in the urine of TCC patients. aFGF was shown to be associated with motility in a rat-derived bladder carcinoma cell line [9, 22, 23], suggesting the possible involvement of aFGF in the progression of bladder tumors. Basic fibroblast growth factor (bFGF) is known to be an angiogenesis stimulating factor [8, 13, 18]. Our aims were to determine which cell compartments in bladder carcinoma show aFGF immunoreactivity and to confirm whether or not this regulatory peptide was involved in the biology of TCC. We also tried to establish any immunohistochemical staining differences between acidic and basic FGF, and in this paper we present data showing that the acidic form and not the basic form is associated with TCC of the bladder.

Materials and methods

Tissue controls for antibody specificity

Frozen tumor sections from native NBT_{II} and NFS14 nude mice xenografts [1] were used as positive controls.

Cell studies

Prior to performing in situ experiments, cytospins were made from 4 established human transitional cell lines: T_{24} [2], 647V [7], RT_4 [16],

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Table 1. Immunostaining intensity of each different cell line using anti-aFGF and anti-bFGF (on cytospins)

	aFGF	bFGF
RT_4	3+	+/−
T_{24}	2+	0
647 V	4+	0
253J	3+	+
Normal cells	+	0

Table 2. Staining intensity of each tumoral sample using anti-aFGF and anti-bFGF.

According to stage

	T1A	T1B	T2	T3A	T3B
aFGF	2+	2+	3+	3+	3+
	2+	+	2+	3+	
	3+	+	2+		
bFGF	0	0	0	+	0
	0	0	0	0	
	0	0	0		

According to grade

	G1	G2	G3		
aFGF	+	2+	2+	3+	3+
	2+		2+	3+	3+
	+		3+	2+	
bFGF	0	0	0	0	0
	0		0	0	0
	0		+	0	

253J [26] and from a cell suspension of a normal bladder obtained by bladder scraping. Slides were then stored at −80°C.

Human studies

All samples were obtained at surgery from patients (TCC) and from cadaveric donors (normal urothelia). Samples were frozen immediately in liquid nitrogen and stored at −80°C until used. Then samples were sliced on a cryostat and air dried. Four normal urothelium samples and 12 TCC (6 superficial and 6 infiltrative tumors with a distribution of 3 grade 1,1 grade 2, 8 grade 3) were used.

Immunohistochemical procedures

Slides were immersed in cold acetone for 15 min and then rehydrated. Slides were then preincubated for 30 min with PBS + 10% normal swine serum (NSS) + 1% bovin serum albumin (BSA), followed by incubation for 60 min with primary antibody. Anti-aFGF (UBI, Lake Placid, N.Y.) and anti-bFGF (BTI, Stoughton, USA) polyclonal rabbit antibodies at 1/50 were used, and as a negative control PBS 0.15 M NaCl + 10% NSS + 1% BSA.

Slides were washed in PBS and, after inhibiting endogenous peroxidase, incubated for 45 min with the second antibody at 1/50 (DAKOPATTS peroxidase-conjugated swine antirabbit immunoglobulins). Slides were washed, incubated for 5 min with diaminobenzidine and then washed in double-distilled water (DDW). Cells were then counterstained, washed in DDW, and dehydrated in increasing ethanol and xylol gradients (70 → 100%) before mounting in resin under coverslips.

Evaluation criteria

Using polyclonal antibodies, a sample was found negative when its immunostaining was the same as the negative control staining. When a sample was found positive, two examiners studied its intensity twice. When there was disagreement, a third examination was done. The final intensity was expressed from 0 to 4+.

Results

Using NBT_{II} and NFS14 nude mice xenografts (before and after aFGF transfection), there was strong immunoperoxidase staining with anti-aFGF, but not with anti-bFGF. All the human bladder cell lines tested demonstrated positive immunostaining with anti-aFGF antibody, but not the normal urothelial cells, except for umbrella cells. The pattern of staining is shown in Table 1.

In all TCC samples, using anti-aFGF, there was immunoperoxidase staining (Table 2) in tumoral cells with occasional clusters of more strongly stained cells in the urothelium (Figs. 1, 2, 5). In this small number of samples there was a reaction-intensity difference between high-grade tumors (the mojority being 3+) and low-grade ones. Using anti-bFGF, there was no staining of tumoral cells, except in one high-grade sample where a very low reaction was observed. There was, however, a very strong specific reaction with stromal vessels and basal membranes in nearly all slides (Figs. 3, 4, 6).

On normal urothelium sections, using anti-aFGF, there was no staining except in umbrella cells (Fig. 7). With anti-bFGF, the same strong reaction described for tumor samples was found in basal membranes and vessels (Fig. 8).

Discussion

From the examination of this limited panel of TCC samples, it was concluded that aFGF and not bFGF was expressed in the epithelial cell compartment of TCC. Normal urothelium did not express aFGF, except at the most superficial layer of a low level.

Using cytospins of different kinds of tumoral and healthy bladder cells, aFGF immunostaining was present in different ways. Tumoral cells were always homogeneously stained, and it is not at all surprising to have such a reaction difference with these 4 types of cell lines, which are at many stages of malignant differentiation. The staining of normal bladder cells was limited to umbrella cells both in cell suspension and histological samples. These preliminary results also demonstrated an increase in the intensity of aFGF staining with increase of tumor grade.

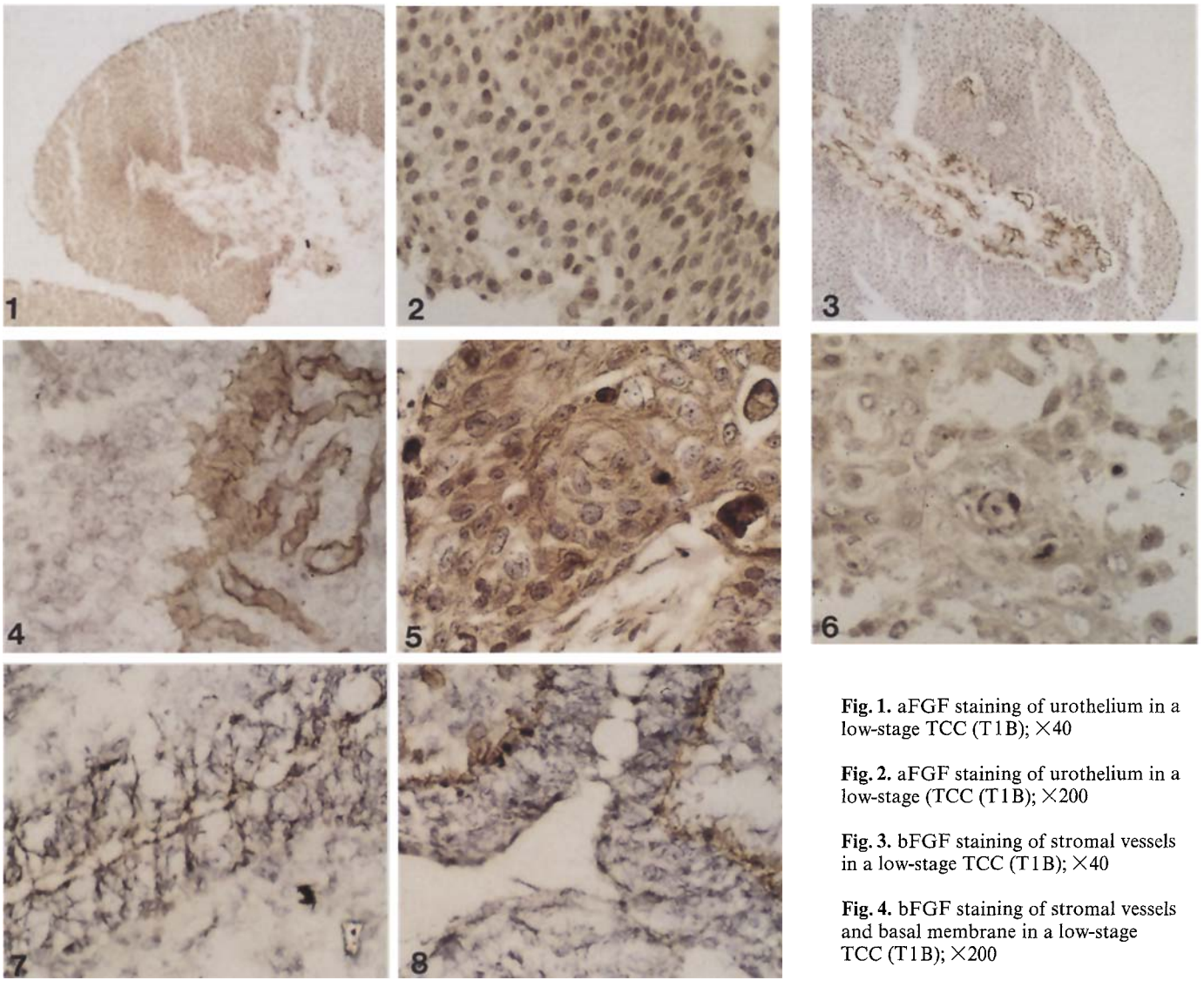

Fig. 1. aFGF staining of urothelium in a low-stage TCC (T1B); ×40

Fig. 2. aFGF staining of urothelium in a low-stage (TCC (T1B); ×200

Fig. 3. bFGF staining of stromal vessels in a low-stage TCC (T1B); ×40

Fig. 4. bFGF staining of stromal vessels and basal membrane in a low-stage TCC (T1B); ×200

Using anti-bFGF, only one tumoral urothelium was slightly positive. However, for all samples (tumoral and healthy), there was a strong reaction with vessels and basal membranes: it has recently been shown that capillary endothelial cells synthesize basic FGF [17, 25] and also that extracellular matrices [24] and basement membranes [8] contain bFGF bound to heparan sulfates.

There are probably at least two genes encoding FGF, corresponding to each form of FGF [10]. FGF bind to two different classes of receptors whose cDNAs have been cloned. The low-affinity receptors use the extracellular heparan sulfate moiety. High-affinity receptors belong to the tyrosine kinase family [14].

The patterns of staining observed in this study could be due to modification of aFGF expression at the transcriptional or translational level during urothelial transformation. It might be interesting to evaluate modifications at the receptor level and the relationship between growth of TCC cells and autocrine secretion of aFGF. We are now following this line of research.

Fig. 5. aFGF staining of urothelium in a high-stage TCC (T3A) with areas of epidermoid metaplasia; ×200

Fig. 6. Very low bFGF staining of urothelium in a high-stage TCC (T3A); ×200

Fig. 7. aFGF staining of the most superficial layer in normal urothelium; ×200

Fig. 8. bFGF staining of basal membrane in normal urothelium; ×200

In conclusion, these data demonstrate aFGF and not bFGF expresion in the epithelial cell compartment of bladder cancer, in spite of their structural homology [11]. The correlation level of aFGF expression according to grade and tumors stage needs confirmation and are continuing to accumulate data.

These observations, added to those which show the involvement of aFGF in proliferation, motility and

differentiation of certain tumoral cells suggest that aFGF has a role in the biology of TCC and could also be a useful marker for bladder cancers.

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Dr. D. K. Chopin
Service d' Urologie
Hôpital Henri Mondor
51 avenue du Mal Delattre de Tassigny
F-94000 Créteil
France