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Eye-derived Growth Factors: Receptors and Early Event Studies

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Eye-derived growth factors I and II (EDGF I and II) have been purified from bovine retina (Barritault et al. 1982; Courty et al. 1985) and represent the two families of heparin-binding growth factors that have been purified to homogeneity by several groups in the last 2 years. We have shown that EDGF I corresponds to basic fibroblast growth factor (bFGF) (Courty et al. 1986) and that EDGF II corresponds to endothelial cell growth factor or acidic FGF (aFGF) (Schreiber et al. 1985).

Although the mitogenic properties of partially purified preparations have been described for over a decade (Armelin 1973; Gospodarowicz 1975), very little information on the mechanism of action of these growth factors is known. We have obtained highly purified preparations of aFGF and bFGF and have studied the interaction of these growth factors with target cells.

Characterization of High-affinity Receptors to EDGF I/bFGF

Binding and receptor studies were made possible by labeling conditions that maintained the growth factors biologically active. We were able to show (Moenner et al. 1986) that ^{125}I -labeled bFGF bound to bovine epithelial lens cells in culture could be displaced by unlabeled bFGF in a dose-dependent manner and that almost 90% of the radioactivity was displaced by a 100 molar excess of unlabeled bFGF. Scatchard analysis indicated an apparent dissociation constant of 5×10^{-11} M. Using cross-linking agents, we bound this growth factor to its putative receptor and could estimate an apparent M_r of 130,000 for this receptor.

Evidence for High- and Low-affinity Receptors

To purify the M_r 130,000 receptors, we looked for cells rich in receptors. The results are presented in Table 1. We also found that the number of high-affinity receptors could vary for the same cells, accord-

Table 1 High-affinity Specific Binding of aFGF (EDGF II) and bFGF (EDGF I) on Mammalian Cells

Cells	Cells/cm ² ($\times 10^{-5}$)	aFGF sites/ cell	Apparent K_d ($M \times 10^{10}$)	bFGF sites/ cell	Apparent K_d ($M \times 10^{10}$)
Bovine epithelial lens	0.4			20,000	0.5
Baby hamster kidney (BHK-21)	2.8	30,000	7.0	15,000	6.0
Chinese hamster lung fibroblasts (CCL39)	0.9	10,000	1.0	50,000	1.0
Mouse fibroblasts (BALB/3T3)	2.9			10,000	0.8
Human fibroblasts (AG1523)	2.5			5,000	0.6
Human fibroblasts (AG1523)	0.2			100,000	0.5
Bovine endothelial cornea	0.4	12,000	0.5	10,000	0.9
Murine lung endothelial capillary (LE II)	0.4			15,000	0.3
Human epidermoid carcinoma A431	1.2	<1,000		<1,000	
Human alveolar carcinoma A549	2.5	<1,000		<1,000	

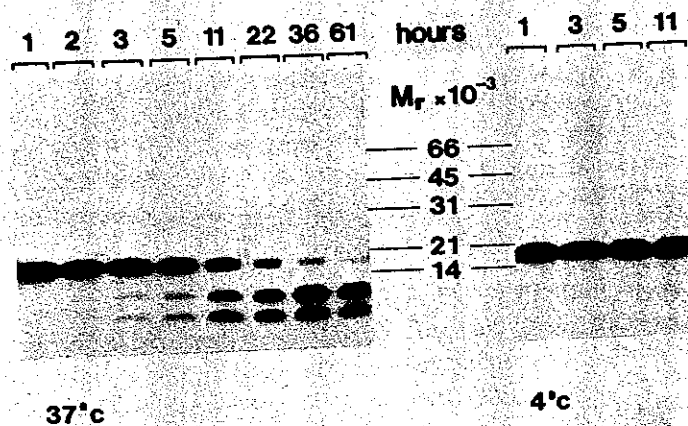
Binding was performed at 4°C. Nonspecific binding was determined in the presence of a large excess of partially purified FGF/EDGF (acidic acid extract; Barritault et al. 1982).

ing to cellular density. Scatchard analysis also indicated the existence of a second class of receptors (400,000 to 1,000,000 per cell) with an apparent dissociation constant of 1×10^{-9} to 2×10^{-9} M (not shown). Furthermore, preliminary experiments (not shown) indicated that this second class of receptors was partially sensitive to heparinase treatment but not to chondroitinase.

EDGF/FGF Does Not Induce Hydrolysis of Polyphosphoinositide

We have shown (Magnaldo et al. 1986) that the addition of aFGF or bFGF to bovine epithelial lens cells or Chinese hamster fibroblasts cells (CCL39) induces a rise of intracellular pH (0.2 to 0.4 units) and free-calcium concentration (240 nM to 400 nM). The origin of the

A Processing kinetics of ^{125}I basic FGF in CCL39 cells



B Kinetic of disappearance of ^{125}I basic FGF from cellular supernatant at 37°C

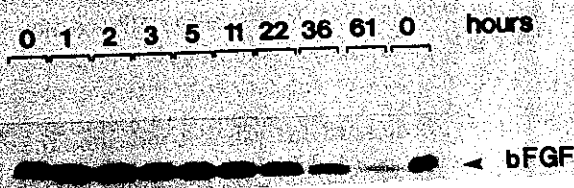


Figure 1 Autoradiograph of the electrophoretic migration of ^{125}I -labeled FGF associated with cellular extracts (A) and present in cellular supernatant (B) at different times of incubation at 37°C and 4°C. ^{125}I -labeled FGF was added at 5 ng/ml of culture medium.

calcium was strictly extracellular. Furthermore, the breakdown of phosphoinositide was not activated, suggesting that another pathway might be involved in the mitogenic response. This hypothesis is supported by a recent report that a tyrosine kinase activity might be associated with the FGF receptor (Huang and Huang 1986).

Processing of EDGF I/bFGF

Study of the fate of EDGF I/bFGF was carried out by the addition of ^{125}I -labeled bFGF to subconfluent G_0 -arrested CCL39 cells maintained in serum-free medium. The doses of ^{125}I -labeled bFGF used ensured maximal stimulation of thymidine incorporation for these cells.

At different incubation times at 4°C and 37°C, the migration of ^{125}I -labeled bFGF associated with the cell lysate, as well as in the culture medium, was studied by gel electrophoresis. Autoradiographs of the electrophoretic migrations are shown in Figure 1. At 4°C, there is no detectable degradation in the cell medium. At 37°C, two faint degradation bands at 10 kD and 8 kD, associated with the cell lysate, are detectable after 2 hours of incubation. These bands become more visible as the incubation time increases. Concomitantly, the 17 kD (intact bFGF) band decreased in intensity in both the cell lysate and culture medium. The radioactivity associated with these bands was measured, and the results are presented in Table 2.

Table 2 Fate of ^{125}I -labeled bFGF Incubated with CCL39 Fibroblasts at 37°C

	Incubation time (hr)								
	1	2	3	5	11	22	36	61	
(a) Repartition of radioactivity in cellular supernatant (%)									
Culture medium	100	102	105	90	60	44	30	21	
Intact FGF (17K)	0	0	0	10	40	56	70	79	
Degraded FGF	(b) Repartition of radioactivity associated with cells (%)								
	Incubation time (hr)								
	1	2	3	5	11	22	36	61	
Cell extract	100	82	72	65	40	25	13	5	
Intact FGF (17K)	0	3	14	15	32	42	54	54	
10K fragment	0	3	14	15	27	29	30	32	
8K fragment									

Radioactivity was counted in the 17K, 10K, and 8K bands after electrophoresis (Fig. 1). Results are given as a percentage of the radioactivity loaded on each track of the gel.

DISCUSSION

Our results indicate that EDGFs/FGFs have most of the properties of other growth factors with regard to high-affinity receptors and mitogenic pathway. However, the processing is slow and depends on the formation of intermediate products. Further investigations are now in progress to characterize these intermediate products and to determine if they play a significant role in the mechanism of action of EDGF/bFGF.

In addition to study of its angiogenic activity, the wound-healing properties of EDGF have been studied in models of corneal and epidermal ulceration (Thompson et al. 1982; Fournanier et al. 1986). It is therefore important to increase our understanding of the mechanism of action of these molecules in the control of cell division and tissue homeostasis in order to elucidate their physiological meaning as well as to use them as wound-healing agents.

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