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EXPRESSION PATTERN OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTOR GENES IN ADULT RAT EYES

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Although the retinal angiogenic and mitogenic factors have been identified to be acidic and basic fibroblast growth factors (aFGF and bFGF), little information has so far been available about the cells producing them and their function in retinal tissues. We found, by in situ hybridization, that the expression pattern of the aFGF gene differed remarkably from that of the bFGF gene in adult rat eyes. Our results demonstrated that the aFGF gene was produced by photoreceptor visual cells, neuronal cells in the inner nuclear layer and ganglion cells of the retina, in addition to pigment epithelial cells of the choroid, iris and ciliary body, and epithelial cells of the cornea, conjunctiva and lens, while bFGF was synthesized solely by the photoreceptor visual cells.

The existence of retinal vasculogenic factors was first proposed by Michaelson (1) and more recently by Patz (2). These retina-derived factors have been isolated on the basis of their activity in vitro to stimulate the proliferation of capillary endothelial cells (3,4). Recently, these were demonstrated to be identical with acidic and basic fibroblast growth factors (aFGF and bFGF) (4). Although the FGFs have been found in various tissues, including the retina, brain, cartilage, vascular endothelial cells, little is known about the cells producing them in vivo and their functions. Thus, it is of primary importance to identify the cells specifically producing the FGFs in these

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tissues. In the present study, we demonstrated for the first time the expression pattern of both FGF genes in adult rat eyes by *in situ* hybridization.

Materials and Methods

Eyes of 6-week rats were enucleated and cut into halves after perfusion from the left ventricle with phosphate-buffered 4% paraformaldehyde (pH7.4) at 4°C, post-fixed for 3 hours in the same fixative, dehydrated with alcohol series at 4 °C, and embedded in paraffin (Paraplast). The sections with 5 μm thickness were pretreated, hybridized and washed, according to Ingham et al.(5) and Noji et al.(6).

We performed *in situ* hybridization on paraffin sections of the rat eyes with single-stranded ^{35}S-labeled riboprobes synthesized from a BamHI fragment of the rat bFGF cDNA (7,8) and a chemically synthesized DNA fragment (454 bp) coding the human aFGF (The sequence will be published elsewhere.), which were generous gifts from Takeda Chemical Industries, LTD. To prepare antisense and sense riboprobes, the chemically synthesized aFGF cDNA and the bFGF cDNA were inserted in a plasmid pGEM and a plasmid pSP6, respectively. Specific activity of the riboprobes labeled with ^{35}S-UTP (400 Ci/mol, Amersham) was about 5×10^8 dpm/μg. Probes for the hybridization were subjected to limited alkaline hydrolysis to shorten the transcripts to about 50-150 base pairs. For autoradiography, the slides were immersed in Kodak NTB2 emulsion (diluted 1:1 with water), air-dried and exposed for 7 days at 4°C. The slides were developed and finally stained with hematoxylin and eosin.

Results and Discussion

Hybridization with sense-strand probes gave a background-level distribution of exposed silver grains at all tissue sections (Fig. 1c, 1d, 2c, 2d, 3c and 3d).

With the antisense riboprobe for aFGF mRNA, extensive accumulation of exposed silver grains were observed in the photoreceptor visual cells at the bottom of Fig. 1b, cells in the inner nuclear layer in the middle of Fig. 1b, and the ganglion cells at the top of Fig. 1b. More precisely, the signals for the the aFGF mRNA were localized on the inner segments of the photoreceptor visual cells. This localization is quite reasonable, because these segments have the ability of protein synthesis. This result presents a possibility that production and secretion of aFGF in the photoreceptor cells are closely related

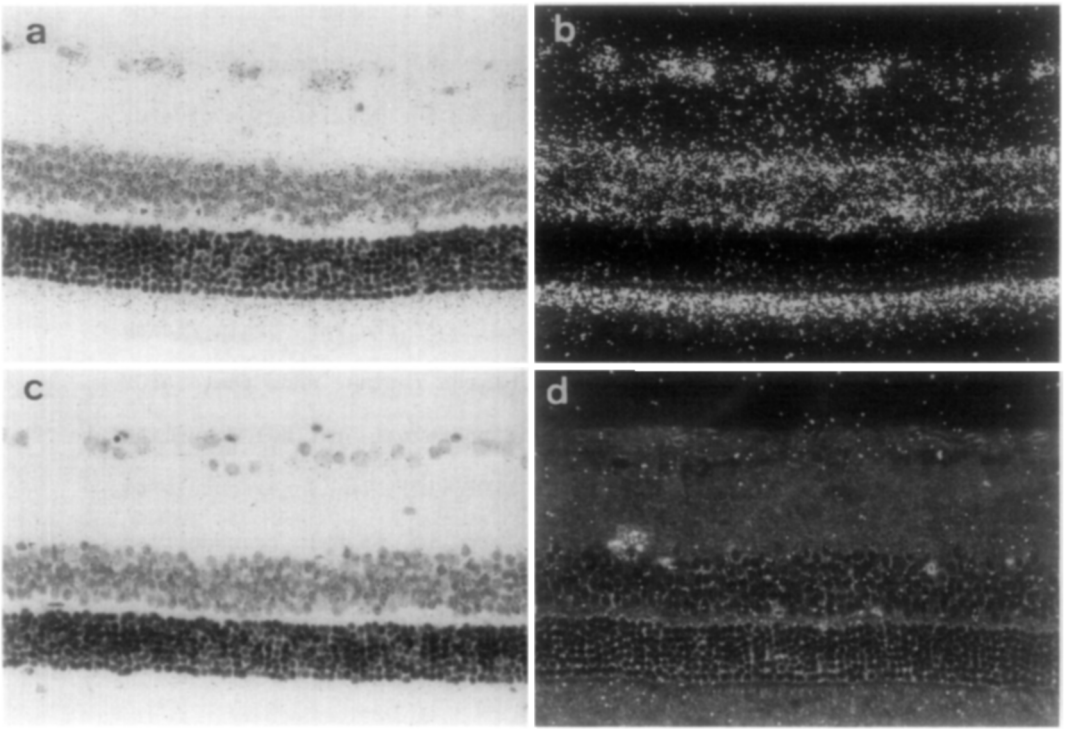

Fig. 1. Expression pattern of the aFGF gene in retina. a and b: Bright and dark field photographs with the antisense probe; c and d: bright and dark field photographs with the sense probe.

to photo-stimulation. The aFGF mRNAs were also detected in the cells of the inner nuclear layer, which consist of amacrine, bipolar, and horizontal cells in addition to supporting cells of Mueller. Since the signal grains are more intensive in the edge of this inner nuclear layer than in its middle, there is a possibility that not all but specific cells in this layer would express the aFGF gene more intensively than did the other cells. Furthermore, the aFGF gene is expressed selectively in ganglion cells (larger cells with large, clear nuclei in ganglion cell layer in Fig. 1a and 1b), whereas no significant expression was observed in glial cells (smaller cells with dark nuclei) around the ganglion cells.

These findings are consistently correlated with the immunohistochemical localization of the aFGF protein: the aFGF

proteins were localized in the inner segments of the photoreceptors, the inner nuclear layer and ganglion cells of the bovine retina (9). This correlation of the localization of aFGF mRNA with that of the corresponding proteins indicates a short-range intercellular interaction among the neuronal cells through aFGF in the rat retina. In view of the facts that aFGF gene is expressed intensively in neuronal cells of the rat brain (data not shown) as well as in those of the retina and that aFGF promotes neuronal outgrowth of the postnatal retinal ganglion cells to a far greater degree than bFGF (10), it is reasonable to suppose that aFGF participates somehow in signal transduction among the retinal neurons and also in maintaining normal neuronal network in the retina.

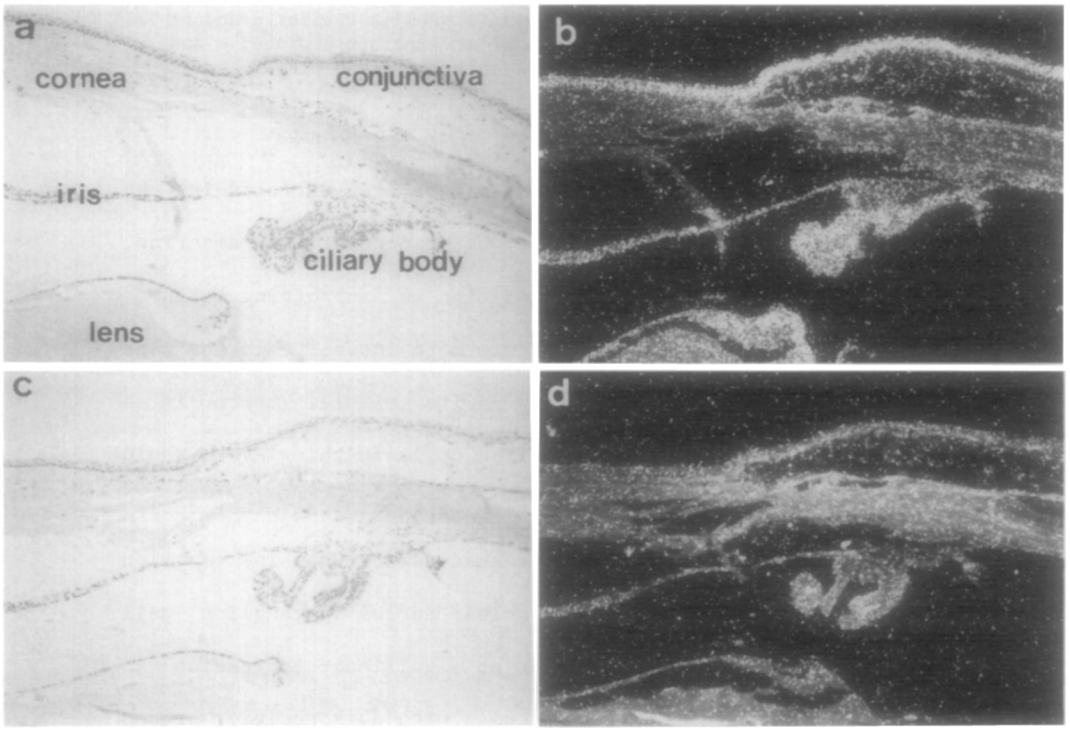

Fig. 2. Expression pattern of the aFGF gene in lens, iris, ciliary body, cornea and conjunctiva of a rat eye. a and b: Bright and dark field photographs with the antisense probe; c and d: bright and dark field photographs with the sense probe.

We also detected the aFGF mRNA in the pigment epithelial cells of the choroid, iris and ciliary body, and the epithelial cells of the cornea, conjunctiva and lens, as shown in Fig. 2. The aFGF produced by the lens epithelial cells is likely to maintain growth and differentiation of themselves as an autocrine factor, based on the fact that aFGF and bFGF promote differentiation of lens epithelial cells in vitro as reported previously (11). In the same manner, aFGF produced by the corneal and conjunctival epithelial cells may stimulate growth and differentiation of themselves and/or their stromal cells. As the function of aFGF in the neuronal cells seems to differ from that in the epithelial cells, the aFGF proteins may be classified into several subtypes created by such a mechanism as alternative splicing of the common aFGF gene.

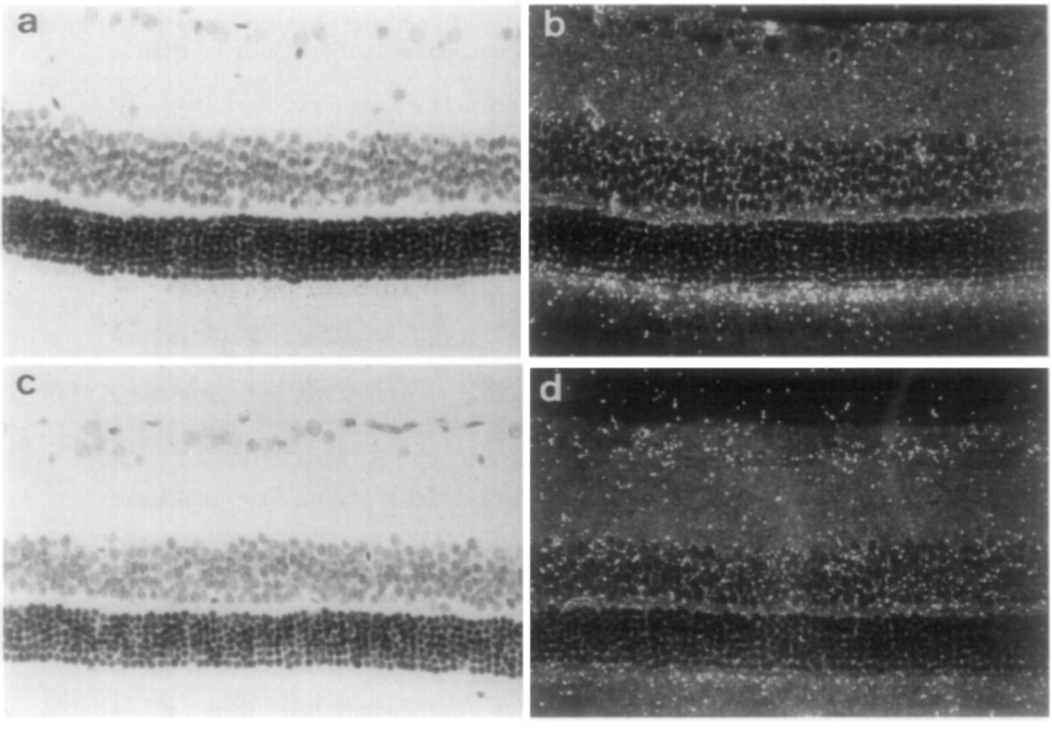

Fig. 3. Expression pattern of the bFGF gene in retina. a and b: Bright and dark field photographs with the antisense probe; c and d: bright and dark field photographs with the sense probe.

In contrast to the results for aFGF, the bFGF gene is expressed exclusively in the inner segments of the photoreceptor visual cells as shown in Fig. 3a and 3b. Since the bFGF proteins were reported to be localized around microvasculature in the inner nuclear layer of the bovine retina (9), the localization of the bFGF mRNA differs from that of the gene product. A possible interpretation of this fact is that the bFGF proteins synthesized in the photoreceptor cells are secreted and diffuse to the target cells in the inner nuclear layer. If that is the case, bFGF should be the angiogenic factor in the retina. More precise analyses are required to clarify this point. On the other hand, the corneal endothelial and/or epithelial cells have been supposed to produce bFGF, because the bFGF proteins were found in the corneal basement membranes (12,13). However, we could not find the detectable amount of the bFGF messenger RNA in the cornea under our experimental conditions. This result implies that the bFGF gene is transcribed not constitutively or not in a large amount in the cornea, but that its transcription may be induced intermittently only when the storage of bFGF is depleted.

Clinically, it is well known that when normal retinal capillaries are obstructed under a pathological condition as diabetic retinopathy, new vessel formation occurs so as to result in serious clinical complications (2). Judged from our results, the obstruction of the retinal capillaries is considered to damage the neuronal cells and this damage leads to abnormal secretion of the FGF proteins. Since FGF is an angiogenic factor, abnormal capillary formation may then occur. The above consideration is substantiated by the fact that a diabetic patient with advanced proliferative retinopathy had nine times more FGF in the ocular fluid than did those without retinopathy (14).

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