Fibroblast Growth Factor Receptor 4 (FGFR4) Is Expressed in Adult Rat and Human Retinal Photoreceptors and Neurons

Veronique Fuhrmann, Norbert Kinkl, Thierry Leveillard, Jos Sahel, and David Hicks*

Laboratoire de Physiopathologie Cellulaire et Moléculaire de la Retine, Clinique Médicale A, INSERM-CHUR-Université Louis Pasteur, Centre Hospitalier Regional Universitaire, BP 426, 1 Place de l’Hôpital, 67091 Strasbourg Cedex, France

Abstract

The fibroblast growth factor (FGF) family, with its prototype members acidic FGF (FGF-1) and basic FGF (FGF-2), binds to four related receptor tyrosine kinases, termed FGFR1, R2, R3, and R4, expressed on most types of cells in tissue culture. In many respects, the FGFR appear similar to other growth factor receptors; thus, dimerization of receptor monomers on ligand binding is likely to be a requisite for activation of the kinase domains, leading to receptor transphosphorylation. Within the central nervous system (CNS), including retina, FGFR1 and R2 have been widely described as the predominant forms. FGFR4 is reported to be strongly expressed only during early stages of development, and apart from one small region (the lateral habenular nucleus) is not detectable in adult CNS. Screening of different neural and nonneural tissues by reverse transcriptase-polymerase chain reaction (RT-PCR) revealed that whereas FGFR1 and R2 were strongly expressed in adult cortex, cerebellum, retina, and kidney, robust FGFR4 expression was only seen in retina and kidney. FGFR4 mRNA was present within fractions of the outer and inner nuclear layers isolated from adult rat retinas, and could also be detected in pure photoreceptor cultures prepared from young rat retinas. On the contrary, FGFR4 mRNA could not be detected in primary cultures of retinal Müller glia or pigment epithelium, indicating specific enrichment in retinal neurons. In situ hybridization studies of adult rat retina showed FGFR4 expression in all retinal cellular layers, especially prominent in the outer nuclear layer. FGFR4 protein was detected by immunoblotting of homogenates of rat retina, with specific antibody binding to bands at 115, 47, and 30 kDa. FGFR4 mRNA and protein were also reliably detected in postmortem adult human retina. The potential roles of these signal transduction molecules in FGF-induced biological responses in the retina are discussed.

Index Entries: Fibroblast growth factor receptors; mRNA; protein; central nervous system; retina; photoreceptors; RT-PCR; in situ hybridization; immunoblotting.

*Author to whom all correspondence and reprint requests should be addressed.
Introduction

The fibroblast growth factor (FGF) family encompasses at present about 20 factors, which are 30–70% identical in their primary sequences (Burgess and Maciag, 1989; see Goldfarb, 1996 for review). Acidic FGF (FGF-1) and basic FGF (FGF-2) lack signal sequences for conventional export out of producer cells, whereas most other FGF family members possess signal sequences, and several of them have been identified as transforming proteins (Grothe and Wewetzer, 1996). FGFs are potent mitogens for a wide variety of cell types in vivo and in vitro, and are implicated in differentiation of endotheial and neuronal cells. FGFs are expressed in a strict temporal and spatial pattern during development and have important roles in patterning and limb formation (Goldfarb, 1996; Grothe and Wewetzer, 1996). The FGFs bind in an overlapping pattern to four structurally related receptor tyrosine kinases, FGF receptors 1–4 (FGFR1–4) (Johnson and Williams, 1993; Klint and Claesson-Welsh, 1999). Like the FGFs these receptors are widely distributed in normal and neoplastic tissues, and share ~55–70% amino acid identity. FGFR1, R2, and R3 exist as multiple variants through the generation of additional isoforms by alternative splicing (Johnson et al., 1990; Reid et al., 1990; Champion Arnau et al., 1991; Eisemann et al., 1991; Miki et al., 1992; Avivi et al., 1993). The high sequence similarity between the FGFR together with the overlapping pattern of FGF binding, i.e., most FGFs bind to all four FGFR (Ornitz et al., 1996), implies redundancy within this growth factor-receptor family (Klint and Claesson-Welsh, 1999). In spite of this redundancy, targeted gene inactivation of different FGF and FGFR members yields a specific phenotype for each factor. Low-affinity receptors in the form of heparan sulfate proteoglycans are also involved in FGF signaling (Yayon et al., 1991).

FGFR1 and R2 are known to be widely expressed in the developing and adult central nervous system (CNS), including the retina (Heuer et al., 1990; Wanaka et al., 1990; Asai et al., 1993; Tcheng et al., 1994; Yazaki et al., 1994; Grothe and Wewetzer, 1996). FGFR4 expression, however, has been reported to be absent from adult CNS (Yazaki et al., 1994; Miyake and Itoh, 1996). The aim of the present study was to examine the expression of FGFR1, R2, and R4 in several structures of the adult rat CNS and nonneural tissues. The data show that within the CNS, adult retina are highly exceptional in expressing high levels of FGFR4, suggesting that this member plays an important role in differentiated retinal function or survival.

Materials and Methods

Biological Material

Adult (3- to 6-mo) Long-Evans rats were anesthetized and killed, and tissue dissected rapidly from different regions of the CNS (cortex, cerebellum, and retina) and kidney (positive control for FGFR4 expression). Slices of outer (photoreceptors) and inner (other neurons and glia) retina were obtained by vibratome sectioning (Fontaine et al., 1998) to isolate specific fractions of adult rat retina. Purified primary cell cultures of retinal pigment epithelium, retinal Müller glia, and photoreceptors were prepared according to the protocols of Edwards (1981), Hicks and Courtois (1990), and Fontaine et al. (1998), respectively.

Small specimens of postmortem human retina were obtained within 24 h following death, and treated either for total RNA extraction or for immunoblotting (see below). The human breast cancer cell line SUM1315M02, which overexpresses FGFR4 (generous gift of S. Ethier, University of Michigan Breast Cell/Tissue Bank and Database, Ann Arbor, MI) was used as another positive control. Cells were cultured in Ham’s F-12 medium supplemented with 5% fetal calf serum, insulin, and epidermal growth factor, washed and prepared as described below.

Preparation of RNA

Total RNA was extracted from each tissue and cell preparation by the acid guanidium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987).

Detection of FGFR1, R2 and R4

DNA fragments of β-actin, R1, R2, and R4 were amplified from total rat and human retinal RNA.