Alternate FGF2-ERK1/2 Signaling Pathways in Retinal Photoreceptor and Glial Cells in Vitro*

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Basic fibroblast growth factor (FGF2) stimulates photoreceptor survival in vivo and in vitro, but the molecular signaling mechanism(s) involved are unknown. Immunohistochemical and immunoblotting analyses of pure photoreceptors, inner retinal neurons, and Müller glial cells (MGC) in vitro revealed differential expression of the high affinity FGF receptors (FGFR1–4), as well as many cytoplasmic signaling intermediates known to mediate the extracellular signal-regulated kinase (ERK1/2) pathway. FGF2-induced tyrosine phosphorylation in vitro exhibited distinct profiles for each culture type, and FGF2-induced ERK1/2 activation was observed for all three preparations. Whereas U0126, a specific inhibitor of ERK kinase (MEK), completely abolished FGF2-induced ERK1/2 tyrosine phosphorylation and survival in cultured photoreceptors, persistent ERK1/2 phosphorylation was observed in cultured inner retinal cells and MGC. Furthermore U0126 treatment entirely blocked nerve growth factor-induced ERK1/2 activation in MGC, as well as FGF2-induced ERK1/2 activation in cerebral glial cells. Taken together, these data indicate that FGF2-induced ERK1/2 activation is entirely mediated by MEK withinphotoreceptors, which is responsible for FGF2-stimulated photoreceptor survival. In contrast, inner retina/glia possess alternative, cell type, and growth factor-specific MEK-independent ERK1/2 activation pathways. Hence signaling and biological effects elicited by FGF2 within retina are mediated by cell type-specific pathways.

Basic fibroblast growth factor (FGF2)† belongs to a family of structurally related polypeptides, encompassing at present over 20 factors (1, 2) that stimulate growth and differentiation of cells of mesodermal and neuroectodermal origin (3). In the central nervous system, FGF2 is expressed widely in neuronal and glial cells (4, 5) and possesses neuroprotective effects both in vivo (6) and in vitro (7). FGF2 mediates its biological effects via binding and activation of specific high affinity tyrosine kinase receptors, named FGFR1–4 (8, 9).

FGF signal transduction has been intensively studied, mostly in non-neuronal tissue, using immortalized cell lines overexpressing one or more FGFR (10–12), and a variety of molecules involved in the FGF signal transduction cascade have been described: phospholipase Cγ1 (PLCγ1), a regulator of phosphatidylinositol mechanism (13); the adaptor protein Shc; son of sevenless (SOS), a guanine nucleotide exchange factor for ras and growth factor receptor-binding protein 2 (Grb2) (12); a phosphotyrosine phosphatase designated as SH-PTP2 (14); and protein kinase B (Akt) (15). These molecules activate downstream signaling pathways, including those of the mitogen-activated protein kinase (16), also known as extracellular signal-regulated kinases (ERK), a family of serine/threonine protein kinases. The ERK signal transduction cascade is common to many different cell types including neurons (17–19). Mammalian ERK1 (p44) and ERK2 (p42) are the best known and best studied members of the MAPK family (20). Activation of ERK1 and ERK2 occurs via phosphorylation by dual-specificity MAPK kinases, MEK1 and MEK2, and is often associated with the stimulation of cell proliferation, differentiation, and survival (21–23).

Within the central nervous system, especially the retina, FGF2 has shown promise for therapeutic treatment of neurodegeneration. FGF2 delays photoreceptor (PR) breakdown in various rat models in vivo (24–26), and PR-targeted FGFR inactivation leads to retinal degeneration in mice (27). In vitro, FGF2 induces PR differentiation (28) and directly stimulates survival of purified PR (29). The complexity of the central nervous system has rendered the analysis of molecular pathways underlying neurotrophic actions very difficult, but their understanding is essential for the development of rational therapeutic strategies. Although recent studies have implicated ERK activation in FGF2 signaling (30, 31), for the moment there is no evidence that PR survival is directly influenced via the ERK pathway. We have exploited primary cultures of different retinal cells (purified PR, inner retina (IR, without PR), and purified Müller glial cells (MGC)) to determine whether FGF2-related signaling molecules have distinct expression patterns between the different cell populations and whether FGF2 possesses multiple pathways for signal transduction in the retina. This is indeed the case, PR survival depending uniquely upon ERK activation via MEK. Whereas in FGF2-treated PR, MEK seems to be the only upstream ERK activator, additional MEK-independent pathways exist in IR and MGC.

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1 The abbreviations used are: FGF2, basic fibroblast growth factor; FGFR,FGF receptor; Akt, protein kinase B; BSA, bovine serum albumin; CD, chemically defined medium; DMEM, Dulbecco’s modified Eagle’s medium; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum;Grb2, growth factor receptor binding protein 2; IR, inner retina; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; MGC, Müller glial cells; NGF, nerve growth factor; PBS, phosphate-buffered saline; PLCγ1, phospholipase Cγ1; PR, photoreceptors; SOS, son of sevenless.

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Alternate FGF2-ERK1/2 Signaling in Retina Cells in Vitro

EXPERIMENTAL PROCEDURES

Materials—Dulbecco's modified Eagle's medium (DMEM), CO₂-independent DMEM (DMEM/CO₂), fetal bovine serum (FBS), trypsin, and molecular weight markers were purchased from Life Technologies, Inc. (Cergy-Pontoise, France). Fovine serum albumin (BSA), desoxyribonuclease 1 (DNase 1), gelatin, laminin, poly-t-lysine, and all other reagents for cell culture were from Sigma-Aldrich (Saint Quentin Fallavier, France). Papain and collagenase were obtained from Worthington (Freehold, NJ). Recombinant human FGF2 was from Stratmann-Biotech (Hamburg, Germany), and nerve growth factor (NGF) was from Chemicon International (Temecula, CA). U1026 was obtained from Promega (Madison, WI). Anti-phosphotyrosine and anti-phos-specific antibodies were from Upstate Biotechnology (Lake Placid, NY). Antibodies to FGF1, FGF2, FGF3, FGF4, SOS1, SOS2, FGFR1, FGFR2, FGFR3, FGFR4, anti-rabbit IgG/Alexa Fluor™ 488 secondary antibodies. Immuno-staining of cultured retinal cells with antisera directed against growth factor receptor. Growth factor or Inhibitor Treatment—PR and IR were cultured for 24 h in DMEM/10% FBS. Then cells were split with DMEM and replaced with a defined medium (CDM) for 48 h. Confluent MGC and brain glial cell cultures grown in DMEM/10% FBS were split with DMEM and replaced in CDM for 48 h. Cultures were stimulated with FGF2 (100 ng/ml) for 30 min to 30 min prior to growth factor stimulation. Two additional trials using U1026 were performed: MGC were preincubated with U1026 (10 μM) for 30 min and stimulated with NGF (10 ng/ml) for 30 min, and mixed cerebral glia were preincubated with U1026 (10 μM) for 30 min and stimulated with FGF2 (100 ng/ml) for 30 min.

Photoreceptor Survival Assay—The survival assay for PR was conducted as described previously (29). Briefly, after 24 h DMEM/10% FBS was replaced by CDM supplemented or not with FGF2 (20 ng/ml) and U1026 (100 nm to 1 μM). FGF2 and U1026 were added again after 72 h. After 5 days in vitro the viability of PR was tested by the Live/Dead assay (37). For each coverslip 25 fields were recorded (using 20× objective for observation) using Visiulab 1000 image analysis software (Bio-Com, Lyon, France), and cells were counted. For each treatment in each experiment two coverslips were counted and the experiment conducted three times. Statistical analysis was performed using the parametric Peritz test according to Harper (38), values of $p < 0.05$ being considered statistically significant.

**Protein Extraction and Western Blotting**—For immunodetection of FGF1–4, Akt, ERK1/2, Grb2, MEK1, MEK2, PLC-y1, Shc, SH-PTP2, SOS1, SOS2, arrestin, and vimentin, cultures of PR, IR (3 d in vitro), and MGC were rinsed with PBS and collected in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM NaF, 1 mM NaVO₃) containing a protease inhibitor mixture. For anti-phosphotyrosine and anti-phospho-ERK1/2 immunoblots, cultures were washed once with PBS and stimulated with growth factors for different times. The reaction was stopped by addition of liquid nitrogen. Cells were then collected in lysis buffer and lysed as above. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. For probing with anti-phosphotyrosine antibody, the membranes were blocked with PBS, 0.2% Tween 20, 3% BSA, 1% free milk powder for 1 h at room temperature. For all other antibodies membranes were blocked PBS, 0.1% Tween 20, 5% fat-free milk powder for 1 h at room temperature. Membranes were then incubated with primary antibodies (anti-FGFR, signaling intermediates, and cell type-specific markers, each 1 μg/ml final concentration) overnight at 4°C. Membranes were then incubated with goat anti-mouse or goat anti-rabbit IgG-horseradish peroxidase secondary antibodies (0.15 μg/ml). Immunoreactive bands were visualized using a Pierce Super Signal West Pico kit according to the manufacturer's instructions. In some trials Western blots probed with anti-phospho-ERK1/2 antibody were stripped and subsequently reprobed with anti-ERK1/2 antibody. Molecular weights were compared with prestained molecular weight markers.

**RESULTS**

FGFR and FGFR-related Signaling Molecules Are Widely Expressed in Retinal Cells in Vitro—Antisera against FGFR1–4 were used to investigate expression and distribution of FGFR and second messengers in the different culture models. FGFR1 and FGFR4 were clearly and uniformly expressed at the surface of cultured PN5 PR, whereas FGFR2 and especially FGFR3 stained only weakly (Fig. 1, A–H). Within cultures of IR, which contain a mixed population of neurons (biopol, amacrine, and ganglion cells) and MGC, all four FGFR were detected (Figs. 1, I–P). FGFR1 stained neurons and glia with equal intensity, while FGFR2–4 labeled neurons more intensely than MGC. If antibodies were used that had been preadsorbed with their corresponding immunizing peptides, staining was completely abolished (data not shown). Immunostaining of cultured retinal cells with antisera directed against signaling molecules (PLC-y1, SOS1, SOS2, ERK, SH-PTP2, SHC) showed uniform PR, neuronal, and glial labeling (data not shown).

Expression of FGFR1–4 and the various signaling molecules were further compared between different culture models using Western blotting techniques. Culture purity was checked using cell type-specific antibodies; arrestin immunoreactivity was not detected (data not shown).
specifically expressed in PR but was absent from IR and MGC cultures, whereas vimentin immunoreactivity was detected in IR and MGC but not in PR cultures (Fig. 2A).

FGFR1 was detected in PR at a molecular mass of ~120 kDa. In IR, immunopositive bands were detected at 140 and 100 kDa and in MGC at 150, 125, and 100 kDa. FGFR2-positive bands were found in PR, IR, and MGC at ~90 kDa. Additional bands were observed in MGC cultures at 130 and 100 kDa. Western blots probed with the FGFR4 antibody revealed a single band at ~120 kDa in PR, IR, and MGC. For all receptors MGC exhibited stronger signals compared with PR and IR (Fig. 2B). If antibodies were preadsorbed with their corresponding immunizing peptides prior to immunoblotting, no positive bands were observed (data not shown). We were not able to obtain satisfactory results on Western blots using the FGFR3 antibody.

Western blots for FGF-related signaling molecules revealed that despite loading of equal total protein concentrations between fractions, PR preparations showed consistently lower relative amounts of each protein examined compared with IR and MGC (Fig. 3). Levels of PLCγ1, SOS2, Akt, and Grb2 were markedly lower in PR than in IR and MGC, while levels of SOS1 and SH-PTP2 were equivalent. Specific differences in expression of ERK1/2 and Shc were observed; while ERK2 was present at similar levels between the three samples, ERK1 was notably reduced in PR, and while three isoforms of Shc at 46, 52, and 66 kDa were observed in both IR and MGC, the 46-kDa isoform was very weak and the 66-kDa isoform undetectable in PR (Fig. 3). SOS1 and Akt were more abundant in pure MGC than in mixed IR cultures.

FGF2 Induces Cell-specific Signal Cascades in Different Populations of Retinal Cells in Vitro—To examine FGF2-induced tyrosine phosphorylation, PR, IR, and MGC cultures were incubated for increasing times with a fixed dose of FGF2 and samples immunoblotted with a phosphotyrosine-specific antibody. In PR cultures, as described previously (29), we observed time-dependent tyrosine phosphorylation of major bands at ~120 kDa in PR, IR, and MGC. For all receptors MGC exhibited stronger signals compared with PR and IR (Fig. 2B). If antibodies were preadsorbed with their corresponding immunizing peptides prior to immunoblotting, no positive bands were observed (data not shown). We were not able to obtain satisfactory results on Western blots using the FGFR3 antibody.

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kDa demonstrated highest staining at 2 min, and the doublet at 55 and 53 kDa became maximally stimulated after 5 min. MGC cultures treated with FGF2 showed the same pattern of tyrosine-phosphorylated bands as IR and one additional band with a molecular mass >200 kDa (Fig. 4C). The band at 120 kDa showed maximal stimulation already after 30 s and maintained this level up to 5 min. The band at 65 kDa showed increased phosphorylation after 30 s and reached its maximum at 1 min. The 90-kDa band showed only a weak increase in phosphorylation between 30 s and 2 min but showed a strong phosphorylation after 5 min. The bands at 180 and >200 kDa increased steadily in intensity over the duration of incubation. The different phosphotyrosine profiles for each culture model are schematized in Fig. 6D to facilitate comparison.

FGF2 Induces Activation of ERK1/2 in Vitro—As the ERK pathway represents a major pathway for FGF2-induced signaling in neurons (19) and glia (39), we investigated whether exogenous application of FGF2 could induce phosphorylation of ERK1 and ERK2 in PR, IR, and MGC cultures in vitro. Cultures were incubated for 15 or 30 min with FGF2, and ERK1/2 activation was monitored by immunoblotting using phospho-ERK1/2-specific antibody. As shown in Fig. 5, after 15 min of stimulation IR and MGC cultures showed an increase in phosphorylated forms of ERK1/2, whereas no ERK activation was observed in PR. Within IR samples the increase was almost completely restricted to ERK2, whereas MGC showed equally intense labeling for both isoforms. After 30-min stimulation the phosphorylation of ERK1/2 had further increased in IR and MGC, and for IR samples was now also elevated in ERK1. Phospho-ERK1/2 was also visible in PR at this time point, predominantly in ERK2, showing the direct activation of the ERK pathway by FGF2 in these cells.

FGF2-induced ERK1/2 Activation Is Blocked Completely by the MEK Inhibitor U0126 in PR but Not in IR and MGC—Since exogenous application of FGF2 led to activation of the ERK pathway in PR, IR, and MGC, we examined whether we could suppress the ERK1/2 activation with the MEK1- and MEK2-specific inhibitor U0126 (40). Cultures of PR, IR, and MGC were preincubated with U0126 and then stimulated with FGF2 for 30 min and phospho-ERK1/2 detected as above. U0126 completely blocked ERK1/2 activation in PR at concentrations of 10 and 20 μM (Fig. 6A). In contrast, in IR and MGC the phosphorylation of ERK1/2 was decreased but not completely blocked (Fig. 6A). U0126 used at 20 μM led to a 2-fold decrease in ERK1/2 phosphorylation, but compared with control cultures, the activation of ERK1/2 was still marked. Even a concentration of 50 μM U0126 did not further decrease ERK1/2 phosphorylation in IR and MGC (data not shown). Use of an-
other MEK inhibitor PD098059 (50 and 100 μM) to block ERK activation in MGC confirmed these results (data not shown).

To verify that the persistent ERK phosphorylation in IR and MGC was not due to incomplete inhibition of MEK, two different controls were performed. Neonatal rat brain glia were treated with FGF2 in the presence or absence of U0126; the inhibitor entirely blocked FGF2-induced ERK1/2 phosphorylation, with only residual background activation remaining (Fig. 6B). In the second, MGC cultures were stimulated with NGF in the presence or absence of U0126; whereas NGF induced sustained ERK1/2 activation in MGC, in cultures preincubated with U0126 this effect was completely inhibited (Fig. 6C). These two results showed clearly that U0126, at the concentrations used, efficiently blocked MEK and inhibited downstream ERK phosphorylation. No differences in protein expression levels of MEK1 and MEK2 were observed in the retinal cultures; Western blots revealed distinct and uniformly expressed bands at 42 and 45 kDa for MEK1 and MEK2, respectively, in PR, IR, and MGC (Fig. 7).

**Survival-promoting Activity of FGF2 Is Mediated by the ERK Pathway in PR in Vitro**—We previously reported that survival of purified PR in vitro is directly stimulated by FGF2 (29). To see whether this effect of FGF2 was mediated by ERK1/2 activation, survival of control and FGF2-treated PR after 1 or 5 days in vitro was monitored using a cytotoxicity assay kit. Survival at 5 days was chosen because the previous study showed the survival-promoting effect of FGF2 to be maximal at this time. At this time point, significantly more PR survived in the presence of 20 ng/ml FGF2 than untreated PR: 60% in the presence of FGF2 compared with 40% in controls or rescue of 50% PR that would have died in the absence of exogenous factor. If PR were cultivated in the presence of different concentrations of the MEK inhibitor U0126 (0.1–1 μM), the survival-promoting effect of FGF2 was completely abolished, and the survival rate dropped to the level of untreated controls (Fig. 8).

**DISCUSSION**

**FGF2-induced Tyrosine Phosphorylation in Retinal Cells in Vitro**—Despite the presence of all FGFR and candidate signaling intermediates in PR, IR, and MGC, FGF2-induced tyrosine phosphorylation profiles and kinetics differed between them. IR and pure MGC had largely comparable patterns of tyrosine phosphorylation, which is normal given that IR contain significant numbers of MGC. There were distinct differences between IR and MGC, however, especially the presence of prominent >200- and 65-kDa phosphoproteins in pure MGC. Such changes may be due to different culture conditions (prolonged exposure to serum and increased proliferation in MGC) or lack of neuron-glia interactions. PR showed a very different pattern with several phosphorylated bands between 74 and 155 kDa, absent from IR and MGC. These various phosphotyrosine proteins could represent differentially expressed FGFR isoforms or signaling molecules, differential recruitment of common signaling molecules, or differential phosphatase activity. Our efforts to assign specific phosphotyrosine-immunoreactive bands to individual candidate proteins were not successful. Particularly the identity of the major phosphotyrosine protein at ~140 kDa in PR is puzzling, since this band potentially represents an FGFR through the rapidity and intensity of FGF-induced activation, yet none of the data obtained for FGFR1, FGFR2, or FGFR4 reveal the presence of such a molecular mass. A second major phosphotyrosine band of ~120 kDa within PR cultures...
also exhibits rapid activation kinetics and may correspond to the Western blotting data for FGFR1 and/or FGFR4. The major phosphotyrosine band in MGC at 110 kDa coincides with both FGFR1- and FGFR4-immunoreactive proteins. In Western blots of FGF-treated IR and MGC, phosphotyrosine-immunoreactive bands at 90 kDa match up with the FGFR2-immunopositive label. Western blots of PR demonstrated FGFR2-immunoreactive bands of the same weight, but we did not observe any FGF-induced activation. The Western blotting data revealed that MGC expressed higher levels of all FGFR, as well as several signaling intermediates, than either PR or IR. Since IR cultures themselves contain many MGC, this upregulation may result from either more prolonged culture conditions or alterations in neuron-glial interactions. In summary to this section, the data demonstrate that even in highly purified and simplified neuronal and glial populations it is very difficult to attribute individual phosphotyrosine proteins to specific identified signaling molecules by such methods. Even so, the numerous differences in phosphorylation patterns and kinetics between cell types indicate the existence of distinct signaling pathways within retinal tissue.

**FIG. 6.** Effect of U0126 on FGF2-induced ERK activation in vitro. A, cultures of PR, IR, and MGC were preincubated with the MEK inhibitor U0126 for 30 min and then stimulated with FGF2 (100 ng/ml) for 30 min. Total proteins (20 μg/lane) were analyzed by Western blot using anti-phospho-ERK antibody. ERK activation was completely blocked in PR in the presence of 10 μM U0126. In IR and MGC, phosphorylated ERK1/2 was still clearly detectable. U0126 at 20 μM led to a 2-fold reduction in intensity of ERK immunolabeling in IR and MGC compared with cultures stimulated with FGF2 alone, but ERK activation was still marked. In each case (PR, IR, MGC), the upper row shows the treatments used (FGF2, U0126 at 10 or 20 μM), the middle row shows immunodetection of phospho-ERK (pERK1/2), and the lower row shows immunodetection of total ERK1/2 (Western blots were stripped and re-probed with antibodies to ERK1/2). Panels are representative of three independent experiments that gave similar results.

**FIG. 7.** Expression of MEK1 and MEK2 in different populations of rat retinal cells in vitro. Western blots of proteins (20 μg/lane) prepared from PR, IR, and MGC cultures were probed with MEK1- and MEK2-specific antibodies. The cultures showed equivalent expression of MEK1 (42 kDa) and MEK2 (45 kDa) proteins. Western blot panels are representative of three different experiments that gave similar results.

**FIG. 8.** FGF2-induced PR survival in vitro is modulated by the ERK pathway. Relative percentages of surviving PR cultured from PN5 rat retinas were compared at 5 days in vitro with the number at 24 h in vitro. After 24 h, DMEM/10% FBS was replaced with CDM with or without FGF2 (20 ng/ml) and the MEK inhibitor U0126 (0.1, 0.5, or 1 μM). FGF2 and U0126 were added again after 72 h in vitro. FGF2-treated PR survived significantly better than untreated control cultures. The survival-promoting effect of FGF2 was completely abolished in the presence of U0126. Error bars are S.E.M. (n = 3). C, control. Statistical analysis: asterisks above FGF2-treated culture with respect to untreated control: ***, p < 0.005. Small circles above FGF2/U0126-treated cells with respect to FGF2 treatment alone: three small circles, p < 0.005; two small circles, p < 0.01; one small circle, p < 0.05.

Alternate FGF2-ERK1/2 Signaling in Retina Cells in Vitro

Also exhibits rapid activation kinetics and may correspond to the Western blotting data for FGFR1 and/or FGFR4. The major phosphotyrosine band in MGC at ~125 kDa coincides with both FGFR1- and FGFR4-immunoreactive proteins. In Western blots of FGF-treated IR and MGC, phosphotyrosine-immunoreactive bands at ~90 kDa match up with the FGFR2-immunopositive label. Western blots of PR demonstrated FGFR2-immunoreactive bands of the same weight, but we did not observe any FGF-induced activation. The Western blotting data revealed that MGC expressed higher levels of all FGFR, as well as several signaling intermediates, than either PR or IR. Since IR cultures themselves contain many MGC, this upregulation may result from either more prolonged culture conditions or alterations in neuron-glial interactions. In summary to this section, the data demonstrate that even in highly purified and simplified neuronal and glial populations it is very difficult to attribute individual phosphotyrosine proteins to specific identified signaling molecules by such methods. Even so, the numerous differences in phosphorylation patterns and kinetics between cell types indicate the existence of distinct signaling pathways within retinal tissue.
ERK Signaling in Retinal Cells in Vitro—ERK1/2 are implicated in a diverse array of cellular functions, such as cell growth and proliferation, differentiation, and apoptosis (41), and activation of the ERK cascade occurs in response to different environmental stimuli, including growth factors (42, 43) and neurotrophins (44, 45). FGF2 application led to time-dependent ERK1/2 phosphorylation in PR, IR, and MGC in vitro. It has been reported previously that FGF2 can induce ERK activation in glia (46, 47) and cortical neurons (48) in vitro. Other groups have demonstrated that FGF2-induced ERK activation regulates neurite outgrowth or neuronal survival in embryonic chicken retina in vitro (30, 49). It has not been shown previously that FGF2 can directly induce ERK activation in PR. Indeed Wahlin et al. (31) were unable to show FGF2-induced activation of intracellular signaling pathways in PR in vitro and concluded that FGF2 exerts its effects by acting indirectly through the activation of MGC or other IR cells. FGF2 clearly induced ERK phosphorylation in purified postmitotic PR in vitro, but with slower kinetics than that observed for other retinal cells. Pretreatment of PR with the specific MEK inhibitor U0126 abolished FGF2-induced ERK1/2 phosphorylation and completely inhibited FGF2-induced PR survival, demonstrating that FGF2-dependent PR survival is mediated entirely by ERK1/2 activation via MEK.

Interestingly, U0126 pretreatment of IR and MGC reduced, but did not abolish, FGF2-induced ERK1/2 phosphorylation in IR and MGC. This was surprising, since ERK1/2 activation has been reported to be uniquely regulated by MEK (50, 51). It has been reported previously that FGF2 can induce ERK phosphorylation and completely inhibited FGF2-induced PR survival, demonstrating that FGF2-dependent PR survival is mediated entirely by ERK1/2 activation via MEK.

ERK1/2 phosphorylation in MGC, were completely blocked in ERK1/2 phosphorylation in brain glial cells, and NGF-induced FGF2 plausibly acts by preventing apoptotic PR cell death, since serum deprivation induces apoptosis in primary neurons (27) or homologous recombination technology. In addition, FGF2 plausibly acts by preventing apoptotic PR cell death, since serum deprivation induces apoptosis in primary neurons in vitro (66, 67), neurotrophic factor withdrawal induces apoptosis during development (68), and FGF2 prevents apoptosis in embryonic chicken retinal cultures (30). Future work will focus on the identification of MEK-independent ERK activators in the retina and on the role of individual signaling molecules in PR survival through the use of dominant-negative (27) or homologous recombination technology. Such studies will address how FGF2 affects pro- and anti-apoptotic pathways. Such studies will provide impetus to the application of such neurotrophic factors as potential therapies for inherited retinal degeneration.

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