Endogenous FGF1-induced Activation and Synthesis of Extracellular Signal-regulated Kinase 2 Reduce Cell Apoptosis in Retinal-pigmented Epithelial Cells*

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Xavier Guillonneau, Marijke Bryckaert‡, Catherine Launay-Longif, Yves Courtois, and Frédéric Mascarelli‡

From the Développement, Vieillissement et Pathologie de la Rétine, INSERM U. 450, Affiliée CNRS, Association Claude Bernard-29, rue Wilhem, 75016, Paris, France and §INSERM U. 348, IFR Circulation, 75010 Paris, France

Retinal-pigmented epithelial (RPE) cell survival is critical to the maintenance of the function of the neural retina and in the development of various retina degenerations. We investigated molecular mechanisms involved in this function by assessing apoptosis in RPE cells following serum deprivation. Apoptosis induced by serum withdrawal is lower in aged RPE cells because of higher endogenous acidic fibroblast growth factor (FGF1) synthesis and secretion. These experiments examined several aspects of FGF signaling and the contribution of endogenous FGF1 to activation of the extracellular signal-regulated kinase 2 (ERK2). In aged RPE cells, FGFFR1 was rapidly activated, and its autophosphorylation followed the kinetics of endogenous FGF1 secretion, before the onset of apoptosis. ERK2 phosphorylation, activity, and de novo synthesis increased at the same time. In marked contrast, no de novo JNK1 synthesis was observed. MEK1 inhibition resulted in lower levels of ERK2 activation and synthesis and higher levels of apoptosis. Treatment with neutralizing anti-FGF1 or blocking anti-FGF1 antibodies mimics these effects. Thus, this study strongly suggests that the survival-increasing effect of FGF1 in aged RPE cells is because of an autocrine/paracrine loop in which the ERK2 cascade plays a pivotal role.

Fibroblast growth factors (FGFs) are a family of at least 15 polypeptides that stimulate growth and differentiation in cells of various mesenchymal and ectodermal origins. (for reviews, see Refs. 1–5). Acidic FGF (FGF1) and basic FGF (FGF2) are the prototype members of this family. FGF1 and 2 lack a classic signal peptide (6, 7), implying that they are not secreted by the classical secretion pathway. There is evidence that FGF1 and 2 are exported from the cell and subsequently act as autocrine or paracrine factors (8, 9). FGF1 and 2 exert their effects via high affinity tyrosine kinase receptors (FGFR1–FGFR4) (for reviews, see Refs. 10–12) and via lower affinity heparan sulfate proteoglycan binding sites (13–15). FGFR activation causes tyrosine phosphorylation of the receptor itself, intracellular proteins including phospholipase Cγ (PLCγ) (16), extracellular signal-regulated kinases (ERKs) (17), and of several uncharacterized proteins of 80–90 kDa (18, 19). All cell layers in normal adult retina produce FGF1, as cells no longer differentiate or proliferate. The pattern of FGF1 production suggests that FGF1 may be involved in the regulation of specific spatiotemporal events, including proliferation, migration, differentiation and survival in the retina. In vivo, despite the presence of FGFs in the retinal interphotoreceptor matrix, RPE cells have a limited proliferation capacity consistent with the normal increase in retinal space associated with growth and age. In adult, RPE cells cannot divide in vivo, but they may still require survival factors to inhibit their apoptosis. Cell survival and proliferation may be determined by the amount of factor available and the amount of receptor produced (20). RPE cells produce FGF1 (21), FGFR (22, 23), and low affinity binding sites (heparan sulfate proteoglycan) (24) in culture. In RPE cells, ERKs, also known as mitogen-activated protein (MAP) kinases, undergo rapid and biphasic activation in response to exogenous FGF1 and FGF2 (24).

Unlike exogenous FGF1, endogenous FGF1 is not a mitogenic factor. It is a survival factor, both for nondifferentiating epithelial cells (25) and for neuronal cells of the PC12 line (26). We have also shown that the FGF2-stimulated release of endogenous FGF1 is associated with lower levels of apoptosis in RPE cells (27), whereas FGF1 secreted by RPE cells and purified from culture medium causes RPE and retinal Müller glial (RMG) cell proliferation and survival via ERK2 activation (28). These data suggested these may be an FGF1 autocrine/paracrine pathway supporting RPE cell survival. FGF activity is regulated in RPE cell subcultures (24). So we used an in vitro approach to investigate several aspects of FGF signaling, including the production and excretion of endogenous FGF1, the expression and autophosphorylation of FGFR1 (the major FGF receptor in RPE cells), and the production and activation of ERK2 in quiescent, confluent RPE cell subcultures, as a function of cell survival. RPE cell cultures from bovine eyes were made to age in vitro by repeated culture passage, an aging strategy termed replicative senescence. This study shows that the amount of FGF1 controls cell survival by FGFR1 activation and by affecting the activation and the production of ERK2. Therefore, long term activation of MAP kinase and up-regulation of its production may be involved in integrating and transmitting transmembrane signals for cell survival.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**Bovine RPE cells were isolated as described previously (24). RPE cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) containing 10% fetal calf serum (Life Technol-
ERK2 Activation and Synthesis in Aged RPE Cells

Aged RPE Cells Are More Resistant to Apoptosis and Synthesize More FGF1 after Serum Withdrawal

Apoptosis Is Lower in Aged RPE Cell Cultures in Serum-Free Conditions—Latter passages (5th to 11th) of RPE cells were less sensitive to apoptosis after serum withdrawal than early passages (Fig. 1A). We used the TUNEL method to identify PCD at the single cell level and found that, until day 3 of culture, there was less than 1.1% TUNEL-labeled nuclei in late RPE cells. This is similar to the value obtained for cells cultured in the presence of serum. In aged RPE cells, the number of cells undergoing PCD doubled on day 5 and was 5.2-fold higher on day 7. Early passages of RPE cell cultures in the absence of serum underwent apoptosis more rapidly than later passages. In early passages, the number of cells undergoing PCD increased and was 2.5-fold higher (2.5% PCD) on day 5, 13-fold higher (10.5% PCD) on day 5, and 21-fold higher (16.8% PCD) on day 7 than the basal level of PCD in RPE cells on day 1 of culture (0.8% PCD). The protein bands detected on the autoradiograph were quantified using a LKB Ultrascan XL laser densitometer (Amersham Pharmacia Biotech).

Statistics—Each figure shows the results of experiments repeated at least three times. All data are expressed as the mean ± S.E. Statistical comparisons were performed using the two-tailed Student’s t test (Gaussian populations with equal S.D.) and the Wilcoxon or Mann and Whitney test (nonparametric).

RESULTS

Aged RPE Cells Are More Resistant to Apoptosis and Synthesize More FGF1 after Serum Withdrawal

Aged RPE cells were incubated in serum-free medium for 7 days. They were washed twice in phosphate-buffered saline and lysed in ice-cold Triton X-100 lysis buffer and centrifuged at 4 °C for 10 min at 10,000 × g. Monoclonal antibody directed against β-actin was used as an internal standard for control of protein loading. For ERK2, JNK1, and FGF1 FGF1 analysis, 30, 80, and 100 μl of Triton X-100 cell lysate, respectively, were mixed with 5× Laemmli’s sample buffer and heated for 5 min at 95 °C. The soluble protein of the cell lysates was separated by SDS-PAGE (12% polyacrylamide gel) (34). Western Blot Analysis—RPE cells were incubated in serum-free medium for 7 days. They were washed twice in phosphate-buffered saline and lysed in ice-cold Triton X-100 lysis buffer and centrifuged at 4 °C for 10 min at 10,000 × g. Monoclonal antibody directed against β-actin was used as an internal standard for control of protein loading. For ERK2, JNK1, and FGF1 FGF1 analysis, 30, 80, and 100 μl of Triton X-100 cell lysate, respectively, were mixed with 5× Laemmli’s sample buffer and heated for 5 min at 95 °C. The soluble protein of the cell lysates was separated by SDS-PAGE (12% polyacrylamide gel for ERK2 and 7% polyacrylamide gel for JNK1 and FGF1 FGF1 analysis). Protein samples were electroblotted onto nitrocellulose filters, and probed with monoclonal antibodies raised against p42 ERK2, p46 JNK1, and FGF1 FGF1 (Santa Cruz). The primary antibodies were detected using a horseradish peroxidase-conjugated goat anti-rabbit second antibody. Enhanced chemiluminescence substrates were used to detect positive bands, according to the manufacturer’s instructions, and the membrane was used to expose Hyperfilm (Amersham Pharmacia Biotech). The protein bands detected on the autoradiograph were quantified using a LKB Ultrascan XL laser densitometer (Amersham Pharmacia Biotech).
of total protein) on day 3 was eight-fold higher in late passage RPE cells than in early passage cells (10^6 ± 2 ng/mg of total protein). Thereafter, the amount of FGF1 decreased on day 4 and then reached a plateau (48 ± 4 ng/mg of total protein). In contrast, the amount of endogenous FGF1 in early passage RPE cells did not change during the first 3 days of culture (Fig. 1C). The concentration of FGF1 then increased significantly and was 2.5-fold higher than the basal level on the 4th day of culture (29 ± 2.5 ng/mg of total protein). It reached a plateau by the 7th day of culture.

In addition, the amount of secreted FGF1 on day 1 in the absence of serum by late passage RPE cells (10 ng/10 ml of culture medium) was higher than that detected in early RPE cells (3.2 ng/10 ml of culture medium) (Fig. 1B). Secretion of FGF1 from aged RPE cells was fairly constant throughout the 7-day culture period (10–13 ng/10 ml of culture medium) and did not follow the kinetics of endogenous FGF1 production. The secretion of FGF1 in early passage cells followed a similar pattern to that of the synthesis of FGF1 (Fig. 1C). Note that the amount of accumulated FGF1 in the cells and in the culture media at the later time points are identical in the late and early passage cells and that the early passage cells begin to accumulate significant amounts of FGF1 as they begin to undergo apoptosis.

Fig. 1. Effects of serum depletion in long term cultures on RPE programmed cell death in early and late RPE cell passages and on endogenous FGF1 content and FGF1 secretion in early and late RPE cell passages. RPE cells were cultured in the presence of 10% fetal calf serum until confluence, and then after 3 days of culture (Day 0), the medium was removed, the cells were washed four times with phosphate-buffered saline, and cultured in serum-free conditions for 7 days. A, in the PCD experiments, values are means of four different experiments, and differences between means were analyzed by using the Mann and Whitney test. *, p < 0.005. B and C, FGF1 protein levels were determined using 3 mg of total protein lysate prepared from 3 x 10^6 RPE cells. FGF1 was purified on heparin-Sepharose column and quantified by EIA as described under “Experimental Procedures.” The data are representative of three independent experiments that gave similar results. Values are means ± S.D. For both endogenous and secreted FGF1 by late-passage RPE cell cultures (B), note extended scales of ordinate compared with endogenous and secreted FGF1 by early RPE cell cultures (C).

Fig. 2. Effects of serum depletion in long-term culture on FGFR1 de novo synthesis, expression, and autophosphorylation in early and late RPE cell passages. RPE cells were cultured in serum-free conditions for 7 days as described for Fig. 1 A, each day, 150 μCi/ml of [3H]cysteine and [35S]methionine were added for 24 h. Subsequently, RPE cells were lysed on days 1, 3, and 5, and equal volumes containing 200 μg of protein of the supernatants were immunoprecipitated with anti-FGFR1 antibody and were analyzed by SDS-7% PAGE and autoradiography. M, 14C-methylated molecular mass markers (200, 92, and 69 kDa). B, lysates from early and late passages were analyzed by immunoblotting with anti-FGFR1 antibody. Each day, early (C) and late (D) RPE cell passages had been incubated with 200 μCi/ml of Na_3 PO_4. Equal volumes containing 200 μg of protein of cell lysates were incubated with protein A-Sepharose CL-4B previously treated with anti-phosphotyrosine antibody. After elution of phosphotyrosine-containing protein with 0.1% Triton X-100 buffer, anti-FGFR1 antibody coupled to protein A-Sepharose was added. After being washed, the adsorbed material was analyzed by SDS-PAGE and autoradiography. Time of exposure of autoradiograph was 3 days. These experiments were repeated three times with similar results.

Serum-deprivation Causes More Rapid FGFR1 Activation by Secreted FGF1 in Late RPE Cells

Cell survival may be controlled by the amount of receptor produced. We analyzed the de novo synthesis of FGFR1 (the only FGF tyrosine-kinase receptor at the bovine RPE cell surface) during serial passage and investigated its production...
during the 7-day culture period after serum withdrawal (Fig. 2,A and B). The de novo synthesis of FGFR1 was constant in both early and late passages during the culture period in the absence of serum, and there was no difference in the level of the de novo synthesis of FGFR1 during the subcultures (Fig. 2A). In addition, FGFR1 production did not vary significantly during the 7-day culture period in early and late RPE cell passages and was unaffected by passage number (Fig. 2B). Because autophosphorylation of FGFR plays a key role in the interactions between activated tyrosine-kinase receptors and downstream signal transduction pathways, we investigated the autophosphorylation of FGFR1 in RPE cells during subcultures in the absence of serum (Fig. 2, C and D). FGFR1 in early passages (Fig. 2C) underwent tyrosine phosphorylation to a lesser extent than in late passages (Fig. 2D). In addition, FGFR1 tyrosine autophosphorylation in early passage RPE cells was weak, did not change during the first three days of culture, and then increased to reach a plateau by the 4th day of culture (Fig. 2C). In contrast, FGFR1 tyrosine autophosphorylation in late passage RPE cells was fairly constant throughout the culture period. Neutralization of secreted FGFR1 in early and late passages resulted in reduction of FGFR1 activation to a constant basal level.

**Sustained Phosphorylation, Activity, and de Novo Synthesis of ERK2 over a 7-Day Culture Period after Serum Deprivation in Late Passage Cells**

Exogenous FGF1 Causes a Sustained ERK2 Activation in Late Passage Cells—We found that there was a relationship between cell survival, FGF1 synthesis and secretion, and FGFR1 activation. This led us to explore the downstream pathways which may be involved in cell survival in late RPE cell passages. We began by investigating the activation of ERK2 by exogenous FGF1 during serial passage (Fig. 3). Activation of FGFR1 resulted in phosphorylation of ERK2 within 5 min, reaching a peak at 15 min in both early and late passages, (Fig. 3, A and B). In early and late passage cells, the first phase of activation was followed by a second sustained phase of activity lasting 12 h. ERK2 activity was determined by measuring phosphorylation of myelin basic protein as substrate (Fig. 3, C and D). As expected, there was a direct correlation between ERK2 phosphorylation and ERK2 activity in both early and late passages. No change in JNK1 expression was detected after FGF1 stimulation in either early or late passage cells (Fig. 3, E and F).

**Sustained Activation and de Novo Synthesis of ERK2 over the 7-Day Culture Period in Late Passage Cells—As exogenous FGF1 induced sustained, high level ERK2 activation in late passage cells, we investigated ERK2 phosphorylation and activity in both early and late RPE cell passages over the 7-day culture period in the absence of serum in which various kinetics and amounts of FGF1 excretion were observed (Fig. 4). There was only weak phosphorylation and activity of ERK2 during the 7-day culture period of cells in early passage (Fig. 4A). Contrary to expectation, we found that the total amount of ERK2 was in late passage cells 4–5-fold higher than that in early passage cells (Fig. 4A). Phosphorylation of ERK2 detected after 24 h was still detected after 7 days of culture, and ERK2 activity was also 4–6-fold higher than in early passage cells (Fig. 4B).

Protein synthesis was monitored using radiolabeled amino acids on each day of the 7 days of culture, to determine whether the increase in ERK2 during serial passage was because of its de novo synthesis, (Fig. 4C). ERK2 synthesis was low in early passage cells and did not significantly change during the 7-day culture period. De novo synthesis of ERK2 was detected on day 1 of culture, at similar levels in early and late-passage cells. After 24 h in late passage RPE cells, de novo synthesis of ERK2 increased and was 3-fold higher on day 2 and 5-fold higher on day 5, whereas de novo synthesis of JNK1 was unaffected (Fig. 4D).

**ERK2 Synthesis and Activation Is Required for RPE Cell Survival—**In a control experiment, 10 μM PD098059 completely inhibited activation of ERK2 in RPE cells by exogenous FGF1 (data not shown). Inhibition of MEK 1 activity resulted in inhibition of ERK2 synthesis and phosphorylation (Figs. 5, A and B). ERK2 synthesis was 7-fold lower than the basal levels in early and late passage cells on day 5, and its activation was completely inhibited in early passage cells and 5-fold lower than basal levels in late passage cells. A single dose of PD098059 led to a rapid 8-fold increase in apoptosis after only 24 h of culture in both early and late cell passages (Fig. 5, C and D). On day 7, cell survival in the presence of the MEK 1 inhibitor was 2-fold lower in early passage cells and 4-fold lower in late passage cells on day 7. There were no nonspecific cytotoxic effects of PD098059 because we detected no significant increase in the number of apoptotic cells in either early or late passages in the presence of serum (Fig. 5, C and D). Furthermore, 10 μM PD098059 did not inhibit total protein synthesis (data not shown).
Lower Levels of Apoptosis via ERK2 Activation Depend on FGF1 Secretion and FGFR1 Activation

We examined the effects of secreted FGF1 on the activation of ERK2 (Fig. 6). Neutralization of secreted FGF1 resulted in inhibition of ERK2 phosphorylation after 3 days of culture in both early and late passage cells (Fig. 6A). It also reduced de novo synthesis of ERK2 by 70% after 5 days of culture in both early and late passage cells (data not shown).

We added a blocking anti-FGFR1 antibody to the culture medium to check that the effects of secreted FGF1 on activation of the MAP kinase pathway and on reduction of RPE cell apoptosis were mediated by an autocrine/paracrine stimulation of the tyrosine-kinase receptor FGFR1. We analyzed the effects of FGFR1 blocking on PCD of RPE cells by the TUNEL method (Fig. 6B). Inactivation of FGFR1 had no significant effect during the first 3 days of culture. On day 5, the number of TUNEL-labeled nuclei doubled in early passage cells and increased by a factor of 12 in late passage cells in the presence of neutralizing antibody. On day 7, TUNEL labeling of cells in both early and late passages indicated levels of PCD similar to those obtained in the presence of the MEK 1 inhibitor (Figs. 5, C and D, and 6B). Inhibition of the binding of secreted FGF1 to FGFR1 in early RPE cell passages resulted in a 3-fold reduction in the de novo synthesis of ERK2 after 3 days of culture (Fig. 6C). In late RPE cell passages, the blocking anti-FGFR1 antibody reduced...
respectively. Similar results were obtained for three independent experiments. ERK2 phosphorylation (B) and de novo synthesis of early and late RPE cell passages. RPE cells were incubated in serum-free medium for 7 days in the presence or absence of neutralizing anti-FGF1 antibody (Ab) at 200 μg/ml (A) and blocking anti-FGFR1 antibody at 100 nM (B, C, and D). PCD (D) was detected and quantified as described in Fig. 1, and ERK2 phosphorylation (B) and de novo synthesis (C and D) were analyzed as described in Figs. 3 and 4, respectively. Similar results were obtained for three independent experiments. B, values are means ± S.D. and differences between means were analyzed by the Mann and Whitney test. *, p < 0.05; **, p < 0.005. C, exposure time of autoradiogram was 5 days; D, exposure time 36 h.

**Fig. 6.** Effects of FGF1 neutralization on ERK2 phosphorylation and of FGFR1 blocking on programmed cell death and de novo ERK2 synthesis of early and late RPE cell passages. RPE cells were incubated in serum-free medium for 7 days in the presence or absence of neutralizing anti-FGF1 antibody (Ab) at 200 μg/ml (A) and blocking anti-FGFR1 antibody at 100 nM (B, C, and D). PCD (D) was detected and quantified as described in Fig. 1, and ERK2 phosphorylation (B) and de novo synthesis (C and D) were analyzed as described in Figs. 3 and 4, respectively. Similar results were obtained for three independent experiments. B, values are means ± S.D. and differences between means were analyzed by the Mann and Whitney test. *, p < 0.05; **, p < 0.005. C, exposure time of autoradiogram was 5 days; D, exposure time 36 h.

de novo synthesis of ERK2 by a factor of 3 after 5 days and by a factor of 8 after 7 days (Fig. 6D). Neutralizing anti-FGF1 and blocking anti-FGFR1 antibodies did not affect PCD in RPE cells cultured in the presence of serum and did not significantly alter total protein synthesis (data not shown). This shows there was no nonspecific cytotoxic effect of the antibodies. These results support the notion that the activation of FGFR1 by secreted FGF1 and the subsequent activation and synthesis of ERK2 were required for secreted FGF1 to increase the survival of RPE cells.

**DISCUSSION**

**FGFR1-mediated Survival Activity of FGF1—**RPE cells are terminally differentiated and are mostly postmitotic by the end of the second week after birth. Consequently, RPE cell survival is critical to RPE function. Various studies have shown that, in experimental and inherited retinal degeneration, exogenous FGFs act as trophic factors improving the survival of cells in the retina (35, 36). However, little is known about the mechanisms of action of exogenous and endogenous FGF-induced cell survival.

In this paper we show that, in the absence of serum, confluent stationary aged RPE cells, which overproduced FGF1 and secreted a sustained high level of FGF1, were more resistant to apoptosis than cells of early RPE cell passages. FGF1 detected in the RPE cell culture medium was not because of release of this growth factor during cell death because 1) the secretion of FGF1 and its increase preceded RPE cell death, 2) no increase in FGF2 secretion was observed during RPE cell culture when PCD increased greatly (27), 3) RPE cells were intact and without swelling or rupture of membranes when viewed by light microscopy, and 4) the programmed cell death observed on days 5 and 7 did not involve cell lysis.

In a previous study, the direct role of endogenously secreted FGF1 in delaying RPE cell death was demonstrated using an anti-FGF1 neutralizing antibody which caused RPE cell apoptosis (27). However, no mechanistic connection between excretion of FGF1 and RPE cell survival was characterized. Several recent studies show that endogenous FGF1 increases the survival of nondividing cells. Injection of specific FGF1 antisense oligonucleotides into confluent quiescent epithelial lens cells leads to cell death (25). In PC12 cells with a high level of FGF1 production achieved by transfection, there is an increase in the number of surviving cells (26). However, these studies do not distinguish between the potential roles of endogenous FGF1 acting via an intracrine pathway and secreted FGF1 acting through tyrosine-kinase receptors. Fox and Shanley (37) demonstrated an autocrine mechanism for FGF2, mediating survival of vascular smooth muscle cells independent of cell proliferation, and highlighted different roles of exogenous and endogenous FGF2.

FGFR1 de novo synthesis was constant during subcultures of RPE cells and was not modified by serum deprivation. This is consistent with previous data demonstrating that FGFRs were constantly turning over in the presence or in the absence of ligand (38). In contrast, we showed that FGFR1 was more rapidly and to a greater extent activated by secreted FGF1 in aged RPE cells than in early passage RPE cells. This suggests that, FGFR1 activation mediated by an autocrine/paracrine loop was the mechanism involved in reducing apoptosis in late passage cells because aged cells accumulated and secreted a higher amount of FGF1 and because inactivation of FGFR1 resulted in an increase in PCD. This is consistent with previous data showing that autocrine stimulation of growth factor receptors makes it possible for cells to resist PCD (39), whereas lower levels of FGFR1 signal transduction are involved in the inability of senescent endothelial cells to respond to exogenous FGF1 (40).

**ERK2 Activation Is Required for FGF1-dependent Inhibition of Apoptosis**—At least two pathways are used to transduce FGF1 signals generated by FGFR1 stimulation, one dependent on Ras and the other is dependent on PLC-γ (41, 42). Raf 1 integrates signals from these two pathways to activate the MAP kinase cascade. ERK2 is vital for integration and trans-
mission of the FGF1 signal in RPE cells (28), JNK1 is implicated in the induction of apoptosis after growth factor depri-

vation (43), and ERK2 and JNK1 have opposing effects on cell death (44). PD098059, which prevents MEK1 activation by Raf 1, does not inhibit JNK1 (45). In our culture system, treatment with PD098059 effectively inhibited ERK2 activation in both early and late passage cells. This led to a rapid increase, after 1 day of culture, in the loss of cell viability, consistent with previous reports on ERK2 inactivation by PD098059 leading to rapid cell death (45) and inhibition of FGF2-protection against apoptosis (46). The effects we observed were specific because the MEK1 inhibitor had no effect on RPE cell apoptosis in the presence of serum although ERK2 was still inactivated (data not shown).

We attempted to define further the molecular mechanisms involved in the regulation of ERK2 expression in serial cultures of RPE cells after serum depletion. We demonstrated that both neutralization of secreted FGF1 and inhibition of FGRF1 in-
hibited ERK2 synthesis and activity resulting in rapid apoptosis. PCD induced by addition of anti-FGF1 neutralizing or anti-FGRF1 blocking antibodies occurred later than PCD induced by MEK1 inhibition, suggesting that ERK2 activation is presumably an early step in cell activation. This is consistent with previous reports of de novo synthesis of FGF1 on days 4 and 5 of the culture period after serum withdrawal (27).

Two mechanisms may be involved in producing the high levels of ERK2 protein detected in later passages. The subculture itself may be important because the amount of ERK2 protein in aged RPE cells cultured in the presence of serum was 4–5 fold higher than in early passage RPE cells. The depletion of serum may also be involved because de novo synthesis of ERK2 in aged RPE cells increases by a factor of 5, 5 days after serum withdrawal. This suggests that both phosphorylation and synthesis of ERK2 may be required for cell survival. Our results are consistent with recent data demonstrating that up-regulation of MAP kinase expression was a critical element of the metastatic potential of various forms of human breast cancer (47). In our model, the increase in ERK2 synthesis was not because of a general increase in total protein synthesis but because synthesis of FGF2 and JNK1 was not affected by serum withdrawal.

The mechanisms by which the ERK2 pathway regulates apoptosis in RPE cells are unclear. Studies with the MEK1 inhibitor PD098059 have shown that activation of ERK iso-

forms is required for growth factor-induced protein synthesis (48). It has been demonstrated that FGF2-induced up-regulation of Bcl2 gene expression delays apoptosis (49). Moreover, in vivo models of retinal degeneration in which FGFs are up-regulated, overexpression of Bcl2 reduces apoptotic photoreceptor cell death (50). This suggests that FGF1 may increase cell survival by activating the ERK2 pathway, leading to transcription of anti-apoptotic genes. This notion is supported by very recent data showing that long-term survival is strongly corre-

lated with the induction of Bclx gene expression, which is dependent on MAP kinase activation (51). Activation of JNK, which antagonizes the anti-apoptotic action of Bcl2 (52) and has opposing effects to ERK2, is also consistent with this. Such mechanisms may account for the effects on survival of FGFs in quiescent and differentiated cells. The synthesis of FGFs and autocrine FGFs signaling may be involved in the survival of the terminally differentiated cells.

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