Graefe's Arch Clin Exp Ophthalmol
(2001) 239:87–95

CLINICAL INVESTIGATION

DOI 10.1007/s004170000235

Nahoko Ogata
Akira Ando
Masanobu Uyama
Miyo Matsumura

Expression of cytokines and transcription factors in photocoagulated human retinal pigment epithelial cells

Received: 18 May 2000
Revised: 4 September 2000
Accepted: 4 October 2000
Published online: 27 February 2001

N. Ogata (✉) · A. Ando · M. Uyama
M. Matsumura
Department of Ophthalmology,
Kansai Medical University,
Fumizono-cho 10-15, Moriguchi,
Osaka 570-8507, Japan
e-mail: ogata@takii.kmu.ac.jp
Tel.: +81-6-69921001 ext. 3324
Fax: +81-6-69932222

Abstract *Background:* It has been shown that scatter photocoagulation induces regression of retinal neovascularization, but the mechanism for this effect is not completely understood. The main focus of our research is to determine the mechanism for the beneficial effects of photocoagulation. In the present study, we quantified the expression of growth factors and transcription factors that inhibit or induce angiogenesis in photocoagulated human retinal pigment epithelial (RPE) cells in vitro. *Methods:* RPE cells were grown to confluence, and RNA was isolated from the RPE cells with or without photocoagulation. The following growth factors, their receptors and transcription factors were examined by reverse transcription polymerase chain reaction (RT-PCR): transforming growth factor (TGF)-β1, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), kinase insert domain-containing receptor (KDR/flk-1), hypoxia-inducible factor (HIF)-1, ETS-1, nuclear factor kappa B (NF-κB), interleukin-8 (IL-8). *Results:* Laser photocoagulation increased the expression of TGF-β1. Expression of angiogenic factors bFGF, VEGF, IL-8 and their transcription factor, ETS1, was also increased. However, the up-regulation of these factors was observed early (6 h) after photocoagulation. Seventy-two hours after photocoagulation, when RPE cells were repaired, the expression of VEGF, IL-8, ETS-1, and NF-κB was decreased to the levels before photocoagulation. *Conclusions:* These results suggest that TGF-β produced by photocoagulated RPE cells and the down-regulation of angiogenic factors in repaired RPE cells, in all likelihood, play an important role in the processes that occur after laser photocoagulation.

Introduction

Laser photocoagulation is one of the most effective treatments for a variety of retinal disorders, especially ischemic retinal diseases such as diabetic retinopathy and retinal vein occlusion [10, 11, 45]. Despite its widespread clinical use, the mechanisms for the therapeutic effect of photocoagulation have not been determined. Many hypotheses, including the reduction of hypoxic areas that produce a vasoformative factor or the production of a factor that induces regression of new vessels, have been postulated to explain the regression of neovascularization after photocoagulation.

The main site of energy absorption in laser photocoagulation is the melanin pigment in the retinal pigment epithelial (RPE) cells and the choroid. We selected to study RPE cells because it has been shown that photocoagulated RPE cells produce growth factors and cytokines that modulate proliferation of vascular endothelial cells, and also because panretinal photocoagulation (PRP) causes RPE cells to change their profile of growth factor and cytokine production [19, 25, 54]. In addition, it has

Table 1 The sequences of the primers

	Sense primer sequence	Antisense primer sequence
bFGF	–AAA ACG GGG GCT TCT TCC TG	–ACT GGT GTA TTT CCT TGA CC
TGF-β1	–GAT CCA CGA GCC CAA GGG C	–TCA GCT GCA CTT GCA GGA G
VEGF	–GAG TAC CCT GAT GAG ATC GAG T	–AAT GCT TTC TCC GCT CTG
KDR/flt-1	–ATC GAA TTC ATC ACA TCC ACT GGT ATT GG	–ATC GAA TTG CCA AGC TTG TAC CAT GTG AG
HIF-1	–ATG ACT TCC AGT TAC G17 CTT T	–GAG GCT GTC CGA CTT TG
ETS-1	–TAT GGA ATG TGC AGA TGT CC	–ATC TCC TGT CCA GCT GAT AA
NF-κB (P65)	–AGC ACA GAT ACC ACC AAG ACC C	–CCC ACG CTG CTC TTC TAT AGG AAC
IL-8	–TCT CTT GGC AGC CTT CCT GAT	–ATT GAC TGC TAA GAT TAT ATT
β-actin	–ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG	–CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC

been reported that photocoagulated RPE cells secrete an inhibitor of vascular endothelial cell proliferation [25, 54]. However, other studies have shown that RPE cells produce angiogenic factors, e.g., basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor, and interleukin-8 (IL-8) [2, 13, 38, 41, 53]. In keeping with RPE's role in angiogenesis, we recently demonstrated that RPE cells promote choroidal neovascularization through the secretion of cytokines [27, 31, 32, 43].

In the present study, we used an in vitro model to determine whether retinal laser photocoagulation alters the expression of growth factors and cytokines and their transcription factors in cultured human RPE cells.

Materials and methods

RPE cells

ARPE-19, a human RPE cell line was used [12]. RPE cells were seeded density of 5×10^5 cells in uncoated, 3.5-cm-diameter dishes. They were maintained for 2 days in complete medium (DME/F12 medium; Gibco-BRL) to obtain confluent cells. Fetal bovine serum (10%, FBS, Hyclone), 15 mM HEPES (Gibco-BRL), 1% (v/v) L-glutamine (Gibco-BRL) in 200 mM solution, and 1% penicillin-streptomycin (100× at 10^4 units/10^4 μg) were added to the medium. The cultures were incubated in 10% CO_2 incubator at 37°C.

Laser photocoagulation

Photocoagulation of confluent RPE cells was performed with a clinical slit-lamp delivery system equipped with a krypton laser (Coherent Radiation Model 900 laser, Palo Alto, Calif.). As RPE cells in culture are depigmented, the photocoagulation procedure required placing a sheet of black paper beneath the dishes as previously described [25]. The following parameters were used: 300 mW of power, 0.1 s duration, 50 μm spot size, and 300 spots per dish. These parameters were necessary to obtain reproducible coagulation spots. Immediately after treatment, the medium was replaced with fresh complete medium, and the cells were returned to the CO_2 incubator. RPE cells without photocoagulation served as control.

Extraction of RNA and cDNA synthesis

The expression of cytokines and transcription factors was analyzed by reverse transcription polymerase chain reaction (RT-PCR). In brief, total RNA from RPE cells was isolated from five separate 3.5-cm-diameter dishes, using the acid guanidinium thiocyanate–phenol–chloroform extraction (AGPC) method [7], at 6, 24, 48, and 72 h after photocoagulation. Total RNA from RPE cells without photocoagulation served as control. The extracted RNA was quantified, and 5 μg was subjected to DNase I digestion (Boehringer Mannheim, Mannheim, Germany) at 37°C for 45 min. After each DNase I digestion, 3 μg of RNA was subjected to reverse transcription using oligo dT primer following the protocol described in cDNA cycle kit (Invitrogen, San Diego, Calif.).

Analysis of mRNA expression by PCR

PCR amplification was performed on 0.5 μg of each sample using a Gene Amp PCR kit from Perkin Elmer Cetus (Norwalk, Conn.) for the expression of cytokines, their receptors, and transcription factors. We selected angiogenic factors that have been shown to promote endothelial cell proliferation, e.g., bFGF [1, 9], VEGF and its receptor (kinase insert domain-containing receptor-1, KDR/flk-1) [34], its transcription factor (hypoxia-inducible factor-1, HIF-1) [15], interleukin-8 (IL-8) [26] and its transcription factor [nuclear factor kappa B, NF-κB (P65)] [29], and ETS-1 [20]. We also selected transforming growth factor-β1 (TGF-β1), which has been shown to inhibit endothelial cell proliferation [30,35].

The sequences of the primers and the optimized PCR conditions are presented in Table 1 and Table 2, respectively. Each PCR reaction included 2 min denaturation at 94°C for one cycle, followed by 30–35 cycles of denaturation, annealing, and extension. This was followed by one cycle of 7 min for additional extension at 72°C. β-Actin was used as an internal control, and each PCR amplification included a negative control containing all the reagents except for the sample. After PCR amplification, PCR products were loaded onto 2% agarose gels (Bio-Rad, Richmond, Calif.) including 0.05% ethidium bromide.

Semiquantification by PCR

A semiquantitative PCR amplification method was performed using the expression of β-actin mRNA as an internal control. The β-actin band in gel acted as a standard band, and the density of the cytokines, their receptors, and transcription factors were evaluated by expressing their density relative to β-actin standard band by densitometry. Thus, densitometry was performed on all gels and each signal was normalized to the corresponding β-actin signal using an NHI imaging system (version 1.16). At time 0 (before photocoagulation as a control), all levels were determined to be 1.0.

Table 2 The optimized PCR conditions

	PCR conditions	Reference or manufacturer and derived sequence of primers
bFGF	Denaturation at 94°C for 45 s; annealing at 50°C for 45 s; extension at 72°C for 90 s; followed by 30 cycles	Clonetech
TGF-B1	Denaturation at 94°C for 30 s; annealing at 60°C for 30 s; extension at 72°C for 90 s; followed by 35 cycles	[8]
VEGF	Denaturation at 94°C for 30 s; annealing at 60°C for 30 s; extension at 72°C for 90 s; followed by 35 cycles	Sense (891–); antisense (–1141)
KDR/flt-1	Denaturation at 94°C for 30 s; annealing at 60°C for 30 s; extension at 72°C for 90 s; followed by 35 cycles	[5]
HIF-1	Denaturation at 94°C for 30 s; annealing at 62°C for 30 s; extension at 72°C for 90 s; followed by 30 cycles	Sense (1737–); antisense (–2015)
ETS-1	Denaturation at 94°C for 45 s annealing at 60°C for 30 s; s extension at 72°C for 90 s; followed by 30 cycle	[20]
NF-κB (P65)	Denaturation at 94°C for 30 s; annealing at 60°C for 30 s; extension at 72°C for 90 s; followed by 35 cycles	Biognostic
IL-8	Denaturation at 94°C for 45 s; annealing at 60°C for 30 s; extension at 72°C for 90 s; followed by 30 cycles	Biognostic
β-Actin	Denaturation at 94°C for 45 s annealing at 60°C for 30 s; extension at 72°C for 90 s followed by 30 cycles	Clonetech

Because the quantitative application of this method is contingent upon the analysis of the PCR products during the amplification phase prior to the plateau, the cycle relationship and delusional curves for cDNA for each of the cytokines, their receptors, transcription factors, and β-actin were determined empirically.

One-way analysis of variance (ANOVA) and the Fisher test were performed to test for significance. A P value <0.05 was taken as significant.

Results

Morphological changes induced by photocoagulation

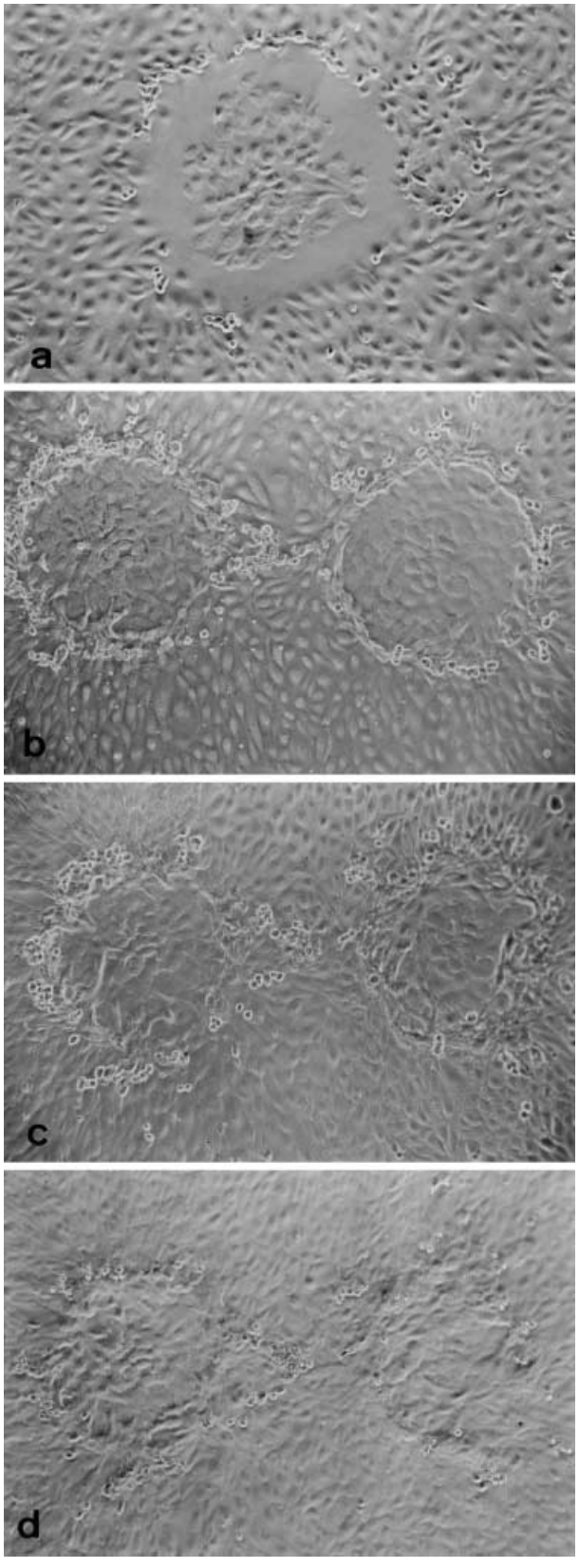
Six hours after photocoagulation, round coagulated areas that appeared decolorized and empty were observed in the RPE cell cultures. By 72 h, a repair process was evident in the coagulated areas from the appearance of RPE cells that proliferated and migrated from the edge of the wound to the denuded spaces (Fig. 1). This pattern of photocoagulation injury and subsequent repair is similar to that reported previously [25, 48].

Gene expression of cytokines, their receptors, and transcription factors

PCR was performed after photocoagulation using the template cDNAs prepared from the ARPE-19 cells. The PCR products of expected sizes were obtained by using specific primers. Nucleotide sequencing revealed that the PCR products were derived from the target cDNA sequences. As stated, semiquantitative PCR analysis was carried out to determine the changes in the level of the expression after photocoagulation. The same levels of β-actin expression was found in all of the samples (Fig. 2). The levels of expression of cytokines and other factors were compared to those before photocoagulation, and the results of semiquantification by RT-PCR are shown in Fig. 3.

Anti-angiogenic factor

The expression of TGF-β1 was lower than the control level at 6 h after photocoagulation, then recovered and was higher than the control level at 48 h, after which the expression level remained nearly constant up to 72 h (Figs. 2, 3). Thus, the expression level of TGF-β1 was 0.80±0.17 (mean ± standard deviation) (P>0.1) times the control levels before photocoagulation at 6 h then increased to 1.26±0.22 (P<0.05) times at 24 h, to 1.42±0.26 (P<0.005) times at 48 h, and to 1.43±0.20 (P<0.005) times at 72 h after photocoagulation (Fig. 3). Although the expression of TGF-β1 was not significantly different from that before photocoagulation at 6 h, the increase of the expression level of TGF-β1 was highly significant at 24–72 h after photocoagulation.



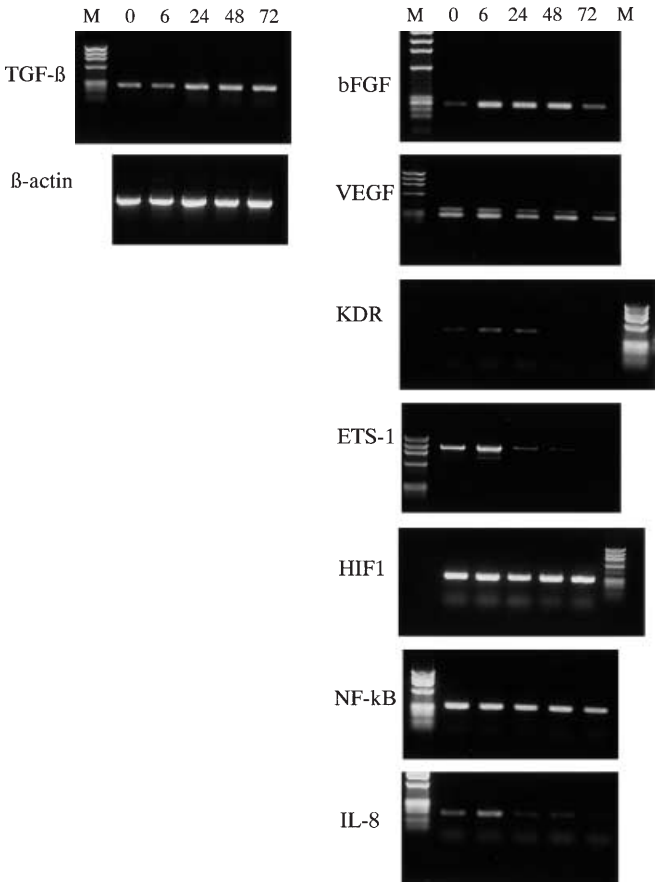


Fig. 2 Gene expression of cytokines, transcription factors, and receptors in RPE cells following laser photocoagulation. PCR amplification followed by gel electrophoresis and ethidium bromide staining of the samples. Lanes: *M* molecular weight marker, X174/HaeIII, *0* gene expression before photocoagulation, *6* gene expression 6 h after photocoagulation. *24* gene expression 24 h after photocoagulation, *48* gene expression 48 h after photocoagulation, *72* gene expression 72 h after photocoagulation. The expression of TGF-β1 increased 24–72 h after photocoagulation. Gene expression of bFGF increased early after photocoagulation, then gradually decreased. The expression of VEGF and KDR had increased at 6 h after photocoagulation, then gradually decreased thereafter. HIF-1 expression did not change significantly. Gene expression of ETS-1 increased early after photocoagulation, then quickly decreased. Expression of NF-κB was slightly decreased after photocoagulation. The expression of IL-8 was higher 6 h after photocoagulation, then decreased to a level lower than that before photocoagulation. The same level of β-actin expression was found in all of the samples

◀ **Fig. 1a–d** Morphological changes following photocoagulation. **a** Six hours after photocoagulation, round coagulated areas that appeared decolorized and empty are observed in RPE cell cultures. **b** Twenty-four hours after photocoagulation. **c** Forty-eight hours after photocoagulation. **d** Seventy-two hours after photocoagulation. There is repair by RPE cells that have migrated from the edge of the wound to the denuded space

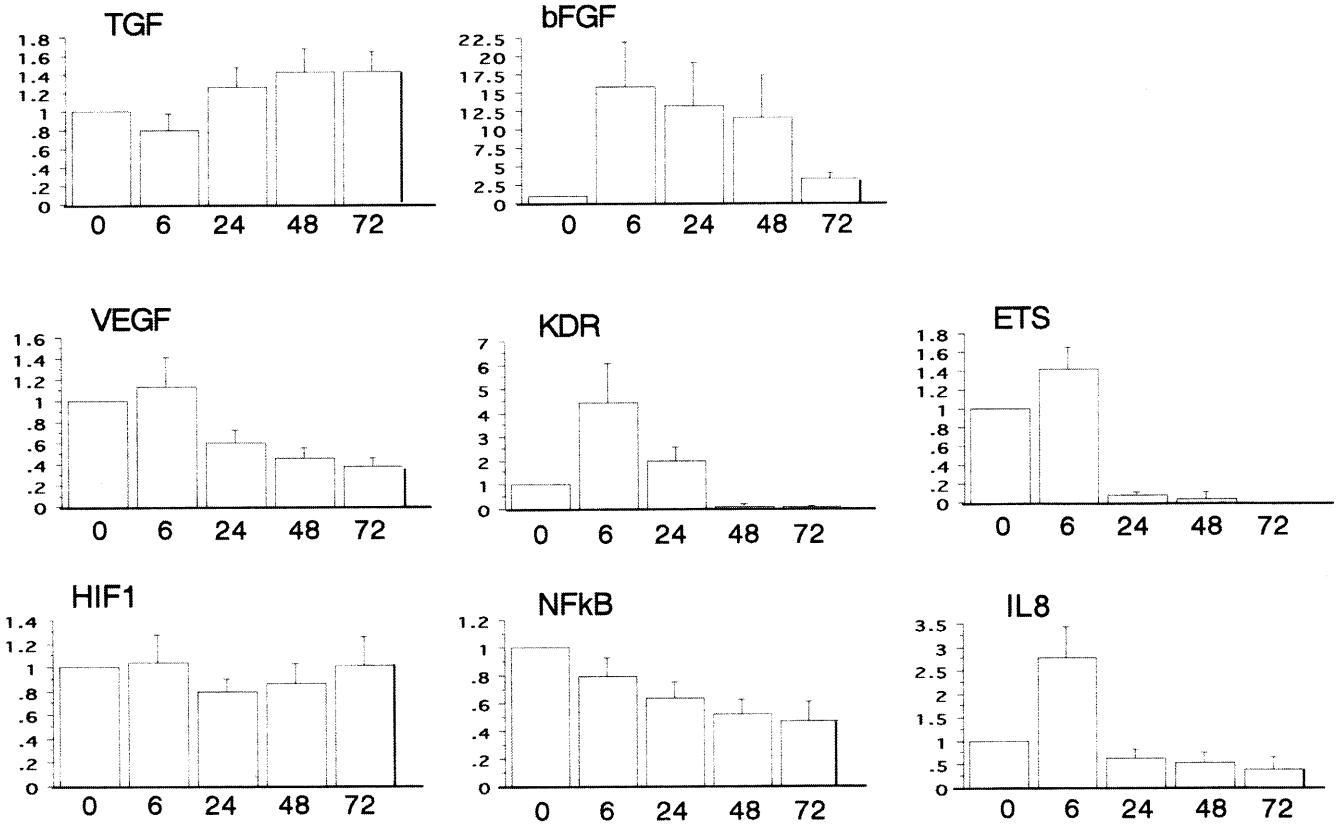


Fig. 3 Semiquantification of the levels of anti-angiogenic factors and angiogenic factors in RPE cells following laser photocoagulation (Abbreviations as in Fig. 2). The density of the bands was evaluated by comparing it with the standard β-actin band. The values in each column represent the mean ± standard deviation (n=5)

Angiogenic factors and their transcription factors

The expression of bFGF markedly increased after photocoagulation reaching a peak as early as 6 h (Fig. 2). Thus, bFGF had increased to 15.81±6.10 (P<0.001) times the control level at 6 h, and decreased to 12.96±5.98 (P<0.005) times at 24 h and to 11.53±5.50 (P<0.002) times at 48 h. The bFGF expression then dropped to 3.23±0.88 (P>0.4) times at 72 h after photocoagulation (Fig. 3). These results demonstrated clearly that the level of bFGF expression was increased after photocoagulation but the level dropped at 72 h.

The gene expression of VEGF had also increased slightly by 6 h, then gradually decreased to a level lower than that before photocoagulation at 72 h (Fig. 2). Thus, the level was 1.13±0.27 (P>0.1, not significant, n.s.) times the control level at 6 h, but then dropped to 0.60±0.12 (P<0.001) times at 24 h, to 0.45±0.10 (P<0.001) times at 48 h, and finally to 0.38±0.08 (P<0.001) times at 72 h (Fig. 3). KDR/flk-1 expression increased significantly at 6 h to 4.42±1.64 (P<0.001) times the control level, and dropped to 2.01±0.56 (P>0.05) times at 24 h. Thereafter the level decreased to a level lower than that before photocoagulation and was barely detectable at 48 and 72 h (Figs. 2, 3). The expression level of KDR was significantly decreased at 24–72 h compared to that at 6 h after photocoagulation (P<0.001). HIF-1 expression did not change significantly after photocoagulation; the level was 1.03±0.23 (P>0.7) times the control level at 6 h, 0.80±0.10 (P>0.05) times at 24 h, 0.87±0.16 (P>0.2) times at 48 h, and 1.01±0.24 (P>0.9) times at 72 h (Figs. 2, 3). Gene expression of ETS-1 had increased by 6 h to 1.41±0.24 (P<0.001) times the control level, then rapidly decreased to 0.07±0.05 (P<0.001) times at 24 h, 0.03±0.07 (P<0.001) times at 48 h, and was barely detectable at 72 h after photocoagulation (P<0.001) (Figs. 2, 3).

The expression of NF-κB decreased slightly after photocoagulation to 0.79±0.13 (P<0.01) times the control level at 6 h, to 0.63±0.12 (P<0.001) times at 24 h, to 0.52±0.10 (P<0.001) times at 48 h, and 0.47±0.14 (P<0.001) times at 72 h (Figs. 2, 3). The expression of IL-8 was 2.78±0.65 (P<0.001) times the control level at 6 h, 0.63±0.20 (P>0.05) times at 24 h, 0.54±0.20 (P<0.05) times at 48 h, and 0.39±0.25 (P<0.02) times at 72 h after photocoagulation (Fig. 2, 3). The expression level of IL-8 was significantly lower at 24–72 h than at 6 h after photocoagulation (P<0.001).

It should be noted that several of the angiogenic and transcription factors – bFGF, VEGF, KDR, ETS-1, and

IL-8 – showed a transient increase of expression at 6 h after photocoagulation and then gradually decreased. Interestingly, VEGF, KDR, ETS-1, NF-κB, and IL-8 decreased to levels that were lower than that before photocoagulation. This was especially true for KDR and ETS-1, whose expression was barely detectable at 72 h after photocoagulation.

Discussion

We have demonstrated that not only anti-angiogenic factors but also angiogenic factors were up-regulated transiently in cultured RPE cells after laser photocoagulation. It has been reported that, following photocoagulation, the regenerating RPE cells behave differently from normal RPE cells in vivo and in vitro [37, 48], and the production of cytokines and growth factors from the cells may be altered in quantity and in quality [19, 25, 48, 49, 50, 51, 54].

We found that regenerating RPE cells express higher levels of TGF-β than normal quiescent RPE cells, as previously reported [19, 25, 54]. We previously found an up-regulation of TGF-β and bFGF in laser-photocoagulated retina of rats [49, 50, 51]. TGF-β is a potent inhibitor of the proliferation of vascular endothelial cells [4,30] and also modulates the bFGF-induced proteolytic and angiogenic properties of endothelial cells in vitro [35]. It has been reported that TGF-β and bFGF have opposing effects on the proliferation of cultured bovine retinal capillary endothelial cells [4]. Thus, TGF-β may play an important role in the regression of neovascularization after photocoagulation.

Wong et al. [46] described the production of a stimulator rather than an inhibitor of vascular endothelial proliferation in cultured RPE cells. In our experiment, the expression of stimulators of vascular endothelial proliferation, viz. bFGF, VEGF, IL-8, ETS-1, was up-regulated early (6 h) after photocoagulation.

This up-regulation of bFGF early after photocoagulation is consistent with previous reports of the elevation of the expression of bFGF in laser-photocoagulated retinas [47, 49, 50]. bFGF is a potent stimulus for the proliferation of various types of cells and has been detected during the healing of wounds [16]. In addition, exogenous bFGF also accelerates wound healing [28]. Therefore, we suggest that bFGF promotes the proliferation of RPE cells and accelerates wound healing in laser-injured RPE cells in culture. It is also well known that bFGF is a strong angiogenic factor. However, the increased expression of bFGF in the laser-treated RPE cells was seen at 6 h and then gradually decreased. Therefore, bFGF would probably affect the proliferation of RPE cells early after photocoagulation. In addition, bFGF may also play a role in the protection of the sensory retina from laser injury, because it is also a neurotrophic factor [14, 44].

Guerrin et al. showed that cultured RPE cells express flt-1 and KDR/flk-1 and contain an autocrine loop for VEGF, a very strong angiogenic factor [18]. We found that the mRNA expression of VEGF and KDR/flk-1 was increased at 6 h after photocoagulation, and then gradually decreased to a level lower than that before photocoagulation by 72 h, when RPE cells were being repaired. Because earlier reports demonstrated that TGF-β1 is a major regulator of the VEGF/flk-1 signal transduction pathway in endothelial cells [24], the increased TGF-β1 after photocoagulation may play a role in inhibiting VEGF and KDR/flk-1 expression in RPE cells.

Although the VEGF and KDR/flk-1 expression was altered following photocoagulation, HIF-1 expression was not altered significantly after photocoagulation. Because HIF-1 is induced by hypoxia and activates the transcription of VEGF [15,40], we can conclude that laser injury does not induce hypoxia in RPE cells. Transient activation of VEGF and KDR/Flt levels may be induced by other factors, such as bFGF, platelet-derived growth factor and TGF-β1 [34, 36].

ETS-1 is a member of the ets gene family of transcription factors that binds to the ETS-binding motif in the *cis*-acting elements and regulates the expression of certain genes. Four typical angiogenic factors, aFGF, bFGF, VEGF, and EGF, have been shown to induce the expression of ETS-1 mRNA in human microvascular endothelial cells [20]. A previous study demonstrated that VEGF stimulates ETS-1 expression, and the ETS-1 gene had a direct role in angiogenesis [6]. We found that the mRNA expression of ETS-1 was higher at 6 h after photocoagulation, then quickly decreased; at 72 h, the expression was lower than that before photocoagulation. The transient up-regulation of ETS-1 could have been induced by VEGF, and the down-regulation of ETS-1 could have induced the down-regulation of angiogenic factors and also may inhibit endothelial cell migration. These processes may constitute the mechanism responsible for the beneficial effects of photocoagulation in neovascular disorders.

NF-κB is an inducible transcription factor that was originally identified as a heterodimeric complex of 50-kDa and 65-kDa (p65) subunits [3]. It is thought to play a central role in the regulation of a number of immune and proliferative response genes [17, 22, 23]. Recent studies have shown that NF-κB may regulate the initiation of angiogenesis in vitro [39, 42]. The down-regulation of NF-κB expression after photocoagulation may also be responsible for the beneficial effects of photocoagulation.

IL-8 is an inflammatory cytokine and a potent angiogenic factor in vitro and in vivo. The promoter of IL-8 gene contains potential binding sites for NF-κB [26, 29,33]. Karakuram et al. showed that hypoxia induces the expression of IL-8 in vascular endothelial cells through the activation of NF-κB [21]. A recent study re-

ported that the IL-8 mediated by NF-κB may contribute to the pathogenesis of intraocular neovascularization [52]. However, we found that the expression of NF-κB decreased slightly following after photocoagulation, although the expression of IL-8 increased at 6 h after photocoagulation. These results suggest that photocoagulation may not participate in the activation of NF-κB because photocoagulation would not induce hypoxia. The expression of IL-8 may be transiently induced as an inflammatory response to the photocoagulation, then decreased to a level lower than that before photocoagulation at 72 h.

By using cultured RPE cells, it may not be possible to identify all the activators and inhibitors of endothelial cell proliferation in the photocoagulated RPE cells. However, we were able to show that in vitro photocoagulation up-regulates angiogenic and angiogenic transcription factors – bFGF, VEGF, IL-8, and ETS-1 – early after photocoagulation. Although these responses were only observed early after photocoagulation, and with longer times, the expression gradually decreased. In fact, at 72 h the expression of VEGF, IL-8, ETS-1, and NF-κB had decreased to levels lower than before photocoagulation.

The mechanisms responsible for the beneficial effects of photocoagulation on areas of retinal capillary non-perfusion are unclear. It has been hypothesized that laser treatment may release potential inhibitors of neovascularization from the cells in the scar tissue or reduce the intraocular levels of stimulator of neovascularization by destroying cells that produce these factors. Our data suggest that photocoagulation may activate potential inhibitors of neovascularization and/or reduce the levels of stimulators of neovascularization from regenerating RPE cells.

Acknowledgements This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education in Japan and the Science Research Promotion Fund of the Japan Private School Promotion Foundation. We thank Prof. L.M. Hjelmeland (Department of Biological Chemistry, University of California, Davis, USA) for providing us with ARPE cells.

References

1. Abraham JA, Mergia A, Whang JL, Tumolo A, Friedmsan J, Hjerrild KA, Gospodarowicz D, Fiddes JC (1986) Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science 233: 545–548
2. Adamis AP, Shima DT, Yeo TK, Brown LF, Berse B, D'Amore PA, Folkman J (1993) Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. Biochem Biophys Res Commun 193: 631–638
3. Baeuerle PA (1991) The inducible transcription activator NF-κB: regulation by distinct protein subunits. Biochem Biophys Acta 1072: 63–80
4. Bensaid M, Malecaza F, Bayard F, Tauber JP (1989) Opposing effects of basic fibroblast growth factor and transforming growth factor-β on the proliferation of cultured bovine retinal capillary endothelial (BREC) cells. Exp Eye Res 48: 791–799
5. Boocock CA, Charnock-Jones DS, Sharkey AM, mcLaren J, Barker PJ, Wright KA, Twentryman PR, Smith SK (1995) Expression of vascular endothelial growth factor and its receptor flt and KDR in ovarian Cartinoma J Natl Cancer Inst 87: 506–516
6. Chen Z, Fisher RJ, Riggs CW, Rhim JS, Lautenberger JA (1997) Inhibition of vascular endothelial growth factor-induced endothelial cell migration by ETS1 antisense oligonucleotides. Cancer Res 57: 2013–2019
7. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159
8. Date M, Matsuzaki K, Matsushita M, Salitani K, Shibano K, Okajima A, Yamamoto C, Ogata N, Okamura T, Seki T, Kubota Y, Kan M, M cKeehan WL, Inoue K (1998) Differentiational expression of transforming growth factor-β and its receptors in hepatocytes and nonparenchymal cells of rat liver after CCL_4 administration. J Hepatol 28:572–581
9. De Juan E, Stefansson E, Ohira A (1990) Basic fibroblast growth factor stimulates ^{3}H-thymidine uptake in retinal venular and capillary endothelial cells in vivo. Invest Ophthalmol Vis Sci 31:1238–1244
10. Diabetic Retinopathy Study Research Group (1978) Photocoagulation treatment of proliferative diabetic neovascular retinopathy: the second report of Diabetic Retinopathy Study findings. Ophthalmology 85: 82–106
11. Doft BH, Blankenship G (1984) Retinopathy risk factor regression after laser panretinal photocoagulation for diabetic retinopathy. Ophthalmology 91: 1453–1457
12. Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM (1996) ARPE-19, A human retinal pigment epithelial cell line with differentiated properties. Exp Eye Res 62:155–169
13. Elner VM, Strieter RM, Elner SG, Baggiolini M, Lindley I, Kunkel SL (1996) Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. Am J Pathol 136: 745–750
14. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM (1990) Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 347: 83–86
15. Forsythe JA, Jiang B, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL (1996) Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 16: 4604–4613
16. Gospodarowicz D, Neufeld G, Schiweigerer L (1986) Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. Cell Differ 19: 1–17

17. Grilli M, Chiu JJ-s, Leonald MJ (1993) NF-κB, and rel : participants in a multiform transcription regulatory system. Int Rev Cytol 143: 1–62
18. Guerrin M, Moukadiri H, Chollet P, Moro F, Dutt K, Malecaze F, Plouet J (1995) Vasculotropin/vascular endothelial growth factor is an autocrine growth factor for human retinal pigment epithelial cells cultured in vitro. J Cell Physiol 164:385–394
19. Ishida K, Yoshimura N, Yoshida M, Honda Y (1998) Upregulation of transforming growth factor-β after panretinal photocoagulation. Invest Ophthalmol Vis Sci 39: 801–807
20. Iwasaka C, Tanaka K, Abe M, Sato Y (1996) Ets-1 regulates angiogenesis by inducing the expression of urokinase-type plasminogen activator and matrix metalloproteinase-1 and the migration of vascular endothelial cells. J Cell Physiol 169: 522–531
21. Karakuram M, Shreeniwas R, Chen J, Pinsky D, Yan S, Anderson M, Sunouch K, Major J, Hamilton T, Kuwabara K, Rot A, Nowygrod R, Sterm D (1994) Hypoxic induction of interleukin-8 gene expression in human endothelial cells. J Clin Invest 93: 1564–1570
22. Lenardo MJ, Baltimore D (1989) NF-κB: a pleiotropic mediator of inducible and tissue specific gene control. Cell 58: 227–229
23. Liao F, Andalibi A, Qiao JH, Allayee H, Fogelman AM, Lusis AJ (1994) Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. J Clin Invest 94: 877–884
24. Mandriota SJ, Menoud P-A, Pepper MS (1996) Transforming growth factor-β1 down regulates vascular endothelial growth factor receptor 2/flk-1 expression in vascular endothelial cells. J Biol Chem 271: 11500–11505
25. Matsumoto M, Yoshimura N, Honda Y (1994) Increased production of transforming growth factor-β2 from cultured human retinal pigment epithelial cells by photocoagulation. Invest Ophthalmol Vis Sci 35:4245–4252
26. Matsusaka T, Fujikawa K, Nishino Y, Mukaida N, Matsushima K, Kishimoto T, Akira S (1993) Transcription factors NF-kappaB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. Proc Natl Acad Sci USA. 90: 10193–10197
27. Matsushima M, Ogata N, Takada Y, Tobe T, Yamada H, Takahashi K, Uyama M (1996) Expression of FGF receptor in the experimental choroidal neovascularization. Jpn J Ophthalmol 40:329–338
28. Mcgee GS, Davidson JM, Buckley A, Sommer A, Woodward SC, Aquino AM, Barbour R, Demetriou AA (1988) Recombinant basic fibroblast growth factor accelerates wound healing. J Surg Res 45: 145–153
29. Mukaida N, Morita M, Ishikawa Y, Rice N, Okamoto S, Kasahara T, Matsushima K (1994) Novel mechanism of glucocorticoid-mediated gene repression: nuclear factor-kappa B is target for glucocorticoid-mediated interleukin 8 gene expression. J Biol Chem 269: 13289 13295
30. Muller G, Behrens J, Nussbaumer U, Bohlen P, Birchmeier W (1987) Inhibitory action of transforming growth factor beta on endothelial cells. Proc Natl Acad Sci USA 48: 5600- 5604
31. Ogata N, Matsushima M, Takada Y, Tobe T, Takahashi K, Yi X, Yamamoto C, Yamada H, Uyama M (1996) Expression of basic fibroblast growth factor mRNA in developing choroidal neovascularization. Curr Eye Res 15:1008–1018
32. Ogata N, Yamamoto C, Miyashiro M, Yamada H, Matsushima M, Uyama M (1997) Expression of transforming growth factor β mRNA in experimental choroidal neovascularizarion. Curr Eye Res 16:9–18
33. Oliveira IC, Mukaida N, Matsushima K, Vilcek J (1994) Transcription inhibition of the interleukin-8 gene by interferon is mediated by the NF-kappaB site. Mol Cell Biol 14: 5300- 5308
34. Patterson C, Perrella MA, Hsieh C, Yoshimizu M, Lee M, Haber E (1996) Cloning and functional analysis of the promoter for KDR/flk-1, a receptor for vascular endothelial growth factor. J Biol Chem 270: 23111–23118
35. Pepper MS, Belin D, Montesano R, Orci L, Vassali JD (1990) Transforming growth factor-β1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol 111: 743–755
36. Pertovaara L, Kaipainen A, Mustonen T, Orpana A, Ferrara N, Saksela O, Alitalo K (1994) Vascular endothelial growth factor is induced in response to transforming growth factor-β in fibroblastic and epithelial cells. J. Biol Chem 269: 6271–6274
37. Roider J, Michaud NA, Flotte TJ, Birngruber R (1992) Response of the retinal pigment epithelium to selective photocoagulation. Arch Ophthalmol 110:1786–1792
38. Schweigerer L, Malerstein B, Neufeld G, Gospodarowicz D (1987) Basic fibroblast growth factor is synthesized in cultured retinal pigment epithelial cells. Biochem Biophys Res Commun 143: 934–940
39. Shono T, Ono M, Izumi H, Jimi S, Matsushima K, Okamoto T, Kohno K, Kuwano M (1996) Involvement of the transcription factor NF-κB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress. Mol Cell Biol 16: 4231–4239
40. Shweiki D, Itin A, Soffer D, Keshet E. (1992) Vascular endothelial growth factor induced by hypoxia may mediates hypoxia-initiated angiogenesis. Nature 359: 843–845
41. Sternfield MD, Robertson JE, Shipley GD, Tsai J, Rosenbaum JT (1989) Cultured human retinal pigment epithelial cells express basic fibroblast growth factor and its receptors. Curr Eye Res 8:1029–1037
42. Stoltz RA, Abraham NG, Laniado-Schwartzman M (1996) The role of NF-kappaB in the angiogenetic response of coronary microvessel endothelial cells. Proc Natl Acad Sci USA 93: 2832–2837
43. Wada M, Ogata N, Otsuji T, Uyama M (1999) Expression of vascular endothelial growth factor and its receptor (KDR/flk-1) mRNA in experimental choroidal neovascularization. Curr Eye Res 18: 203–213
44. Walicke P, Cowan WM, Ueno N, Baird A, Guillemin R (1986) Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. Proc Natl Acad Sci USA 83:3012–3016
45. Weiter JJ, Zuckerman R (1980) The influence of the photoreceptor-RPE complex on the inner retina: an explanation for the benefical effects of photocoagulation. Ophthalmology 87: 1133–1139
46. Wong HC, Boulton M, McLeod D, Bayly M, Clark P, Marshall J (1988) Retinal pigment epithelial cells in culture produce retinal vascular mitogens. Arch Ophthalmol 106:1439- 1443
47. Xiao M, Sastry SM, Li Z-Y, Possin DE, Chang JH, Klock IB, Milam AH (1998) Effects of retinal laser photocoagulation on photoreceptor basic fibroblast growth factor and survival. Invest Ophthalmol Vis Sci 39: 618–630

48. Yamakawa R, Shirakawa H, Yoshimura N, Asayama M, Ogino N (1998) Involvement of fibronectin in vitro regeneration of retinal pigment epithelium. Graefe's Arch Clin Exp Ophthalmol 226:11–14
49. Yamamoto C, Ogata N, Matsushima M, Takahashi K, Miyashiro M, Yamada H, Uyama M (1996) Gene expression of basic fibroblast growth factor and its receptor in healing of rat retina after laser photocoagulation. Jpn J Ophthalmol 40: 480–480
50. Yamamoto C, Ogata N, Yi X, Takahashi K, Miyashiro M, Yamada H, Uyama M, Matsuzaki K (1996) Immunolocalization of basic fibroblast growth factor during wound repair in rat retina after laser photocoagulation. Graefe's Arch Clin Exp Ophthalmol 234: 695–702
51. Yamamoto C, Ogata N, Yi X, Takahashi K, Miyashiro M, Yamada H, Uyama M, Matsuzaki K (1998) Immunolocalization of transforming growth factor β during wound repair in rat retina after laser photocoagulation. Graefe's Arch Clin Exp Ophthalmol 236: 41–46
52. Yoshida A, Yoshida S, Khalil AK, Ishibashi T, Inomata H (1998) Role of NF-κB mediated interleukin-8 expression in intraocular neovascularization. Invest Ophthalmol Vis Sci 39: 1097–1106
53. Yoshida M, Tanihara H, Yoshimura N (1992) Platelet-derived growth factor gene expression in cultured human retinal pigment epithelial cells. Biochem Biophys Res Commun 189: 66–72
54. Yoshimura N, Matsumono M, Shimizu H, Mandai M, Hata Y, Ishiba T (1995) Photocoagulated human retinal pigment epithelial cells produce an inhibitor of vascular endothelial cell proliferation. Invest Ophthalmol Vis Sci 36: 1686–1691