Biochemical and Biophysical Research Communications 377 (2008) 104–108

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Up-regulation of semaphorin 3A in human corneal fibroblasts by epidermal growth factor released from cocultured human corneal epithelial cells

Ji-Ae Ko *, Naoyuki Morishige, Ryoji Yanai, Teruo Nishida

Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube City, Yamaguchi 755-8505, Japan

ARTICLE INFO

Article history:
Received 18 September 2008
Available online 1 October 2008

Keywords:
Coculture
Corneal fibroblasts
Corneal epithelial cells
EGF
Sema3A

ABSTRACT

Semaphorins are a family of glycoproteins that play an important role in repulsive axon guidance during embryogenesis. We have now investigated the effect of corneal epithelial cells on the expression of Sema3A in corneal fibroblasts with the use of a coculture system in which the two cell types are separated by a collagen vitrigel membrane. Reverse transcription-polymerase chain reaction and immunoblot analyses revealed that the presence of immortalized human corneal epithelial (HCE) cells increased the expression of Sema3A in human corneal fibroblasts at both the mRNA and protein levels. This effect of HCE cells was mimicked by recombinant human epidermal growth factor (EGF) in a concentration- and time-dependent manner. An inhibitor of the tyrosine kinase activity of the EGF receptor, PD153035, blocked the EGF-induced up-regulation of both Sema3A mRNA and protein in corneal fibroblasts. Depletion of EGF in HCE cells by RNA interference largely abolished the effect of these cells on Sema3A expression in corneal fibroblasts. These findings indicate that EGF released from corneal epithelial cells up-regulates the expression of Sema3A in corneal fibroblasts. This effect of EGF may play an important role in maintenance of corneal structure and repair of corneal damage.

The cornea is unique in terms of its structure and biological properties, consisting of simple layers of epithelial cells, fibroblasts, and endothelial cells as well as being avascular, richly innervated, and transparent. Fibroblasts in the corneal stroma are often activated in individuals with persistent corneal epithelial defects, suggesting that epithelial–mesenchymal interactions may play an important role in maintenance of corneal structure and function. And, cytokine-mediated signaling may thus occur either unidirectionally, from epithelial cells to fibroblasts or from fibroblasts to epithelial cells, or bidirectionally [1].

We recently established an in vitro model based on the coculture of corneal epithelial cells and fibroblasts separated by a collagen membrane for studies of epithelial–mesenchymal interactions. With this model, we showed that the presence of corneal fibroblasts induced up-regulation of the expression of tight-junction proteins in corneal epithelial cells [2], suggesting that corneal fibroblasts might play an important role in maintenance of the barrier function of the corneal epithelium. Furthermore, we found that the presence of corneal epithelial cells up-regulated the expression of connexin43, a component of gap junctions, in corneal fibroblasts [3]. The secretion of insulin-like growth factor-1 from the epithelial cells appeared to contribute to this effect [3]. Epidermal growth factor (EGF) has been shown to affect the proliferation or migration of corneal epithelial cells and fibroblasts [3–5], and its receptor (EGFR) has been shown to be expressed in corneal fibroblasts [6]. The EGF-EGFR axis may thus also participate in epithelial–mesenchymal interactions in the cornea.

The proper distribution of nerve fibers during tissue innervation and development is achieved in part by the action of neural guidance proteins. Such proteins include the semaphorins [7,8], a large family of structurally distinct secreted or transmembrane glycoproteins characterized by the presence of a conserved sema domain of ~500 amino acids [9,10]. Several secreted semaphorins (group-3 semaphorins in vertebrates), such as Sema3A, -B, -C, and -F [11], induce the collapse of certain populations of growth cones in cultured neurons and thus function as inhibitory or repulsive cues in axonal guidance [9,12,13]. Evidence implicating secreted semaphorins in chemorepulsion came initially from the finding that chick Sema3A induces the collapse of growth cones of chick dorsal root ganglion neurons [14]. Sema3A was subsequently shown to act as a chemorepellent for a variety of sensory and motor axons in mammals [15,16]. We recently showed that Sema3A is expressed in all cells of the rat cornea with the exception of superficial epithelial cells [17]. Sema3 proteins and their receptors are also expressed in the neonatal and adult rat retina

* Corresponding author. Fax: +81 836 22 2334.
E-mail address: jiae0831@yamaguchi-u.ac.jp (J.-A. Ko).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.085

[18]. Moreover, lens-derived Sema3A regulates sensory innervation of the cornea [19].

The cornea is the most densely innervated tissue of the human body, containing primarily sensory nerves and some autonomic nerves [20]. In primates, most corneal nerves originate from the ophthalmic branch of the trigeminal nerve and enter the corneal stroma in a radial pattern from the limbus [20,21]. The cell bodies of the sensory nerves of the cornea are located in the trigeminal ganglion [22]. In addition to their role in sensing insults to or irritants in the cornea, corneal nerves possess trophic properties that contribute to maintenance of a healthy ocular surface. Characterization of the regulation of Sema3A expression in corneal fibroblasts might be expected to provide insight into the possible role of Sema3A produced by these cells in nerve growth in the cornea.

We have now investigated the effect of corneal epithelial cells on the expression of Sema3 by corneal fibroblasts in our coculture system. Furthermore, to identify the factors responsible for signaling from corneal epithelial cells to fibroblasts, we examined the effect of EGF released from epithelial cells on the expression of Sema3A in fibroblasts. Our results suggest that expression of Sema3A in corneal fibroblasts is regulated by EGF released from corneal epithelial cells.

Materials and methods

Antibodies and reagents. Rabbit polyclonal antibodies to Sema3A were obtained from Abcam (Cambridge, UK), and mouse monoclonal antibodies to EGF were obtained from R&D Systems (Minneapolis, MN), those to α-tubulin were from Sigma (St. Louis, MO). Recombinant human forms of EGF were obtained from R&D System, Inc. (Minneapolis, MN), and a small interfering RNA (siRNA) specific for human EGF mRNA and the transfection reagent siPORT *NeoFX* were obtained from Ambion (Foster, CA). PD153035 were obtained from Calbiochem (San Diego, CA).

Cells. (1) Human corneal fibroblasts; Human corneal fibroblasts were prepared from the tissue remaining after corneal transplantation surgery and were cultured as described previously [23]. In brief, human corneas were obtained from Mid-America Transplant Service (St. Louis, MO), Northwest Lions Eye Bank (Seattle, WA), or the Eye Bank of Wisconsin (Madison, WI). The donors were white males and females ranging in age from 4 to 65 years.

(2) Human corneal epithelial cells; Simian virus 40-transformed human corneal epithelial (HCE) cells [24] were obtained from RIKEN Biosource Center (Tsukuba, Japan).

Coculture of HCE cells and corneal fibroblasts on a collagen membrane. Corneal fibroblasts (1×10^5) were seeded on a 30-mm-diameter collagen vitrigel membrane (Asahi Technoglass, Tokyo, Japan) in DMEM supplemented with 10% heat-inactivated FBS. After 24 h, the membrane was turned upside down in another dish and HCE cells (2×10^5) were seeded on the empty side of the membrane, also in DMEM supplemented with 10% heat-inactivated FBS. After an additional 6–8 h, the membrane was again turned upside down in another dish containing DMEM supplemented with 0.5% heat-inactivated FBS. As a control, corneal fibroblasts were seeded on a vitrigel membrane without HCE cells. The effects of recombinant human EGF on corneal fibroblasts were determined with cells cultured in 30-mm dishes and deprived of serum for 24 h.

Immunoblot analysis. Corneal fibroblasts were lysed in 200 μl of a solution containing 150 mM NaCl, 2% SDS, 5 mM EDTA, and 20 mM Tris–HCl (pH 7.5). Cell lysates were fractionated by SDS–polyacrylamide gel electrophoresis, and the separated proteins were transferred to a nitrocellulose membrane and exposed consecutively to primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Immune complexes were detected with enhanced chemiluminescence reagents.

RT-PCR analysis. Total RNA was isolated from corneal fibroblasts with the use of an RNeasy kit (Qiagen, Valencia, CA), and portions (0.5 μg) of the RNA were subjected to reverse transcription (RT) and polymerase chain reaction (PCR) analysis with a One-Step RT-PCR kit based on the Platinum Taq system (Invitrogen, Carlsbad, CA). The PCR protocol was designed to maintain amplification in the exponential phase. The sequences of the PCR primers (sense and antisense, respectively) were 5′-CTGTCTTTTCTGGGGAGTATT ACTT-3′ and 5′-AATTAGCACATTCTTTCAGGATGTC-3′ for Sema3A, 5′-GACATTGGTACTGAGTGCATGAACT-3′ and 5′-TCCAAAATACCTT

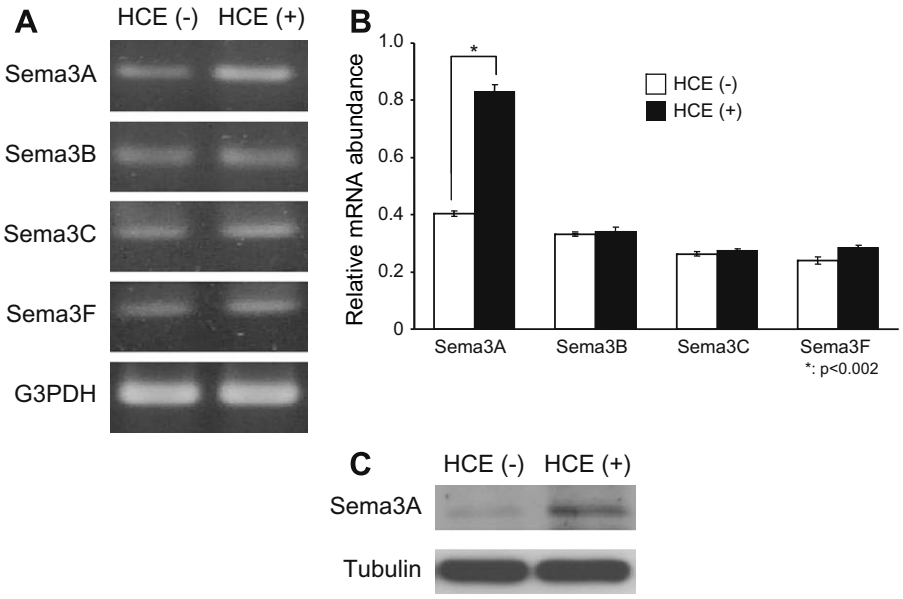

Fig. 1. Effect of HCE cells on Sema3 expression in human corneal fibroblasts. (A) Total RNA prepared from corneal fibroblasts cultured for 12 h on a collagen vitrigel membrane in the absence or presence of HCE cells was subjected to RT-PCR analysis of Sema3A, -B, -C, and -F mRNAs. (B) The abundance of Sema3 mRNAs in experiments similar to that shown in (A) was quantified by image analysis and normalized by the corresponding amount of G3PDH mRNA. Data are means ± SE from three separate experiments. $^*P < 0.002$ versus the corresponding value for corneal fibroblasts incubated in the absence of HCE cells. (C) Lysates of corneal fibroblasts cultured for 24 h on a collagen vitrigel membrane in the absence or presence of HCE cells were subjected to immunoblot analysis with antibodies to Sema3A and to α-tubulin.

GACAAACTTGGG-3′ for Sema3B, 5′-GCGTGTTGGTTGGAGTATTT ATTTG-3′ and 5′-ATTCAGGGAAAGAATGTGATCTTTG-3′ for Sema3C, 5′-ATGCTGGTGTGTACATCGATTTTAT-3′ and 5′-CTCACGGAAGAA GAAGTAAAGCTTA-3′ for Sema3F, and 5′-ACCACAGTCCACGCCAT CAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′ for glyceraldehyde-3-phosphate dehydrogenase (G3PDH, internal control). RT was performed at 50 °C for 30 min, and the PCR cycle comprised incubations at 94 °C for 2 min, 58 °C for 30 s, and 72 °C for 1 min.

RNA interference. HCE cells (2×10^5) were seeded in 30-mm dishes and cultured for 24 h to 50–60% confluence. A small interfering RNA (siRNA) specific for human EGF mRNA (1 μM; Ambion, Foster, CA) was mixed with 5 μl of transfection reagent (siPORT *NeoFX*, Ambion) and then diluted with 200 μl of OPTI-MEM (Invitrogen-Gibco, Carlsbad, CA). After incubation for 10 min at room temperature, the mixture was added to culture dishes and the cells were cultured for an additional 48 h.

Statistical analysis. Quantitative data are presented as means ± SE from three independent experiments and were analyzed by Student's *t*-test. A *P* value of <0.05 was considered statistically significant.

Results

Effects of HCE cells on Sema3 expression in human corneal fibroblasts

We first investigated the effects of the presence of HCE cells on expression of the group-3 semaphorins Sema3A, -B, -C, and -F in human corneal fibroblasts. RT-PCR analysis revealed that the amount of Sema3A mRNA in corneal fibroblasts cultured for 12 h in the presence of HCE cells was about twice that in those cultured without HCE cells (Fig. 1A and B). In contrast, the presence of HCE cells had no effect on the amounts of Sema3B, -C, or -F mRNAs. Immunoblot analysis also revealed that the amount of Sema3A protein in corneal fibroblasts cultured for 24 h in the presence of HCE cells was markedly increased compared with that in those cultured alone (Fig. 1C).

Effect of EGF on Sema3A expression in corneal fibroblasts

To examine the possible role of EGF in the effect of HCE cells on Sema3A expression in corneal fibroblasts, we first determined the effect of exogenous EGF on the abundance of Sema3A mRNA in these latter cells. RT-PCR analysis revealed that EGF increased the amount of Sema3A mRNA in corneal fibroblasts in a concentration-dependent manner; whereas the abundance of the mRNA increased with EGF concentration up to 50 ng/ml, it had decreased to below basal levels in the presence of EGF at 100 ng/ml (Fig. 2A). The effect of EGF (10 ng/ml) on the abundance of Sema3A mRNA was also time-dependent, with the number of transcripts being maximal at 12 h and having returned to control values by 48 h (Fig. 2B). Incubation of corneal fibroblasts alone for 12 h had no effect on the abundance of Sema3A mRNA.

Immunoblot analysis showed that EGF also increased the amount of Sema3A protein in corneal fibroblasts in a concentration-dependent manner; this effect was maximal at an EGF concentration of 50 ng/ml but was no longer apparent at 100 ng/ml (Fig. 2C). The amount of Sema3A was maximal after exposure to EGF (10 ng/ml) for 24 or 48 h but had returned to basal levels by 72 h (Fig. 2D). Incubation of corneal fibroblasts alone for 24 h had no effect on the abundance of Sema3A.

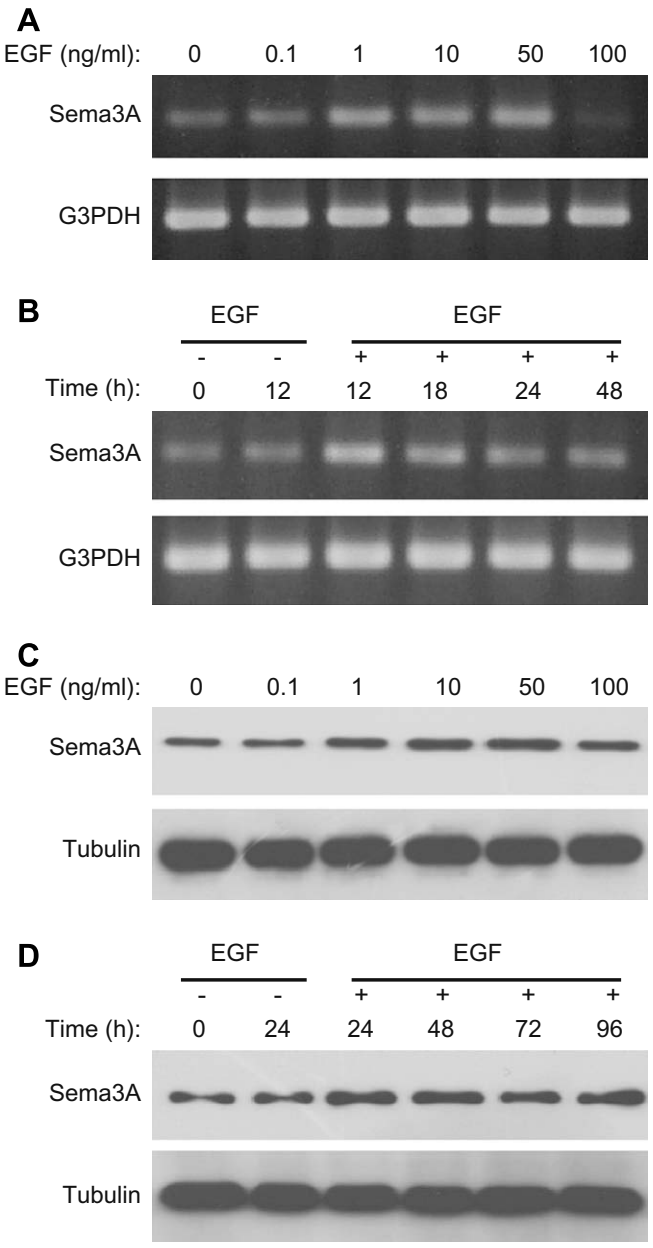

Fig. 2. Concentration dependence and time course of the effect of EGF on the abundance of Sema3A in corneal fibroblasts. Corneal fibroblasts were deprived of serum for 24 h and then incubated either for 12 h in the presence of the indicated concentrations of EGF (A) or for the indicated times in the absence or presence of EGF at 10 ng/ml (B). Total RNA was then isolated from the cells and subjected to RT-PCR analysis of Sema3A and G3PDH mRNAs. Corneal fibroblasts were deprived of serum for 24 h and then incubated either for 24 h in the presence of the indicated concentrations of EGF (C) or for the indicated times in the absence or presence of EGF at 10 ng/ml (D). Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to Sema3A and to α-tubulin.

Role of EGFR in the up-regulation of Sema3A expression in corneal fibroblasts by EGF

To determine whether the effect of EGF on Sema3A expression in corneal fibroblasts was mediated by the EGFR, we exposed the cells to PD153035, a specific inhibitor of the tyrosine kinase activity of EGFR. PD153035 blocked the EGF-induced up-regulation of both Sema3A mRNA (Fig. 3A) and protein (Fig. 3B) in corneal fibroblasts, confirming that the effect of EGF was mediated by EGFR.

Role of EGF in the up-regulation of Sema3A expression in corneal fibroblasts by HCE cells

Finally, we examined the effect of depletion of EGF in HCE cells by RNA interference on the up-regulation by these cells of Sema3A

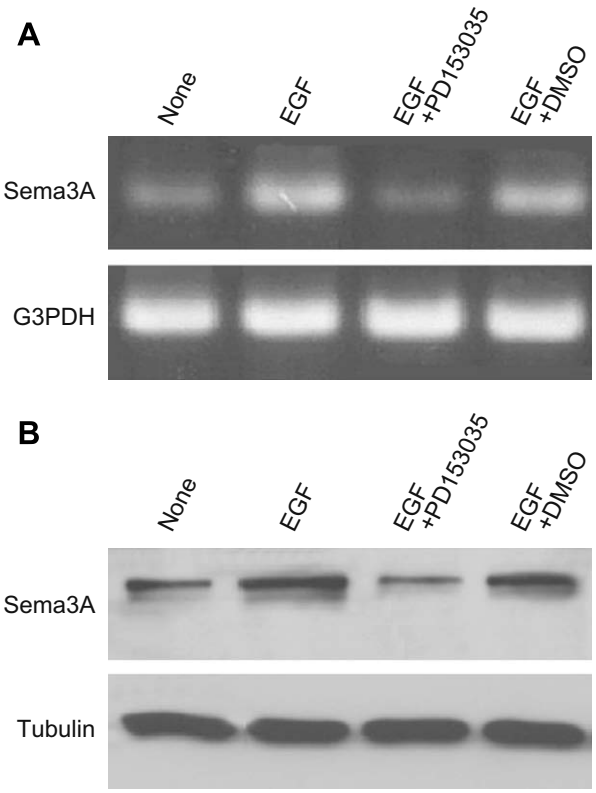

Fig. 3. Effect of the EGFR antagonist PD153035 on the EGF-induced up-regulation of Sema3A expression in corneal fibroblasts. (A) Corneal fibroblasts were deprived of serum for 24 h and then incubated in the absence or presence of EGF (10 ng/ml) or PD153035 (10 μg/ml) for 12 h. Cells were exposed to dimethyl sulfoxide (DMSO) as a control for the PD153035 vehicle. Total RNA was then isolated from the cells and subjected to RT-PCR analysis of Sema3A and G3PDH mRNAs. (B) Cells treated as in (A) but incubated with EGF or PD153035 for 24 h were lysed and subjected to immunoblot analysis with antibodies to Sema3A and to α-tubulin.

expression in corneal fibroblasts. The time course of EGF depletion in HCE cells revealed that the effect of the EGF siRNA was most pronounced after transfection for 48 h (Fig. 4A). Immunoblot analysis revealed that such depletion of EGF in HCE cells largely abolished the stimulatory effect of these cells on Sema3A expression in corneal fibroblasts (Fig. 4B). These results thus indicated that EGF released from HCE cells mediates the effect of these cells on Sema3A expression in corneal fibroblasts.

Discussion

With the use of a coculture system based on a collagen vitrigel membrane, we have shown that the presence of corneal epithelial cells up-regulated the expression of Sema3A in corneal fibroblasts. Depletion of EGF by RNA interference in HCE cells largely abolished the stimulatory effect of these cells on Sema3A expression in corneal fibroblasts, indicating that EGF released from HCE cells is responsible, at least in part, for the observed up-regulation of Sema3A in corneal fibroblasts. Consistent with these results, direct addition of EGF to corneal fibroblasts increased the amounts of Sema3A mRNA and protein in a concentration-, time-, and EGFR-dependent manner. Our observations thus suggest that EGF released from corneal epithelial cells may contribute to homeostasis in the corneal stroma through regulation of Sema3A expression in corneal fibroblasts.

Interactions between two different types of cells have been studied by examining the effects of conditioned medium from one cell type on the function of the other cell type [3,25]. Medium conditioned by corneal fibroblasts has thus previously been shown to stimulate the proliferation of corneal epithelial cells but not that of corneal fibroblasts themselves [25]. In contrast, medium conditioned by corneal epithelial cells did not have any effect on the proliferation of corneal fibroblasts [3]. As an alternative to this system, we have developed a coculture model in which corneal epithelial cells and corneal fibroblasts are cultured on opposite sides of a collagen membrane. No direct interactions between the two cell types are possible in this model, but humoral factors, such as cytokines, released from one cell type may affect the function of the other cell type.

With this system, we have now shown that RNA interference-mediated depletion of EGF in HCE cells prevented the stimulatory effect of these cells on Sema3A expression in corneal fibroblasts. Various growth factors or cytokines have previously been shown to affect the biological functions of corneal epithelial cells or fibroblasts in monolayer culture [3,5,6]. For example, EGF and basic fibroblast growth factor (bFGF) each increased, whereas transforming growth factor-β reduced, the proliferation rate of both cell types in a concentration-dependent manner [3,25]. Transforming growth factor-β was also previously found to up-regulate bFGF expression in keratocytes [26], and the newly synthesized bFGF may then stimulate proliferation in an autocrine manner [27,28]. The stimulatory effect of EGF on the proliferation of corneal fibroblasts suggested a role for this growth factor in corneal maintenance and repair [29,30].

Several secreted semaphorins, including Sema3A, -E, and -F, as well as membrane-bound semaphorins such as Sema4 C, -6A, and

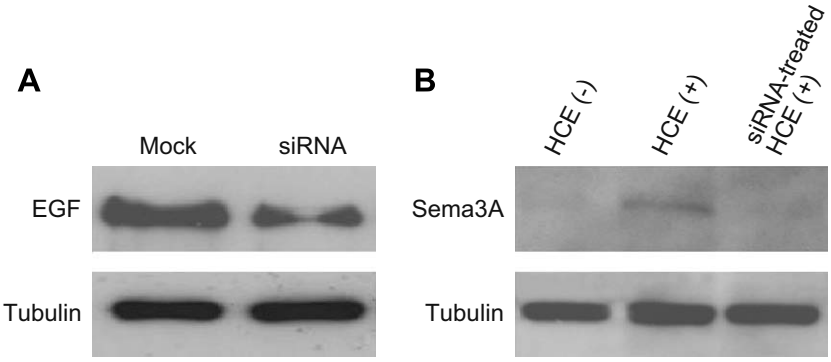

Fig. 4. Effect of EGF depletion in HCE cells on the up-regulation by these cells of Sema3A expression in corneal fibroblasts. (A) HCE cells were either transfected for 48 h with an siRNA specific for EGF mRNA or subjected to mock transfection, after which cell lysates were subjected to immunoblot analysis. (B) Corneal fibroblasts were cultured for 24 h on a collagen vitrigel membrane in the absence or presence of normal HCE cells or of HCE cells that had been transfected with the EGF siRNA for 48 h. Lysates of the corneal fibroblasts were then subjected to immunoblot analysis.

-7A [11] have been shown to be expressed in the cornea at various stages of development [31]. Semaphorin 3A (Sema3A), one of the most well characterized members of this family, also increases the expression of cell adhesion molecules and regulates cell–cell and cell-extracellular matrix interactions as well as cell motility in fibroblasts [32,33]. In particular, the expression of Sema3A and its role in the timing and pattern of sensory innervation in the cornea have been described [19]. We have now shown that, among the group-3 semaphorins examined, the expression of only Sema3A was increased in corneal fibroblasts by the presence of HCE cells and that this effect was mediated by EGF acting at EGFR. EGF has also been shown to up-regulate the expression of neuropilin-1, which is a receptor for Sema3A, in keratinocytes [34]. The EGF-EGFR axis may thus participate in epithelial–mesenchymal interactions in the cornea. Further characterization of the functions of Sema3A and its mechanisms of action in the cornea will provide insight into the role of such interactions in corneal homeostasis.

Acknowledgments

We thank Shizuka Murata, Yukari Mizuno, and Yasumiko Akamatsu for technical assistance.

References

[1] D.Q. Li, S. Tseng, Three patterns of cytokine expression potentially involved in epithelial fibroblasts interactions of human ocular surface, J. Cell. Physiol. 163 (1995) 61–79.
[2] J.-A. Ko, Y. Liu, R. Yanai, T. Chikama, T. Takezawa, T. Nishida, Upregulation of tight-junctional proteins in corneal epithelial cells by corneal fibroblasts in collagen vitrigel cultures, Invest. Ophthalmol. Vis. Sci. 49 (2008) 113–119.
[3] J.-A. Ko, R. Yanai, N. Morishige, T. Takezawa, T. Nishida, Up-regulation of connexin43 expression in corneal fibroblasts by corneal epithelial cells, Invest. Ophthalmol. Vis. Sci., in press.
[4] S.R. Sandeman, M.C. Allen, C. Liu, R.G. Faragher, A.W. Lloyd, Human keratocyte migration into collagen gels declines with in vitro ageing, Mech. Ageing Dev. 119 (2000) 149–157.
[5] M. Haber, Z. Cao, N. Panjwani, D. Bedenice, W.W. Li, P.J. Provost, Effects of growth factors (EGF, PDGF-BB and TGF-β1) on cultured equine epithelial cells and keratocytes: implications for wound healing, Vet. Ophthalmol. 6 (2003) 211–217.
[6] D.Q. Li, S.C.G. Tseng, Differential regulation of cytokine and receptor transcript expression in human corneal and limbal fibroblasts by epidermal growth factor, transforming growth factor-α, platelet-derived growth factor B, and interleukin-1β, Invest. Ophthalmol. Vis. Sci. 37 (1996) 2068–2080.
[7] Y. Goshima, T. Ito, F. Nakamura, Semaphorins as signals for cell repulsion and invasion, J. Clin. Invest. 109 (2002) 993–998.
[8] F. Nakamura, R.G. Kalb, S.M. Strittmatter, Molecular basis of semaphorin-mediated axon guidance, J. Neurobiol. 44 (2000) 219–229.
[9] M. Tessier-Lavigne, C.S. Goodman, The molecular biology of axon guidance, Science 274 (1996) 1123–1133.
[10] A.L. Kolodkin, D.J. Matthes, C.S. Goodman, The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules, Cell 75 (1993) 1389–1399.
[11] Semaphorin Nomenclature Committee, Unified nomenclature for the semaphorins/collapsins, Cell 97 (1999) 551–552.
[12] N. Miyazaki, T. Furuyama, T. Sakai, S. Fujioka, T. Mori, Y. Ohoka, N. Takeda, T. Kubo, S. Inagaki, Developmental localization of semaphorin H messenger RNA acting as a collapsing factor on sensory axons in the mouse brain, Neuroscience 93 (1999) 401–408.
[13] N. Miyazaki, T. Furuyama, M. Amasaki, H. Sugimoto, T. Sakai, N. Takeda, T. Kubo, S. Inagaki, Mouse semaphorin H inhibits neurite outgrowth from sensory neurons, Neurosci. Res. 33 (1999) 269–274.
[14] Y. Luo, D. Raible, J.A. Raper, Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones, Cell 75 (1993) 217–227.
[15] R.H. Adams, M. Lohrum, A. Klostermann, H. Betz, A.W. Puschel, The chemorepulsive activity of secreted semaphorins is regulated by furin-dependent proteolytic processing, EMBO J. 16 (1997) 6077–6086.
[16] H. Kobayashi, A.M. Koppel, Y. Luo, J.A. Raper, A role for collapsin-1 in olfactory and cranial sensory axon guidance, J. Neurosci. 17 (1997) 8339–8352.
[17] N. Morishige, J.-A. Ko, Y. Liu, T. Chikama, T. Nishida, Localization of semaphorin 3A in the rat cornea, Exp. Eye Res. 86 (2008) 669–674.
[18] F. Winter, Q. Cui, N. Symons, J. Verhaagen, A.R. Harvey, Expression of class-3 semaphorins and their receptors in the neonatal and adult rat retina, Invest. Ophthalmol. Vis. Sci. 45 (2004) 4554–4562.
[19] P.Y. Lwigale, M. Bronner-Fraser, Lens-derived semaphorin3A regulates sensory innervation of the cornea, Dev. Biol. 306 (2007) 750–759.
[20] C.F. Marfurt, L.C. Ellis, Immunohistochemical localization of tyrosine hydroxylase in corneal nerves, J. Comp. Neurol. 336 (1993) 517–531.
[21] C.F. Marfurt, S.F. Echtenkamp, Central projections and trigeminal ganglion location of corneal afferent neurons in the monkey, *Macaca fascicularis*, J. Comp. Neurol. 272 (1988) 370–382.
[22] L.J. Muller, G.F. Vrensen, B. Willekens, Architecture of human corneal nerves, Invest. Ophthalmol. Vis. Sci. 38 (1997) 985–994.
[23] Y. Lu, Y. Liu, K. Fukuda, Y. Nakamura, N. Kumagai, T. Nishida, Inhibition by triptolide of chemokine, proinflammatory cytokine, and adhesion molecule expression induced by lipopolysaccharide in corneal fibroblasts, Invest. Ophthalmol. Vis. Sci. 47 (2006) 3796–3800.
[24] K. Araki-Sasaki, Y. Ohashi, T. Sasabe, K. Hayashi, H. Watanabe, Y. Tano, H. Handa, An SV40-immortalized human corneal epithelial cell line and its characterization, Invest. Ophthalmol. Vis. Sci. 36 (1995) 614–621.
[25] K. Chan, R. Haschke, Epithelial–stromal interactions: specific stimulation of corneal epithelial cells in vitro by a factor(s) from cultured stromal fibroblasts, Exp. Eye Res. 36 (1983) 231–246.
[26] S. Tseng, J. Merritt, D. Li, Mechanism of TGF-β paradox in epithelial growth, Invest. Ophthalmol. Vis. Sci. 34 (Suppl.) (1993) 1302.
[27] S. Wilson, Y. He, S. Lloyd, EGF, basic FGF, and TGFβ-1 and IL-1α mRNA in human corneal epithelial cells and stromal fibroblasts, Invest. Ophthalmol. Vis. Sci. 33 (1992) 1756–1765.
[28] S. Wilson, Y.G. He, G. Schultz, Effects of epidermal growth factor, hepatocyte growth factor, and keratinocyte growth factor, on proliferation, motility and differentiation of human corneal epithelial cells, Exp. Eye Res. 59 (1994) 665–678.
[29] M. Hongo, M. Itoi, N. Yamaguchi, J. Imanishi, Distribution of epidermal growth factor (EGF) receptors in rabbit corneal epithelial cells, keratocytes and endothelial cells, and the changes induced by transforming growth factor-β1, Exp. Eye Res. 54 (1992) 9–16.
[30] M. Ohji, N. SunderRaj, R. Thoft, Transforming growth factor-β stimulates collagen and fibronectin synthesis by human corneal stromal fibroblasts in vitro, Curr. Eye Res. 12 (1993) 703–709.
[31] A.H. Conrad, J.M. Strafuss, M.D. Wittman, S. Conway, G.W. Conrad, Thyroxine increases the rate but does not alter the pattern of innervation during embryonic chick corneal development, Invest. Ophthalmol. Vis. Sci. 49 (2008) 139–153.
[32] G. Serini, D. Valdembri, S. Zanivan, G. Morterra, C. Burkhardt, F. Caccavari, L. Zammataro, L. Primo, L. Tamagnone, M. Logan, M. Tessier-Lavigne, M. Taniguchi, A.W. Puschel, F. Bussolino, Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function, Nature 424 (2003) 391–397.
[33] J. De Wit, F. De Winter, J. Klooster, J. Verhaagen, Semaphorin 3A displays a punctuate distribution on the surface of neural cells and interacts with proteoglycans in the extracellular matrix, Mol. Cell. Neurosci. 29 (2005) 40–55.
[34] P. Kurschat, D. Bielenberg, M. Rossignol-Tallandier, A. Stahl, M. Klagsbrun, Neuron restrictive silencer factor NRSF/REST is a transcriptional repressor of neuropilin-1 and diminishes the ability of semaphorin 3A to inhibit keratinocyte migration, J. Biol. Chem. 281 (2006) 2721–2729.