Expression of TGF-β superfamily receptors in the retinal pigmented epithelium

Expressão de receptores da superfamília de TGF-β no epitélio pigmentário da retina

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SUMMARY

Background: **Retinal pigment epithelial (RPE) cells play an important role in the inflammatory response of the eye. Transforming growth factor-beta (TGF-β) and other members of the TGF-β superfamily are described to regulate some RPE cells functions. In this study the expression of TGF-β superfamily receptors in RPE cells at mRNA level was investigated**

Methods: **RT-PCR technique was performed using mRNAs from D407 RPE cells (human RPE cell line) and HaCatTcells (human keratinocyte cell line used as positive control).**

Results: **Expression of 6 type I receptors (TGF-β type I receptor, ALK-1, Activin type I receptor, activin type IB receptor, BMP type IA receptor, BMP type IB receptor), and 4 type II receptors (TGF-β type II receptor, activin type II receptor, activin type IIB receptor, BMP type II receptor) were studied. The results demonstrated that TGF-β, activins and BMPs express their own specific receptors at mRNA level.**

Conclusions: **The present study suggests that TGF-β superfamily members can exert effects on D407 RPE cells through their specific receptors.**

Key words: TGF-β superfamily receptor, Retinal pigment epithelial cells, Polymerase chain reaction.

INTRODUCTION

Retinal pigment epithelial (RPE) cells play a very important role in the normal function of the retina. They are responsible for the formation of the outer blood-ocular barrier, phagocytosis of rod and cone outer segments, vitamin A metabolism, and important regulator of posterior segment ocular inflammatory responses (Yamashita H, 1986; Liversidge J, 1993). Proliferation and migration of RPE cells in pathological situations may contribute to the pathogenesis of proliferative vitreoretinopathy, submacular neovascularization, pigment epitheliopathy and other diseases (Machemer R, 1977).

Transforming growth factors (TGF) were first identified as small polypeptides that caused transformation and induced proliferation of non-neoplastic cells in culture. However, these cytokines are now known to be multifunctional proteins with different effects on many target cells and tissues, being involved in the regulation of inflammation, immune

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http://dx.doi.org/10.5935/0004-2749.19980071

Table 1. Oligonucleotide primers sequence, corresponding bases in the receptor sequence, annealing temperature and predicted size of the PCR product.

Receptors	Primers Sequence Sense/Antisense	Bases Temp (°C)	Annealing Size (bp)	Predicted
TGF-b_1 typeIR	5'-AGATTACCAACTGCCTTATT-3'	1342-1361	55	330
	5'-TATCCTTCTGTTCCCTCTCA-3'	1652-1672		
ALK1	5'-CGTCAACCACTACTGCTGCG-3'	534-553	60	813
	5'-GGTAATCGCTGCCCTGTGAG-3'	1328-1347		
Actvin typeIR	5'-AAGATGAGAAGCCCAAGGTC-3'	168-187	59	340
	5'-GCAGGCAGGCTAAAAGACAT-3'	489-508		
BMP typeIAR	5'-TAGCACCAGAGGATACCTTGC-3'	458-478	55	427
	5'-AATGCTTCATCCTGTTCCAAA-3'	875-885		
Activin typeIBR	5'-GTGGTGATGTGGCTGTGAAA-3'	683-702	65	408
	5'-GGCAATGTCAATGGTGTCAG-3'	1082-1091		
BMP typeIBR	5'-GATGACTCTGGGTTGCCTGT-3'	493-512	65	275
	5'-CGAGGTCTGGTTTCTTGTCTT-3'	748-768		
TGF-b_1 typeIIR	5'-GCAGTGGGAGAAGTAAAAGA-3'	1722-1741	55	287
	5'-TGTTTAGGGAGCCGTCTTCA-3'	1990-2009		
ActivinA typeIIBR	5'-TTTCCCTCATCGTCCTGCTG-3'	450-469	65	623
	5'-CGTCTCGTGCCTACCTGTCC-3'	1054-1073		
ActivinA typeIIR	5'-TACACCTAAGCCACCCTATT-3'	563-582	59	458
	5'-CAGTTCATTCCAAGAGACCA-3'	1002-1021		
BMP typeII	5'-CAGAATCAAGAACGGCTATG-3'	244-263	55	442
	5'-TTGTTTACGGTCTCCTGTCA-3'	667-686		

responses and tissue repair (Wahl SM, 1989). Among TGF there is the TGFβ superfamily that includes TGFβ isoforms (TGFβ1, TGFβ2, TGFβ3) as well as other structurally related multifunctional proteins such as activins and bone morphogenic proteins (BMPs) which are expressed in the eye and it has been reported to exert some effects on the RPE functions (Leschey KH, 1990; Sheu SJ, 1994; Murphy TL, 1995; Gabrielian K, 1994; Osusky R, 1994).

It has been described that RPE cells produce TGFβ superfamily members (Lutty GA, 1993; Pfeffer BA, 1994; Anderson DH, 1995) and express their mRNA (Tanihara H, 1993; Jaffe GJ, 1994; Kvanta A, 1994).

Our purpose in the current study was to investigate the expression of mRNA transcripts for TGFβ superfamily receptors in cultured human RPE cells.

MATERIALS AND METHODS

Cell cultures

- A spontaneously arising, transformed cell line from human RPE cells, D407 (Davis AA, 1995) (obtained from Dr. R. C. Hunt from the University of South Carolina, USA) was used and cultured in Eagle's minimum essential medium (EMEM, Nikken Biomedical Lab., Kyoto, Japan) containing 15% fetal bovine serum (FBS, Gibco BRL, Gaithersbutg, MD, USA) and gentamicin (20 μg/ml, Bio Whittaker, MD, USA). These cells were incubated in a humidified incubator in a 5% CO_2 atmosphere at 37°C, and the culture medium was changed every 48 hours. As it is a cell line, it mantains its characteristics even after several passages. In this study, cells were seeded at passage 261 and the experiment was repeated over 5 times.

- Human keratinocyte cell line (HaCaT), (received from Prof. Dr. Norbert E. Fusenig, head of the Division of Differentiation and Carcinogenesis of the German Cancer Research Center in Heidelberg, Germany) (Boukamp P, 1988) was used as the positive control of TGF-β superfamily receptor expression (TGF-β1 and TGF-β2 receptors: Game S M, 1992; activin and BMP receptors: personal communication from Dr. H. Yamashita of the Department of Ophthalmology, Faculty of Medicine, University of Tokyo, Japan). HaCat cells were maintained in EMEM with addition of 10% FBS.

Expression of TGF-β superfamily receptors

1μg of messenger RNA (mRNA) was extracted via 0.05 trypsin-EDTA (Gibco-Grand Island, NY) digestion (enzyme action was neutralized by serum-supplemented media) from subconfluent culture of RPE cells using Quick Prep Micro mRNA Purification Kit (Pharmacia Biotech, Uppsala, Sweden) and reverse transcribed into first-stranded complementary DNA (cDNA) by First-strand cDNA Synthesis Kit (Pharmacia Biotech). Human keratinocyte cell line (HaCat cells) was used as positive control.

cDNAs from RPE cells and HaCat cells were used as templates for PCR. PCR was performed with 0.5ml of cDNA template, 10pmol of sense, 10pmol of antisense, 10ml of 10X PCR buffer, 8ml of 20mM dNTTPs, 2.5U Taq polymerase (Takara, Japan) and sterile water to 100ml, using Astec Program Temperature Control System, PC-700, according to the following: 1 cycle of denaturation at 95°C (5min), annealing temperature of 55-65°C, depending on primers used (2min), extension at 72°C (2min); 28 cycles of94°C (1min), 55-65°C, depending on primers (2min), 72°C (2min), followed by 1 cycle of 94°C (1min), 55-65°C, depending on primers (2min)

and 72°C (10min). Oligonucleotide primers (obtained from Dr. K. Miyazono of the Department of Biochemistry, the Cancer Institute, Japan), predicted size for RT-PCR products and annealing temperature are listed in Table 1. The same experiment was performed using a greater amount of sterile water instead of cDNA as negative control. After PCR, each sample was electrophoresed (Mupid/Advance Co Ltd 0.4X3, 100V) through a 1.5% (wt/vol) agarose gel and stained with ethidium bromide.

RESULTS

Expression of TGF-β superfamily receptors

In order to confirm the expression of TGF-β superfamily receptors in RPE cells, RT-PCR was performed using mRNAs from RPE cells and HaCat cells. Among the examined receptors, the predicted PCR products of 5 type I receptors (TGF-β type I receptor, Activin type I receptor, activin type IB receptor, BMP type IA receptor, BMP type IB receptor) (Fig. 1), and 3 type II receptors (TGF-β type II receptor, activin type II receptor, BMP type II receptor) (Fig. 2) were detected in RPE cells and HaCaT cells. Activin receptor-like kinase 1 (ALK-1) receptors were not detected neither in RPE cells nor in HaCaT cells.

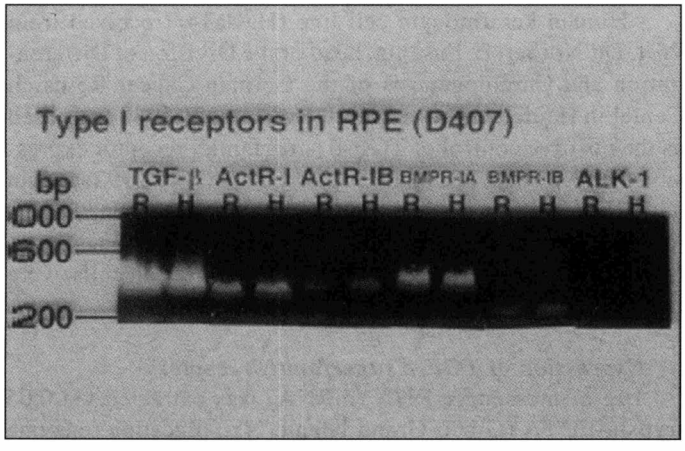

Fig. 1 - Expression of TGF-β superfamily type I receptors. C: HaCat control, R: RPE cell line (D407)

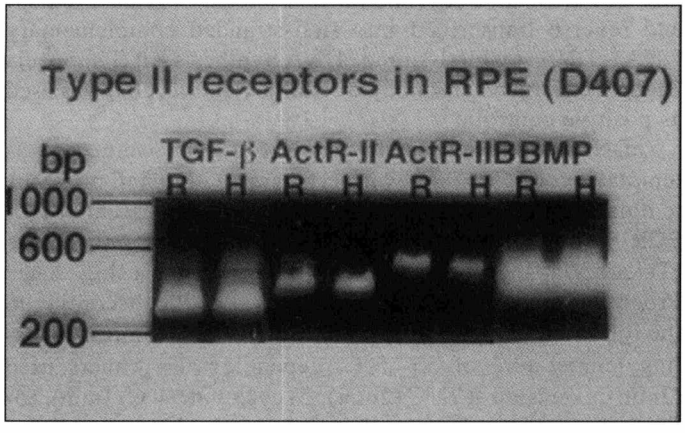

Fig. 2 - Expression of TGF-β superfamily type II receptors. C: HaCat control, R: RPE cell line (D407)

DISCUSSION

The response of RPE cells in many pathological situations is very important for the evolution of the disease (resolution or complications) (Yamashita H, 1986; Liversidge J, 1993) and growth factors, such as TGF-β superfamily members, seem to play an important role on this response (Yoshimura N, 1995; Matsumoto M, 1994).

It is likely that the TGF-β superfamily members receptors have a serine/threonine kinase activity domain in common that is very important for signal transduction. TGF-β signal transduction depends on two types of receptors, type I and type II, which have a cytoplasmic protein serine/threonine kinase activity. Both types I and II receptors must interact with each other in a receptor complex for the signal transduction: receptor type II binds TGF-ß (ligand) and receptor type I transduces the signal (Miyazono K, 1994, Miyazono K, 1994).

Fetal human RPE cells were cultured for this experiment, however the amount of cells during the first three passages was insufficient for mRNA extraction. After the fourth passage, cells lost their characteristics and D407, a human cell line, was chosen.

Transformed and nontransformed human RPE cells express and secrete TGF-β (Kvanta A, 1994), and D407, RPE cell line used in this study, expressed 5 TGF-β superfamily type I receptors and 3 type II receptors, demonstrated through RT-PCR method, meaning that this occured at mRNA level. Each of all type I and type II receptors obtained from RPE showed the same size as those from HaCat cells (positive control). We also investigated the expression of ALK-1 receptor that has not been described yet. However its mRNA was not detected by RT-PCR in cultured human D407 RPE cells nor in HaCaT cell line.

The present results demonstrated that TGF-β, activins and BMPs express their own specific receptors at mRNA level and these growth factors can exert effects on D407 RPE through them. It suggests that TGF-β superfamily members act directly on the cell surface activating some intracellular signal transduction pathway. Our aim in the next study is to investigate this pathway since the ligand biding to its receptor, the intracellular messengers involved in the signal transduction to the evidence of the growth factor influence in cell migration and proliferation.

RESUMO

Objetivo: *O epitélio pigmentário da retina (EPR) desempenha um importante papel na resposta inflamatória ocular. "Transforming growth factor-beta" (TGF-β) e outros membros de sua superfamília têm sido descritos*

como reguladores de certas funções do EPR. Neste estudo, os autores investigaram a expressão de receptores da superfamília de TGF-β superfamily nas células do EPR a nível de RNA mensageiro.

Métodos: *Técnica de RT-PCR foi usada com RNA mensageiro de D407 (linhagem de células do EPR humano) e HaCatT (linhagem de queratócitos humanos usados como controle positivo).*

Resultados: *A expressão de 6 receptores tipo I (TGF-β receptor tipo I, ALK-1, activina receptor tipo I, activina receptor tipo IB, BMP receptor tipo IA, BMP receptor tipo IB), e 4 receptores tipo II (TGF-β receptor tipo II, activina receptor tipo II, activina receptor tipo IIB, BMP receptor tipo II) foi investigada. Os resultados demonstraram que TGF-β, activinas e BMPs expressam receptores específicos a nível de RNA mensageiro em células do EPR.*

Conclusões: *O presente estudo sugere que membros da superfamília de TGF-β podem exercer efeitos nas células de EPR D407 através de seus receptores específicos.*

Palavras chave: *Receptor da superfamília TGF-β; Células do epitélio pigmentário da retina; Reação em cadeia da polimerase.*

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