in vivo *25*: 179-184 (2011)

Expression of the Insulin-like Growth Factor 1 (IGF-1) and Type I IGF Receptor mRNAs in Human HLE-B3 Lens Epithelial Cells

MARILITA M. MOSCHOS[1,2], ATHANASIOS ARMAKOLAS[2], ANASTASIOS PHILIPPOU[2], NIKOLAOS PISSIMISSIS[2], ZACHAROULA PANTELEAKOU[2], ADRIANOS NEZOS[2], MARIA KAPARELOU[2] and MICHAEL KOUTSILIERIS[2]

Departments of [1]Ophalmology and [2]Experimental Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Abstract. *Background/Aim: The E peptide of the IGF-1Ec transcript has been documented to stimulate the growth of different cell lines, via a type I IGF-1 receptor (IGF-1R)-independent mechanism. The aim of the present study was to determine the implication of the IGF-1Ec isoform into the posterior capsule opacification process in human lens epithelium. Materials and Methods: The expression of the IGF-1 system was characterized in human HLE-B3 lens epithelium cells and the mitogenic activity of IGF-1 and synthetic E peptide and the effects of growth hormone (GH) and dihydrotestosterone (DHT) were examined, using qualitative real-time PCR, RT-PCR, Western blot analysis and trypan blue exclusion assays in wild-type and IGF-1R knock-out HLE-B3 cells. Results: The data showed that HLE-B3 cells express only the IGF-1Ea and IGF-1R transcripts. GH increased the expression of IGF-1Ea and of the previously undetectable IGF-1Eb mRNA. Finally, IGF-1 did not present any activity in the knock-out cells. Conclusion: The IGF-1Ea isoform is the main source for the formation of mature IGF-1 in HLE-B3 cells. The effects of exogenous IGF-1 depend on the existence of IGF-1R. IGF-1 Ec is not expressed even in the presence of GH or DHT nor has it any effect on cell proliferation.*

There are several lines of evidence suggesting that the insulin-like growth factor (IGF-1) is implicated in mechanisms involving lens cells proliferation and differentiation (1). The *IGF-1* gene encodes different IGF-1 isoforms by alternative splicing (namely, IGF-1Ea, IGF-1Eb and IGF-1Ec) (2), which are regulated by growth hormone (GH) (3, 4). The post-translational cleavage of all the IGF-1 precursor polypeptides results in the formation of the mature IGF-1 peptide (exons 3 and 4) and in different carboxy-terminal E peptides (exons 5 and 6) (2).

Recently, it has been suggested that the preferential expression of the IGF-1Ec transcript generates a specific E peptide, which has been associated with skeletal muscle regeneration processes (5-12), myocardium remodelling (13), endometriosis (14), even cancer biology (15). A synthetic E peptide was demonstrated to stimulate cell growth acting *via* a type I IGF-1 receptor (IGF-1R)-independent and an insulin receptor (IR)-independent mechanism *in vitro* (14, 15). These data supported the notion that preferential expression of IGF-1Ec isoform produces two bioactive products, namely IGF-1 and E peptide, however, each one acting *via* a distinct receptor. This issue has not been addressed as yet in human lens epithelium cells.

This study assessed the expression of the IGF-1 isoforms and of IGF-1R, at both the mRNA and protein level and examined the proliferative effects of mature IGF-1 and synthetic E peptide in human HLE-B3 lens epithelial cells and in genetically engineered IGF-1R knock-out (KO) HLE-B3 cells, using silencing methods (KO HLE-B3 cells).

Materials and Methods

Cell cultures. The HLE-B3 cells, a human lens epithelial cell line, were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained as sub-confluent monolayers cultures using Eagle's minimum Essential medium (EMEM) which contained 2 mM L-glutamine (Cambrex, Walkerville, MD, USA) supplemented with 20% heat-inactivated foetal bovine serum (FBS; Biochrom, Berlin, Germany) and 100 U/ml penicillin/streptomycin (Cambrex) at 37˚C in a humidified atmosphere with 5% CO_2. Cell culture media were replaced every three days.

RNA extraction and semi-quantitative PCR and quantitative real-time PCR. Total RNA was isolated from HLE-B3 cells using TRI-reagent (MRC, Amsterdam, Holland) according to the manufacturer's

Correspondence to: Dr. Michael Koutsilieris, MD, Ph.D, Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, 75 Micras Asias, Goudi-Athens, 115 27, Greece. Tel: +30 2107462597, Fax: +30 2107462571, e-mail: mkoutsil@med.uoa.gr

Key Words: IGF-1, lens epithelium cells, IGF-1R.

protocol, as previously described (15). Each reverse transcriptase (RT) reaction was carried out as previously described (15).

Briefly, 2 mg of RNA were mixed with 10 mM dNTPs (HT Biotechnology Cambridge, UK), 3 mg/ml Random Hexamer Primers (Invitrogen, Carlsbad, CA, USA) and 40 U human placental ribonuclease inhibitor (HT biotechnology). The reaction was then heated at 65˚C for 5 min and quick-chilled on ice. The RT buffer containing 200 U/ml of MMLUV Reverse Transcriptase (Finzymes, Espoo, Finland) was then added and the reactants were incubated at 42˚C for 50 min and 70˚C for 20 min. The obtained cDNA was amplified by RT-PCR and quantitative RT–PCR.

Different pairs of primers were designed using the Primer Select computer program (DNAStar; GIBCO, Carlsbad, CA, USA) and obtained from Invitrogen; each set of primers was designed to include sequences from different exons to ensure the detection of specifically one of the IGF-1 transcripts (9), avoiding amplification of genomic DNA and after testing for their compatibility with the alternate 18S primers (Table I). For each primer set, PCR conditions were set to optimal conditions and normalised according to the results of linearity tests for each target mRNA and 18S.

The expected sizes of the specific PCR products were initially verified by electrophoretic separation on agarose gel and all target sequences were identified by sequencing analysis to ensure specificity of the primers and to further verify each target mRNA.

A relative quantitative PCR method, using 18S ribosomal RNA as internal standard, was applied to study the expression of mRNAs for the three IGF-1 transcripts and for IGF-1R in HLE-B3 cells. Each target cDNA was co-amplified with 18S internal standard (Ambion, Austin, TX, USA). The PCR was obtained using to the HotStartTaq DNA Polymerase Kit according to manufacturer's instructions (Qiagen, Valencia, CA, USA). The reactions were carried out in a PTC-200 Peltier Thermal Cycler, (MJ Research, Waltham, MA, USA). The PCR products were separated on a 2% agarose gel by electrophoresis, stained with ethidium bromide and analysed with Kodak EDAS 290 imaging system (Carestream Health, Rochester, NY, USA). Each target cDNA signal was normalised to its corresponding ribosomal 18S and expressed as fold of change from the values of controls, as previously described and validated (5, 8, 13).

The obtained cDNA was also examined by quantitative real-time PCR, as previously described (15). Each reaction was obtained in 25 μl using 12 μl SYBR green Supermix (Biorad Laboratories, Hercules, CA, USA), 0.5 μg/ml oligo dTs (Fermentas, Glen Burnie, MD, USA), 2 μl cDNA, and 0.3 μM primers for the IR and for the IGF-1R. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-actin were used as internal controls in each case. The validation of the product identity and expression was obtained by the melting curve analysis and Ct analysis.

Western blot analysis. Total proteins were extracted from confluent HLE-B3 cells using RIPA buffer (50 mM Tris-HCl, 150 mM NaCl; Sigma, St. Louis, MO, USA) containing protease and phosphatise inhibitors (Sigma). The extracts were analysed for total protein concentration using a kit based on the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA). HLE-B3 cells were analysed by Western blot analysis for IGF-1Ec (mechano growth factor, MGF), mature IGF-1 and IGF-1R, as previously described (13-15). The primary antibodies used were: a rabbit anti-human MGF polyclonal antibody (1:10,000 dilution) (16); IGF-1, a mouse monoclonal anti-IGF-1 (1:1,000 dilution) (MS-1508, Thermo Scientific, Fremont, CA, USA; molecular weight of antigen: 7.6 kDa, approximately) and IGF-1R. Membranes were then incubated with a horseradish peroxidase-conjugated secondary anti-rabbit IgG (goat anti-rabbit, 1:2,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-mouse IgG (goat anti-mouse, 1:2,000 dilution; Santa Cruz Biotechnology), for 1 h at room temperature. GAPDH was used as an internal control to correct for potential variation in the protein loading and to normalise the protein measurements on the same immunoblot. A mouse monoclonal primary antibody was used for GAPDH (1:2,000 dilution; Santa Cruz Biotechnology), with a horseradish peroxidase-conjugated secondary anti-mouse IgG (goat anti-mouse, 1:2,000 dilution; Santa Cruz Biotechnology). Specific band(s) were visualised by exposure the membrane to X-ray film, after incubation with an enhanced chemiluminecent (ECL) substrate according to the manufacturer's protocol (SuperSignal; Pierce Biotechnology, Rockford, IL, USA). The films were captured under white light in a Kodak EDAS 290 imaging system (Carestream Health).

Table I. *The sequence of the specific sets of primers used in mRNA RT-PCR analyses*.

Target mRNA	PCR primer sequence	Product size (bp)
IGF-1Ea	5'-GTGGAGACAGGGGCTTTTATTTC-3'	
	5'-CTTGTTTCCTGCACTCCCTCTACT-3'	251
IGF-1Eb	5'-ATGTCCTCCTCGCATCTCT-3'	
	5'-CCTCCTTCTGTTCCCTC-3'	411
IGF-1Ec	5'-CGAAGTCTCAGAGAAGGAAAGG-3'	
	5'-ACAGGTAACTCGTGCAGAGC-3'	150
IGF-1R	5'-ACCCGGAGTACTTCAGCGC-3'	
	5'-CACAGAAGCTTCGTTGAGAA-3'	230

Cell proliferation assays. Trypan blue exclusion assays were used to measure the number of viable cells, as described elsewhere (14, 15, 17, 18). HLE-B3 cells were plated at a cell density of approximately 2.3×10^4 cell/well in 6-well plates and grown with EMEM:F12 medium containing 10% FBS. Twenty-four hours after plating, the media was changed to EMEM:F12 containing 0.5% FBS in order to reveal the full effect of the mitogens used. In addition, HLE-B3 cells were exposed to either 50 ng/ml of GH or 50 ng/ml of mature IGF-1 peptide (rhIGF-1; Chemicon International, Temecula, CA, USA) or 50 ng/ml dihydrotestosterone (DHT) for 24 and 48 h. A synthetic E peptide corresponding to the last 24 amino acids of the *C*-terminal of the IGF-1Ec E-peptide (parts of exons 5 and 6), as previously described (14, 15), was also used in an identical manner for HLE-B3 cells. Control HLE-B3 cell cultures were treated with phosphate-buffered saline (PBS).

IGF-1R siRNA KO. In order to further investigate the role of IGF-1R in HLE-B3 cells proliferation and to assess whether the synthetic MGF E peptide acts on HLE-B3 cells *via* the IGF-1R-mediated pathway, the IGF-1R expression of HLE-B3 cells was silenced using the commercially available Stealth siRNA technology (Invitrogen), as described previously (14, 15). Briefly, three different 25mer siRNA molecules were examined for their potential to knock out the expression of IGF-1R in HLE-B3 cells. It was determined that the most efficient KO of the IGF-1R was obtained by using the UCUUCAAGGGCAAUUUGCUCAUUAA siRNA duplex, at a

concentration of 50 pmol, using reverse transfection according to the manufacturer's instructions. HLE-B3 cells, both wild-type and treated with a universal negative control stealth siRNA, were used as controls. In brief, HLE-B3 cells were grown in EMEM 2 mM L-glutamine media supplemented with 20% FBS. The transfection mixture was obtained by diluting the 40 pmol of the siRNA duplex in 100 μl OptiMem serum-free medium (Invitrogen) in a well of a 24-well plate, followed by the addition of 2 μl lipofectamine RNAiMAX (Invitrogen). After 20 min, 500 μl of the trypsinised HLE-B3 cells were added to the mixture. Forty-eight hours after the KO, the media switched to Dulbecco's modified Eagle's medium (DMEM) 0.5% FBS and, after 24 h, the IGF-1R KO cells were exposed to mature IGF-1 or MGF E peptide for 24 and 48 h in triplicate. The viable cells were counted using the trypan blue exclusion assay.

Charaterization of the IGF-1R KO HLE-B3 cells. The expression level of IGF-1R transcript, after the siRNA IGF-1R KO in HLE-B3 cells, was assessed by quantitative real time-PCR (qRT-PCR). The KO mRNA levels were determined 48 h after the siRNA KO according to the manufacturer's instructions. GAPDH and β-actin were used as internal controls. The validation of the product identity was obtained by the melting curve. Prior to and after the siRNA IGF-1R in HLE-B3 cells, qRT-PCR was performed to examine the levels of expression of IGF-1R. Briefly, quantification analysis of RT-PCR data was carried out in the forms of melting and amplification curves, Ct values and normalised gene expression (ddCt), using the Bio-Rad's IQ5 optical software 2.0. The primers used in the reactions were generated using FastPCR software (Primerdigital, Helsinki, Finland) and were forward: ACCCGGAG TACTTCAGCGC; reverse: CACAGAAGCTTCGTTGAGAA. The PCR conditions were as follows: 95˚C for 30 s ×1 cycle, 94˚C for 20 s, 60˚C for 30 s, 72˚C for 30 s × 35 cycles and 72˚C for 5 min.

Statistical analysis. Changes in cell numbers were assessed with two-way analysis of variance (ANOVA) using SPSS v. 11 (SPSS Inc., Chicago, USA). Where significant F ratios were found for main effects or interaction ($p<0.05$), the means were compared using Tukey's *post-hoc* tests. All data are presented as mean±standard error of the mean. The level of significance was set at $p<0.05$.

Results

IGF-1 and IGF-1R mRNA expression in HLE-B3 cells. The HLE-B3 cells did express the IGF-1Ea transcript (Figure 1A); however, they did not express either the IGF-1Eb or the IGF-1Ec transcript (Figures 1B and 1C, respectively). HLE-B3 cells did express mature IGF-1 (Figure 2A) and IGF-1R, as detected by Western blot analysis (Figure 2B). The lack of the expression of the IGF-1Ec transcript was also verified by real-time PCR (Figure 2C).

Exogenous administration of GH for 24 and 48 h produced a two-fold increase of the expression of the IGF-1Ea transcript (Figure 3A), while it induced the previously undetectable expression of IGF-1Eb (Figure 3B). Interestingly, under the experimental conditions used, the expression of IGF-1Ec was not detected in HLE-B3 cells either at the mRNA level or at protein level (Figure 3C). IGF-1R expression was not affected by GH in HLE-B3 cells (Figure 3D). Moreover, DHT did not alter the expression of IGF-1 and IGF-1R mRNAs in HLE-B3 cells (data not shown).

HLE-B3 cell proliferation. Exogenous administration of mature IGF-1 increased the number of living HLE-B3 cells. E peptide and GH did not stimulate the growth of HLE-B3 cells *in vitro* (Figure 4). Silencing of the IGF-1R expression in HLE-B3 cells blocked the mitogenic effect of IGF-1 in IGF-1R KO HLE-B3 cells. E peptide did not stimulate the growth of the IGF-1-R KO HLE-B3 cells (Figure 5). These data suggest that IGF-1 stimulated the growth of HLE-B3 cells *via* an IGF-1R mechanism and HLE-B3 cells did not possess the putative E peptide-specific receptor.

Discussion

Posterior capsule opacification (PCO) is one of the most commonly encountered complications after cataract extraction, with an occurrence rate of 50% in adults and 100% in children at 2-5 years after surgery, often leading to a substantial decrease in visual acuity (19). It reflects the wound-healing process of lens epithelial cells (LECs) in the capsular bag after cataract surgery. It has been reported that the opacification is a result of LEC proliferation, migration and metaplasia, along with collagen and basal lamina production in the hypolemmal part of the anterior capsule and at the equator (20-22). The LEC proliferation may start just a few hours after surgery and it is one of the main causes of PCO, thus it may be a possible target in the prevention of PCO (23). Within a wide range of therapeutic targets, growth factors and receptors may play an important role since they are expressed by LECs and influence their post-operative proliferation (24).

This pathogenesis is believed to reflect the wound-healing process of LECs that occurs in the capsular bag after cataract surgery (19). There is evidence that inappropriate TGFβ1 signalling in the anterior lens epithelial cells results in an epithelial–mesenchymal transition (EMT) of human lens epithelial cells, such as the HLE-B3 cells. Previous studies have demonstrated that fibronectin has been detected among subcapsular plaques in rat lens cultured with TGFβ1, as well as in LECs transformed into mesenchymal-like cells in type I collagen gel culture systems. IGF-1 is implicated in mechanisms involving lens cell proliferation and differentiation (1, 25) and it is also responsible for TGFβ1-mediated fibronectin accumulation in human LECs. This suggests that IGF-1 plays a major role in the EMT phenomenon that bears morphological and molecular resemblance to human cataracts, including anterior subcapsular and posterior capsule opacification.

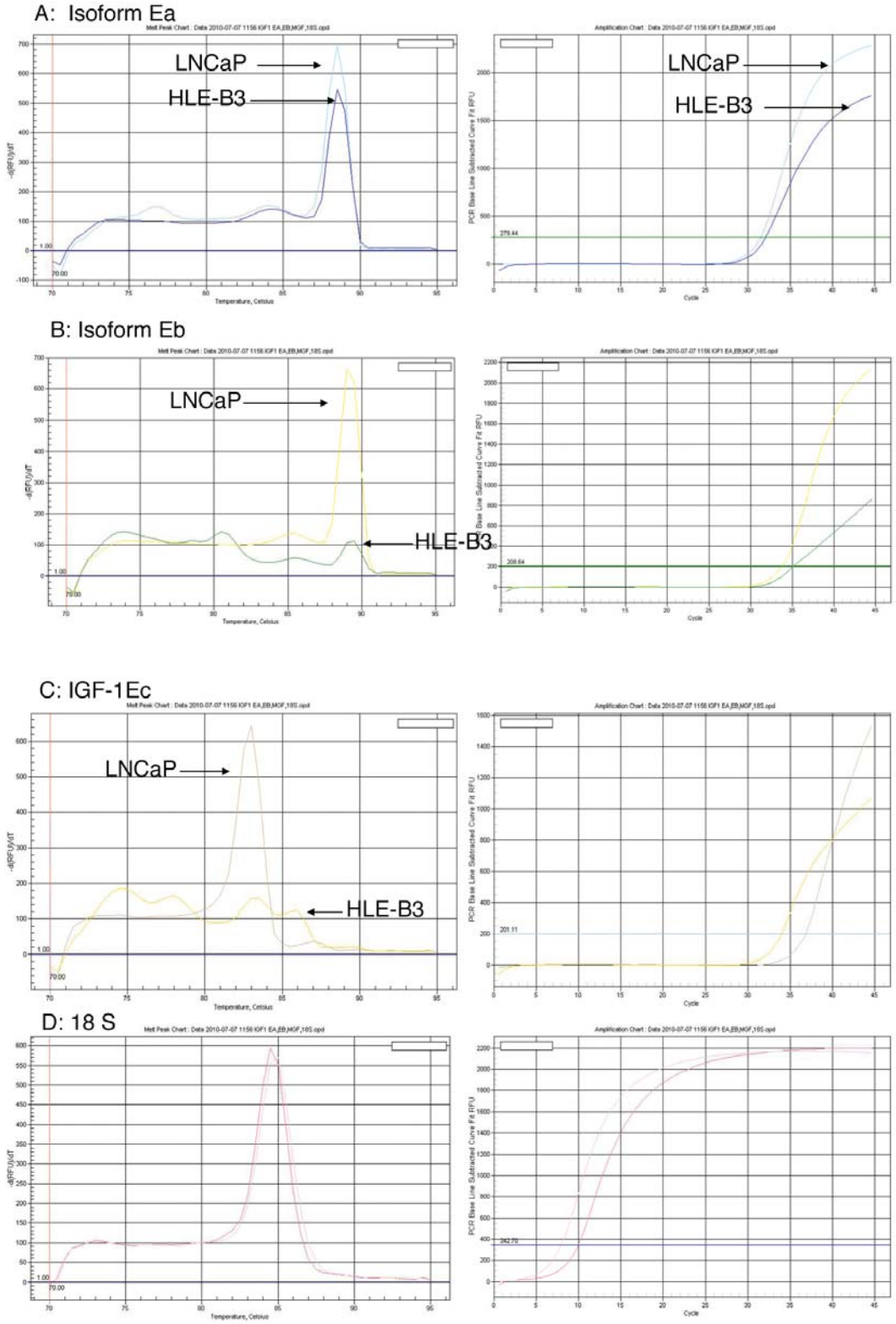

Figure 1. *Transcriptional expression determined with qRT-PCR, of the different IGF-1 isoforms in HLE-B3 cells. HLE B3 cells express only the isoform IGF1-Ea (A). LNCaP prostate cancer cells were used as a positive control for the three isoforms. Note that no mRNA expression of the IGF-1Eb (B) and IGF-1Ec (MGF) (C) isoforms was detected in the HLE-B3 cells. The normalisation was obtained by using 18S (D).*

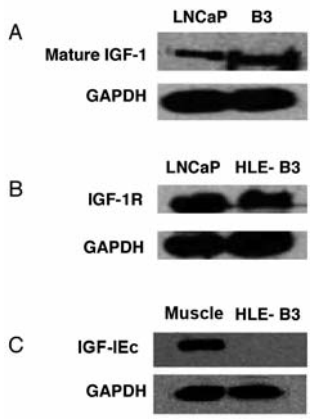

Figure 2. *In order to examine and characterize the IGF-1 bioactive system in HLE-B3 cells, Western blot analysis was performed, revealing that these cells express mature IGF-1 (A) and also express the IGF-1R (B). The absence of the isoform IGF-1Ec previously detected by qRT-PCR was also verified at the protein level. A human skeletal muscle sample was used as a positive control, since the expression of this particular IGF-1 isoform in human muscle has been previously confirmed.*

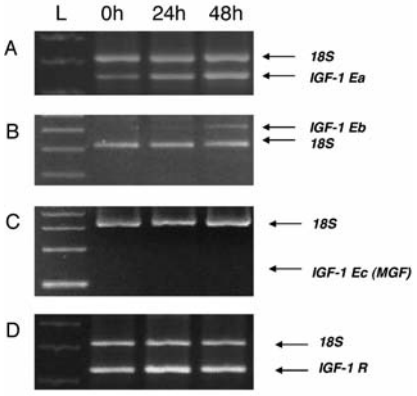

Figure 3. *GH effect on the expression of IGF-1 Ea, Eb, Ec and IGF-1R after 24 and 48 h was associated with Eb (B) isoform expression and with an at least 2-fold increase of the Ea (A) isoform at 24 and 48 h. GH administration did not affect the IGF-Ec isoform (C) nor IGF-1R (D) expression.*

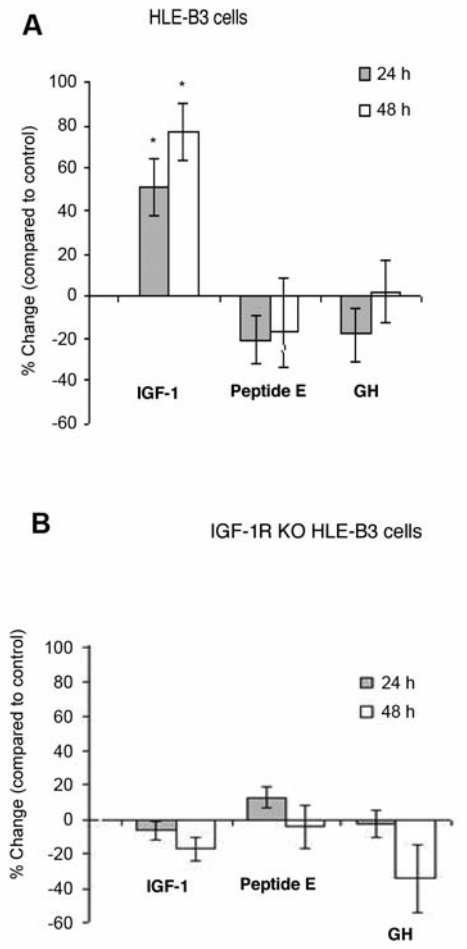

Figure 4. *(A) Treatment with mature IGF-1 and peptide E on HLE-B3 cell proliferation as assessed by trypan blue exclusion assays. In all the control cultures, HLE-B3 cells were treated under the same procedure using PBS (solvent of the treatment factors). (B) The effects of 24 and 48 h treatment with mature IGF-1 peptide E and GH on HLE-B3 cell proliferation as assessed by trypan blue exclusion assays The mitogenic activity of IGF-1 was blocked in IGF-1R KO HLE-B3 cells. Data are presented as the mean±standard error of the mean of three different wells from three repeated experiments.*

The present study analysed the expression of the three different isoforms of IGF-1 and revealed that HLE-B3 cells express only the IGF-1Ea transcript. Since mature IGF-1 has been suggested to be involved in many different ways in the pathogenesis of subcuptular cataracts, the study examined whether the proliferative mode of action of IGF-1 is generated *via* IGF-1R. Recently, there have been data from this group showing that E peptide can stimulate the proliferation of various cell type *via* an IGF-1R- and IR-independent mechanism (14, 15). Since the IGF-1Ec transcript was not expressed in HLE-B3 cells, the study further examined whether these cells possess the putative E peptide receptor. Thus, it was investigated whether HLE-B3 cells respond to exogenous administration of the synthetic E peptide. The data demonstrated that IGF-1 acts *via* IGF-1R and that E peptide does not affect HLE-B3 cell proliferation.

Experiments using the IGF-1R KO HLE-B3 cells have confirmed that IGF-1 acts mainly *via* IGF-1R in HLE-B3 cells. In addition, the present study showed that the exogenous administration of GH enhances the expression of IGF-1Ea and induces the expression of IGF-1Eb isoform in HLE-B3 cells. Notably, DHT did not affect the expression of any IGF-1 isoforms and IGF-1R in HLE-B3 cells. In

conclusion, IGF-1Ec and E peptide probably have no biological role in HLE-B3 cells and for such cells the only bioactive product of *IGF1* gene is the mature IGF-1 peptide.

Acknowledgements

This work was supported by Alcon, Ltd, Hellas. The sponsor had no role in the design or conduct of this research.

References

1 Klok E, Lubsen NH, Chamberlain CG and McAvoy JW: Induction and maintenance of differentiation of rat lens epithelium by FGF-2, insulin and IGF-1. Exp Eye Res *67*: 425-431, 1998.

2 Matheny RW Jr., Nindl BC and Adamo ML: Minireview: Mechano growth factor: a putative product of IGF-I gene expression involved in tissue repair and regeneration. Endocrinology *151(3)*: 865-875, 2010.

3 Shavlakadze T, Winn N, Rosenthal N and Grounds MD: Reconciling data from transgenic mice that overexpress IGF-I specifically in skeletal muscle. Growth Horm IGF Res *15*: 4-18, 2005.

4 Imanaka M, Iida K, Murawaki A, Nishizawa H, Fukuoka H, Takeno R, Takahashi Y, Okimura Y, Kaji H and Chihara K: Growth hormone stimulates mechano growth factor expression and activates myoblast transformation in C_2C_{12} cells. Kobe J Med Sci *54*: 46-54, 2008.

5 Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD and Goldspink G: The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. J Physiol *555*: 231-240, 2004.

6 Bickel CS, Slade JM, Haddad F, Adams GR and Dudley GA: Acute molecular responses of skeletal muscle to resistance exercise in able-bodied and spinal cord-injured subjects. J Appl Physiol *94*: 2255-2262, 2003.

7 Barton ER, DeMeo J and Lei H: The insulin-like growth factor (IGF)-I E-peptides are required for isoform-specific gene expression and muscle hypertrophy after local IGF-I production. J Appl Physiol *108*: 1069-1076, 2010.

8 Kim JS, Cross JM and Bamman MM: Impact of resistance loading on myostatin expression and cell cycle regulation in young and older men and women. Am J Physiol Endocrinol Metab *288*: 1110-1119, 2005.

9 Philippou A, Papageorgiou E, Bogdanis G, Halapas A, Sourla A, MAridaki M, Pissimissis N and Koutsilieris M: Expression of IGF-1 isoforms after exercise-induced muscle damage in humans: characterization of the MGF E peptide actions *in vitro*. In Vivo *23*: 567-575, 2009.

10 Hameed M, Orrell RW, Cobbold M, Goldspink G and Harridge SD: Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol *547*: 247-254, 2003.

11 Chew SL, Lavender P, Clark AJ and Ross RJ: An alternatively spliced human insulin-like growth factor-I transcript with hepatic tissue expression that diverts away from the mitogenic IBE1 peptide. Endocrinology *136*: 1939-1944, 1995.

12 Hill M and Goldspink G: Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. J Physiol *549*: 409-418, 2003.

13 Stavropoulou A, Halapas A, Sourla A, Philippou A, PApageorgiou E, Papalois A and Koutsilieris M: IGF-1 expression in infarcted myocardium and MGF E peptide actions in rat cardiomyocytes *in vitro*. Mol Med *15*: 127-135, 2009.

14 Milingos DS, Philippou A, Armakolas A, Papageorgiou E, Sourla A, Protopapas A, Liapi A, Antsaklis A, Mastrominas M and Koutsilieris M: Insulin-like growth factor-1Ec (MGF) expression in eutopic and ectopic endometrium: characterization of the MGF E peptide actions *in vitro*. Mol Med *17*: 21-28, 2011.

15 Armakolas A, Philippou A, Panteleakou Z, Nezos A, Sourla A, Petraki C and Koutsilieris M: Preferential expression of IGF-1Ec (MGF) transcript in cancerous tissues of human prostate: Evidence for a novel and autonomous growth factor activity of MGF E peptide in human prostate cancer cells. Prostate *11*: 1233-1242, 2010.

16 Philippou A, Stavropoulou A, Sourla A, Pissimissis N, Halapas A, Maridaki M and Koutsilieris M: Characterization of a rabbit antihuman mechano growth factor (MGF) polyclonal antibody against the last 24 amino acids of the E domain. In Vivo *22*: 27-35, 2008.

17 Koutsilieris M, Rabbani SA, Bennett HP and Goltzman D: Characteristics of prostate-derived growth factors for cells of the osteoblast phenotype. J Clin. Invest *80*: 941-946, 1987.

18 Choki I, Sourla A, Reyes-Moreno C and Koutsilieris M: Osteoblast-derived growth factors enhance adriamycin-cytostasis of MCF-7 human breast cancer cells. Anticancer Res *18(6A)*: 4213-4224, 1998.

19 Hodge WG: Posterior capsule opacification after cataract surgery. Ophthalmology *105*: 943-944, 1998.

20 Nishi O and Nishi K: Preventing posterior capsule opacification by creating a discontinuous sharp bend in the capsule. J Cataract Refract Surg *25*: 521-526, 1999.

21 Awasthi N, Guo S and Wagner BJ: Posterior capsular opacification: a problem reduced but not yet eradicated. Arch Ophthalmol *127*: 555-562, 2009.

22 Bertelmann E and Kojetinsky C: Posterior capsule opacification and anterior capsule opacification. Curr Opin Ophthalmol *12*: 35-40, 2001.

23 Lu L, Reinach PS and Kao WW: Corneal epithelial wound healing. Exp Biol Med (Maywood) *226*: 653-664, 2001.

24 Kampmeier J, Baldysiak-Figiel A, de Jong-Hesse Y, Lang GK and Lang GE: Effect of growth factors on proliferation and expression of growth factor receptors in a human lens epithelial cell line. J Cataract Refract Surg *32*: 510-514, 2006.

25 Lang RA: Which factors stimulate lens fiber cell differentiation *in vivo*? Invest Ophthalmol Vis Sci *40*: 3075-3078, 1999.

Received December 10, 2010
Revised January 24, 2011
Accepted January 25, 2011