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## A-Abstract

Recent studies have demonstrated the existence of two estrogen receptor subtypes  $\alpha$  (ER $\alpha$ ) and  $\beta$  (ER $\beta$ ) with significant differences of expression among organs. Since important pathologies of human eye could be linked to hormonal status, we looked for the expression of ER $\beta$  in ocular posterior segment.

Immunohistochemical localization of ER $\beta$  and ER $\alpha$  protein and detection of ER mRNAs by reverse transcription-polymerase chain reaction (RT-PCR) were performed in macular and extramacular regions of the retina and in the choroid on male and female donors eyes. ER $\beta$  protein was localized in the ganglion cell layer and in the choroid. At the transcriptional level, mRNA for ER $\beta$  and for ER $\alpha$  were both present. Local differences in the expression level were however observed suggesting the possibility of variation in the ratio of ER $\alpha$  vs ER $\beta$ .

The coexistence of two estrogen receptor subtypes in the human ocular posterior segment raises acute questions about their potential physiological role, but offers a perspective for preferential targeting of a specific receptor subtype.

## B- MATERIALS AND METHODS

### Tissue collection

Human male and female donor eyes (8 males and 5 females) with a limited post-mortem enucleation time (1h-15h) were collected from the Cornea Bank, (University of Liège, Belgium). Mean age was 65 yrs (range 46-82). After removal of the anterior segment structures, 5 mm diameter punches were made in the macular region and in the peripheral retina. Neural retina was then separated from the retinal pigmented epithelium and from the choroid (RPE-choroid complex) and tissues were stored at -80°C. Alternatively, posterior segments were fixed in 4% formalin, dehydrated and embedded in paraffin.

### Immunocytochemical localization of ER $\beta$

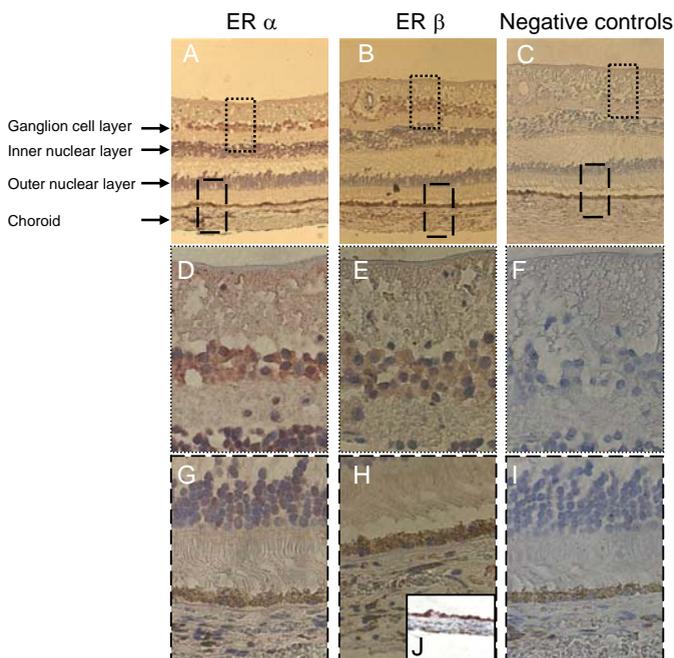
Frozen sections of 5mm were fixed 5 min in acetone at room temperature, air-dried, and covered 1hr with 3% normal goat serum. Then, rabbit anti-human estrogen  $\alpha$  or  $\beta$  receptor (Santa Cruz Biotechnology, CA) diluted 1/25 were applied for 1 hr, sections were rinsed in Tris/ HCl pH 7.4 saline, and covered with one drop of EnVisionTM (DAKO, ready to use goat anti-rabbit peroxidase-conjugated antibody) for 30 min. After rinsing in Tris/HCl, one drop of AEC+ (DAKO, 3-amino-9-ethylcarbazole) was added. Sections were washed in H<sub>2</sub>O, counterstained 1 min in haematoxylin and mounted in Aquamount. Deparaffinized sections were similarly treated excepting for a preliminary microwave (350 W, four times for 5 min in 10 mM sodium citrate buffer, pH 6.0) step to unmask antigenic sites. Negative controls were obtained by omitting the primary antibody, while for positive controls, uterine tissue known to express estrogen  $\beta$  was used (data not shown).

### Detection of ER $\alpha$ and ER $\beta$ by RT-PCR

Total RNA from 5 mm punches were extracted using RNeasyMini Kit (QIAGEN) as described by the manufacturer. 28S rRNA, ER $\alpha$  and ER $\beta$  mRNA were measured in 10ng aliquots of total RNA using the GeneAmp Thermostable rTth reverse transcriptase RNA PCR kit (Perkin Elmer) and three pairs of primers (Gibco BRL - Life Technologies) (Sense: 5'-GTTTCCCCCACTCAACAGCGT-3' and Reverse : 5'-ACTTCCCTTGTCATTGGTACGGC-3' for ER $\alpha$  mRNA, Sense: 5'-TTCCAGCAATGTCTACTAACT-3' and Reverse : 5'-CTCTTTGAACCTGGACCAGTA-3' for ER $\beta$  mRNA and, Sense: 5'-GTTCCACCACTAATAGGGAACGTGA-3' and Reverse: 5'-GGATTCTGACTTAGAGCGTTCACT-3' for 28S rRNA). Reverse transcription was performed at 70°C for 15 min, followed by 2 min. incubation at 95°C for denaturation of RNA-DNA heteroduplexes. Amplification started by 15 sec. at 94°C, 20 sec at 58°C and 15 sec. at 72°C (35 cycles for ER $\alpha$  and ER $\beta$  and 19 cycles for 28S) and terminated by 2 min. at 72°C. RT-PCR products were resolved on 10% acrylamide gels and analysed using a Fluor-S Multimager (BioRad) after staining with Gelstar dye (FMC BioProducts).

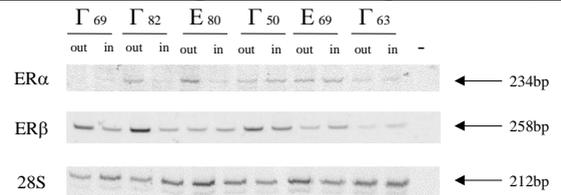
## C- RESULTS

### 1) ER immunolocalization on posterior segment sections

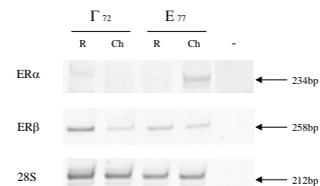


- Immunolocalization of ER $\alpha$  is widespread in the neural retina with more intensity in the ganglion cell layer whereas ER $\beta$  is found in the ganglion cell layer of the retina and in the choroid.
- The staining was similar in tissues of both sexes

### 2) ER mRNA expression in the posterior segment



- ER $\alpha$  and ER $\beta$  mRNA expression was detected in ocular tissues from different age and gender patients, regardless of the original location of the sample (inside or outside the macula)
- The expression of ER $\beta$  mRNA was relatively constant between different donors, while there was more variation with ER $\alpha$ .



- Both receptor subtypes mRNA were detected in the RPE-choroid complex, but ER $\alpha$  was inequally distributed between the retina and the RPE-choroid

## D- CONCLUSIONS

- We demonstrate the **presence of ER $\beta$  in human male and female ocular posterior segment** by immunohistochemistry and RT-PCR analysis. At the transcriptional level, while the expression of ER $\beta$  was relatively constant, much more variability between different specimens was observed for ER $\alpha$ .
- Our results contribute to explain, at least partly, the beneficial effects observed in retinal pathology with genistein treatment. Genistein is a naturally occurring phytoestrogen with a 20-fold affinity difference for ER $\beta$  vs ER $\alpha$ .
- Our observations suggest that under certain circumstances, **ER $\beta$ /ER $\alpha$  expression ratio could vary in the eye**. This provides a mechanism by which estrogen could exert different effects on the same cell type.