Exploring the role of a long non-coding RNA in the retinal pigment epithelium and the oxidative stress response, a major disease mechanism in retinal diseases

E. Delanote[I,II], K. Van Acker[I,II], E. De Bruycker[I,II], A. Dueñas Rey[I,II], M. Bouckaert[I,II], H. Lenaerts[I,II], L. Delhaye[I,III,IV], S. Lefever[I,III], P. Mestdagh[I,III], F. Coppieters[I,II,V]

[I]Center for Medical Genetics Ghent, Ghent University, C. Heymanslaan 10, Ghent, Belgium, [II]Therapy and RNA Group Ghent, Department of Biomolecular Medicine, Ghent University, C. Heymanslaan 10, Gent, Belgium, [III]OncoRNALab, Cancer Research Institute Ghent, C. Heymanslaan 10, Gent, Belgium, [IV]VIB-UGent Center for Medical Biotechnology, VIB, Technologiepark-Zwijnaarde 75, Gent, Belgium, [V]Department of Pharmaceutics, Ghent University, Ottergemsesteenweg 460, Gent, Belgium

The oxidative stress response is a major disease mechanism underlying rare inherited as well as common multifactorial retinal diseases. We identified a long non-coding RNA (lncRNA) specifically expressed in the retinal pigment epithelium (RPE), the layer supporting the photoreceptors, with a potential role in this key pathway. LncRNAs are fascinating molecules that regulate gene expression at the right time and place, yet little is known about their function in the human retina. Specifically, this RPE-specific lncRNA is upregulated in age-related macular degeneration and upon oxidative stress. We hypothesize that it is involved in normal functioning of the RPE by playing a key role in the oxidative stress response and by regulating a lysosomal flippase. This flippase is the only protein-coding gene located in the same topologically associating domain as the lncRNA that is expressed in the RPE. To investigate this link, lncRNA knockdown using gapmer antisense oligonucleotides (ASOs) was performed in ARPE-19 cells, resulting in decreased expression of the protein-coding gene (RT-qPCR), suggesting that it is indeed regulated by the lncRNA. Furthermore, poly(A) RNA-seq was performed on these cells. In total, 2,745 genes were significantly differential expressed in ASO-treated cells compared to negative control-ASO-treated cells. Gene Ontology enrichment analysis of the upregulated differential genes revealed terms related to endosomes, oxygen levels and endoplasmic reticulum stress. Single-molecule RNA in situ hybridisation of the lncRNA revealed accumulation in RPE-cell nuclei. Finally, knockdown in basal and upon oxidative stress conditions is ongoing in ARPE-19 cells differentiated to a more RPE-like phenotype, followed by RNA-seq. In this gene-focused study, fundamental insights in the functionality of the lncRNA in the human RPE are acquired. Further research is ongoing to investigate the role of the lncRNA in the disease mechanism of retinal degenerative diseases.