Gene expression analysis using RNA sequencing reveals novel insights into pathological alterations in human vocal fold fibroblasts from patients with Reinke's edema

Magdalena Grill[1]*, Isaac Lazzeri[2], Tanja Grossmann[1], Ellen Heitzer[2], Markus Gugatschka[1]

[1]Division of Phoniatrics, Department of Otorhinolaryngology, Medical University of Graz, Graz, Austria
[2]Department of Human Genetics, Medical University of Graz, Graz, Austria

Keywords: Reinke's edema; vocal fold fibroblasts; RNAseq

Introduction:

Reinke's edema (RE) is a smoking- and voice-abuse associated benign lesion of the vocal folds, defined by an edema of the Reinke space [1][2][3]. Vocal fold fibroblasts (VFF) are the main cell type of the lamina propria and thought to have a key role in the ongoing of the disease [4][5][6]. In addition, changes in the microvasculature and increased infiltration of immune cells have been described [7]. Current treatment strategies are restricted to treatment of symptoms and there is an urge need for a better understanding of the molecular causes of the disease. In the current study, we investigated differential expression profiles of RE and control VFF by means of RNAseq.

Methods:

Human vocal fold tissue samples from patients with RE and healthy controls were collected during phonosurgical procedures, or received post mortem (Diagnostic and Research Institute of Pathology, Medical University of Graz) (RE n=18, controls n=9). Tissue samples were allowed to adhere to 12-well plates and cultured until outgrowth of fibroblastoid cells. Near-primary VFF were cultured in T25 flasks and RNA isolation was performed using Qiazol and miRNeasy mini kit (Qiagen). Sequencing library was prepared using 500 ng RNA and the TrueSeq® Stranded total RNA HMR kit (Illumina) and sequencing was performed on a Nextseq 550 sequencer. Normalized read counts (TPM) were generated by running Salmon on RNA-seq data. Results from Salmon were used for differential expression analysis by DeSeq2.

Results and Discussion:

We identified 81 differentially regulated genes in total (log2FC > +1 or < -1; adjusted p-value < 0.05), of which 21 genes were upregulated and 60 downregulated.

We subjected the set of differentially regulated genes to gene set enrichment analysis using FGSEA in order to obtain biological processes enriched in VFF of RE patients compared to controls. The top 5 terms for upregulated KEGG pathways revealed lysosome, glycosaminoglycan biosynthesis chondroitin sulfate, glycosaminoglycan degradation, complement and coagulation cascades and ECM receptor interaction.

Gene ontology enrichment analysis of molecular function also showed an increase in extracellular matrix structural constituent, proteoglycan binding, platelet derived growth factor binding, hydrolase activity hydrolyzing o-glycosyl compounds and collagen binding (adjusted $p<0.05$).

Top 5 relevant downregulated KEGG pathways were DNA replication, oxidative phosphorylation, proteasome, cell cycle and ribosome.

The current study addressed for the first time a direct comparison of RE to control VFF, showing a major increase in pathways associated with extracellular matrix components and a decrease in pathways associated with cell growth and proliferation.

Acknowledgements

Thanks to Christine Beichler for expert technical assistance. This work is funded by the Austrian Science Fund FWF grant P30496 awarded to M. Gugatschka.

References

[1] Zeitels et al. Otolaryngol Head Neck Surg. Apr;126(4):333-348. 2002
[2] Dikkers et al. Ann Otol Rhinol Laryngol 1995 Sep;104(9 Pt 1):698-703.
[3] Marcotullio et al. Am J Otolaryngol 2002 Mar-Apr;23(2):81-84.
[4] Hirano et al. Acta Otolaryngol 1999 Mar;119(2):271-276.
[5] Buckley et al. Trends Immunol 2001 Apr;22(4):199-204.
[6] Martins et al. J Voice 2016 Jul 21.
[7] Sato et al. Ann Otol Rhinol Laryngol 1999 Nov;108(11 Pt 1):1068-1072.

* ma.grill@medunigraz.at