P795 CHARACTERIZATION OF ERCC6L2 SYNDROME PATIENTS’ TRANSCRIPTOME

**Topic**: Bone marrow failure syndromes incl. PNH - Biology & Translational Research

Suvi P.M. Douglas, Ilse Kaaja, Tuulia H. Räisänen, Esa Pitkänen, Outi Kilpivaara, Ulla Wartiovaara-Kauto

**Background**: Biallelic germline mutations in ERCC6L2 cause a bone marrow failure syndrome with a tendency for acquiring somatic TP53 mutations and possible progression to acute myeloid leukemia with erythroid characteristics. The disease and some functions of ERCC6L2 have only recently been characterized but a lot remains uncovered. ERCC6L2 affects DNA repair, but also plays a role in immunoglobulin class-switching and mitochondrial function. Although the biallelic ERCC6L2 mutation is in all the patient’s cells, it seems to affect the hematopoietic cells most drastically. However, we have seen differences also in the metabolism and growth of the ERCC6L2-deficient patients’ skin fibroblasts. Constitutional changes in RNA expression levels and altered metabolism due to ERCC6L2 deficiency may also affect the bone marrow niche and help us understand the disease more profoundly.

**Aims**: We aimed to characterize ERCC6L2 syndrome on the transcriptome level using patient-derived samples (blood, skin fibroblasts, lymphoblastoid cell lines [LCLs]).

**Methods**: 32 blood samples were collected from 15 ERCC6L2 patients (some in multiple time points), and 6 healthy controls. Skin fibroblast cell lines from 4 ERCC6L2 patients, and 3 healthy controls were grown in duplicates. In total 14 samples were grown in high-glucose (25mM) media until about 80% confluence and cells were harvested after six hours of changing the media.

LCLs derived from EBV-transformed PBMCs from 6 ERCC6L2 patients and 4 healthy controls were grown in duplicates in two different media compositions (in total 20 samples).

3’ RNA sequencing was performed on RNA extracted from all patient samples. Differential expression analysis was done with DESeq2 and pathway enrichment analysis with PathfindR.

**Results**: ERCC6L2-patients and controls differ significantly in their mRNA expression levels in multiple cell types and enriched pathways differ between cell types.

In the patient blood samples, global genome nucleotide excision repair and transcription-coupled nucleotide excision repair (TC-NER) pathways are overrepresented compared to healthy controls. ERCC6L2 has been suggested to be involved both in TC-NER and non-homologous end joining of DNA double strand breaks. Also neddylation, which is a ubiquitin-like posttranslational modification affecting gene expression, is highly overrepresented. Cellular response to chemical stress is also upregulated, which demonstrates the reported sensitivity of ERCC6L2-deficient cells to some chemicals.

The patients’ skin fibroblasts have different expression patterns compared to healthy controls in pathways related to cell differentiation, response to stimuli and extracellular matrix composition. These results support our previous
observations about impaired fibroblast growth and metabolism. Expression changes in fibroblasts can tell us about effects that the germline ERCC6L2 mutation can have on extra-hematopoietic cells and tissues in the absence of somatic TP53 mutations.

LCLs from ERCC6L2 patients’ cells differ from healthy controls by various immune system pathways: the enrichment of complement activation, phagocytosis, B-cell receptor regulation, but also in their response to metal ions.

**Summary/Conclusion:** Multiple cell types of ERCC6L2 patients’ cells differ significantly from healthy controls’ cells in pathways related to DNA repair, stress response, cell morphology and immune function. These enriched pathways demonstrate the diverse effects the germline ERCC6L2 mutation has on the patients’ cells.