Unraveling the Role of HDAC10 in the Molecular Background of Sézary Syndrome

Monika Pieniawska[1], Karolina Rassek[1], Bogumiła Skwara[1], Magdalena Żurawek[1], Iwona Ziółkowska-Suchanek[1], Natalia Rozwadowska[1], Katarzyna Iżykowska[1]

[1] Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Sézary Syndrome (SS) is an aggressive, leukemic, and incurable variant of Cutaneous T-cell lymphoma (CTCL) marked by severe erythroderma and the presence of malignant T lymphocytes in both skin lesions and peripheral blood. One of the therapeutic approaches for SS involves histone deacetylase inhibitors (HDACi), a class of epigenetic drugs designed to target histone deacetylases (HDACs) - enzymes responsible for removing acetyl groups from histones, thereby remodeling chromatin and repressing transcription.

Two CTCL cell lines (Hut78, SeAx) with HDAC10 overexpression were used in the study. The HDAC10 overexpression was introduced using lentiviral expression vectors and verified on mRNA/protein levels using qRT-PCR and Western Blot. The cellular localization of HDAC10 was analyzed using immunofluorescence (IF), confocal microscopy, and cellular fractionation. To study the potential protein interactions, Co-Immunoprecipitation (Co-IP) with Mass Spectrometry was used. Whole transcriptomes of CTCL cells with HDAC10 overexpression were analyzed using RNA-seq to identify deregulated genes and pathways. Various functional assays to measure cell cycle (BrdU/7AAD), cell viability and proliferation (MTS), and apoptosis (AnxV/7AAD), were conducted. The analysis of inhibition of HDAC10 using the recently discovered, selective inhibitor DKFZ-748 was performed using cell viability assay (MTS).

Our previous study indicated that HDAC10 is overexpressed in SS patients (relative expression SS: 30,4E-04, n=6 vs HD: 5,65E-04, n=7; p=0,0005). This work aims to analyze the impact of HDAC10 on the transcriptome and biology of malignant cells in SS. Cellular fractionation and IF analysis confirmed mainly cytoplasmatic localization of HDAC10 in CTCL cell lines and primary SS cells. Only a small fraction was detected in the nucleoplasm and bound to chromatin, and the Co-IP/MS analysis confirmed both nuclear and cytosolic proteins associated with HDAC10. The RNA-seq analysis revealed alterations in the global expression of genes and the modulation of cellular pathways under the influence of HDAC10. Particularly noteworthy was the downregulation of the potential tumor suppressors like *DUPS6*, and genes involved in pathways associated with cancer (*JAG1, CXCL8, LEF1, F2RL3*), transcriptional misregulation (*ETV4*), T cell receptor signaling (*IL10, PTPN6, CTLA4, ICOS*), and the JAK-STAT pathway (*IL19, PTPN6*). There was no effect of HDAC10 overexpression on cellular processes including cell proliferation, cell cycle, and apoptosis in SS cell lines, however, in primary T-cells, there was a slight change in the S phase of the cell cycle. Moreover, when exposed to the pro-apoptotic drug Camptothecin (CPT), an inhibitory impact on the progression of apoptosis was observed in cells with HDAC10 overexpression (apoptotic cells: 39,5% vs 55,4% in controls, 6μM CPT; 48h). Furthermore, the evaluation of the effects of the selective HDAC10i (DKFZ-748) was performed, indicating elevated IC_{50} values in the SS cell lines with introduced HDAC10 overexpression relative to the control group.

In conclusion, our studies have shown that HDAC10 is an important player in the biology of Sézary cells. We proved that HDAC10 overexpression significantly disrupts the gene expression patterns, and affects the cell sensitivity to possible treatment. The extended knowledge of HDAC10 overexpression in SS has the potential to enhance comprehension of advanced future therapeutic strategies.