Abstract

Estrogens are as important in the development of the male reproductive system as androgens. The proper production of estrogens, which concentration in the male body is much lower than that of androgens, is crucial for the proper functioning of the male gonad. There are three types of receptors that mediate estrogen signaling, i.e.: canonical estrogen receptors (ER), membrane G-protein coupled estrogen receptor (GPER) and related receptors estrogen receptors (ERR). Importantly, these receptors can interact with each other or with peroxisome proliferator-activated receptors (PPAR), which regulate cellular metabolism. Disruption of estrogen signaling might affect the functioning of gonadal cells and even lead to the development of cancer. The aim of this study was to determine the effect of non-classical estrogen signaling on the function of testicular cells, including the regulation of gene expression and epigenetic processes.

As research material, the following were used: the C57BL/6 mice strain in a *in vivo* model, fragments of immature pig testes in an *ex vivo* model, fragments of human Leydig cell tumors and healthy human Leydig cells cultured *in vitro*, and a mouse line of tumor Leydig cells MA-10 in an *in vitro* model. In order to determine the function of the tested receptor in the testicular cells, depending on the experiment, the selected receptor was pharmacologically blocked or activated using: a ERRα antagonist (XCT790), a GPER antagonist (G-15), a ERRβ/γ agonist (DY 131) and a selective PPARα antagonist (GW6471) and a PPARγ antagonist (T0070907). Various concentrations of sodium selenite were used to describe the effect of metaloestrogen on mouse Leydig cells. All used doses were selected based on the available literature data and pilot experiments.

The study used the RNA sequencing technique (RNA-sequencing), which allows to comprehensively describe total mRNA expressed in the tested sample, supplemented with bioinformatical functional analysis to describe the function of the identified genes, their

interactions, and the interaction of their protein products. Microscopic, molecular and biochemical analyzes were also used to describe changes in mRNA expression (qRT-PCR), changes in protein expression (Western Blot), visualize the location of proteins in the cell (immunocytofluorescence) and determine the concentration of secreted hormones (ELISA).

As a result of the conducted studies, it was found that pharmacological blockage or activation of ERR or GPER caused changes in the transcriptome of mouse testicular cells. It was shown that the blockage of ERRα resulted in an increased expression of genes involved in the immune response, and activation of ERRβ/γ increased expression of genes related to the post-translational modification of proteins. Blockage of GPER activity affected the expression of single genes that could not be collectively assigned to one process **[Publication 1]**. On the other hand, in the pig testis explants, only modulation of PPARγ activity resulted in changes in the expression of groups of genes related to metabolism, adhesion and tubule formation. Blocking the activity of PPARα and GPER led to changes in the expression of genes not involved in any meaningful cellular process **[Publication 2]**. Transcriptomic comparison of the healthy human Leydig cells with the Leydig cell tumors showed that the expression of genes involved in estrogen signaling, apoptosis and blood vessel development is disrupted in the tumors. Importantly, the aberrant expression of homologous genes has also been reported in the mouse transcriptome after pharmacological modulation of non-canonical estrogen signaling **[Publication 3]**. The research carried out in the in vitro model, for the first time, proved that selenium as a metalloestrogen affects the GPER expression. In addition, high concentrations of selenium caused changes in the epigenetic status of cells, and in a number of cells resulted in DNA breaks **[Publication 4]**.

Based on the conducted studies, it can be concluded that the disturbance of signal transmission via estrogens significantly affects the transcriptome of testicular cells and may be associated with disease. Metaloestrogens affect the epigenome and modulate the expression of

classical and non-classical estrogen receptors. The results obtained in this work may help in explaining the cellular processes of the gonads regulated by ERR, GPER and PPAR. In the future, these studies may contribute to the development of therapeutic approach and diagnostics of hormone-dependent testicular cancers.