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Rozprawa doktorska

**Rola białek RBMXL3 oraz DDX53 w regulacji
metabolizmu RNA w komórkach linii płciowej człowieka**

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Abstract

Human spermatogenesis is a highly organized and dynamic biological process, the full recapitulation of which under *in vitro* conditions has not yet been achieved. The proper progression of this process requires precise temporal and spatial coordination of gene expression, molecular signalling, and extensive epigenetic reprogramming. Despite significant advances, many key molecular regulators involved in male germ cell differentiation remain unidentified. Genetic studies using whole-genome sequencing (WGS) and whole-exome sequencing (WES) have identified the genes *RBMXL3* and *DDX53* as potentially associated with human spermatogenesis.

The aim of this doctoral thesis, comprising three scientific publications, was to investigate the network and molecular targets of the *RBMXL3* and *DDX53* proteins in the context of male germ cells development and human spermatogenesis. The introductory chapter describes processes related to RNA metabolism and the roles of selected proteins in male gamete formation that have been partially characterised to date. The first publication provides an overview of research strategies relevant to studies of human germ cells and spermatogenesis. The remaining two publications are original research articles focused on the investigation of *RBMXL3* and *DDX53* proteins. To assess the relevance of these proteins in the context of male germ cells development and human spermatogenesis, the TCam-2 human testicular germ cell tumour cell line was used. Appropriate cellular models were established using TCam-2 cells to achieve the expression of *RBMXL3*, *DDX53*, and GFP proteins (FLAG-tagged). The expression of the studied proteins was confirmed at both the RNA and protein levels using molecular techniques, including RT-qPCR, Western blotting, and immunofluorescence. Investigation of the interaction networks and molecular targets of *RBMXL3* and *DDX53* was performed using RNA-seq, eCLIP, and Co-IP-MS. The obtained results enabled the identification of differentially expressed genes, alternative splicing events, as well as RNA molecules and proteins directly interacting with *RBMXL3* and *DDX53*. The interpretation of the results focused on molecular targets described in the literature as being involved in male germ cell differentiation. Subcellular localisation of the investigated proteins was also analysed using cell fractionation combined with Western blotting and double immunofluorescence staining. Additionally, the presence of both proteins was confirmed in human testicular sections by immunohistochemistry using specific antibodies.

Collectively, the findings of this doctoral thesis support the role of *RBMXL3* and *DDX53* proteins in the regulation of processes underlying human male germ cell development.