SUMMARY

THE INFLUENCE OF CHEMERIN ON THE TRANSCRIPTOME OF OVARIAN LUTEAL CELLS OF THE DOMESTIC PIG (*SUS SCROFA DOMESTICA* L.)

Karol G. Makowczenko, M.Sc.

Doctoral thesis was carried out at the Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn under the guidance of prof. dr hab. Tadeusz Kaminski (supervisor) and Jan Pawel Jastrzebski, PhD (ancillary supervisor).

The doctoral thesis comprises two papers published in 2020 and 2022 (1 – *Genes* 2020; 11:651. doi: 10.3390/genes11060651; 2 – *Cells* 2022; 11:715. doi: 10.3390/cells11040715), focused on the influence of chemerin on genes and long non-coding RNAs expression and modifications of alternative splicing and allel-specific expression processes in ovarian luteal cells of the domestic pig (*Sus scrofa domestica* L.) during the mid-luteal phase of the estrous cycle.

Many studies indicate the existence of an endocrine system that simultaneously controls metabolic processes and the functioning of the reproductive system in mammalian organisms. According to recently published reports, one of the key elements of this system, which also interacts with the immune system, seems to be a lately discovered hormone – chemerin. Due to the functions performed by this peptide, related to the initiation or silencing of the local inflammatory response and chemotaxis of selected immune cell groups, it is categorized as a chemokine. In addition, due to the chemerin's production mainly by adipocytes and its involvement in the regulation of metabolic processes, this hormone is also included in the adipokine group. Available data indicate that chemerin is also involved in the control of the mammalian reproductive system, including an inhibitory effect on the ovarian steroidogenesis. Transcripts and proteins of chemerin and its receptors have also been found in structures of the endocrine regulatory hypothalamic-pituitary-gonadal axis (HPG) in humans, rats, pigs, cattle and sheep. Chemerin interacts with target cells through three receptors: CMKLR1, GPR1 and CCRL2. The first two, by binding to G proteins, generate a cellular response, including transcription of selected genes. The last receptor does not cause signal transduction, but it is responsible for the local aggregation of chemerin molecules and

sharing them with other receptors. Fragmentary information on the activity of chemerin in mammalian organisms and the aforementioned observations were the premis for the research described in the doctoral thesis.

The hypothesis formulated in this dissertation assumed, that chemerin influences expression profiles of genes and long non-coding RNAs, and modifies the alternative splicing and allel-specific expression processes in the ovarian luteal cells of the domestic pig (*S. scrofa domestica* L.) during the mid-luteal phase of the estrous cycle (days 10–12 of the cycle, when the secretory activity of the cells is the highest). High-throughput transcriptome sequencing (RNA-Seq) methods were used to conduct the study, which, combined with advanced *in silico* analyses of the acquired data, provided detailed insight into the molecular mechanisms initiated by the studied hormone.

The research was conducted using *in vitro* cultured luteal cells obtained from sexually mature female domestic pigs. RNA, isolated from cells treated with chemerin and from control samples, was subjected to RNA-Seq sequencing, generating a bioinformatically processed data set. During the analyses, significant changes in the expression of 509 genes and 24 transcripts classified as long non-coding RNAs were observed, as well as 137 cases of modifications in the alternative splicing process and 18 cases of allel-specific expression variation. Based on the results, detailed functional analyses were performed, resulting in the assignment of genes (affected by the observed transcriptomic phenomena) to such intracellular processes as cytokine interactions with cytokine receptors, apoptosis, arachidonic acid metabolism and signal transduction pathways of chemokines, TNF, MAPK and others. Based on the acquired functional data, detailed maps showing the unified effects of chemokine on signaling and metabolic pathways in domestic pig luteal cells were prepared. The results obtained by *in silico* methods were validated on the basis of the material used for RNA-Seq, using quantitative real-time polymerase chain reaction (qPCR).

The obtained results expanded the knowledge of the chemerin effect on the transcription process and accompanying molecular mechanisms in luteal cells. The observed transcriptomic changes may lead to activation of MAPK and JAK/STAT signal transduction pathways, and stimulation of both pro-apoptotic and pro-survival mechanisms in luteal cells. In addition, chemerin, by modifying the luteal cells' transcriptome, may control the corpus luteum functions through the regulation of steroidogenesis and angiogenesis, as well as the

production and secretion of pro-inflammatory factors and prostaglandins. The conducted studies suggest that chemerin is the hormone linking the regulation of energy homeostasis and the reproductive system in mammalian organisms.

This research was supported by National Science Centre (Project no.: 2015/17/B/NZ9/03595)