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WYDZIAŁ MEDYCYNY WETERYNARYJNEJ I NAUK O ZWIERZĘTACH
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Review of the doctoral thesis of Sanaz Hassani

Conducted in the Institute of Molecular Biology and Biotechnology - Adam Mickiewicz University in Poznan
Under the supervision of prof. dr hab. Hans A.R. Bluyssen

The submitted doctoral thesis entitled "The role of ISRE and GAS composite containing genes in long-term IFN-I and IFN-II responsiveness" is a 190-page work containing a typical structure consisting of chapters: introduction, hypothesis and objectives, material and methods, results, discussion, references, lists of figures and tables, supplementary information, summaries in a foreign and Polish language, list of acronyms, and information on research funding. Additionally, the Author included information of co-authored manuscripts already published and those prior submission as a result of PhD thesis.

The interferon (IFN) signaling pathway is an essential component of mammalian innate antiviral defense. IFN induction is initiated by recognition of specific pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors. Upon interaction of IFNs with receptors, phosphorylation of the JAK1 and TYK2 kinases occurs following the formation of ISGF3, GAF and GAF-like complexes. These complexes are composed of STATs proteins which phosphorylated form heterodimers (STAT1/STAT2) or homodimers (STAT1). Together with IRFs after nucleus entry activate the interferon-stimulated response element (ISRE) and gamma activated sites (GAS) to regulate the expression of IFN-stimulated genes (ISGs). Therefore, JAK/STAT is one of the most important regulatory signal cascades of a series of cellular processes that are triggered by numerous types of ligands. The dissertation presents new approach based on the use of high-throughput technique data to elucidate the number of composite-sites containing genes and their transcriptional regulation as a response to IFN-I and IFN-II unraveling the potential mechanism in anti-viral action. A number of genes, particularly involved in viral defense and inflammation, possess both ISRE and GAS elements within their promoters, making them responsive to more than a single signaling pathway. This is referred to as a composite promoter, which integrates the signals of multiple transcription factors in response to diverse stimuli. As described in the PhD thesis, ISRE and GAS elements in a composite

promoter permit integrative response to cytokines and interferons. Interestingly genes containing composite promoters are involved not only in immune response but also in pathways regulating stress response or cell death (e.g. apoptosis, ferroptosis). They might be also utilized and activated during cellular differentiation to orchestrate hormonal signaling, cell cycle and metabolic demands.

The dissertation introduction is a lengthy chapter but very clear and informative. Author describes the role of interferons, types and pathways. Meticulously define the STATs proteins, Interferon regulatory factors (IRFs) and Interferon stimulated gens together with very well-prepared graphics, figures and tables. The only one comment here concern the consequence in drawing the mechanisms of action of IFNs through receptors – possibly it easier to follow every figure if specific IFN is always at the same position (if possible). The most interesting part consider chapter "Modulation of IFN response over time" introducing the reader directly to the purpose of the work and defining the most intriguing problems to be solved in IFN signaling pathway.

Research hypothesis is clear and states that transcription of composite genes depends on ISRE and GAS elements composition and differential binding of IFN-I and IFN-II signaling related complexes. Eight research objectives were presented in a format ranging from the most general to the more in-depth formula.

In the Material and Methods section, the Author provide necessary information on basic material, cell lines (hepatocellular carcinoma) with knock outs (KO)of *STAT1, STAT2, IRF9, IRF1* and *IRF1.9* providing cell culture conditions and interferon alpha and gamma treatment. Subsection of qPCR description (3.4) needs some clarification where molar concentrations rather than volumes of reagents should be indicated (e.g. table 2). Although the primer sequences used for qPCR have been listed, there is no information about the length of the amplicons and the reaction conditions. Taking into account the MIQE guidelines, a study for performing qPCR analyses, which aims to standardize methods and ensure the repeatability of published results, in my opinion the dissertation should have included additional important information. First, the Author indicates that the calculations were done using only one reference gene - *GAPDH*. Was the stability of the expression of the reference gene validated and why only one gene was picked? It would be also worth supplementing, especially when the mRNA expression of the studied genes is compared with one to the other, the reaction efficiencies for individual amplicons (including slope and Y-intercept). There is also no mention on the statistical methods used for intergroup comparisons or in time intervals. The number of biological repeats is rather low (n=2), but on charts no statistical significance is pointed in example between different time points of cell culture even though for some genes a massive upregulation has been found. Other methods like luciferase report assay, antiviral assay were described. RNA seq and Chip-seq experiments have been conducted by team members at earlier stages of project, while PhD candidate worked on data using all computational and bioinformatics tools. At this point it is worth mentioning the honest indication by the PhD student of the people who participated in the research, helped with the analyses, or made raw data available for further use. Concerning entire dissertation, PhD candidate should follow the Gene/Protein Nomenclature

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Guidelines and Requirements, as gene symbols for the human are italicized with all letters in upper case.

The results are described in details and very lengthy. Although divided into multiple chapters I think they could be described in slightly more concise way leaving the most important observations and sharing some of them within discussion. The Author prepared a complete workflow figure describing step by step analysis from generating the output data into detailed analysis with the final list of preselected genes meeting experimental conditions. Although Chip-Seq and RNA seq data brought necessary data for starting analysis, others like luciferase assay allowed for functional description of active sites for transcriptional factors and between-gene variations. The question arises considering data filtration from RNA seq and selection for only the protein coding genes. A more epigenetic insights in the regulation of expression would be interesting.

The discussion addresses all research objectives and summarizes the obtained results on 21 pages. At this part of the doctoral thesis Author primarily aimed at describing the scientific achievement and at the same time discussed it with the current knowledge on the regulation of the IFN Iand IFN II-dependent signaling pathway, which are distinct but show some overlapping mechanisms. This part raises some important questions. Considering the analysis of single ISRE and GAS genes as well as those with multiple ISRE and GAS sites does the activation of particular sites might be cell type specific? Some differences in pathway are also species-specific but mostly rodents have been analyzed. Are there any data on other mammalian species that might be used as a model? Additionally, depending on two active ISRE sites with different activities is it possible to track the switching mechanism if only one site is predominantly used? In composite genes are there any data concerning polymorphisms in ISRE or GAS sites which disturb binding profiles and consequently the expression? To sum up, I believe that the presented doctoral thesis has a high substantive value, documented by valuable results that should be reflected in the original scientific publication. In addition, it was written meticulously with professional language and a very good stylistic structure. Having more often the opportunity to read PhD thesis based on published manuscripts and therefore lacking extensive descriptions and discussions, I admit that I read it with pleasure.

Having aforementioned in mind, I declare that the doctoral dissertation of Sanaz Hassani meets the requirements set for doctoral theses in accordance with Article 187 of the Act of July 20, 2018, the Law on Higher Education and Science (Journal of Laws of 2018, item 1668) and I request the Discipline Council of Biological Sciences of Adam Mickiewicz University in Poznań to admit Ms. Sanaz Hassani to further stages of the proceedings for awarding with the degree of doctor (PhD) in the discipline of biological sciences.

KIEROWNIK KATEDRY

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