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Review of the Erkut Ilaslan PhD thesis titled 'Distinct roles of NANOS paralogues in post-transcriptional mRNA regulation in human male germ cells'.

The PhD thesis I have reviewed describes and discusses the results of experiments performed by Erkut Ilaslan in the Institute of Human Genetics PAS in Poznan, Poland. The research presented in the thesis was supervised by Dr. Kamila Kusz-Zamelczyk. This dissertation is composed of three scientific articles that were published in International Journal of Molecular Sciences. In one of these papers Erkut Ilaslan is the second author, in two others he is first, and in addition serves as a co-corresponding author, together with his supervisor Dr. Kamila Kusz-Zamelczyk. All co-authors of the three papers presented as the Erkut Ilaslan PhD thesis provided signed permissions, confirming that he was allowed to use these articles in his PhD dissertation. Two papers show original experimental results and present their interpretation, the third is a kind of review, but contains also interesting computational analyses of global data carried out by the PhD candidate. The involvement of Erkut Ilaslan in preparation all three articles was clearly described. We read that he not only participated in planning and

performing the experiments presented in his PhD thesis, but also in analyses of the results obtained, as well as writing the manuscripts and preparing figures. As I have already mentioned, he is a co-corresponding author of two articles included in his dissertation that is the best evidence of the leading role he had while working on those studies. This is rather unusual situation and, in my opinion, requires underlining as well as needs a special appreciation.

It was known that NANOS proteins have roles in germ cell development. However, a role of individual NANOS paralogues in humans, namely: NANOS1, NANOS2 and NANOS3, in this process remained elusive. Therefore, Erkut Ilaslan decided to identify mRNA targets that are recognized controlled by each NANOS. Comparison between the pools of mRNAs recognized by these specific RNA-binding proteins should help to establish its function in germ cell development, as well as propose the molecular mechanisms in which they are involved during this process. Upon characterization of mRNA pools regulated by each NANOS, Erkut Ilaslan designed and performed experiments to uncover the biological implications of such NANOS-mediated regulation to germ cell development and/or pathologies as infertility and germ cell cancers. The goal of the studies was precisely stated and clearly described. The TCam-2 cell line was used to reach both these scientific aims. This is a unique line originated from primary testicular seminoma, which maintains the characteristic feature of seminomas and does not undergo apoptosis. Developmentally, TCam-2 cells correspond to a prenatal post migratory stage of the primordial germ cells (PGCs), thus seems to be a good choice to study the role of each NANOS paralogue in germ cells development in humans.

The first paper that is part of the Erkut Ilaslan thesis described the results of experiments aiming at understanding of molecular effects of the *NANOS1* mutation that was reported to be associated with the most severe male infertility, Sertoli cell only syndrome (SCOS). Men having this particular mutation within the *NANOS 1* gene do not have germ cells

inside the seminiferous tubules in testis. To understand why is that Erkut Ilaslan expressed wild-type NANOS1 and its mutated version in TCam-2 cells, and test proliferation, cell cycle and apoptosis in the cells transfected with both NANOS1 variants. Surprisingly, expression of wild-type as well as mutated NANOS 1 affects the cell cycle in the same way, increasing the G0 cell population while decreasing the S phase cell population. However, overexpression of wild-type NANOS led to the increased viable cell number and higher proliferation rate while the mutated NANOS1 present in TCam-2 cells led to the decreased number of cells and their lower proliferation rate. Moreover, the overexpression of wild-type NANOS in TCam-2 cells reduced the number of apoptotic cells. In contrast, expression of the mutated version of NANOS in TCam-2 led to the increased number of apoptotic cells. Using RNA-seq Erkut Ilaslan identified four apoptosis-related genes which mRNAs were downregulated by NANOS1, but not by the mutated NANOS1 protein, suggesting that NANOS inhibits apoptosis and stimulates proliferation. Thus, the mutation of NANOS 1 identified in infertile patients alters its function. The authors proposed that during germ cell development, mutated NANOS is not able to protect the germ cells from apoptosis, which in consequence results in the absence of them in testis of patients with the mutation identified in some of infertile men. Reading this part of the thesis I thought about an experiment in which expression of the four selected genes which products are connected with apoptosis, and the levels of their mRNAs are controlled by NANOS1, could be silenced in TCam-2 cells. Will the effect of such downregulation on cell proliferation and apoptosis be the same as in the case of NANOS1 overexpression? Are there patients with infertility described as having mutations in one of those four genes?

The second paper that is included in Erkun Ilslan PhD dissertation concerns characterization of specific mRNA targets of NANOS1 and NANOS3 proteins. To identify such targets he overexpressed NANOS \1 and NANOS3 proteins in TCam-2 cells. As I already mentioned, this line corresponds to human primordial germ cells and shows low levels of

NANOS expression. Then, he isolated RNA and performed RNA-seq to find mRNAs that are differentially expressed in the transfected cells in comparison to control. The results of global sequencing were analyzed using special software developed by Erkut Ilslan called Biological Clustering Analysis (BCA). The computational studies on transcriptomes were combined with cell cycle analyses using flow-cytometry. The results showed that NANOS1 and NANOS3 differently influence the cell cycle: NANOS1 is involved in the G1/S phase transition, and NANOS 3 in the G2/M phase transition. Moreover, Erkut Ilslan identified that TAF1 and FOXM1 are crucial for the regulation of mRNA levels in the G1/s and G2/M phase transition respectively. Next, Erkut wanted to answer the question how NANOS3 and FOXM1 regulate expression of genes during the G2/M phase transition. The obtained results showed that the PUMILIO (PUM) protein binds to 3'UTR of FOXM1 mRNA, recruits NANOS3 reducing the level of FOXM1 in the cell. This effect expression of many genes connected with the G2/M cell cycle phase transition. Interestingly, Erkur found also that FOXM1 regulates expression of NANOS3, PUM as well as its own gene. He suggested that deregulation of the NANOS3-PUM1-FOXM1 axis may have a role in testis carcinogenesis. Was similar research conducted on NANOS1- and the TAF1-mediated regulation of gene expression?

In the third and the last article that was used in the Ercut Ilslan thesis he and his colleagues reviewed the published results regarding a role of NANOS proteins in a broad range of cancers. In addition, he described the results of his own computational studies of publicly available expression data from seventeen different tissues from both control and cancer samples. The study presented in this interesting review revealed that overexpression of NANOS1 and NANOS3 is observed in many human cancers. In contrast, the lower expression level of NANOS2 is a characteristic feature of testicular cancer. The authors of this work proposed that the mRNA targets for each NANOS paralogue could provide valuable information helping to design novel therapeutic strategies against cancers.

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I have no doubts that Erkut Ilaslan presented in his PhD dissertation novel, original results which allowed him to suggest interesting models of NANOS proteins activity. This is an excellent research, and the results obtained are already published in three solid papers. In my opinion, Erkut Ilaslan proved to be able not only to design and carry out molecular and cell biology experiments, but also he can interpret the results of the experiments performed. Therefore, I recommend the Scientific Board of the Institute of Human Genetics to proceed with all necessary procedural steps to confer Erkut Ilaslan a PhD degree. Because of original and important scientific findings presented in the thesis, I also recommend the Scientific Board to award this work with a special prize.

Signed by / Podpisano przez:

Artur Michał Jarmołowski

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