Objective of the project

Exploration of processes responsible for germ cell specification and development is crucial for understanding causes of human infertility which denotes a serious medical problem worldwide. We have previously identified an abnormal NANOS1 protein in association with absence of the germ cells in testes of patients. Additionally, applying TCam-2 cells as a model of human male germ cells in culture, we have demonstrated that abnormal NANOS1 caused a ~50 % decrease of the cell number. This finding was in line with what we found in infertile patients – the absence of germ cells in testes. Moreover, the abnormal NANOS1 protein significantly induced TCam-2 programmed cell death called apoptosis, which could be one of mechanisms contributing to that decrease of the germ cell number. These preliminary results indicate that NANOS1 protein might be important for a normal germ cell functioning which ensures human fertility. NANOS1 protein interacts with RNAs which encode proteins and with few protein partners. Importantly, the *NANOS1* gene mutation affects interaction with GEMIN3 protein. Therefore, the general objective of this project is to get comprehensive insight into RNA/protein interaction networks called RNP interactome controlled by NANOS1 and their dynamics during specification/early germ cell development. In parallel to the normal NANOS1-controlled protein interactome, we will study the abnormal one built by mutated NANOS1, originating from infertile patients. We expect that by comparing the normal NANOS1 functioning with the mutated one, we will be able to select pathways that are the most important for specification/early stages of human germ cell development.

Research to be carried out

Knowing that NANOS1 has ability to bind various protein and RNA molecules, we plan to investigate the above processes, first by identification of NANOS1-bound RNAs and proteins. However, considering the ethical issues, we cannot perform such studies in humans and even less in human embryos in whom those processes take place. Therefore, we will use two models mimicking specification and early stages of human germ cell development. One of them is *in vitro* growing human embryonic stem cells (hESCs) which we will differentiate to get 3 consecutive germ cell developmental stages. The second one is TCam-2 cells which we have been using for several years now and which represent a slightly later but still early developmental stage of germ cells and differentiating towards male germ cells and we call it stage 4. We will apply cutting edge methodologies for global identification of RNA molecules that the normal NANOS1 protein binds to, during those stages, to check which among them are stage specific. Importantly, we will perform that experiment for both, the wild-type and the abnormal NANOS1. As the next step, by using cutting edge methodology, we will globally identify proteins which bind to the normal NANOS1 protein and those binding/nonbinding the abnormal one. Data obtained from two above approaches we will used to obtain networks of RNA and protein interacting molecules controlled by NANOS1which we call RNA-interactome, to get insight into their potential functional interactions. Pertinent bioinformatic tools will be implemented for that purpose. We expect that these networks will be at least partially different in specific developmental 0-4 stages (hESCs up to TCam-2 cells) but also for the normal and abnormal NANOS1 protein. Finally, the most interesting elements of those networks such as proteins encoded by RNAs bound to NANOS1 will be tested: does their quantity depend on NANOS1 presence. If so, we will study influence of those elements on such processes as cell division, apoptosis or other, which potentially could underlie the phenotype of the infertile patients carrying abnormal NANOS1 protein.

Reason for choosing the research topic

Infertility affects approximately 15% of couples worldwide. Genetic abnormalities account for 15%–30% of male factor infertility. Notably, male infertility is a risk factor for developing testis germ cell tumor (TGCT). We expect that this study will enable to get a global insight into processes responsible for a proper specification and early germ cell development, which are controlled by NANOS1 protein. Severity of the patients infertility characteristics (absence of germ cells in testes) indicates that those processes are crucial for human fertility. Within this project we plan to identify molecules, such as RNAs and proteins which are involved in those processes. They may be used in the future to elaborate genetic diagnosis tools for couples which cannot conceive and are seeking to undergo *in vitro* fertilization. We expect that our findings will also be of value for future therapies of male infertility. We anticipate that our findings will shed light on mechanisms underlying origins of TGCT, given that this type of tumor affects a growing number of young men at the reproductive age.