Functional consequences of loss of chromosome Y (LOY) in immune cells in the context of cancer; *ex-vivo* and *in vitro* studies of human cells

Cancer is one of the leading causes of death in modern societies. Notably, the male sex is a risk factor for many common types of cancer. In addition, malignant tumors develop earlier and have higher mortality rates in males. These sex disparities cannot be fully explained by known risk factors. Loss of chromosome Y (LOY) in peripheral blood leukocytes is the most frequent post-zygotic mutation in aging males. Many epidemiological studies have already shown the association between LOY in circulating immune cells and the increased risk of different age-related diseases, including hematologic and non-hematologic cancers. Moreover, it has been shown that LOY in leukocytes causes dysregulation of many autosomal genes, including genes crucial for the proper function of immune system. Nevertheless, the lack of extensive research showing the functional consequences of LOY in leukocytes for the development of malignant tumors in humans presents a serious gap in our understanding of the process of cancer formation. The main hypothesis for this research project is that LOY in immune cells disturbs the process of immune-surveillance, which is crucial for the clearance of cancer cells. The main aim of this study is to analyze the functional consequences of LOY in leukocytes in relation to cancer development on the molecular and cellular level. We would like to propose a series of experiments using both *ex-vivo* human leukocytes collected from cancer patients and healthy controls as well as *in vitro* models of LOY-leukocytes. Our previous research has shown that CD4+ T cells isolated from blood are preferentially affected by LOY in prostate cancer (PC) patients, which suggested that this type of cells may be responsible for cancer development when affected by LOY. Our further studies have shown that CD4+ T regulatory cells (Tregs) have the highest level of LOY of all leukocytes in colorectal cancer (CRC) patients and that LOY-Tregs have higher expression of immunosuppressive genes. The higher immunosuppressive activity of Tregs has been associated with more favorable microenvironment for tumor development and progression. Here we would like to study the functional consequences of LOY differential gene expression analysis in different types of leukocytes isolated by applying fluorescent activated cell sorting (FACS) from the whole blood of 60 CRC and 60 PC patients as well as 60 age-matched healthy controls, with the special focus on CD4+ T helper cells (Ths), CD4+ Tregs, CD8+ cytotoxic T cells (CTLs) and myeloid derived suppressor cells (MDSCs). MDSCs are the type of suppressor cells which have already been associated with cancer and may also be affected by LOY. Additionally, it has been shown that Tregs and MDSCs co-regulate each other within tumor microenvironment (TME). To more comprehensively describe the influence of LOY in immune cells on the process of carcinogenesis, we would also like to analyze leukocytes isolated from tumor microenvironment. As around 50% of patients with CRC develop liver metastases, we will perform tissue dissociations of 5-10 metastatic tumors and use FACS to isolate tumor infiltrating Ths, Tregs and CTLs. We will analyze bulk gene expression in each sorted leukocyte subtype in LOY- vs. non-LOY individuals using SMARTer RNAseq. Furthermore, to extend our results on the consequences of LOY for the immunosuppressive activity of Tregs, we will create an *in vitro* model of LOY-Tregs using CRISPR/Cas9 system. We will test the immunosuppressive activity of LOY-Tregs by analyzing the spectrum of cytokine production. In addition, we will create 2D and 3D co-cultures of LOY- vs non-LOY-Tregs with effector immune cells (e.g. CTLs) as well as colon- and prostate- cancer cell lines and cancer spheroids. We expect that the results of this study may be valuable for the development of new anti-cancer immunotherapies. Currently many patients do not respond properly to immunotherapy and this may be connected to enhanced immunosuppressive phenotype of leukocytes in TME due to LOY.