“Cardiovascular miRNA fingerprint of anthracyclines - study in vitro and in vivo in acute lymphoblastic leukemia long-term survivors“

Advances in cancer treatment have led to the significant improvement in survival rates of childhood malignancies. Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and it accounts for about 30 % of all childhood cancer. A 5-year survival rate in children treated for acute lymphoblastic leukemia increased to about 87% compared to 57% in the seventies of the twentieth century, however improved outcomes are accompanied by substantial risk of late, and potentially long-term, adverse effects of anti-cancer treatment. Although the occurrence of acute cardiotoxicity has been radically reduced in modern treatment protocols, chronic cardiotoxic side effects are among most serious consequences in these patients, what is reflected in the fact that the leading non-cancer cause of morbidity and mortality in long-term survivors are cardiovascular diseases. Among survivors of childhood leukemia the relative hazard of congestive heart failure, myocardial infarction and late-occurring stroke is increased 4.2 fold, 3.3 fold and 6.4 fold respectively, with the reference to the sibling control groups, and standardized cardiovascular mortality ratio is elevated 4.2-fold.
The excessive cardiovascular risk in long-term survivors of childhood cancer is a result of specific therapeutic regiments, particularly anthracyclines based, resulting in cardiomyopathy and endothelial dysfunction. It has been proved that some changes in cardiovascular system are anthracycline dose-dependent. Anthracycline antibiotics are very important and effective drugs used for ALL, breast, ovarian, uterine and lung cancer-treatment.
Micro RNAs, targeting the 3’ untranslated region of mRNA are powerful regulators of translation, acting not only inside the cell but also having an impact on cell-to-cell communication. It has been found that cells may select and export particular miRNAs to circulation, where they may circulate for prolonged time, packed in lipid vesicles protecting them from cleavage by RNAses, whereas other miRNAs are retained in cells. Many studies show that miRNA pattern could be injury- or disease-specific. Dysregulation of microRNAs system has been reported in many cardiovascular diseases however its role of in cardiovascular complications developing in cancer survivors as well as in signaling between cardiac and endothelial cells remain uncovered. Changed expression patterns of circulating miRNA may be associated with anthracyclines-induced end organ injury and DNA damage (DNA binding and alkylation, DNA cross-linking, interference with DNA, strand separation and helicase activity changes) also in progenitors cells which are source of next generations of cells.
We expect that ALL patients with prior anthracyclines treatment have more pronounced cardiac and endothelial changes with reflection in exosomal miRNA content in plasma (particular miRNAs expression levels are expected to correlate with the severity of cardiac/endothelial dysfunction). We also suggest that cultured cardiomyocytes as well as endothelial cells change the set of miRNAs released in exosomes to environment as a result of anthracyclin-induced injury. Moreover we expect that miRNAs released upon anthracyclin treatment in vitro can alter gene expression in cultured cells via paracrine/autocrine manner.

To verify these assumptions we have designed the set of experiments in vivo and in vitro with use of the most up-to date new high-throughput technologies like next-generation sequencing and which enable broad searching of all micro RNAs with treatment-changed expression levels what is desirable especially in the case of uncovered research area. We are going to measure expression of plasma proteins related to cardiovascular disease with Proximity Extension Assay technique which enables measurement of up to 92 proteins in as small amount of plasma as 1ul. The use of this techniques is very reasonable in the case of studies on drugs acting in pleiotropic manner.
To verify these assumptions we have designed the set of experiments in vivo and in vitro with use of the most up-to date Next Generation Sequencing (NGS) technology, which enables searching the full spectrum of existing micro RNAs (in contrast to microarrays technology or qPCR) secreted to circulation or cell culture media. The advantage of this study consist of tight cooperation between experienced clinicians from selected Units of Jagiellonian University Hospital with well-trained molecular biologists and bioinformaticians, working in the Omicron Center for Medical Genomics at the Faculty of Medicine Jagiellonian University Medical College.
After a child finishes treatment for cancer, it will need regular, at least once a year, follow-up screening to confirm the absence of cancer and diagnose of any potential late effects. Long-term follow-up care, which should be continued throughout adulthood, proving that survivors of childhood cancer stay healthy. We hope that our study will help to find easy to monitor miRNA marker candidates and explain the role of miRNA in anthracyclines action in cardiovascular system.