Metabolic and proteomic analysis of oxidative stress effects in heart diseases

-Progress Report-

Projektnummer: 201901

Forschungseinrichtung: Medizinische Universität Graz, Institut für Pathologie

Projektleitung: TOMIN Tamara Dr.

Brief summary of the proposed aims and methodology

Main focus of this project is to dissect the effects of oxidative stress in failing hearts by addressing the redox state of key thiol containing antioxidative metabolites as well as protein thiols.

To achieve that, in the **first aim** we proposed to extend the scope of our liquid chromatography coupled to mass spectrometry (LC-MS/MS) approach for measurement of reduced and oxidized glutathione (GSH and GSSG, respectively) to also cover other relevant redox couples. Our hypothesis is that not only ratio of GSH/GSSG could be used for prediction of heart pathologies, but also ratios of other small molecule thiol pairs (e.g. cysteine/cystine).

Furthermore, as changes in reduction/oxidation status of protein thiols can affect proteins' conformation and activity (e.g. by altering disulfide bonds) and since some of those changes can contribute to heart dysfunction, in the **second aim** we proposed to address the redox state of proteome in failing compared to non-failing hearts. Therefore, as one of the expected outcomes of this project, we suggested development of a complementary redox proteomics method which can be applied to heart tissue samples of dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM) patients, in order to clearly delineate the impact of oxidative stress on diseased heart tissue on protein level.

Lastly, in the **third aim** we proposed to validate our findings in an *in vitro* approach by inducing oxidative stress in isolated cardiomyocytes.

Current status of the project

By this end we have set up redox proteomics approach which we then applied to failing and non-failing human hearts. For the analysis of protein thiols, we carried out a two-step alkylation protocol in the sample preparation procedure for subsequent analysis by LC-MS/MS. After initial quenching of all free protein thiol groups with "light" N-ethylmaleimide (NEM), disulfide bonds were reduced and newly formed free thiols alkylated with isotopically ("heavy") labelled NEM (d5-NEM). In this way "light" to "heavy" (L/H) NEM ratios for each cysteine can be determined, which denotes the oxidative state of the

respective cysteine (higher ratio reflects lower oxidative state) independent of protein amount. Additional benefit of the approach is that protein quantitation based on the protein abundance can also be performed on the same data set, just by changing parameters in the data post-processing.

We applied this redox proteomics approach to left-ventricular tissue samples of ten explanted hearts from patients suffering from end-stage DCM (n=5, male, age 63 ± 1.5 years, left ventricular ejection fraction (EF) 25 ± 2.8 %) or ICM (n=5, male, age 63 ± 1.6 years, EF 37 ± 6.3 %) and non-failing control donor hearts (n=5, male, age 62 ± 0.8 years, EF 62 ± 1 %). We have already determined that these failing hearts have significantly reduced (Students' t-test p-value < 0.05) GSH/GSSG ratios (DCM: 188 ± 16, ICM: 220 ± 27) compared to control samples (318 ± 30), indicating a greater extent of myocardial oxidative stress. As a result of the redox proteomics analysis of the heart tissues, we obtained around 3000 cysteine containing peptides with reported L/H ratios and we are currently performing the data analysis and interpretation.

In parallel, we started establishing the "extended" redox couple method. Optimization of MS transitions is already done and now special attention will be dedicated to optimization of LC parameters as well as extraction procedure. To achieve even higher quality of chromatography of redox pairs, we needed to purchase a new LC column (more details in the accounting section). Completion of this part of the project is expected by the end of January/mid-February. We are also currently in the process of obtaining more failing and non-failing heart tissue samples which could be used for redox analyses.

Lastly, *in vitro* validation studies with induction of oxidative stress will be carried in the last month of the project. For this purpose, we will either use isolated cardiomyocytes from rat or mouse, or purchase human cardiomyocyte cell line. This decision will mainly depend on the outcome of the proteomics redox study and to which extent significantly differentially oxidized cysteines we detect in failing hearts are conserved across the species.

Current accounting of the Herzfonds funds

Since October 2019, project leader Dr Tamara Tomin has been employed 30% via the project *Metabolic and proteomic analysis of oxidative stress effects in heart diseases* at the Medical University of Graz. Dr Tomin's addendum to the existing contract with Medical University of Graz is enclosed.

From material costs, by this end one LC column has been purchased. The column is, as mentioned above, used for the extended redox couple method establishment. Original bill from the company (Agilent) is also attached. In following weeks additional material will be ordered and the accounting will be presented at the end of the project. Summary is shown in the following table.

	Initial	Spent	Remaining
Personal Costs (€)	10150	5075	5075
Material Costs (€)	4760	644,88	4115,12

Publications

In August 2019 we published a review with Austrian Herzfonds mentioned in the acknowledgement:

Tomin T, Schittmayer M, Honeder S, Heininger C, Birner-Gruenberger R. Irreversible oxidative post-translational modifications in heart disease. *Expert Rev Proteomics*. 2019;16(8):681–693. doi:10.1080/14789450.2019.1645602.