Metabolic and proteomic analysis of oxidative stress effects in heart diseases

-Final 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.

Final report 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 additional number of left-ventricular tissue samples so by this end we analyzed total of fifteen explanted hearts (+5 compared to progress report) from patients suffering from end-stage DCM (n = 3 (all male), mean age 63 ± 3.9 years, EF 22 ± 5.8 %) or ICM (n = 12, all male, mean age 63 ± 5 years, EF 33 ± 11 %) and non-failing control donor hearts (n=10 (+5 compared to progress report), all male, mean age = 63 ± 3, EF = 63 ± 4.3 %). We have already determined that these failing hearts have significantly reduced GSH/GSSG ratio (Students' t-test p-value < 0.01), 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 on which we performed data analysis and interpretation. In addition, redox dataset was complemented with label free proteome quantification, which revealed distinctive proteome changes between failing and non-failing human heart tissue. The manuscript is in preparation.

In parallel, we establishing the "extended" redox couple method. To achieve even higher quality of chromatography of redox pairs, we needed to purchase a new LC column, which was used for this setup. The method needs some additional optimization steps but once fully established it will be published as a stand-alone method manuscript and will be applied to heart tissue as well as cell culture samples.

Lastly, we carried out *in vitro* validation studies in last months. Due to COVID-19 this part of the project was greatly delayed but we managed to obtain the cells and perform the experiments. For this purpose, we acquired a human model cardiomyocytes (AC16 cells, Sigma) which were treated with 25 µM hydrogen peroxide and harvested for redox and label free proteomic as well as redox couple metabolomic analysis. Sample processing from this part of the project is currently ongoing.

Final accounting of the Herzfonds funds

From October 2019 to March 2020, 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.

Material costs were used to purchase LC column and other LC-MS/MS related materials, primary cardiomyocyte cell line (AC16) and corresponding cell culture medium. All original bills are included.

Publications

Over the duration of the project we published the following papers 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.

Tomin T, Schittmayer M, Birner-Gruenberger R. Addressing Glutathione Redox Status in Clinical Samples by Two-Step Alkylation with N-ethylmaleimide Isotopologues. Metabolites. 2020;10(2):71. Published 2020 Feb 16. doi:10.3390/metabo10020071

Olivieri O, Speziali G, Castagna A, Pattini P, Udali S, Pizzolo F, Liesinger L, Gindlhuber J, Tomin T, Schittmayer M, Birner-Gruenberger R, Cecconi D, Girelli D, Friso S, Martinelli N. The Positive Association between Plasma Myristic Acid and ApoCIII Concentrations in Cardiovascular Disease Patients Is Supported by the Effects of Myristic Acid in HepG2 Cells [published online ahead of print, 2020 Jul 25]. J Nutr. 2020;nxaa202. doi:10.1093/jn/nxaa202