A systems biology approach to investigate the impact of metabolic dysregulation on heart and kidney cytoskeletons

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Project outline

Background:
Under the influence of systemic stress that may derive from metabolic syndromes and inflammatory mediators, proteases become active through the action of free radicals and other enzymes and through a sequence of proteolytic events can cause extensive tissue damage and multiple pathological conditions such as cancer, inflammatory and cardiovascular and kidney diseases. Accordingly, many proteases are a major focus of attention as potential drug targets or as diagnostic and prognostic biomarkers. Targeted protein degradation is a promising emerging therapeutic approach for a number of diseases. Proteolytic processing of proteins is a crucial mechanism that regulate physiological and pathophysiological processes. Regulated protease activity is essential for physiological cellular maintenance.

Hypothesis: Diabetic conditions and metabolic syndromes activate a range of proteases in the cellular microenvironment, causing remodelling of extracellular matrixes such as proteolytic degradation of key cytoskeletal proteins integral to sustain the healthy function of organs such as kidney & heart and alterations of collagens turnover – a key pathway of fibrosis.

Aims: We aim to investigate the protein proteolytic mechanisms using degradomics and metabolomics. Analysis of these datasets using machine learning approaches and bioinformatics will lead to identification of the key enzymes causing cytoskeletal degradation in kidney. To identify metabolomics and proteolytic interactions we will use human kidney cellular models, organoids and an ex vivo model of human kidney precision slices to validate and screen initial inhibitors as potential therapeutics.

Resources: We aim to investigate proteolytic processing pathways using multi-omics technologies (metabolomics and degradomics) in addition to systems biology and bioinformatics approach to discover novel targets to inhibit damaging proteolytic activities. Clinical samples with complete metadata from the Quality in Organ Donation (QUOD) UK wide biobank will be analysed to investigate how diabetes, obesity, ageing alters the kidney & heart tissue degradome and how these alterations impact the function of these organs. QUOD collects clinical samples (longitudinal blood, urine and

biopsies from all the organs) from deceased donors in UK. Biopsies are obtained from donor organs prior to transplantation and posttransplant outcome data reflects organ function.

Aim 1: Investigation of the interrelationship between metabolic dysregulation in diabetes / metabolic syndromes and protein degradation in kidney and heart tissue. Donor blood samples and kidney biopsies will be obtained from the QUOD biobank. Associated metadata will allow to select donor groups with history of diabetes, high BMI and with kidney functional parameters posttransplant. Blood and kidney biopsies from control healthy groups will be obtained from the Oxford Transplant living donor biobank (OTB). Donor groups will be matched for all other demographic and clinical characteristics, as previously described (Vaughan et al., AJT 2021). Deceased donor plasma samples will be analysed by Gas Chromatography - Mass Spectrometry (GC-MS) metabolomics to discover changes in systemic profiles that may reveal the degree of injury of donor organs. Biopsies of donor kidneys will also be analysed by degradomics techniques and MS-based metabolomics to study metabolic pathways, such as glycolysis, oxidative phosphorylation, beta-oxidation and specific catabolites (Yu et al. 2019). MS-based techniques that will be used are Protein Topography and Migration Analysis Platform (PROTOMAP), high-efficiency undecanal-based N-termini (HUNTER) workflows (Vaughan et al. 2022; Leeuwen et al. 2022), as well as associated metabolomics (Yu et al. 2017) to study affected organ cytoskeletons and extracellular membrane integrity.

Aim 2: Systems biology approach will be used to develop associations of degradomics profiles and clinical characteristics related to kidney/heart cytoskeleton networks. Systems biology will be applied to identify causal key mechanistic alterations related to metabolic disturbances in deceased donors and organ damage with focus on the cytoskeletal damage that impacts the donor organ function and may lead to transplant dysfunction in the recipient. More specifically, we will investigate the interrelationship between protein degradation (degradomics) and catabolism, such as polyamines (amino acid catabolism)(Yu et al. 2019), for the discovery of novel molecular traits in tissue material derived from multiple tissues (i.e., kidneys/liver/heart) of the same donor.

Aim 3: Identify key enzymes that are activated in kidney/heart biopsies and cleave cytoskeletal proteins. Bioinformatic analysis of the mass spectrometry raw data to identify the cleavage site analysis of differentially abundant peptides and prediction of active proteases. MS analysis software such as MaxQuant and Fragpipe to identify which peptides are present in the sample after processing, followed by N-terminomics analysis software.

Aim 4. Validation phase to confirm mechanistic pathways relevant to cytoskeletal degradation that affect kidney/heart tissue integrity. Novel discoveries can then be followed up and validated in complementary in vitro and ex vivo models (Precision cut human kidney slices). In vitro assays will involve human immortalised human kidney podocyte, proximal tubular cells, to confirm degradation, staining of actin cytoskeleton and confocal analysis, functional assays, enzymatic assays will also be performed. Mapping of all the neo-peptides will performed by mass spectrometry.

Expected outcomes: To this end, this proposed project will investigate the interaction of metabolic changes in diabetes, high BMI in driving kidney/heart tissue degradation. As the analysis will involve clinical samples with kidney functional data this work will lead to identify associations of metabolic and degradomics profiles to kidney function, which may lead to the identification of new targets to slow the progression to kidney & heart dysfunction. The multidisciplinary teams and this collaborative project will offer excellent training opportunities in the application of multiomics technologies, metabolic proteolytic/ degradation biology and drug discovery.

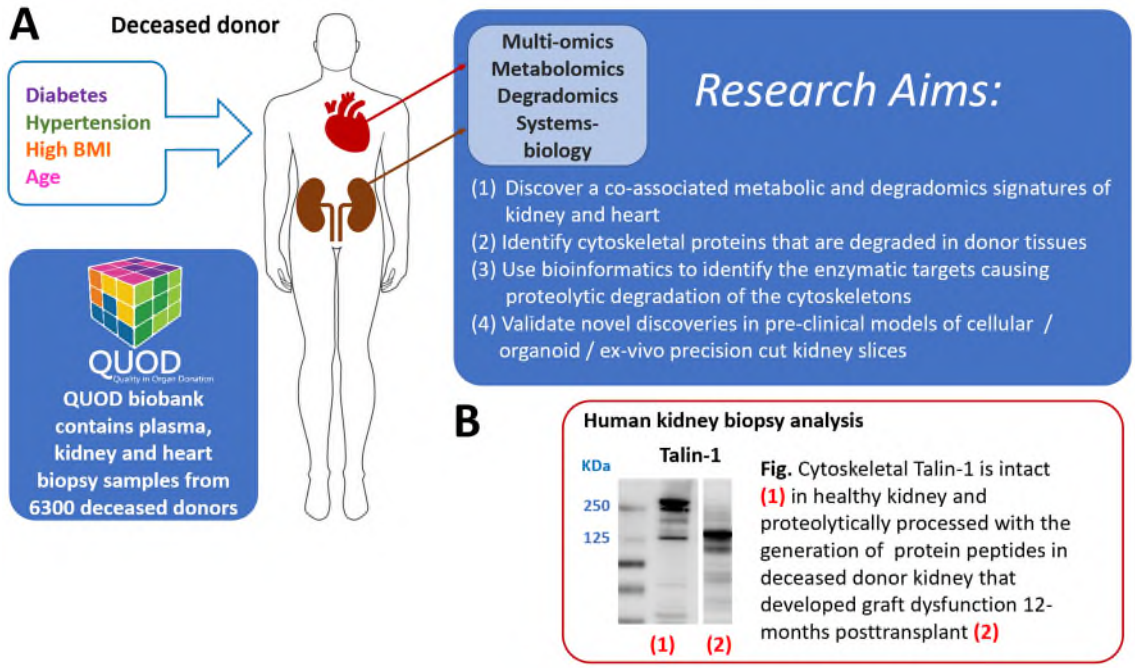

Figure 1. Research aims of the fellowship. (A) *Using biopsy samples derived from kidney organ available through the QUOD biobank, we shall perform metabolomics and degradomics experiments to discovery molecular signatures associated with donor conditions (diabetes, hypertension, BMI, age) and subsequent organ function and integrity.* ***(B)*** *Cytoskeletal degradation reflects a hallmark in donor kidneys with suboptimal function (Vaughan et al., AJT 2021).*

Supervisor's recent relevant publications:

Maria Kaisar

1. Vaughan RH, Kresse J, Farmer LK, Thézénas ML, Kessler BM, Lindeman JHN, Sharples EJ, Welsh GI, Norregaard R, Ploeg RJ, **Kaisar M**. Cytoskeletal protein degradation in brain death donor kidneys associates with adverse post-transplant outcomes. Am J Transplant. 2021; February 2022:1–15.

Benedikt Kessler

2. Leeuwen, L Van, Venema, LH, Heilig, R, Leuvenink, HGD. & **Kessler, BM**. Doxycycline Alters the Porcine Renal Proteome and Degradome during Hypothermic Machine Perfusion. 559–577 (2022).
3. Yu Z, Huang H, Reim A, Charles PD, Northage A, Jackson D, Parry I, **Kessler BM**. Optimizing 2D gas chromatography mass spectrometry for robust tissue, serum and urine metabolite profiling. Talanta. 2017 Apr 1;165:685-691.
4. Yu Z, Huang H, Zhang H, **Kessler BM**. Improved profiling of polyamines using two-dimensional gas chromatography mass spectrometry. Talanta. 2019 Jul 1;199:184-188.
5. Huang H, van Dullemen LFA, Akhtar MZ, Faro ML, Yu Z, Valli A, Dona A, Thézénas ML, Charles PD, Fischer R, Kaisar M, Leuvenink HGD, Ploeg RJ, **Kessler BM**. Proteo-metabolomics reveals compensation between ischemic and non-injured contralateral kidneys after reperfusion. Sci Rep. 2018 Jun 4;8(1):8539.
6. Weiss-Sadan T, Itzhak G, Kaschani F, Yu Z, Mahameed M, Anaki A, Ben-Nun Y, Merquiol E, Tirosh B, **Kessler B**, Kaiser M, Blum G. Cathepsin L Regulates Metabolic Networks Controlling Rapid Cell Growth and Proliferation. Mol Cell Proteomics. 2019 Jul;18(7):1330-1344