BHF Centre of Research Excellence Clinical PhD

Microvascular remodelling in the heart: role of endothelial-mesenchymal transition

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Background

Ischaemic heart disease is a leading cause of death[1-3]. Two thirds of patients with angina do not have obstructive coronary atherosclerosis, but rather small vessel disease (SVD) of the heart causing microvascular angina (MVA)[1,5,6]. SVD is a multisystem disorder. MVA is linked with vascular dementia and heart failure with preserved ejection fraction (HFPEF)[5-11]. The mechanisms of SVD in the heart are incompletely understood [2,3].

Cardiac SVD is a complex trait with a mid-life onset; sex-differences, vascular risk factors and genetics are implicated[6-37]. The pathophysiology of MVA involves impairments of coronary and systemic vasodilator reserve and increases in coronary reactivity and resistance[6-37]. Endothelin-1 (ET-1) is a highly potent endogenous constrictor of human coronary arteries[38,39]. Constitutive release of ET-1 maintains vascular tone *in vivo*[40,41]. ETA receptor (ETAR) activation mediates coronary constriction whereas ETB mediates vasodilatation[39,40]. ET-1 dysregulation is implicated in the pathogenesis of microvascular dysfunction[40-47]. ET-1 mediated activation of the G protein-coupled ETAR on vascular smooth muscle cells (VSMC) induces endothelial cell (EC) dysfunction, inflammation and vasoproliferation[38,39]. In patients, circulating ET-1 concentrations are inversely associated with coronary flow responses *in vivo* and vasconstriction to ET-1[42-45].

Endothelial mesenchymal transition (EndMT) is a pathological process implicated in multiple cardiovascular disorders[46]. We[47] and others[46, for review] have identified EndMT as a mediator of vascular remodelling in models of cardiovascular disease[47] and human vascular tissues[47]. We identified that EndMT is mediated by TGFβ1/SMAD2 signalling in vascular cells [47]. EndMT is also induced by hypoxia[48] and vascular risk factors such as high glucose/diabetes[49]. EndMT may be relevant to the pathogenesis of SVD in microvascular angina. ET-1 induces EndMT in microvascular cells[50] via an ETA receptor-dependent mechanism[51]. To date, there are no mechanistic studies of EndMT in cardiac SVD. Microvascular angina is also associated with vascular fibrosis. Cardiac fibrosis is stimulated by oxidative stress is mediated by EndMT[52]. Some[53], but not all studies[54,55] have directly implicated EndMT in cardiac fibrosis.

The role of EndMT in the pathogenesis of cardiac SVD is incompletely understood. Most of the relevant work to date has been undertaken in cultured cells rather than intact tissues. Widyantoro[49] and Murdoch[52] used mouse models to study cardiac pathophysiology. Their studies focused on cardiac fibrosis in relation to hypertension and diabetes.

In this study, we propose to focus on EndMT and SVD in the heart, predicated on a central hypothesis that EndMT is an underpinning cause of cardiac SVD induced by endothelin via TGFβ1/SMAD2. We propose to specifically focus on the role of endothelin and EndMT in relation to cardiac pathophysiology. Specifically, does endothelin trigger cardiac EndMT in vascular tissues of animal models of 'microvacsular angina/HFPEF' and in human vascular cells/arterioles? If so, does EndMT lead to SVD e.g. microvascular remodelling, vascular rarefaction? If so, what are the physiological consequences *in vivo.* For example, is EndMT implicated in the development and maintenance of chronic myocardial ischaemia? Does EndMT

have a role in the transition from microvascular angina (chronic ischaemia) to HFPEF? Do inhibitors of EndMT arrest the progression of SVD, and thereby have therapeutic potential?

Study design

We propose a translational research study to assess the role of for EndMT in cardiac SVD. We will exploit relevant animal models and clinical samples from the CorMicA study [10,15,25].

In the animal models, we will place a focus on integrating cardiac pathophysiology with vascular pathology. In a collaboration with Prof Rhian Touyz (Co-Supervisor), Prof Chris Loughrey (Co-Supervisor) and Prof Stuart Nicklin (collaborator), we propose to study EndMT in a model of heart failure with preserved ejection fraction (HFPEF)[56]. This model is currently being developed through as a prioritised strategy in the BHF CoRE. In parallel, we propose to develop work using an *in vivo* mouse model for endothelial lineage tracing system e.g. VE-Cad/CreLox mouse. Whilst this model is not currently available in our centre, we have strong collaborative links with other centres e.g. Andrew Baker, University of Edinburgh; Jason Kovacic, Mount Sinai; Manfred Boehm, US NIH. We therefore propose to initiate a new collaboration with options to either undertake the work as part of a secondment or alternatively, should funding permit, to develop the model in Glasgow (Years 1 & 2) with a view to undertaking lab work in Year 3. This new model is intended to serve as a novel resource for future research beyond the lifetime of the current project.

Methods

Animal models: We will undertake immunostaining of endothelial and mesenchymal proteins in cardiac sections. Time-course studies will be undertaken to assess for the presence and extent of EndMT markers during temporal evolution over an approximate 5-week period from exposure to vascular risk factors (hypertension), high fat diet, and the transition to HFPEF.

We will use qRT-PCR to screen for expression of EndoMT genes and Western Blotting to assess for protein expression, and assess the effects of exogenously applied EndMT inhibitors in cultured cells (BMP7, SMAD7)

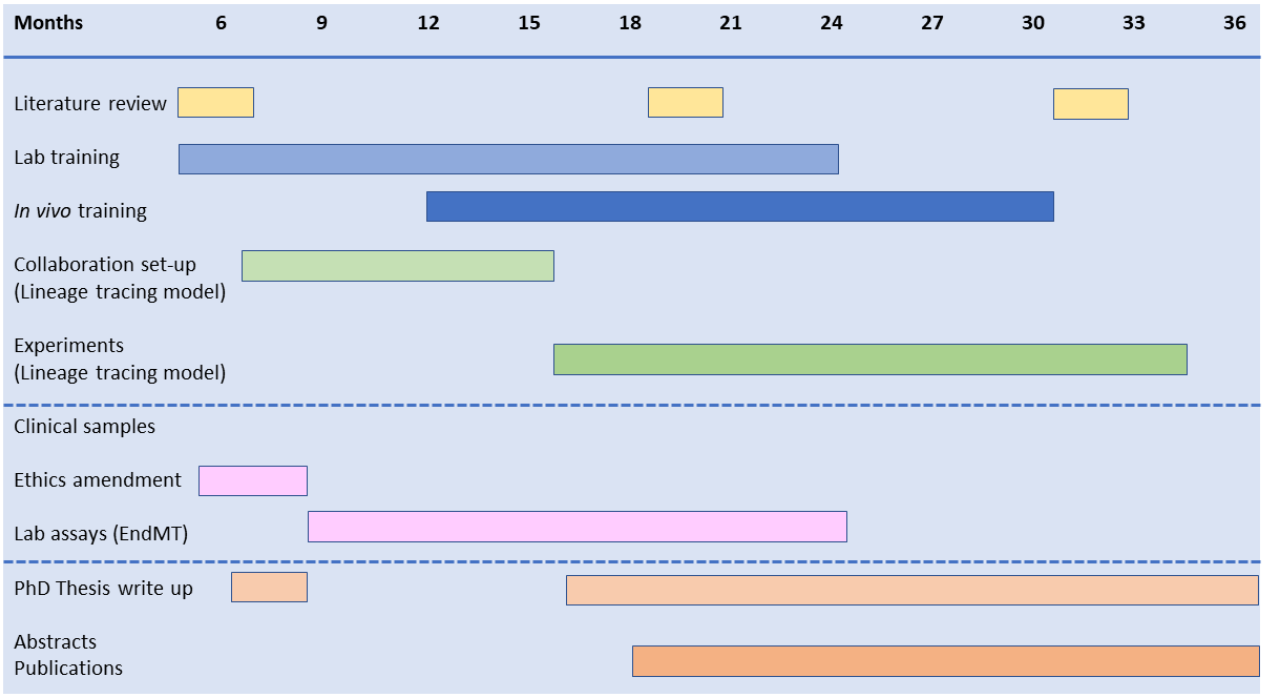
Clinical studies: Patient samples A) plasma/serum – measure circulating biomarkers of EndMT in biobanked samples obtained from patients with microvascular angina vs. controls; B) Resistance arterioles – gene and protein expression studies of EndMT markers, patients vs. controls.

Clinical research: The student will gain clinical observer experience in the Golden Jubilee National Hospital, including to observe cardiovascular procedures. The student will also gain experience in Patient and Public Involvement (PPI), by organising and taking part in patient events.

Value

This translational study will provide excellent training in cardiovascular science. The research will provide new knowledge on disease mechanisms relevant to microvascular angina and HFPEF. The research has transferable relevant to support therapy development for patients with these conditions. By the end of this project, the student will be fully equipped to progress to the next stage in their career in cardiovascular biomedicine.

Figure. Gantt chart of the work plan.



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