Investigating extracellular vesicle-mediated signalling in vascular and cardiac remodelling and hypertensive heart disease (Nicklin, Delles and Loughrey)

Extracellular vesicles (EVs) are membrane bound signalling particles that are secreted by all cells and tissues to mediate intercellular communication. EVs include microvesicles (MVs) 100-1000 nm in size which are produced by blebbing of the plasma membrane and exosomes of 30-130 nm in size and actively produced from multivesicular bodies within the cell[1]. EVs are released by most cells[1] and have been reported to contain DNA, RNA, microRNA and proteins thus they act as important intercellular messengers in both physiology and pathophysiology. There are now numerous reports of how EVs contribute to cardiovascular disease (CVD) development. E.g., cardiomyocyte EV release triples after 2 hr of hypoxia[2]. EVs derived from cardiac myocytes and fibroblasts have been demonstrated to alter the gene expression profile of recipient cells[3] and EVs isolated from plasma and cardiac progenitor cells mediate therapeutic effects in ischaemia-reperfusion injury and myocardial infarction[4], while exosomes derived from cardiac fibroblasts are linked to cardiac disease[5].

While chronic renin-angiotensin-system (RAS) activation elevates angiotensin (Ang) II levels, leading to vascular remodelling and fibrosis and end-organ damage in tissues such as the heart, whether RAS signalling can be mediated via EV transport and signalling is only beginning to be investigated. For example, angiotensin converting enzyme (ACE) and ACE2 are found in urinary exosomes[6] and exosomes isolated from cardiomyocytes after pressure overload had increased AT_1R levels[7]. Cardiac fibroblasts release exosomes that activate the RAS in recipient cardiomyocytes, stimulating hypertrophy[8]. In unpublished data we identified that EVs released from Ang II-stimulated cardiac fibroblasts contain Ang II and fluorescently labelled Ang II can be imaged in EVs. Importantly we compared healthy controls to patients with coronary artery disease (CAD) from the VASCAB study[9] and characterised isolated EV's by Nanosight tracking analysis and measured Ang II levels via specific ELISA. EVs were significantly smaller in CAD patients compared to control healthy subjects and were released at significantly lower concentrations than those found in healthy controls. These data suggest potential for different EV populations to be released in disease. Ang II levels in EVs were quantified and Ang II was significantly higher in CAD patients compared to healthy controls. These data highlight that CAD patients have fewer EVs, but higher Ang II levels, demonstrating they transport more Ang II per EV, and suggest that transport of RAS components via EVs may be a novel mechanism to further our understanding of the (patho)physiological signalling mechanisms of the RAS in CVD.

Hypotheses

- EVs are released from cells and tissues in CVD and contribute to pathological signalling mechanisms in the setting of hypertensive heart disease.
- Using cell culture models and in vivo rodent models the role of RAS signalling via EVs in vascular and cardiac hypertrophy and fibrosis can be dissected.

Methods

<u>In vitro methods</u>

We will utilise established primary cell culture models of vascular smooth muscle cells, endothelial cells, myocytes and fibroblasts to characterise EV populations released from each cell type using Nanosight Tracking Analysis, western immunoblotting for specific EV markers and transmission electron microscopy. We will utilise EVs released from control cells versus EVs isolated from Ang II and Ang-(1-7) stimulated cells to study pathways that contribute to adverse remodelling by studying processes in vitro such as proliferation, migration and hypertrophy.

<u>In vivo models</u>

We will utilise established rodent models of hypertensive heart disease (Ang II-infusion model) and isolate and characterise EVs released in the blood, as well as those released from tissues cultured ex vivo (e.g. aorta and heart explants). We will infuse EVs isolated from AngII infused animals to assess the signalling effects in recipient animals in relation to vascular function (blood pressure, myography) and cardiac function (echocardiography) and vascular and cardiac remodelling (histological and molecular analyses).

<u>Patient samples</u>

Using matched cohorts of healthy controls and patient serum samples we will study the effects of EVs on the cell phenotypes described above and furthermore we will assess content of isolated EVs

with a focus on characterising RAS components as well as performing unbiased screens, e.g. proteomics screen and small RNA content sequencing to identify novel candidate molecules that could contribute to CVD signalling mechanisms. Identified candidate targets will be validated in the in vitro and in vivo models highlighted above.

References

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