Extracellular vesicles as biomarkers and biovectors in primary aldosteronism

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All cell types are capable of generating small heterogeneous vesicles, called extracellular vesicles (EVs). EVs are released from activated or stressed parent cells into the extracellular space such as plasma, cerebrovascular fluid, breast milk, urine, and saliva and reflect the activation status and phenotype of the parent cell from which they were derived[1]. Based on biogenesis, content and size, EVs are classified as exosomes (40 to 100 nm), microparticles (also termed microvesicles) (100-1000 nm) and apoptotic bodies (1-5 μm)[2]. Extracellular vesicles were initially identified as cellular "dust" and considered to be "cell debris". However there is now good evidence in experimental and clinical studies demonstrating that EVs are biomarkers of disease including cancer, metabolic disorders and cardiovascular diseases[3, 4]. Moreover it has become increasingly apparent that they are biologically active and are important signalling vehicles and regulators of local and distant cell-cell communication[1]. EVs act as biovectors carrying cargo such as proteins, lipids, receptors, RNA, microRNA (miR) and enzymes, which are derived primarily from parent cells. They communicate with target cells, through diverse mechanisms, including release of cargo into the extracellular space, cell membrane binding, cell membrane fusion and endocytosis by target cells. Through these interactions and transfer of cargo, EVs influence the function of other cells[2]. For example, neutrophil-derived microvesicles carry cytokines, which function as pro-inflammatory mediators[1]. Cancer cell-derived microparticles release metalloproteases and promote tumor invasion and metastases and platelet-derived microparticles transfer miR-142 into endothelial cells causing endothelial dysfunction[3, 5] .

As biomarkers in cardiovascular disease, platelet microparticles correlate with the development of hypertension, levels of endothelial cell-derived microparticles associate with type 2 diabetes and hypertension and circulating exosomes associate with myocardial infarction, cardiomyopathies and pulmonary hypertension[5]. In hypertension, EV levels also correlate with arterial stiffness, endothelial dysfunction and impaired vasoreactivity and

accordingly have been suggested to reflect underlying vascular status. Beyond their role as vascular biomarkers, EVs directly regulate endothelial and vascular smooth muscle cell function by influencing production of nitric oxide and reactive oxygen species, pro-inflammatory signaling, apoptosis and senescence[2, 6]. These processes have been implicated in various experimental models of hypertension, including Ang II-infused, SHRSP, DOCA-salt, SHR and in patients with essential hypertension.

In the current issue of the journal, Monticone et al[7] advance the field by demonstrating that the number of EVs derived mainly from leucocytes and endothelial cells is significantly increased in patients with primary aldosteronism when compared to individuals with essential hypertension and those with normal blood pressure, effects that were normalized post-adrenelectomy. Moreover, findings from this study showed a strong correlation between serum aldosterone levels and number of circulating EVs. Similar findings have been observed in experimental models of aldosterone-salt-induced hypertension (8). Patients with primary aldosteronism have a higher prevalence of target organ damage and cardiovascular events compared to patients with essential hypertension and endothelial dysfunction is associated, at least partially, with the increased cardiovascular risk observed in these patients[9, 10]. Therefore, considering EVs as surrogate markers of endothelial dysfunction and vascular injury, these data support the notion that the greater the magnitude of endothelial dysfunction in primary aldosteronism the higher the levels of circulating EVs. Renal-derived urinary exosomes have also been shown to be increased in patients with hyperaldosteronism and have been suggested to be markers of aldosterone-induced renal disease[11-13].

Despite the accumulating evidence showing a relationship between hyperaldosteronism and EV formation, it remains unclear whether increased production is a primary effect of high levels of aldosterone or whether increased generation is secondary to

aldosterone-induced vascular injury and hypertension. Also, it may be possible that changes in plasma K^+ due to hyperaldosteronism influence EV production, as previously suggested[12]. Shear stress and elevated blood pressure probably contribute, at least in part to increased circulating EVs, but it may be possible that aldosterone itself can stimulate generation of EVs from various cell types. We previously demonstrated that generation of endothelial cell-derived microparticles depends on mechanisms involving Rho kinase, cholesterol-rich microdomains and cytoskeletal organization[2], processes that are influenced by aldosterone signalling through mineralocorticoid receptors[14]. Hence, it may be possible that in primary aldosteronism, aldosterone itself directly induces production of EVs.

Monticone et al further characterised EVs in primary aldosteronism and demonstrated that mRNA expression of caspase-1 (CASP1) and pre-pro-ET-1 (EDN1) is increased[7]. These vesicles were functionally active, since they promoted endothelial apoptosis and reduced angiogenesis in *in vitro* studies, indicating that they could themselves be involved in vascular dysfunction in primary aldosteronism. Previous studies showed that microparticles stimulate endothelial ROS formation and inflammatory responses via the endothelin-1 (ET-1) system and hence may be a molecular mechanism underlying EV-associated vascular damage in hyperaldosteronism. However, Monticone et al failed to demonstrate any effect of bosentan, a non-selective antagonist of ETA and ETB receptors, and a caspase-1 inhibitor, on EVs-stimulated endothelial cell angiogenesis and apoptosis[7]. Therefore, it is likely that CASP1- and EDN1-loaded EVs induce vascular dysfunction through pathways independent of caspase and ETAR/ETBR (**Figure 1**). Considering the wide array of cargo carried, it is likely that many factors influence EV-induced endothelial dysfunction in primary aldosteronism.

The present study adds to the concept that EVs are biomarkers of endothelial dysfunction in hypertension and that excess aldosterone amplifies this process. The findings also support previous studies showing that EVs influence intercellular communication by

regulating endothelial cell apoptosis and angiogenesis. However, there are a number of unanswered questions and limitations of the study that warrant further consideration. Firstly, it still remains unclear whether aldosterone itself or vascular dysfunction secondary to hyperaldosteronism causes the increase in circulating endothelial EVs. Secondly, reasons why leukocyte-derived EVs are increased in patients with primary aldosteronism were not explored. Thirdly, the significance of increased expression of CASP and EDN1 specifically in EVs from patients with primary aldosteronism is unclear. Finally, there is no attempt to examine how EVs from primary aldosteronism patients interact with target endothelial cells. Nevertheless, some new concepts of clinical relevance are suggested by Monticone et al[7], specifically that CASP- and EDN1- containing EVs may be putative biomarkers of hyperaldosteronism. Further large clinical studies are needed for confirmation.

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Figure legend

Figure 1. Potential mechanisms linking primary aldosteronism (PA) and extracellular vesicle (EV) formation, and how they may impair vascular homeostasis, leading to

cardiovascular disease. Hyperaldosteronism promotes increased generation of *EDN1* and *CASP1*-containing EVs. High levels of aldosterone may influence EV production through direct and indirect mechanisms. Once formed, EVs interact with target vascular cells and induce activation of signalling pathways that influence endothelial/vascular function. Amplification of this process in primary aldosteronism may contribute to endothelial/vascular injury, which contributes to target organ damage and increased cardiovascular risk.