Minireview

Importance of extracellular vesicles in hypertension

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Impact statement

Hypertension is the leading global risk factor for cardiovascular, cerebrovascular, and kidney diseases. There is emerging evidence that circulating and urinary extracellular vesicles are associated with increased blood pressure, suggesting that extracellular vesicles may be biomarkers and also involved in the pathogenesis and progression of hypertension. However, the reviews about the role of extracellular vesicles on hypertension are relatively limited and need to be updated, thus we highlight and discuss the recent studies on the pathophysiological role of extracellular vesicles in the progression of hypertension, with emphasis on the artery and kidney. These may advance our understanding of the pathophysiology of hypertension, find the suitable biomarkers, which could be used to detect the early onset of hypertension before the development of target organ damage, and develop potential therapeutic method by studying role of extracellular vesicles in hypertension.

Abstract

Hypertension affects approximately 1.13 billion adults worldwide and is the leading global risk factor for cardiovascular, cerebrovascular, and kidney diseases. There is emerging evidence that extracellular vesicles participate in the development and progression of hypertension. Extracellular vesicles are membrane-enclosed structures released from nearly all types of eukaryotic cells. During their formation, extracellular vesicles incorporate various parent cell components, including proteins, lipids, and nucleic acids that can be transferred to recipient cells. Extracellular vesicles mediate cell-to-cell communication in a variety of physiological and pathophysiological processes. Therefore, studying the role of circulating and urinary extracellular vesicles in hypertension has the potential to identify novel noninvasive biomarkers and therapeutic targets of different hypertension phenotypes. This review discusses the classification and biogenesis of three EV subcategories (exosomes, microvesicles, and apoptotic bodies) and provides a summary of recent discoveries in the potential impact of extracellular vesicles on hypertension with a specific focus on their role in the blood pressure regulation by organs—artery and kidney, as well as renin-angiotensin-system.

Keywords: Hypertension, extracellular vesicles, endothelium, vascular smooth muscle cells, sodium transporter, RAS

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Introduction

Hypertension, a silent disease, affects approximately 1.13 billion adults worldwide in 2015 and continues to increase.[1,2] According to the American Heart Association, nearly half of U.S. adults have hypertension that is uncontrolled in 45.6%. This scenario is perhaps worse in developing and third world countries. Uncontrolled hypertension is the leading global risk factor for cardiovascular, cerebrovascular, and kidney diseases.[3–5] For those patients with truly drug-resistant hypertension, nonpharmacological therapies with excellent tolerability profiles may be needed.[6,7] Extracellular vesicles (EVs), one of small particles containing various molecules that mediate cell-to-cell communications, have the potential to be such new therapy. There is emerging evidence that hypertension is associated with increased release of EVs

and/or different cargoes sorted into EVs in the urine and blood.[8–13] Given this fact, EVs may serve as biomarkers for the diagnosis and potential targets for therapeutic intervention in hypertension. In this review, we briefly introduce EVs classification, recipient cell interactions, and biological functions, then discuss the evolving understanding of the role of EVs in hypertension and summarize the current knowledge of EV-mediated regulatory mechanisms. These may advance our understanding of the pathophysiology of hypertension and provide novel insights into the field of translational medicine.

EVs classification

According to the International Society for Extracellular Vesicles, EV is "the generic term for particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate, i.e. do not contain a functional nucleus".[14] These small membrane-enclosed structures are shed from nearly all types of eukaryotic cells and carry information from their parent cells, including membrane receptors, soluble proteins, metabolites, nucleic acids, and lipids. According to their sizes and site of origins, EVs can be divided into three subcategories: exosomes, microvesicles, and apoptotic bodies.[10,15]

Exosome

Exosomes are the smallest EVs with diameters from 40 to 160 nm.[16,17] The formation of exosomes is multi-step- and multi-mechanism-involved process, including endosomal sorting complex-required transport (ESCRT)-dependent and ESCRT-independent pathways. ESCRT-dependent pathway is the most common pathway regulated by ESCRT-0, -I, -II, and -III. Exosomes are formed when membrane proteins are endocytosed by inward budding of the cell membrane and transferred to early endosomes. Then, the ESCRT-0 complex recruits ubiquitinated proteins and ESCRT-I, -II, -III to invaginate early endosome to form intraluminal vesicles. During the invagination, cytosolic proteins, mRNAs and miRNAs, DNA fragments, and metabolites are incorporated into the intraluminal vesicles. Finally, ESCRT-III induces the fission of intraluminal vesicles, resulting in the formation of multivesicular bodies. When multivesicular bodies fuse with the plasma membrane, intraluminal vesicles are released into the extracellular space and are then referred to as exosomes. Exosome release appears to be controlled by RAB GTPase in the process of vesicular trafficking, endosome recycling, and vesicular plasma membrane fusion.[18] If the multivesicular bodies fuse with lysosomes instead of the plasma membrane, then multivesicular bodies undergo degradation (Figure 1).

Microvesicles

Unlike exosomes, which are released following the exocytosis of multivesicular bodies, microvesicles, with diameters from 100 nm to 1 μm,[16,17] assemble at and are released from the plasma membrane. Cellular stress, which results in an increase in cytosolic calcium, induces specific membrane changes and loosens the cytoskeleton, which leads to the outward protrusion of the plasma membrane and the formation of microvesicles. Lipidic proteins (myristoylated, palmitoylated) in the lumen may play a role by promoting

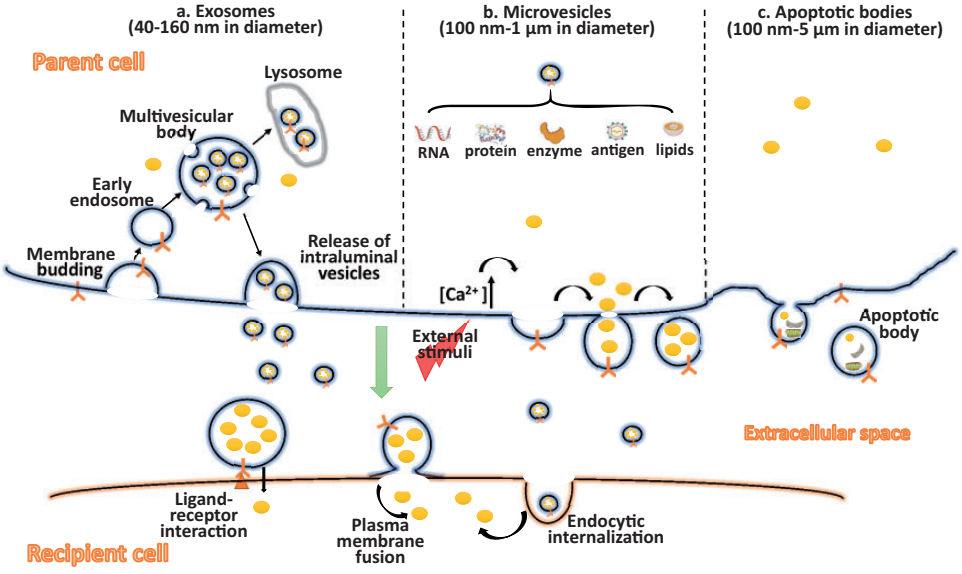

Figure 1. EV biogenesis and interaction with recipient cells. (a) Exosome formation starts with inward budding of the plasma membrane and transfer of early endosomes. During early endosome invagination, cytosolic proteins, RNAs, enzyme, antigen, lipids, and other components are incorporated into the intraluminal vesicles. When multivesicular bodies fuse with the lysosome, the protein contents get degraded. When multivesicular bodies fuse with the plasma membrane, exosomes are released. (b) Microvesicles: external stress, e.g. oxidative stress, hypoxia, increases the cytosolic concentration of calcium to induce specific membrane changes and loosen the cytoskeleton, which leads to outward protrusion of the plasma membrane and the formation of microvesicles. (c) Apoptotic bodies: These are released during the late stage of programmed cell death. They contain several intracellular fragments and damaged cellular organelles, as well as other molecules similar to those inside microvesicles. EVs mediate cell-to-cell communication through different mechanisms: (1) ligand-receptor interaction causes the accumulation of EV contents, e.g. inflammatory cytokines, angiogenic factors, growth factors, and extracellular matrix proteins. (2) EV-plasma membrane fusion causes the release of EV content into the recipient cell cytoplasm. 3) Receptor cell internalization causes the endocytosis of EVs to deliver their contents. EVs: extracellular vesicles.

membrane curvature[19]; some ESCRT subunits, e.g. I/II/III, participate in the assembly and budding of microvesicles.[15,20] Microvesicle release is a scission step similar to the final stage of cytokinesis. The activation of acidic sphingomyelinase, leading to ceramide generation, is associated with the plasma membrane release. Plasma membrane protein aggregation is another important factor for microvesicle release. Overexpression leading to higher-order oligomerization in plasma membrane accelerates HIV Gag budding/exosomal sorting and is sufficient to increase protein targeting to microvesicles.[21]

Apoptotic bodies

Apoptotic bodies (100 nm–5 μm) are released during the late stage of programmed cell death that is controlled by caspase-mediated cleavage, and subsequent activation of Rho-associated protein kinases.[15,17,22] They contain several intracellular fragments and damaged cellular organelles, as well as other molecules similar to those inside microvesicles. Apoptotic bodies are smaller than cells and may provide an easier system for phagocytosis. However, the role of apoptotic bodies in intercellular communication is currently unclear. Therefore, the effect of apoptotic bodies in hypertension is not covered here and the term EVs in this review relate to exosomes and microvesicles.

After the release of exosomes and microvesicles into the extracellular space, they bind and fuse similarly to their recipient cells (Figure 1). In general, EVs communicate with recipient cells via three different types of interactions: (1) classical ligand-receptor interaction; (2) EV-plasma membrane fusion; and (3) EV endocytic internalization. EVs selectively bind to specific cell surface receptors located on the plasma membrane of cells.[23] Such ligand-receptor interactions likely account for many EV-secreted contents, including EV-carried inflammatory cytokines, angiogenic factors, growth factors, and extracellular matrix proteins that activate immediate responses. EVs can directly fuse with the plasma membrane of cells fusion and release the contents into the cell.[24] Exosomes produced by renal proximal tubule cells can be taken up by cells distal to the proximal tubule, such as the distal convoluted and collecting duct cells.[25] The receptor cell internalization of EVs by endocytic mechanisms, includes clathrin-/caveola-mediated endocytosis, micropinocytosis, and phagocytosis. The latter two interactions enable the delivery of RNAs or cytoplasmic proteins from EVs to recipient cells[26–28] (Figure 1).

Preparation and characteristics of EVs

EVs can be isolated based on their physicochemical properties including buoyant density, shape, size, charge, and surface composition. Therefore, multiple approaches have been developed to isolate and purify EVs, such as ultracentrifugation, sucrose density gradient isolation, size-exclusion chromatography, antibody-based affinity capture, ultrafiltration, and polymer-based precipitation.[10,14] More and more investigators realized that each approach has their own advantages and limitations; it is regrettable that there is no technical standard available until now. Researchers choose the appropriate isolation methods mainly based on the type of samples and the forthcoming application of the purified EVs. Generally, if EVs are used as a source of diagnostic biomarker, getting maximal EVs is the major concern. In case of EVs used as therapeutic vehicles, their structure integrity and purification should be the first-priority determinants.

Isolation method

Ultracentrifugation

Among these approaches, differential centrifugation is the most widely accepted method. The sequential centrifugations are initiated with low centrifugal force (g) to remove cells and debris (<1500*g*), and subsequent higher centrifugal force to remove aggregates of biopolymers and apoptotic bodies (<100,000*g*), and then ultracentrifuge force to pellet large EVs (10,000–20,000*g*), and small EVs (100,000–200,000*g*).[29,30] However, contaminants with similar buoyant density, such as fragments of apoptotic cells, lipoproteins, protein aggregates, or proteins, are always presented to prevent the complete purification of EVs and disturb its function.[31–33] For example, during the isolation of urinary EVs, contamination of uromodulin is very common. Uromodulin is the most abundant protein in the urine, which could form into polymers to shroud EVs, and influence EV purification and its function, such as the communication to the recipient cells.[34] In order to remove the unwanted uromodulin, adding the reducing agent dithiothreitol or the zwitterionic detergent CHAPS to disrupt the polymeric uromodulin is a commonly used method.[34]

Filtration

According to the varied EV size, different filtration methods are developed in the past years, including ultrafiltration, hydrostatic dialysis, and size-exclusion chromatography. Currently, commercial kits with pores of various diameters are available for EVs extraction process. EVs could be purified by filtration alone or combination of filtration with other methods. As an additional step of ultracentrifugation, it can increase the purity of EVs and save the isolation time. Size-exclusion chromatography uses a gravity flow for separation, which maintains best the vesicle structure, integrity, and biological activity of EVs.[35]

The combination of ultracentrifugation and filtration might be an ideal way to isolate and purify EVs. First, both methods offer chemical-free handling, which eliminate potential interference due to chemicals. Second, only pipetting and gravity/gravitational force application preserve the EVs' structure and bioactivity function better. Furthermore, taking the advantage of the ability to deal with large volume, highly diluted samples, e.g. urine, can be centrifugated then concentrated by filtration to a specified EV size. However, adding steps to EV preparation means a loss of EVs and increased preparation time, optimizing each step is necessary in order to obtain the best result.

Other preparation methods

Other EVs isolation methods such as utilizing the EV solubility, aggregation, or affinity are also under intensive investigations. It should be noted that a particular method may influence the activity of EVs. By comparing the impact of different methods on EV preparations, Gámez-Valero *et al.* found that polyethylene glycol and PRotein Organic Solvent Precipitation-based EV isolation reduced recipient cell viability *in vitro,*[36] probably due to acetone interferes with the functional properties of vesicular membranes. Therefore, the technique using the organic solvent needs further validation.

Characterization

EVs carry different cell type-specific marker proteins from their parent cells, which are used to identify the origin of EVs after isolation of EVs.[37] For example, endothelial cell-derived EVs are identified by the presence of CD105, CD144, and CD62e proteins. CD3, CD4, and CD8 are used to identify the lymphocyte-derived EVs. When an enough marker protein is exposed, the cellular origin of EV can be determined by using antibodies directed against such cell-type specific surface antigens. Nevertheless, the assessment of EV purification after isolation is technically complicated because of their heterogeneity and various sizes, as well as the difficulty to distinguish the impurities due to nucleic acids, proteins, and lipid contamination. Nanoparticle tracking analysis and nano-flow cytometry are newly developed methods to characterize the properties of EV size distribution, particle concentration, purity, and phenotype, which might be helpful to determine the purity of isolated EVs.[38,39]

Biological functions of EVs

Increasing evidence supports the notion that EVs participate in a wide range of biological processes via cell-to-cell communications, which delineates the most critical function of EVs. As stated earlier, EVs can transfer many cellular components from the parent cell to recipient cell, and therefore are able to change the composition and function of the recipient cells. Several databases, such as EVpedia (www.evpedia.info), Vesiclepedia (www.microvesicles.org), and ExoCarta (www.exocarta.org), have online resources that index EV data, including proteomics and transcriptomics. The known cargos of EVs participate in several physiological processes, such as waste management, immune regulation, and cellular homeostasis modulation.

Waste management

EVs were first observed to be released from activated platelets in 1967 and thought to be inert cellular debris and named as "platelet-dust".[40] Since then waste management has become an essential biological function of EVs. EVs can carry redundant intracellular components, thus acting as cellular waste disposal bags by releasing them from the cell. The inhibition of EV secretion results in the accumulation of nuclear DNA fragments in the cytoplasm, which leads to apoptotic cell death in human cells.[41] EVs containing cellular waste are especially equipped to facilitate their clearance. For example, apoptotic bodies are easy targets for phagocytosis because of the externalized phosphatidylserine and/or their cargos act as chemotactic signal.[22,42,43] The spleen may play a role in accelerating the clearance of EVs. The activity of breast carcinoma- or pancreatic cancer-derived microparticles was detected 5 min after their intravenous injection into mice, but the activity became undetected 2 h after injection. The clearance of the microparticles from circulation is delayed in splenectomized mice.[43] However, the underlying mechanisms by which the spleen participates in the clearance of EVs have not been determined.

Immune regulation

The role of EVs in immune regulation is under intense investigation. EVs can impair or enhance immunity and inflammation through their interchange among multiple types of cells. EVs shed from sites of intestinal inflammation in patients with inflammatory bowel disease have increased mRNA and protein levels of anti-inflammatory IL-10, pro- or anti-inflammatory IL-6, and pro-inflammatory IL-8, and TNF-α.[44–46] These EVs increase the translation of IL-8 in recipient colonic epithelial cells and induce the migration of macrophages.[44] Synovial fluid of rheumatoid arthritis patients contains strong pro-inflammatory and coagulant leukocyte-derived EVs, which trigger autologous fibroblast-like synoviocytes to produce and secrete inflammatory mediators, such as monocyte chemoattractant protein-1, IL-8, IL-6, RANTES, ICAM-1, and VEGF.[47] Therefore, EVs can influence the release of chemokines and cytokines and modulate synovial and intestinal inflammation. EVs from TNF-α-induced inflammation of endothelial cells have pro-inflammatory proteins, such as ICAM-1, CCL-2, IL-6, IL-8, CXCL-10, CCL-5, and TNF-α, which mediate inflammation and promote the adhesion and migration of monocytes.[48] EVs can also directly modulate various immune cells. Tumor-derived EVs inhibit natural killer cell function by decreasing the activity of CD107a, NKG2D, TNF-α, and INF-γ, and impairing glucose uptake.[49] Platelet-derived EVs can also activate monocytes via the RANTES pathway inducing monocyte migration and recruitment to sites of inflammation.[50] It should be emphasized that EVs modulate immune responses not only by stimulating the pro-inflammatory cytokines but also triggering the release of anti-inflammatory mediators.[48] Human neutrophil-derived EVs have no pro-inflammatory activity on human macrophages but increase the release of transforming growth factor beta 1 (TGF-β1), suggesting that EVs down-modulate macrophage activation and can behave as anti-inflammation effectors.[51] However, TGF-β1 can be pro-inflammatory by promoting the secretion of pro-inflammatory cytokines such as IL-17.[52] By contrast, the anti-inflammatory interleukin, IL-10, contained in the cargo of EVs derived from adipose tissue-derived autologous mesenchymal stem cells was found to localize and

protect renal tubule cells from a porcine model of metabolic syndrome and renal artery stenosis.[53]

Cellular homeostasis modulation

One aspect of cellular homeostasis is regulated by the balance of EV-associated cell proliferation, apoptosis, and autophagy. EVs from the serum of healthy human volunteers increase the proliferation of H9C2 cardiomyocytes by up-regulating miR-17-3p which inhibits TIMP3 expression.[54] TIMP3, aka, tissue inhibitor of metalloproteinases 3, belongs to the TIMP family, which inhibits matrix metalloproteinases. Metalloproteinases promote cell proliferation, as a response to acute kidney injury, for example.[55] Mesenchymal stem cell (MSC)-derived EVs enhance the survival of cisplatin-induced acute kidney injury in a mouse model by increasing the expression of anti-apoptotic genes, such as Bcl-xL, Bcl2, and BIRC8, and downregulating the expression of pro-apoptotic genes, such as Casp1, Casp8, and LTA.[56] Autophagy induced by EVs also plays a role in cell survival. MSC-derived EVs increase the expression of the autophagy marker LC3 and beclin-1 but decrease the expression of mTOR and fibrotic marker expression in renal tissue, mechanisms that are involved in the improvement renal function and histology of streptozotocin-induced diabetic nephropathy in rats.[57] However, the ability of EVs to promote cell survival is not beneficial in cancer because the release of EVs may support tumor cell survival by reducing the chemotherapeutic drug concentration within the tumor cell. Experiments show that after treatment of cultured cancer cell lines with chemotherapeutic agents such as cisplatin and doxorubicin, the cells release EVs which contain the drugs.[58,59] The shedding of the EVs is a mechanism for getting rid of the drugs, resulting in drug resistance.

EVs have many other physiological functions which cannot be fully covered in this minireview. In the following sections, we highlight and discuss the recent studies on the pathophysiological role of EVs in the progression of hypertension, with emphasis on the artery and kidney.

EVs and hypertension

Recent clinical studies revealed that circulating and urinary EVs are associated with increased blood pressure, suggesting that EVs may be biomarkers and also involved in the pathogenesis and progression of hypertension.[13,60–64] Circulating EVs are derived from the endothelium, platelets, and immune cells, whereas urinary EVs are derived from the kidney and urinary tract. Patient with severe hypertension and even hypertensive patients with well-controlled blood pressure have increased circulating endothelial and platelet microparticles.[13,61] Urinary endothelial microparticles are increased in essential and renovascular hypertensive patients, relative to normotensive controls.[64] By contrast, endothelial microparticles in renal vein and systemic levels were not different between subjects with essential or renovascular hypertension and normotensive subjects.[64] Three months after treatment of renovascular hypertension with or without stenting, the urinary levels of peritubular capillary endothelial microparticles correlated inversely with renal function. The authors suggested that urinary capillary endothelial microparticles may reflect renal microcirculation injury and serve as biomarkers of intrarenal capillary loss.[64]

In 2018, Otani *et al.* reported that plasma exosomes can modulate systemic blood pressure in a rat model of hypertension.[65] They showed that the intraperitoneal injection of plasma exosomes from spontaneously hypertensive rats (SHRs) increased systolic blood pressure of normotensive Wistar-Kyoto (WKY) rats. By contrast, WKY-derived plasma exosomes decreased the blood pressure of SHRs. Abnormal increase of endothelial EVs in SHRs is associated with endothelial dysfunction and arterial stiffness.[66] Good *et al.* observed that circulating EVs from WKY rats reduced vasodilation of isolated WKY mesenteric arteries but had no effect on SHR mesenteric arteries. However, EVs from hypertensive SHRs failed to reduce vasodilation from both WKY and SHRs.[67] These data support the idea that a blood pressure regulating effect of EVs changes after the development of hypertension.

Mechanisms of EV-mediated regulation of blood pressure

EVs and artery

Increased peripheral vascular resistance, a key feature of hypertension, is due to vasoconstriction, impaired vasodilation, and vascular remodeling.[68–71] Arteries are classified into: (1) large, elastic, conducting arteries; (2) medium-sized, muscular, distributing arteries; and (3) arterioles. All arteries have three distinct layers: an innermost single layer of endothelial cells; layers of vascular smooth muscle cells (VSMCs); and an outermost layer of connective tissue, primarily comprised of collagen and elastin fibers. Endothelium-dependent vessel dilation and vascular remodeling play important roles in the pathogenesis and maintenance of hypertension.[68,71]

EVs and endothelium. Endothelial dysfunction, mainly caused by impaired production of endothelial nitric oxide (NO) and increased production of superoxide, is associated with the development and progression of arterial hypertension.[71,72] Hypertensive individuals have high levels of endothelial EVs, and endothelial EV levels correlate positively with systolic blood pressure, arterial diameter, and pulse wave velocity and inversely with wall shear stress and microvascular dysfunction.[13,61,62,73,74] The arterial vascular endothelium is one of the primary targets of circulating EVs, specifically platelet- and leukocyte-derived EVs. EVs can impair NO release, trigger endothelial inflammation, and alter endothelial cell survival and angiogenesis, to influence arteriolar reactivity.[75] EVs from platelets and leukocytes can affect both proinflammatory and anti-inflammatory processes in endothelial cells. *In vitro*, leukocyte or platelet microparticles release cytokines IL-1β, IL-6, and IL-8, and increase the expression of ICAM-1, VCAM-1, and E-selectin. These factors activate endothelial cell adhesion molecules with/without ERK1/2- and

NF-κB-dependent pathways, promoting inflammatory responses in endothelial cells.[76,77] However, neutrophil microparticles also contain anti-inflammatory proteins, such as annexin-1, and may attenuate vascular contraction.[78] Microparticles from human monocytes cause hypertension and endothelial dysfunction in rats by impairing angiotensin 1–7-mediated vasodilation in mesenteric arteries, which is aggravated by EVs from lipopolysaccharide-treated monocytes. These effects are associated with reduced endothelial NO phosphorylation and Mas receptor expression, via dysregulation of monocyte miRNA-27a.[79] Thus, the ability of EVs to increase endothelial inflammation synergizes with the decreased production of NO and endothelial cell proliferation, increasing blood pressure.

EVs and VSMCs. Numerous studies have documented increased thickness of smooth muscle layers and media-to-lumen ratio of arteries in hypertension,[68,80,81] which may be the result of maladaptive alterations in VSMCs and the components of the adventitia.[82,83] EVs are involved in the thickening of vascular smooth muscle layers and altering of components of the adventitia by different mechanisms that may be related to their different cellular origins.[84–89] For example, platelet-derived EVs exert a strong immunomodulatory activity by increasing monocyte adhesion to VSMCs and interacting with increased CD40- and P-selectin, inducing a switch towards a pro-inflammatory phenotype, stimulating VSMC proliferation and migration.[89] Tong *et al.* showed that arterial adventitial fibroblast-derived exosome from SHRs promoted VSMC migration by transferring angiotensin-converting enzyme (ACE) to VSMCs. ACE knockdown in adventitial fibroblasts reduced ACE contents and activity in its exosome and inhibited the migration of VSMCs.[90] Recently, Ren *et al.* found that miR155-5p is involved in the function of aortic adventitial fibroblast-derived EVs. EVs from SHRs, relative to those from WKY rats, had decreased miR155-5p but increased ACE contents. Aortic adventitial fibroblast-derived EVs were able to transfer miR155-5p and ACE at the same time. WKY-EVs or miR155-5p attenuated while SHR-EVs promoted VSMC proliferation, vascular remodeling, and hypertension in both SHRs and WKY rats.[84]

EVs and kidney

The kidney plays a very important role in the regulation of blood pressure. Besides the role of renal macrovessels and microvessels (afferent and efferent arterioles, vasa recta), renal tubules maintain fluid and electrolyte balance to keep the blood pressure in the normal range.[68,83,91,92] After a first discovery of EVs in urine in 2004,[93] the interest in urinary EVs in the pathogenesis and diagnosis of hypertension has grown exponentially. In focal segmental glomerulosclerosis and diabetes patients, urinary exosomal Wilms' tumor-1 was significantly increased.[94,95] In animal models with podocyte damage (streptozotocin-treated mice, OVE26, and Akita mice), urinary podocyte extracellular vesicles were significantly increased and correlated with the severity of the glomerular injury. The increased biomarkers result from an increased amount of urinary extracellular vesicles and/or increased contents in each vesicle.[94,96]

Urinary EVs are secreted by various cells of the urinary tract. These EVs contain cell-specific marker proteins from every segment of the nephron. For example, sodium/hydrogen exchanger type 3 (NHE3) and aquaporin-1 from the proximal tubule, sodium potassium chloride cotransporter (NKCC) from the thick ascending limb, sodium chloride cotransporter (NCC) from the distal convoluted tubule, aquaporin-2 from the collecting duct, podocalyxin, podoplanin, and WT-1 from podocytes were all detected in the urinary EVs.[9,97] Na^+-K^+/ATPase is not expressed in intestinal epithelial exosomes.[98] However, Na^+-K^+/ATPase β1 subunit has been reported in urine from healthy human volunteers.[93,99] Extracellular vesicles can be filtered in functional nephrons and are found in the urine in healthy subjects due to their small sizes. Since extracellular vesicles circulating in a dynamic process and the lack of effective technology to monitor EV fate, it is difficult to determine to what extent EVs undergo glomerular filtration. Other urinary exosome proteins found in healthy human volunteers can be found in the ESBL urinary exosome protein database (https://hpcwebapps.cit.nih.gov/ESBL/Database/Exosome/).

EVs and sodium transporters. Urinary EVs have been analyzed in various hypertensive disorders and mostly focused on NCC, the sodium chloride cotransporter in the distal convoluted tubule. Urinary total and phosphorylated NCC is increased in EVs of patients with hypertension due to pseudo hypoaldosteronism type II (Gordon's syndrome) and post-kidney transplant patients taking tacrolimus.[100–102] Over-activated NCC leads to the increased renal tubular reabsorption of sodium chloride and subsequently hypertension. Studies have determined whether the abundance of sodium transporters in urinary EVs correlates with sodium reabsorption under physiological conditions. For example, Zachar *et al.* reported that in healthy humans, total and phosphorylated NCC are present in urinary EVs but not affected by sodium intake.[103] Another study also found that the excretions of NCC and NKCC2 in urinary EVs are not associated with the increase in blood pressure in a small number ($n = 6$) of salt-sensitive humans.[104] In rats (strain not given), urinary excretion of exosomal NKCC2 and NCC correlated with their renal abundance. The urinary EVs in women with pre-eclampsia contain NKCC2 with increased phosphorylation at the activating S130 site, and NCC with decreased phosphorylation at the activating T60 site.[105] NKCC2 in urinary exosomes has also been reported to be increased in patients with renal dysfunction and American cutaneous leishmaniasis.[106]

Epithelial sodium channel (ENaC) is responsible for the reabsorption of sodium through the apical membrane of the connecting tubule and the collecting duct. Patients with diabetic nephropathy and hypertension have increased proteolytically cleaved γ-ENaC in their urinary EVs.[107] In the aforementioned women with pre-eclampsia, their urinary EVs have decreased phosphorylation of the

activating T60 site in NCC.[105] Dietary sodium restriction in hypertensive patients or acute aldosterone infusion in healthy humans similarly significantly increased urinary EVs with γENaC, whereas NCC and α-ENaC concentrations were unchanged. Thus, γENaC concentration in EVs may be a useful biomarker of ENaC activation.[108] Urinary exosomal miRNA has also been reported to be correlated with an individual's response to sodium intake.[109] Several of these miRNAs, i.e. hsa-miR-4516, are involved in signaling pathways that regulate renal sodium transporters, such as NCC and ENaC.[110,111]

NHE3 is the major apical sodium exchange in the renal proximal tubule. In Caucasian males and post-menopausal women with type 2 diabetes mellitus, their urinary phosphorylated NHE3 in EVs were higher with glucagon-like peptide receptor agonist, lixisenatide, than insulin glulisine treatment.[112] Patients with American cutaneous leishmaniasis, mentioned above, also have increased excretion of urinary exosomes with NHE3.[106]

EVs and RAS. Abnormalities in sodium and water handling caused by the renin-angiotensin system (RAS) is a common pathophysiology of hypertension.[113,114] The RAS is now classified into the classical pathway and the non-classical or counter-regulatory pathway.[115,116] The classical pathway starts with angiotensinogen, its conversion to angiotensin I by renin, and conversion of angiotensin I to angiotensin II by angiotensin-converting enzyme. This pathway causes vasoconstriction and stimulation of renal sodium transport. In the non-classical or counter-regulatory pathway, angiotensin-converting enzyme 2 converts angiotensin II from the classical pathway to angiotensin 1–7 or alamandine from angiotensin A and in general, opposes the effects of the classical pathway. There are three angiotensin II receptors: AT_1R, AT_2R, and AT_4R, and two angiotensin 1–7 receptors, MrgD and Mas. AT_1R is a vasoconstrictor, while AT_2R, AT_4R, MrgD, and Mas are vasodilators. Components of the RAS can be transferred by EVs and affect the activity in the recipient cells. As mentioned earlier, ACE contents, but not angiotensin II and AT_1R, in adventitial fibroblast EVs are much higher in SHRs than in WKY rats.[84,90] The increased ACE in EVs from SHRs increased angiotensin II levels, activated AT_1R, and promoted VSMC migration. Biomechanical stress, like osmotic stretch or cardiac pressure overload, induces secretion of AT_1R-enriched exosomes.[117] AT_1R-enriched exosomes traffic to cardiac and skeletal myocytes, and resistance vessels in AT_1R knock-out mice to restore Ang II-induced blood pressure response. Other components of the RAS, such as angiotensin 1–7 and Mas receptor, have also been studied. For example, *in vivo* tail injection of EVs from monocytes to rats impairs angiotensin 1–7-mediated vasodilation in mesenteric arteries, accompanied by decreased Mas receptor expression and eNOS phosphorylation in the endothelium.[79] The RAS per se functions as potent stimuli to increase the formation of EVs. Burger *et al.* found that angiotensin II increased microparticle formation in endothelial cells *in vivo* and in *vitro*, accompanied by increased NADPH oxidase-derived ROS generation and Rho kinase activity.[118] Endothelial microparticles are enriched in lipid rafts/caveolae, which themselves contribute to generation of new microparticles. These studies indicate that EVs carry the information from RAS into the recipient cells to mediate the known actions of RAS. In turn, the action of RAS increases the formation of EVs. EVs can increase VSMC proliferation, renal sodium reabsorption, vascular remodeling, and finally results in the development of hypertension (Figure 2).

Prognostic and therapeutic potential of EVs in hypertension

The prognostic and therapeutic potential of EVs have gained interest because of their special characteristics. First, as biomarkers for prognosis, EVs can be obtained by noninvasive (urinary EVs) or minimally invasive (circulating EVs) methods and inform the clinician about the possible cause of the hypertension (EVs carry components from their parent cells). EVs are suggested to be surrogate biomarkers for endothelial dysfunction, vascular damage, and increased activity of renal sodium transporters/exchangers in hypertension.[9,13,61,62] Moreover, EVs can be used as biomarkers of improved vascular endothelial function. Hypertensive patients on hemodialysis treated with aliskiren, a direct renin inhibitor, for 12 weeks show improved levels of platelet-derived EVs and flow-mediated dilation.[119] Subsequent studies aim to analyze the whole spectrum of EVs, including the functional biomolecules within EVs. Proteomic analysis of circulating EVs in albuminuric hypertensive patients showed that two proteins, kalirin and chromodomain-helicase-DNA-binding protein 7, increased with RAS suppression.[120] Because expression of these proteins in circulating EVs positively correlates with the endothelial activation marker E-selectin, it is used to monitor the vascular condition of these patients. EV RNA cargo in hypertension is another area of intensive studies. miRNAs are enriched in the urinary EVs of essential hypertensive subjects.[109] Kidney nephron-derived exosome miRNAs may be associated with renal sodium transport, and therefore regulation of blood pressure. Low miR-146a expression in EVs is associated with the presence of albuminuria and high miR-27a in EVs may cause hypertension.[79,121] Urinary exosome miRNA can be linked to salt sensitivity or inverse salt sensitivity; the latter is a state in which blood pressure is increased by a low salt diet.[109]

EVs have advantages as therapeutic agents. EV-based therapies have a potential to bypass many hurdles of cell-based therapies because EVs have low immunogenicity.[122] EVs are relatively easy to isolate and when frozen are stable over a long period of time, making them available as off-the-shelf products. EVs are able to deliver multiple bioactive molecules that may or may not act synergistically, which allows the tailoring of the method of delivery to optimize their therapeutic effects.[123] There are extensive studies reporting the use of EVs in cardiovascular diseases.[16,124] In addition, the inhibition of exosome secretion not only has some physiological consequences,[41] but also

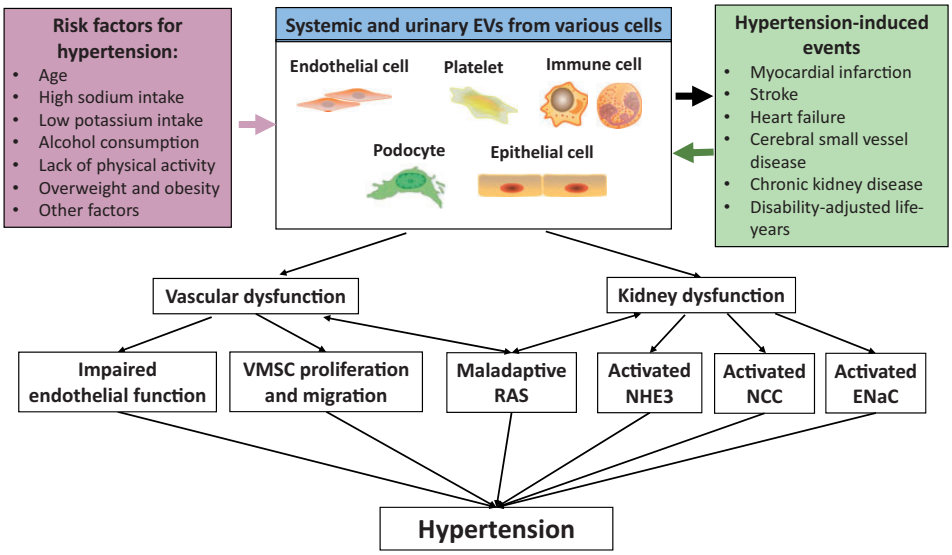

Figure 2. Role of EVs in the development and progression of hypertension. Risk factors for hypertension-induced events cause the release of systemic and urinary EVs from various cells. EV activation contributes to the pathogenesis of hypertension and hypertension-induced events, such as vascular and kidney dysfunction, including impaired endothelial function, VSMC proliferation and migration, altered NHE3, NCC, ENaC activity, and maladaptive of RAS. These processes collectively contribute to the development and progression of hypertension. EVs: extracellular vesicles; VSMCs: vascular smooth muscle cells; NHE3: sodium/hydrogen exchanger type 3; NCC: sodium chloride cotransporter; ENaC: epithelial sodium channel; RAS: renin-angiotensin-system.

may have some effects to certain diseases. For example, in cancers, tumor cells secrete vast amounts of immune inhibitory exosomes that hinder anti-cancer immune responses. Removal circulating tumor EVs is proposed to inhibit disease progression.[125] However, to the best of our knowledge, there is no report that tries to block exosome production in any model of hypertension, which needs to be studied in the future. It should be noted that the therapeutic potential of EVs in hypertension is still in its early phase. An *ex vivo* study demonstrated plasma-poor circulating EVs from WKY and SHRs can differentially modulate vasoreactivity of isolated mesenteric vessels.[67] There is one animal study that demonstrated the potential of plasma EVs to regulate systemic blood pressure with beneficial effect on end-organ damage in hypertension.[65] Future studies will shed more light on EV-mediated therapeutics on blood pressure regulation.

Conclusion and prospects

Hypertension is a strong and independent predictor of risk and future incidence of cardiovascular, cerebrovascular, and kidney diseases.[3–5] In resistant hypertension, an increasing number of patients are unable to achieve adequate control of their blood pressure despite taking more than three antihypertensive drugs.[7] As discussed in this review, EVs play an important role in the development and progression of various hypertensive disorders. The underlying mechanisms include EV-mediated vascular dysfunction, renal sodium and water transporters abnormalities, and RAS disorder. Distinct EVs cause different blood pressure outcomes, which depend upon their origin and stage of disease. Studies are needed to uncover the role of EVs in pathogenesis of hypertension and find EV components that regulate blood pressure, prevent or treat hypertension, and to find out suitable biomarkers, which could be used to detect the early onset of hypertension before the development of target organ damage.

AUTHORS' CONTRIBUTIONS

ZZL: prepared figures and drafted manuscript; PJ, JY, and CZ: manuscript edition and review.

DECLARATION OF CONFLICTING INTERESTS

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