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Microvesicles: ROS scavengers and ROS producers

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ABSTRACT

This review analyzes the relationship between microvesicles and reactive oxygen species (ROS). This relationship is bidirectional; on the one hand, the number and content of microvesicles produced by the cells are affected by oxidative stress conditions; on the other hand, microvesicles can directly and/or indirectly modify the ROS content in the extra- as well as the intracellular compartments. In this regard, microvesicles contain a pro-oxidant or antioxidant machinery that may produce or scavenge ROS: direct effect. This mechanism is especially suitable for eliminating ROS in the extracellular compartment. Endothelial microvesicles, in particular, contain a specific and well-developed antioxidant machinery. On the other hand, the molecules included in microvesicles can modify (activate or inhibit) ROS metabolism in their target cells: indirect effect. This can be achieved by the incorporation into the cells of ROS metabolic enzymes included in the microvesicles, or by the regulation of signaling pathways involved in ROS metabolism. Proteins, as well as miRNAs, are involved in this last effect.

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Microvesicles (MVs)

Extracellular vesicles are membranous subcellular structures produced by the cells; they are located in the extracellular space and are especially abundant in blood, urine, milk, saliva, semen, synovial or cerebrospinal fluid, among other liquids. According to their origin, size and biochemical composition, they are usually classified into three categories: exosomes, microvesicles (MVs) (also microparticles or ectosomes) and apoptotic bodies. The exosomes are 40–120 nm vesicles included in multi-vesicular bodies which are released to the extracellular space after fusion of these multi-vesicular bodies with the plasma membrane. MVs arise through budding and fission of the plasma membrane and are larger (50–1000 nm) than the exosomes [1]. Both types of vesicles are produced by all cells and have different functions [2]; however, it is not always easy to distinguish them [3,4]. Apoptotic bodies are the largest extracellular vesicles (1–5 um) and are formed during the late stages of apoptosis [1].

MVs represent an extraordinarily heterogeneous population of extracellular vesicles [5]. Not only are they heterogeneous in size (ranging from 0.1 to 1.5 μm) but also in their origin, biochemical content and, obviously, function. Blood MVs, in particular, are a paradigm of this heterogeneity; they can be secreted by erythrocytes, leucocytes, platelets or endothelial cells, each one with a different content and function [6]. Obtaining samples containing homogeneous populations of MVs is one of the main methodological challenges for the future.

The main function ascribed to MVs is a role in intercellular communication; however, many specific functions have also been associated with them depending on their cellular origin. Cell adhesion and migration [7,8], waste management [9], vascular function [10], coagulation [11], reticulocyte maturation [12], modulation of the immune response [13], fertilization [14], embryonic development [15], bone calcification [16] and tissue repair [17,18] are activities where MVs, as well as exosomes, have been involved. Moreover, an increase of MV production has been associated with pathological states [19,20], tumor growth, metastasis and angiogenesis [21,22]. They have also been postulated to be biomarkers and/or therapeutic targets [23], especially those derived from mesenchymal stem cells [24]. In fact, a significant therapeutic effect of mesenchymal stem cell-derived extracellular vesicles is the reduction of oxidative stress [25].

Oxidative stress and reactive oxygen species (ROS)

Under normal conditions, ROS production and ROS elimination are balanced; however, oxidative stress

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represents an imbalance due to an increase of ROS [26]. Thus, ROS production and ROS detoxification must be continuously adapted in order to respond to changes and alterations that occur during the cell´s lifespan. The ROS increase has been associated with stress conditions and has been shown to be the causal agent of different pathologies such as neurodegenerative disorders (Alzheimer´s disease, Parkinson´s disease and amyotrophic lateral sclerosis) [27,28], cardiovascular diseases [29,30] and carcinogenesis [31]. In general, ROS production increases with ageing [32], although it has also been proposed to be an adaptive response [33] that may be physiologically modified by age, sex (menopause), tumour growth or stress conditions.

ROS are a group of molecules and free radicals (atoms or molecules with an unpaired valence electron) derived from oxygen. They can be of exogenous (xenobiotics, radiation, pollutants …) or endogenous origin. The latter is a result of the cell's own metabolism: mitochondrial respiration (especially ubiquinone and complex III of the electron transport chain) [34,35], oxidoreductase activities, metal-catalyzed oxidation and NADPH oxidase activity, especially in the respiratory burst of phagocytes [36].

ROS have numerous toxic and harmful effects due to their high reactivity. They are involved in different pathologies and, hence, cells have developed a complete antioxidant machinery for ROS scavenging (Figure 1). This antioxidant system includes enzymatic components, such as superoxide dismutases, catalase, peroxidases, reductases, and non-enzymatic components, such as vitamins C, E and A, glutathione, peroxiredoxins, thioredoxins, etc. It also includes enzymatic activities such as heme-oxygenase or catechol-O-methyltransferase directed at preventing ROS formation. Because the primary source of endogenous ROS is the intracellular compartment (mitochondria, peroxisomes and reticulum), cells have a stronger antioxidant machinery than the extracellular compartment.

ROS have two faces: good and bad. The first involves low ROS levels whereas the second involves high ROS levels [37]. It is well known that ROS are associated with a high number of different pathologies (previous paragraph) because they cause damage to biomolecules (lipids, proteins and DNA) and subcellular structures; however, ROS are also involved in maintaining physiological conditions [38,39]. Reactive nitrogen species (RNS) such as nitric oxide and nitrogen dioxide, among others, can also cause oxidative damage and act as components of intracellular signaling cascades [40]. In fact, the possibility that MVs act as scavengers of nitric oxide has also been proposed [41]. Nonetheless, the relationship between MVs and RNS is not considered in this review.

Under physiological conditions, ROS are involved in cell signaling, regulating a wide variety of functions [39]. These include activation of gene transcription

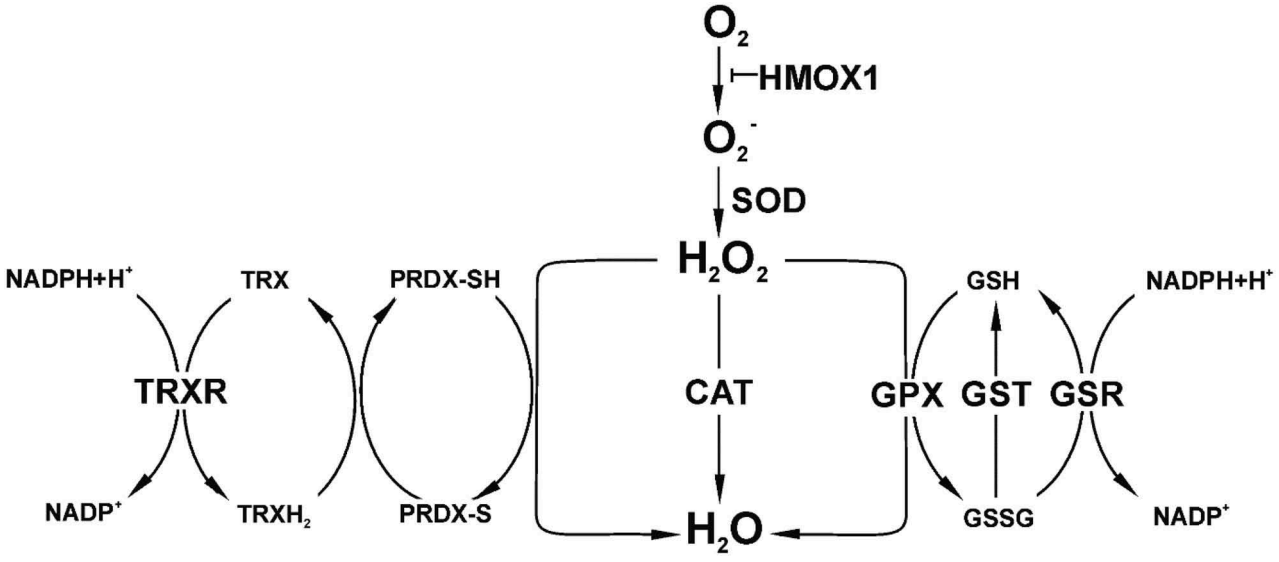

Figure 1. Diagram showing the two main ROS (superoxide radical, O_2^-, and hydrogen peroxide, H_2O_2) and the more essential enzymes and peptides of the antioxidant machinery. CAT: catalase; GPX: glutathione peroxidase; GSH and GSSH: reduced and oxidized glutathione; GSR: glutathione reductase; GST: glutathione S-transferase (detoxify xenobiotics); HMOX: heme oxygenase; PRDX: peroxiredoxin; SOD: superoxide dismutase; TRX: thioredoxin; TRXR: thioredoxin reductase. Other important ROS not included in the diagram are: hydroxyl (OH^-), peroxyl (RO_2^-), alkoxyl (RO^-) and hydroperoxyl (HO_2^-) radicals, as well as ozone (O_3) and singlet oxygen ($^1O_2$) as non-radical ROS. Note that the fuel that nourishes the antioxidant machinery is NADPH.

(directly activating transcription factors, or indirectly activating MAPK cascades) [42,43], regulation of intracellular signaling pathways [44], modulation of calcium signaling [45], apoptosis [46,47], autophagy [48,49], cellular growth [50], and embryonic development as a consequence of their role in proliferation, differentiation, and apoptosis [51]. ROS are also involved in the destruction of pathogens [9] and in inflammatory processes [52,53], blood pressure control [54] and response to physical exercise [55].

ROS are involved in a high number of essential physiological processes, but while low ROS levels are related with physiological conditions, high ROS levels and the subsequent oxidative stress are generally associated with pathological conditions [56]. Therefore, ROS levels have to be finely regulated in the intracellular as well as the extracellular compartment. The biochemical, physiological and structural differences of both compartments make it impossible for them to have identical or similar mechanisms for regulating these levels. The differential distribution and expression of SOD subtypes in these two compartments is a good example of this. SOD1 is located in the cytosol, SOD2 in mitochondria and SOD3 in the extracellular compartment. To maintain proper ROS levels, the activity of these enzymes has to be finely regulated, as can be demonstrated by the fact that a single amino acid substitution in human SOD1 is associated with familial amyotrophic lateral sclerosis whereas the knockout of the SOD2 gene is related to lethal cardiomyopathy in mice [57]. The loss of SOD3 expression has also been associated with a pancreatic ductal adenocarcinoma [58]. This differential system of ROS elimination among intra- and extracellular compartments raises a special interest in analyzing the relationship between extracellular vesicles and the adjustment of ROS levels in the extracellular space.

Microvesicles and ROS

MVs and ROS are closely interrelated, not only because MVs can produce or detoxify ROS, but also because ROS are involved in the production of MVs. Pro-oxidant conditions seem to induce extracellular vesicle release [59]; in fact, NADPH oxidase and nitric oxide synthase-2 (NOS-2) inhibitors inhibit the production of MVs in neutrophils [60]. It is worthy to point out that tumoural [61] and senescent cells [62] also produce a higher number of MVs, and both types have altered redox balances with elevated ROS levels [63,64]). MVs can also serve as an alternative mechanism to remove oxidized proteins after oxidative stress, enabling their use as biomarkers for oxidative stress [65].

The effect of MVs on ROS depends on both the conditions of the cell that originates these vesicles and those of the target cell, as well as the environmental conditions. In fact, oxidative conditions affect the content of MVs [65]; for example, MVs of ischemic muscle [66] or those produced after a high-fat diet [67] produce more ROS than controls. In addition, endothelial-derived MVs obtained after starvation or apoptotic stress have different effects on endothelial cells after hypoxic stress: the former show beneficial effects whereas the latter exhibit detrimental effects [68]. In endothelial cells, specifically human umbilical vein endothelial cells (HUVEC), high glucose conditions have been shown to induce a three-fold increase in MV production, with differences in the molecular composition of these vesicles [69], whereas neutrophils produce diverse MVs in response to different activators [70]. Moreover, direct and indirect effects can be carried out by the same MVs: T-lymphocyte-derived MVs exert a potentially beneficial effect on HUVEC, acting both as ROS scavengers (they carry SOD2 and catalase) and inducing the expression of SOD-1 in these cells [71].

As far as we know, the first evidence that MVs could be involved in ROS metabolism was the location of Cu, Zn superoxide dismutase (SOD2), a cytosolic enzyme that destroys ROS, in MVs from neuroblastoma [72]. On the other hand, tumor-derived MVs and lymphocyte-derived MVs induced the production of ROS in human monocytes [73] and endothelial cells [74]. These early studies already pointed out the different roles and mechanistic procedures that MVs can maintain with ROS: MVs can directly scavenge or produce ROS [41] but they can also act on ROS indirectly, modifying the ROS content of their target cells (Figure 2).

MVs can carry different antioxidant enzymes involved in ROS scavenging: GPX, GST, PRDX, SOD2 or CAT [71,75–78]. Our group recently found a complete list of antioxidant activities and related molecules in MVs derived from HUVEC by proteomic analysis [79]. The fuel that nourishes the antioxidant machinery is NADPH. We also demonstrated that HUVEC-derived MVs contain the enzymatic machinery necessary to synthesize NADPH using blood metabolites to feed different biosynthetic pathways [80]. This last possibility seems to convert the HUVEC-derived MVs into an autonomous extracellular organelle devoted to scavenging ROS from blood and maintaining the redox status in plasma. A protective role of epididymosomes (a type of MV

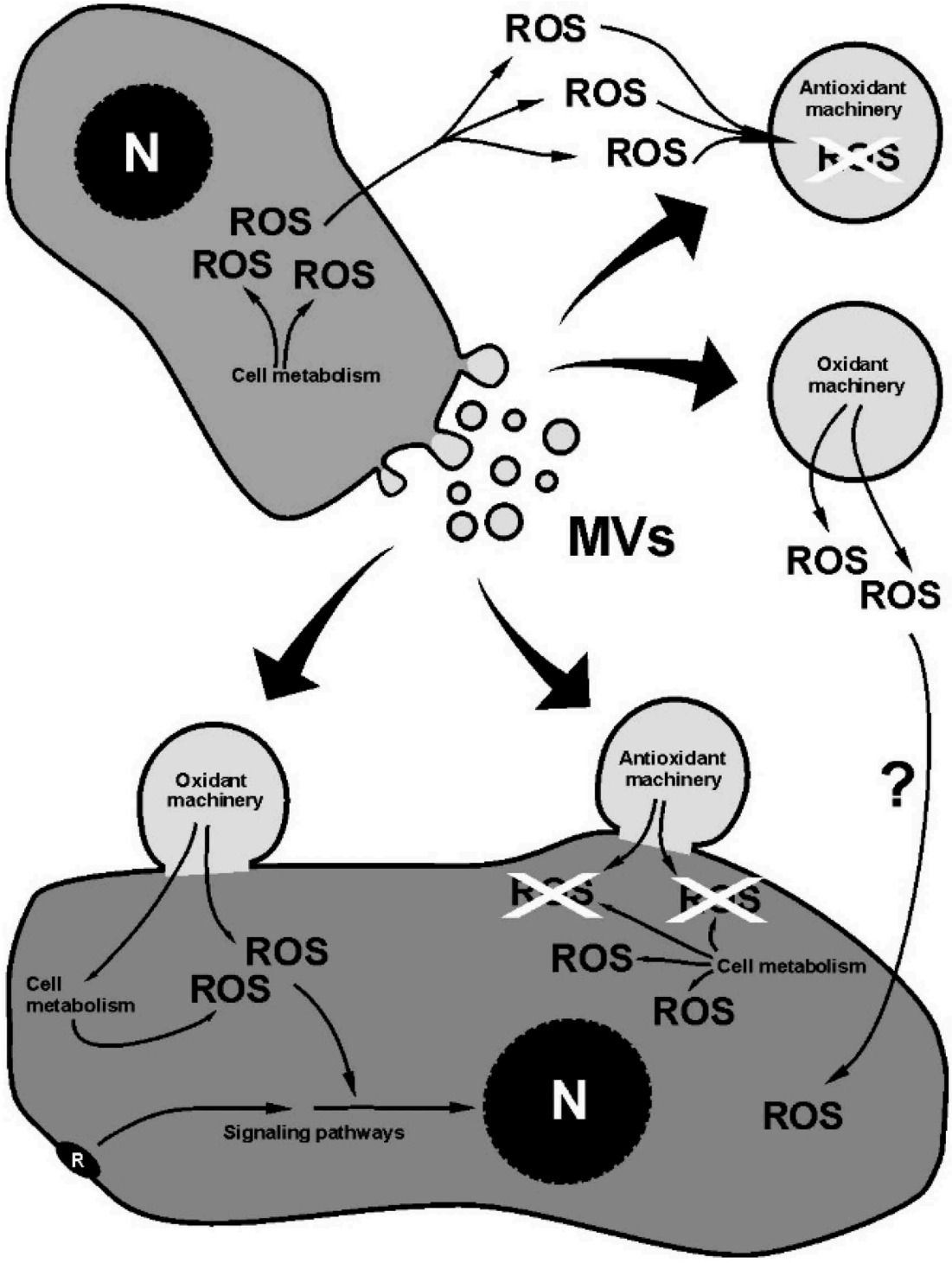

Figure 2. Schematic drawing showing the main effects of MVs on ROS. At the top, the direct effect of MVs; at the bottom, the indirect (cell-mediated) effect. The effect of ROS- regulated signaling pathways on the ROS content of the cell is not included.

originated from epididymal cells) for epididymal spermatozoa against ROS released by dying cells has also been suggested [81]. On the other hand, the existence of NADPH oxidase, an enzyme that synthesizes ROS, has also been demonstrated in MVs [66,82]. Taking all this into account, it is possible to assume that a group of MVs can act as ROS scavengers in the extracellular compartment, and others as ROS producers when their content is incorporated into the target cells. As far as we know, the possibility that MVs can directly increase ROS in the extracellular compartment has not been

demonstrated, although their ability to produce ROS has been assessed [82].

Although the direct effect of MVs on ROS has been analyzed in the previous paragraph, MVs, under physiological conditions, can also have an indirect effect, inducing changes in their target cells that can result in a compensatory mechanism against the effects of oxidative stress or causing detrimental effects [65]. This indirect effect can be achieved by (a) the incorporation to the cells of the enzymatic components included in MVs involved in ROS production/destruction, or (b) the incorporation of signaling molecules that can modify cellular activities and/or gene expression involved in the regulation of redox processes, which finally affect the cellular ROS content. The induction of antioxidant enzyme expression [71,83] and the ROS increase [74,82,84] have been demonstrated. The precise molecular mechanisms activated by the MVs in the target cells are not well known. However, NF-κB, JNK or PI3K/Akt-dependent signaling pathways are usually involved in ROS production in the target cells [68,74,84] whereas suppression of NOX expression [85] or inhibition of NF-κB [86] are molecular mechanisms used to decrease ROS. A common response to oxidative stress involves two transcription factors, Nrf2 and MAFG, as well as the activation of target genes via antioxidant response elements (AREs) [87]. As far as we know, however, these transcription factors have not been found in MVs, although they can be included in exosomes (see last paragraph). MicroRNAs (miRNAs) are molecules involved in the control of oxidative stress [37,88–91] and MVs represent transport vehicles for these [92]. In particular, miR-126, as well as miR-21 [93,94], miR-128 [95], miR-144 [94], miR-34a-5p [96], miR-1915-3p [97] and miR-638 [98], are all involved in oxidative stress and have been found in MVs.

Similar results have been reported for MVs derived from cells subjected to stress or under pathological conditions. MVs derived from ischemic muscles (56), plasma of patients with lupus [99] or neutrophils infected with *Mycobacterium tuberculosis* [70] were shown to induce ROS in mononuclear cells, neutrophils and macrophages, respectively. However, MVs derived from cells exposed to ROS induced ROS tolerance in PC12 cells [100]. MVs derived from tumour cells showed a similar behavior; they can contain antioxidant enzymes [72] or can modulate the activity of human monocytes by increasing ROS, among other effects [73].

Although MVs produced after a hypoxia/reoxygenation treatment contain ROS and may promote apoptosis and oxidative stress in the myocardium [101], the use of MVs as therapeutic tools has been demonstrated. Treatments with MVs reduced oxidative stress in injured kidneys [85,102] and in experimental colitis [86]. In addition, it has been recently demonstrated that MVs derived from mesenchymal cells downregulate oxidative stress in osteoarthritic chondrocytes [103] and that those secreted by genistein (a polyphenol)-treated cells have a protective effect against oxidative stress [104]. On the contrary, in cultured glomerular endothelial cells, platelet microparticles have been shown to induce ROS production and may contribute to glomerular endothelial injury associated with diabetic nephropathy [104].

A question of interest is whether MVs having an antagonistic relationship with ROS (scavenging or production) may (1) coexist in the same place and (2) be produced by the same cell. Plasma MVs can be a paradigm to respond to these questions. We have demonstrated that cultured endothelial cells synthesize MVs that act as ROS scavengers [79,80], but it has also been suggested that MVs produce ROS as part of the signaling processes in endothelial cells [82]. This raises the possibility that MVs involved in ROS scavenging and ROS production may be found simultaneously in plasma, the first ones acting as autonomous structures independent of target cells and the second ones acting on target cells; obviously, both types of MVs should have specific mechanisms of cargo.

The underlying molecular mechanisms of aging appear to be related to increased free radical release [105]; senescence has been equally associated with an increase of oxidative stress. Two possible mechanisms may lead to this oxidative status: a malfunctioning of the antioxidant machinery or an increase of oxidative processes by metabolic alterations of the cells. As previously stated (section 3, first paragraph), senescent cells produce more MVs probably due to their high ROS levels. Senescent endothelial cell-derived MVs exhibit an inductive effect of ROS on endothelial cells [62,106]. However, we have recently demonstrated that senescent HUVEC-derived MVs have a functional and more developed antioxidant machinery, suggesting that the increase of the antioxidant machinery is not able to compensate the higher production of ROS in senescence [79]. In this context, a recent study has demonstrated that the culture time induces changes in microRNAs related to genes involved in ROS production [107]. On the other hand, MVs could modify the senescence status, a condition with high oxidative stress. In this regard, MVs of interleukin-1β-stimulated mesenchymal stem cells have been demonstrated to downregulate β-galactosidase activity, a marker of senescence [108].

In spite of their possible separate origin and functions, MVs and exosomes can have common functional mechanisms; for example, exosomes have also

been involved in ROS metabolism [109]. However, the actual multivesicular body origin of the small EVs is not clearly demonstrated, and the functions described may be contained in exosomes and/or in co-isolated small MVs [3]. Thus, although this review is mainly directed at studying the interrelationship between MVs and ROS, this final paragraph is dedicated to the relationship between exosomes (and co-isolated small MVs) and ROS. Enzymes such as NADPH oxidase, involved in ROS production, have been found in platelet-derived exosomes [110]. In addition, exosomes carry cytochrome P450 [111], a protein family involved in ROS generation [112]. In eosinophils, an increase in ROS production by eosinophil-derived exosomes has been demonstrated in patients with asthma [113]. Production of ROS in axonal regeneration induced by macrophage-derived exosomes containing NADPH oxidase has also been recently demonstrated [114]; in this case, the ROS generated by NADPH oxidase served as an activator of the Akt-dependent signaling pathway involved in regeneration. In the scientific literature, however, it is easier to find studies pointing towards an antioxidant role of exosomes, although this antioxidant role can be carried out by different mechanisms. Cells treated with exosomes derived from stem cells ameliorate their oxidative stress [115] or reduce ROS production [116]. Several studies have demonstrated a protective function of exosomes against oxidative stress [117,118] as well as an increase of antioxidants in exosomes derived from cells treated with ROS, and the subsequent induction of antioxidant mechanisms in cells treated with these exosomes [119,120]. The presence of Nrf2 mRNA, a transcription factor involved in the anti-oxidant stress response, and miRNAs involved in the oxidative stress response has also been demonstrated in exosomes from granulose cells subjected to hydrogen peroxide [121]. In addition, exosomes can diminish cellular oxidative stress by secreting harmful molecules that can promote ROS elevation and induce senescence, as has been demonstrated in the case of nuclear DNA accumulation in the cytoplasm [122]. The interrelationship of ROS and exosomes is phylogenetically preserved; the existence of antioxidant molecules (SOD2, TRX, TRXR and catalase) has also been demonstrated in exosomes from the yeast, *Cryptococcus neoformans* [123].

Conclusions

MVs, or at least a group of them, maintain a close interrelationship with ROS and, subsequently, with oxidative stress. This interrelationship can be described as either direct or indirect. MVs, by themselves (direct effect), can act as ROS scavengers, reducing oxidative stress, since they carry antioxidant enzymes and molecules that form part of the cellular antioxidant machinery as well as enzymes involved in ROS production. After interacting with their target cells (indirect effect or horizontal transfer), MVs can also increase or reduce ROS levels by transferring enzymatic components or signaling molecules that can modify cell metabolism and/or gene expression involved in the regulation of redox processes. This direct and indirect capacity of ROS production or scavenging by the MVs can be affected by the physiological conditions of the cell that produces the MVs. Stress or pathological conditions, as well as aging or senescence, also modify the effect of MVs on ROS metabolism. Since oxidative stress is involved in different pathological conditions and aging, the use of MVs with antioxidant activity could be a useful strategy to prevent the deleterious effects of ROS. The adaptive response consisting of modifying the number and content of MVs related with ROS metabolism under these circumstances supports this possibility. Finally, the direct and indirect capacity of ROS production or scavenging implies the existence of different types of MVs. The mechanisms that regulate the synthesis of each type, their mechanisms of cargo and the mechanism that guides them to fuse with their target cells or to stay in the extracellular space are all interesting subjects for future explorations.

Disclosure statement

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