BMB
Reports

BMB Rep. 2014; 47(10): 531-539
www.bmbreports.org

Invited Mini Review

Extracellular vesicles as emerging intercellular communicasomes

Yae Jin Yoon[1,2], *Oh Youn Kim*[1] *& Yong Song Gho*[1,*]

[1]Department of Life Sciences, Pohang University of Science and Technology, [2]Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang 790-784, Korea

All living cells release extracellular vesicles having pleiotropic functions in intercellular communication. Mammalian extracellular vesicles, also known as exosomes and microvesicles, are spherical bilayered proteolipids composed of various bioactive molecules, including RNAs, DNAs, proteins, and lipids. Extracellular vesicles directly and indirectly control a diverse range of biological processes by transferring membrane proteins, signaling molecules, mRNAs, and miRNAs, and activating receptors of recipient cells. The active interaction of extracellular vesicles with other cells regulates various physiological and pathological conditions, including cancer, infectious diseases, and neurodegenerative disorders. Recent developments in high-throughput proteomics, transcriptomics, and lipidomics tools have provided ample data on the common and specific components of various types of extracellular vesicles. These studies may contribute to the understanding of the molecular mechanism involved in vesicular cargo sorting and the biogenesis of extracellular vesicles, and, further, to the identification of disease-specific biomarkers. This review focuses on the components, functions, and therapeutic and diagnostic potential of extracellular vesicles under various pathophysiological conditions. [BMB Reports 2014; 47(10): 531-539]

INTRODUCTION

Intercommunication among cells is crucial in all living organisms. Such cell-to-cell communication is achieved through direct interactions or via secretion of soluble factors (1, 2). Recently, extracellular vesicles (EVs) have gained attention as mediators of cellular communications. The release of EVs is an evolutionarily conserved process, from archaea, Gram-negative and Gram-positive bacteria, to eukaryotic cells (3, 4). EVs are lipid bilayer-enclosed vesicles, 30-2,000 nm in diameter (1-4). These EVs harbor various proteins, lipids, and nucleic acids, influencing neighboring and distant cells (1-4). EVs are classified based on their biogenesis or cellular origin and biological function (5, 6). Specifically, EVs are categorized into ectosomes (neutrophils or monocytes), microparticles (platelets or endothelial cells), tolerosomes (serum of antigen-fed mice), prostatosomes (seminal fluid), cardiosomes (cardiomyocytes), and vexosomes (adeno-associated virus vectors) (5, 7). On the basis of biogenesis, EVs are further categorized into exosomes and microvesicles (1, 2) (Fig. 1). However, exosomes and microvesicles, having similar properties, are difficult to separate despite various efforts to characterize and isolate by density, size, morphology, and protein and lipid composition (6, 8).

Exosomes are budded out from the fusion of plasma membranes with the multivesicular bodies (MVBs), the large endosomal structures with multiple vesicles in the cytosol (6). The released exosomes are homogenous vesicles, of around 40-100 nm in diameter, with a density of 1.13-1.19 g/ml (9). Exosomes contain endosomal proteins such as tetraspanins (CD9, CD63), Alix, and TSG101, which are used as exosomal markers (9, 10). Unlike exosomes, microvesicles, also known as ectosomes, shedding vesicles, microparticles, and plasma membrane-derived vesicles, originate from the plasma membrane by outward budding and fission (11). Microvesicles are about 50-1,000 nm in diameter, but their density is undefined (7, 11). The major differences between exosomes and microvesicles arise from differences in their composition. Microvesicles are generated by budding from the plasma membrane and thus resemble the plasma membrane composition of the parent cell. On the other hand, exosomes generated by inward budding and fission with MVBs, are composed of various cytosolic proteins related to endolysosomal pathways. Although exosomes and microvesicles contain specific markers, these markers are not sufficiently specific to distinguish among them.

COMPONENTS OF EXTRACELLULAR VESICLES

Proteins

Extensive investigations of extracellular vesicular proteins have been carried out using mass spectrometry (MS)-based proteomic analyses, Western blotting, and immune-electron microscopy (12, 13). MS-based proteomic studies of EVs have provided a high-throughput vesicular proteome dataset in various

*Corresponding author. Tel: +82-54-279-2345, Fax: +82-54-279-8609; E-mail: ysgho@postech.ac.kr

http://dx.doi.org/10.5483/BMBRep.2014.47.10.164

Received 18 July 2014

Keywords: Cancer, Component, Diagnosis, Exosome, Function, Microvesicle, Therapy, Tumor

ISSN: 1976-670X (electronic edition)

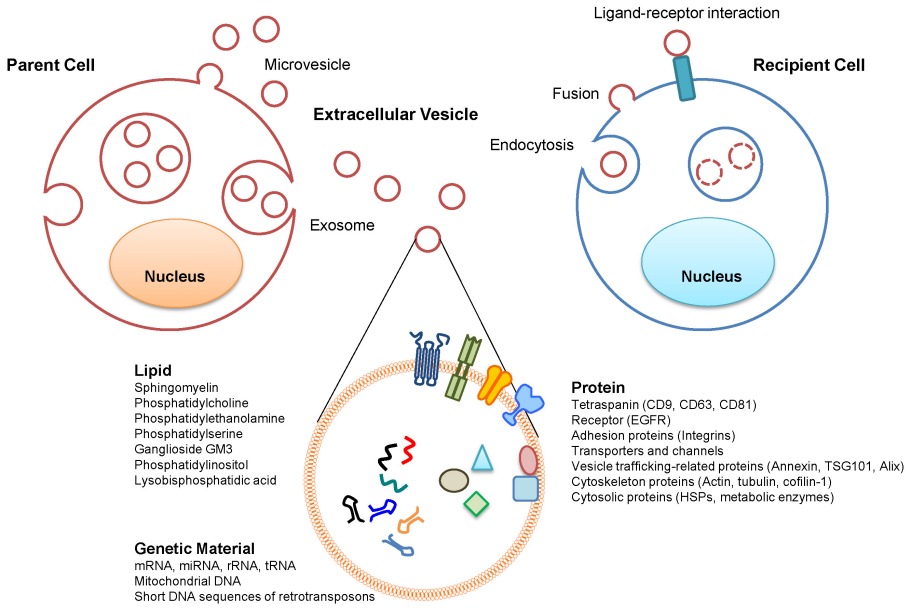

Fig. 1. Intercellular communication via extracellular vesicles. EVs are lipid-bilayered vesicles of 30-2,000 nm in diameter. Mammalian EVs are classified into exosomes and microvesicles, based on their biogenesis. Exosomes and microvesicles are generated by the fusion of multivesicular bodies with the plasma membrane and budding from the plasma membrane, respectively. EVs are intercellular communicasomes, harboring diverse bioactive materials, including RNAs, DNAs, proteins, and lipids. EVs regulate a diverse range of pathophysiological functions by activating receptors or transferring membrane proteins, signaling molecules, mRNAs, and miRNAs. These EVs can interact with recipient cells by ligand-receptor interactions, fusion, and internalization via receptor-mediated endocytosis or macropinocytosis.

cell types and body fluids (13). Proteomic studies on EVs of various origins suggest a controlled protein-sorting mechanism, rather than the random packaging of EV proteins, because EVs from different cell types contain common vesicular proteins. These common vesicular proteins are mainly involved in vesicle structure, biogenesis, and trafficking: tetraspanins (CD9, CD63, and CD81), integrins, heat shock proteins (Hsp60, Hsp70, and Hsp90), the endosomal sorting complexes required for the transport complex (TSG101 and Alix), annexins, cytoskeleton proteins (actins, cofilin-1, ezrin/radixin/moesin, profilin-1, and tubulins), metabolic enzymes, and ribosomal proteins (2) (Fig. 1). The proteins located in the plasma membrane and cytoplasm are more commonly sorted into EVs compared with proteins in the nucleus and mitochondria (13, 14). Several studies have proposed mechanisms for vesicular protein sorting (2, 14). Vesicular proteins are sorted by the endosomal-sorting complexes or by protein and lipid interactions or by the internalization of cytosolic proteins (2). A recent report has shown the co-sorting of cytoplasmic proteins with vesicular cargo proteins via protein-protein interactions in colorectal cancer cells (14). EVs also contain cell type-specific proteins. For example, melanoma-derived EVs contain the tumor-associated antigen, MART1, while epithelial cell-derived EVs contain epithelial cell adhesion molecule, EpCAM (15, 16). Additionally, vesicular EGFRVIII, detected in the plasma of glioblastoma patients, induces the activation of transforming signaling pathways and thus increases the anchorage-independent growth capacity (17). Moreover, docetaxel-resistant prostate cancer cells were found to release MDR-1, the drug transporter, via EVs and transfer drug resistance to non-resistant prostate cancer cells (18).

Lipids

Extracellular vesicular lipids are known to play important roles in the rigidity, stability, function, and intracellular fusion and budding processes of EVs. Membrane lipids (sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, ganglioside GM3, and phosphatidylinositol), prostaglandins (E_2, F_2, J_2, and D_2), and lysobisphosphatidic acid are lipid components of EVs (19-22). Although the specific ratios of these lipids in EVs vary according to the originating cell, generally, EVs are enriched in sphingomyelin, cholesterol, GM3, and phosphatidylserine (21) (Fig. 1). Sphingomyelin and cholesterol allow the tight packing of lipid bilayers and increase overall rigidity and stability (23, 24). GM3 also increases the stability of EVs and prevents the recognition of EVs by blood components and uptake by the reticuloendothelial system (25). Moreover, conical-shaped phosphatidylserine helps to assemble the curved vesicular shape of EVs and facilitates the fusion and fission of the EVs (26). Sphingomyelin and prostaglandins also have functional roles other than maintaining the structure of EVs. Sphingomyelin has a proangiogenic

character that can promote endothelial cell migration, tube formation, and neovascularization (19). Moreover, EV-bound prostaglandins were found to activate signaling pathways in rat basophil leukemia cells (22). Furthermore, lipids modulating intracellular fusion and budding process, such as the lysobisphosphatidic acid are involved in EV biogenesis (21).

Nucleic acids

EVs found in various cells and body fluids, such as plasma, saliva, and breast milk, contain mRNAs and miRNAs (2) (Fig. 1). Using microarray- and 'next-generation' sequencing-based systematic approaches, significant quantities of vesicular mRNA and miRNA have been reported. Horizontal transfer of vesicular mRNAs and miRNAs can lead to epigenetic reprogramming of recipient cells. Also, differences in the profiling of vesicular mRNAs and miRNAs in diseased conditions may potentially be useful as diagnostic tools (27, 28). Furthermore, in addition to mRNA and miRNA, EVs also contain rRNA, tRNA, mitochondrial DNA, and short DNA sequences of retrotransposons (29-31) (Fig. 1). Extracellular miRNA exist in various forms: enclosed in EVs or high density lipoprotein particles, or associated with Ago2 proteins (32). Because body fluids contain mixtures of RNases, miRNA should be enclosed in some vesicular structure to escape RNase degradation. Recent studies have shown the presence of miRNA after treatment with RNases to the solution, suggesting the miRNA does exists within EVs (27, 33). Although the sorting mechanism of miRNA into EVs remains unknown, several reports have shown that certain miRNAs are enriched in EVs, compared with the originating cells (27, 31). Extracellular vesicular miRNAs downregulate target mRNAs in recipient cells (31). Additionally, vesicular miRNAs can directly activate signaling molecules and receptors of the target cells. For example, exosomal miR-21 and miR-29a activate TLR7 and TLR8 receptors in immune cells, leading to tumor growth and metastasis (34, 35).

FUNCTIONS OF EXTRACELLULAR VESICLES

EVs regulate a diverse range of biological processes by transferring membrane proteins, signaling molecules, mRNAs, and

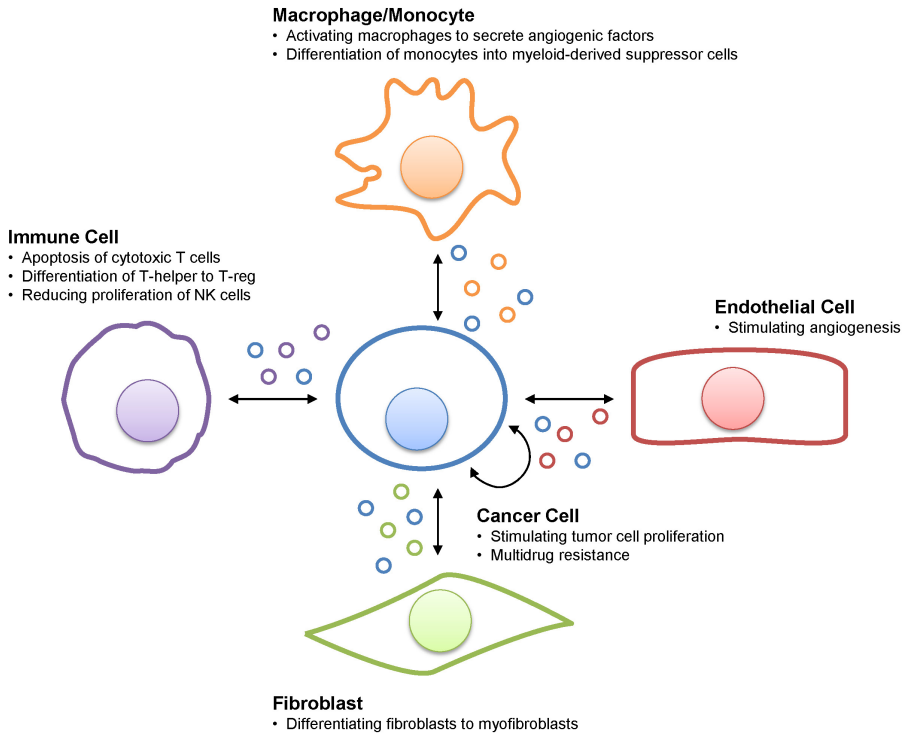

Fig. 2. Multiple functions of extracellular vesicles in the tumor microenvironment. The tumor microenvironment consists of a diverse range of cells including cancer cells, endothelial cells, fibroblasts, macrophages/monocytes, and immune cells. This heterogeneous population of cells secretes EVs into the tumor microenvironment. These EVs make an environment favorable for tumor progression. Cancer cell-derived EVs promote angiogenesis by modulating endothelial cell proliferation, migration, and invasion directly. Also, these EVs stimulate angiogenesis by activating macrophages to secrete proangiogenic factors and by promoting the induction of fibroblast differentiation into myofibroblasts. Moreover, cancer cell-derived EVs suppress immune responses by promoting the differentiation of monocytes into myeloid-derived suppressor cells and by inducing apoptosis of cytotoxic T lymphocytes.

miRNAs, and activating receptors of recipient cells (6, 36) (Fig. 1). The detailed mechanism(s) as to how these EVs interact with target cells remain(s) unknown. Some studies have suggested that EVs can fuse with the plasma membrane of recipient cells and be internalized by the receptor-mediated endocytosis or macropinocytosis (37-39). Local and long-distance interactions of EVs with target cells control normal physiology and disease pathogenesis (40, 41).

EVs exert pleiotropic effects in the maintenance of normal physiology, including tissue repair, stem cell maintenance, and blood coagulation (8, 41). EVs harboring membrane-bound morphogens, such as Wnt and Dll4, stimulate counterpart receptors and induce signal transduction involved in embryonic development and carcinogenesis (42, 43). Morphogen-associated EVs directly activate cell surface receptors, rather than the forming a morphogen gradient in the tissue (44). EVs also have been implicated in stem cell maintenance and cell plasticity. Various studies have reported a pivotal role of stem cell-derived EVs in tissue regeneration after injury and in modulating cellular phenotypes (45-47). EVs derived from embryonic stem cells modulate the pluripotency and the undifferentiated phenotype of stem cells (45). EVs derived from endothelial progenitor cells also activate quiescent endothelial cells, switching on angiogenic processes by transferring mRNAs (46).

In addition to the role of EVs during physiological processes, EVs participate in diverse pathological conditions, including cancer, infectious diseases, autoimmunity, and neurodegeneration (40, 41). In the brain, neurons, oligodendroglial cells, and microglia release EVs, which modulate various neurobiological functions (48-50). In addition to the synaptic neurotransmission, EVs containing neurotransmitter receptors participate in the synaptic plasticity by enhancing glutamatergic activity (49). Also, these EVs can regulate myelin formation, neurite outgrowth, and neuronal survival (51, 52). In the pathogenesis of neuronal disease, EVs are associated with several pathogenic proteins, such as prions, β-amyloid peptide, superoxide dismutase, and α-synuclein (53-55).

EVs can also enhance or suppress autoimmunity and inflammation. In the synovial fluid of rheumatoid arthritis patients, EVs activate autologous fibroblast-like synoviocytes to release proinflammatory mediators and promote monocyte recruitment to the inflammatory sites (56, 57). EVs from tumor cells induce immune response by transferring tumor antigens to dendritic cells (58). In contrast, tumor cell-derived EVs suppress immune responses by inducing apoptosis of activated cytotoxic T lymphocytes or promoting the differentiation of regulatory T lymphocytes (59). In addition to regulating the immune responses, tumor-derived EVs play diverse roles in tumor progression, including growth, invasion, metastasis, and angiogenesis (8) (Fig. 2). This review is focused on the roles of EVs in tumor progression.

Effects of cancer cell-derived EVs on endothelial cells

Several studies have reported the activation of *in vitro* and *in vivo* angiogenesis by EVs derived from cancer cells (19, 60) (Fig. 2). For example, a vesicular lipid component, sphingomyelin, triggers endothelial cell migration, tube formation, and neovascularization (19). Also, cell cycle-related mRNAs belonging to the M-phase are enriched in the transcripts of cancer cell-derived EVs and promote endothelial cell proliferation after internalization (60). In addition to the mRNA, proteins are also transferred to recipient cells. For example, activated EGFR in EVs is transferred to EGFR-negative endothelial cells and, in turn, triggers endothelial cell activation via the MAPK and AKT signal transduction pathways (61). EVs from glioblastomas also contain angiogenic proteins, such as angiogenin, FGF1, IL-6, IL-8, TIMP-1, TIMP-2, and VEGF (28, 62).

Effects of cancer cell-derived EVs on macrophages

Macrophages play an important role in vessel formation during wound repair, inflammation, and tumor growth (63). A significant reduction in neovascularization and tumor growth was observed in monocyte-depleted mice and in nude mice (64, 65). Also, macrophages isolated from tumors have angiogenic activities in endothelial cells *in vitro* and *in vivo* (66). In tumors, potential stimulators of macrophages are low oxygen tensions, wound-like concentrations of lactate, pyruvate, or hydrogen ions, or cytokines such as IFN-γ, granulocyte macrophage colony-stimulating factor, platelet-activating factor, or monocyte chemotactic protein (63). Macrophages activated by such tumor stimuli release secretory products and are able to promote all phases of the angiogenic process: destruction of the local extracellular matrix, endothelial cell migration, proliferation, and differentiation (67). Macrophage-derived factors include proteases, growth factors, and monokines, such as collagenases, tissue plasminogen activator, urokinase-type plasminogen activator, FGF2, VEGF, IL-8, IL-6, and TNF-α (63). These factors promote angiogenesis, but act in an indirect manner through attracting or activating other angiogenic cells. In tumors, endothelial cells are activated and produce endothelial adhesion molecules and cytokines that stimulate the migration and activation of monocytes and macrophages (68).

Cancer cell-derived EVs modulate the tumor microenvironment, which is favorable for tumor growth and invasion, by promoting tumor angiogenesis and immune suppression (19, 69). Recently, several groups have investigated the immunosuppressive roles of cancer cell-derived EVs (69-71) (Fig. 2). Melanoma- or colorectal cancer cell-derived EVs promoted the differentiation of monocytes into myeloid-derived suppressor cells (MDSCs) and inhibited T cell proliferation and effector functions and thus suppressed the antitumor immune response (69). Furthermore, EVs derived from B16 melanoma blocked their differentiation into mature dendritic cells by activating the accumulation of $CD11b^+Gr\text{-}1^+$ MDSCs (71). These $CD11b^+Gr\text{-}1^+$ cells release cytokines and chemokines, such as TNF-α, IL-6, and CCL2, and promote lung metastasis (71). Additionally, conditioned medium from tumor cells can induce the migration of monocytes and mediate M2 polarization

of macrophages (69). When human blood monocytes are cultured with conditioned media from different cancer cells, several genes related to M2-polarized macrophages were upregulated (70). The results of these studies suggest that cancer cell-derived EVs activate tumor-infiltrating macrophages and MDSCs to secret angiogenic factors, leading to tumor growth, metastasis, and angiogenesis (Fig. 2).

Effects of cancer cell-derived EVs on fibroblasts

Cancer-associated fibroblasts are abundant in the tumor stroma and they stimulate inflammation and angiogenesis (72, 73). Stromal myofibroblasts are characterized by a contractile cell type and the expression of smooth muscle actin (α-SMA) (74). Myofibroblasts are important in solid cancers with an altered stroma, promoting angiogenesis and local extracellular matrix remodeling (75). Stromal fibroblasts participate in tumor angiogenesis by secreting proangiogenic growth factors (e.g. VEGF, FGF2, TGF-β, PDGF, HGF, and IL-8) and matrix remodeling proteins (e.g. MMP-1, MMP-2, MMP-3, MMP-7, MMP-11, and MTMMP1) (72, 73). The recruited myofibroblasts act as the main source of VEGF and compensate for the loss of VEGF in the tumor cells (76). Proinflammatory cytokines and chemokines released by myofibroblasts recruit immune cells, including macrophages, neutrophils, and mast cells to the local site (75, 77). Although myofibroblasts are important components of the tumor stroma, the interactions between cancer cells and stromal fibroblasts have not been investigated in detail. A recent study revealed that cancer cell-derived EVs promote the induction of fibroblast differentiation to myofibroblasts (78) (Fig. 2). Prostate cancer cell-derived EVs contain TGF-β protein at the surface, associated with the type-3 TGF-β receptor, betaglycan (78). In EVs, TGF-β exists in a biologically active form and elicits SMAD-dependent signaling, elevating α-SMA expression, inducing a myofibroblastic phenotype (78). Moreover, fibroblast-derived EVs were found to facilitate breast cancer cell protrusion, motility, and metastasis through Wnt-planar cell polarity signaling (79). The Wnt signaling pathway is triggered by the tetraspanin CD81 and Wnt11 loaded inside the EVs (79).

CLINICAL APPLICATIONS OF EXTRACELLULAR VESICLES

EVs are potential biomarkers for the detection, diagnosis, and prognosis of patients, particularly in cancer. Several studies have revealed that EVs can be detected in tumor tissue and body fluids, such as malignant effusions, serum, and urine of cancer patients (80, 81). In cancer patients, the quantity of EVs is elevated and the composition of vesicular proteins, mRNAs, and miRNAs varies significantly by disease state (40). Recent progress in high-throughput tools has provided valuable information on disease-specific vesicular proteins, mRNAs, and miRNAs. EVs can be detected using non-invasive methods in the body fluids of cancer patients (82). Furthermore, among the vesicular biomarkers, proteins and RNAs of luminal cargos are protected from hydrolytic and enzymatic degradation in the extracellular environment (83). Thus, EVs are a good source of proteins and RNAs biomarkers of tumor progression.

In addition to the diagnostic potential of EVs in tumor biology, recent studies have drawn attention to the potential of EVs as therapeutic agents by inhibiting EVs biogenesis, release, cell uptake, or specific vesicular components (8, 82). Because the level of circulating EVs increases in various body fluids of cancer patients, the reduction of circulating EVs may inhibit tumorigenesis (80, 81). The production of EVs can be inhibited by blocking the target of microtubule assembly and stability, endosomal sorting pathways, or proton pumps (84-86). For example, ceramide, tetraspanin, and syndecan proteoglycans are crucial for the formation of EVs (87-89). Small-molecule inhibitors of sphingomyelinase or amiloride attenuate endosomal sorting and endocytic vesicle recycling and vesicle production, and, consequently, lead to a reduction in tumor growth (87, 88). Because the release of EVs depends on the small GTPase RAB27A, RNA interference treatment for RAB27A can also reduce tumor growth and metastasis (85). EVs derived from tumors harbor several functional vesicular proteins, mRNAs, and miRNAs involved in cancer progression. By blocking or depleting the functional cargos with small-molecule inhibitors and RNA interference, EV-mediated tumorigenesis can be attenuated (8, 82). Furthermore, EVs can be used as delivery systems for drugs, proteins, miRNA/siRNA, and other molecules (82, 90). By using intrinsic EVs or bioengineered exosome-mimetics, therapeutic agents can be delivered to diverse target cells, especially endothelial cells, and further lead to the attenuation of tumor growth and metastasis (90-92).

CONCLUDING REMARKS

In recent decades, EVs (exosomes and microvesicles) were regarded as just cellular debris (93). However, more recent findings on cargo sorting, biogenesis, components, and pathophysiological roles of the EVs have suggested that EVs are, in fact, complex extracellular organelles mediating intercellular communication ('communicasomes') (1, 2, 14). Indeed, EVs harbor complex vesicular components regulating a diverse range of physiological and pathological functions (2). In this review, we focused on the components, their functions, and the therapeutic and diagnostic potential of EVs derived from mammalian cells. As the release of EVs is an evolutionarily conserved process occurring from archaea, Gram-negative and Gram-positive bacteria, to eukaryotic cells, the role of EVs may be expanded as communicasomes in interspecies and even interkingdom communications (3, 4). Thus, explaining the complexity of EVs in relation to intercellular communications could provide hints to as-yet unknown mechanisms to various pathophysiological conditions and apply in the development of novel diagnostic and therapeutic tools for understanding, detecting, and treating diseases.

ACKNOWLEDGEMENTS

This work was supported by Mid-Career Researcher Program through NRF grant funded by the MEST (No. 2014023004).

REFERENCES

1. Choi, D. S., Kim, D. K., Kim, Y. K. and Gho, Y. S. (2014) Proteomics of extracellular vesicles: Exosomes and ectosomes. *Mass Spectrom. Rev.* [Epub ahead of print].
2. Choi, D. S., Kim, D. K., Kim, Y. K. and Gho, Y. S. (2013) Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. *Proteomics* **13**, 1554-1571.
3. Lee, E. Y., Choi, D. Y., Kim, D. K., Kim, J. W., Park, J. O., Kim, S., Kim, S. H., Desiderio, D. M., Kim, Y. K., Kim, K. P. and Gho, Y. S. (2009) Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. *Proteomics* **9**, 5425-5436.
4. Deatherage, B. L. and Cookson, B. T. (2012) Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. *Infect. Immun.* **80**, 1948-1957.
5. El Andaloussi, S., Mager, I., Breakefield, X. O. and Wood, M. J. (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov.* **12**, 347-357.
6. Raposo, G. and Stoorvogel, W. (2013) Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* **200**, 373-383.
7. Thery, C., Ostrowski, M. and Segura, E. (2009) Membrane vesicles as conveyors of immune responses. *Nat. Rev. Immunol.* **9**, 581-593.
8. S, E. L. A., Mager, I., Breakefield, X. O. and Wood, M. J. (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov.* **12**, 347-357.
9. Simons, M. and Raposo, G. (2009) Exosomes--vesicular carriers for intercellular communication. *Curr. Opin. Cell Biol.* **21**, 575-581.
10. Simpson, R. J., Jensen, S. S. and Lim, J. W. (2008) Proteomic profiling of exosomes: current perspectives. *Proteomics* **8**, 4083-4099.
11. Muralidharan-Chari, V., Clancy, J. W., Sedgwick, A. and D'Souza-Schorey, C. (2010) Microvesicles: mediators of extracellular communication during cancer progression. *J. Cell Sci.* **123**, 1603-1611.
12. Choi, D. S., Lee, J. M., Park, G. W., Lim, H. W., Bang, J. Y., Kim, Y. K., Kwon, K. H., Kwon, H. J., Kim, K. P. and Gho, Y. S. (2007) Proteomic analysis of microvesicles derived from human colorectal cancer cells. *J. Proteome Res.* **6**, 4646-4655.
13. Raimondo, F., Morosi, L., Chinello, C., Magni, F. and Pitto, M. (2011) Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. *Proteomics* **11**, 709-720.
14. Choi, D. S., Yang, J. S., Choi, E. J., Jang, S. C., Park, S., Kim, O. Y., Hwang, D., Kim, K. P., Kim, Y. K., Kim, S. and Gho, Y. S. (2012) The protein interaction network of extracellular vesicles derived from human colorectal cancer cells. *J. Proteome Res.* **11**, 1144-1151.
15. Andre, F., Schartz, N. E., Movassagh, M., Flament, C., Pautier, P., Morice, P., Pomel, C., Lhomme, C., Escudier, B., Le Chevalier, T., Tursz, T., Amigorena, S., Raposo, G., Angevin, E. and Zitvogel, L. (2002) Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* **360**, 295-305.
16. Runz, S., Keller, S., Rupp, C., Stoeck, A., Issa, Y., Koensgen, D., Mustea, A., Sehouli, J., Kristiansen, G. and Altevogt, P. (2007) Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. *Gynecol. Oncol.* **107**, 563-571.
17. Al-Nedawi, K., Meehan, B., Micallef, J., Lhotak, V., May, L., Guha, A. and Rak, J. (2008) Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat. Cell Biol.* **10**, 619-624.
18. Corcoran, C., Rani, S., O'Brien, K., O'Neill, A., Prencipe, M., Sheikh, R., Webb, G., McDermott, R., Watson, W., Crown, J. and O'Driscoll, L. (2012) Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes. *PLoS One* **7**, e50999.
19. Kim, C. W., Lee, H. M., Lee, T. H., Kang, C., Kleinman, H. K. and Gho, Y. S. (2002) Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin. *Cancer Res.* **62**, 6312-6317.
20. Laulagnier, K., Motta, C., Hamdi, S., Roy, S., Fauvelle, F., Pageaux, J. F., Kobayashi, T., Salles, J. P., Perret, B., Bonnerot, C. and Record, M. (2004) Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. *Biochem. J.* **380**, 161-171.
21. Subra, C., Laulagnier, K., Perret, B. and Record, M. (2007) Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie* **89**, 205-212.
22. Subra, C., Grand, D., Laulagnier, K., Stella, A., Lambeau, G., Paillasse, M., De Medina, P., Monsarrat, B., Perret, B., Silvente-Poirot, S., Poirot, M. and Record, M. (2010) Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J. Lipid Res.* **51**, 2105-2120.
23. Needham, D. and Nunn, R. S. (1990) Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* **58**, 997-1009.
24. Ramstedt, B. and Slotte, J. P. (2002) Membrane properties of sphingomyelins. *FEBS Lett.* **531**, 33-37.
25. Allen, T. M., Austin, G. A., Chonn, A., Lin, L. and Lee, K. C. (1991) Uptake of liposomes by cultured mouse bone marrow macrophages: influence of liposome composition and size. *Biochim. Biophys. Acta.* **1061**, 56-64.
26. Chernomordik, L. V. and Kozlov, M. M. (2003) Protein-lipid interplay in fusion and fission of biological membranes. *Annu. Rev. Biochem.* **72**, 175-207.
27. Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J. and Lotvall, J. O. (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **9**, 654-659.
28. Skog, J., Wurdinger, T., van Rijn, S., Meijer, D. H., Gainche, L., Sena-Esteves, M., Curry, W. T., Jr., Carter, B.

S., Krichevsky, A. M. and Breakefield, X. O. (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol.* **10**, 1470-1476.
29. Gibbings, D. J., Ciaudo, C., Erhardt, M. and Voinnet, O. (2009) Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat. Cell Biol.* **11**, 1143-1149.
30. Balaj, L., Lessard, R., Dai, L., Cho, Y. J., Pomeroy, S. L., Breakefield, X. O. and Skog, J. (2011) Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nat. Commun.* **2**, 180.
31. Kogure, T., Lin, W. L., Yan, I. K., Braconi, C. and Patel, T. (2011) Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology* **54**, 1237-1248.
32. Hessvik, N. P., Sandvig, K. and Llorente, A. (2013) Exosomal miRNAs as Biomarkers for Prostate Cancer. *Front. Genet.* **4**, 36.
33. Turchinovich, A., Weiz, L., Langheinz, A. and Burwinkel, B. (2011) Characterization of extracellular circulating microRNA. *Nucleic Acids Res.* **39**, 7223-7233.
34. Fabbri, M., Paone, A., Calore, F., Galli, R., Gaudio, E., Santhanam, R., Lovat, F., Fadda, P., Mao, C., Nuovo, G. J., Zanesi, N., Crawford, M., Ozer, G. H., Wernicke, D., Alder, H., Caligiuri, M. A., Nana-Sinkam, P., Perrotti, D. and Croce, C. M. (2012) MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc. Natl. Acad. Sci. U. S. A.* **109**, E2110-2116.
35. Lehmann, S. M., Kruger, C., Park, B., Derkow, K., Rosenberger, K., Baumgart, J., Trimbuch, T., Eom, G., Hinz, M., Kaul, D., Habbel, P., Kalin, R., Franzoni, E., Rybak, A., Nguyen, D., Veh, R., Ninnemann, O., Peters, O., Nitsch, R., Heppner, F. L., Golenbock, D., Schott, E., Ploegh, H. L., Wulczyn, F. G. and Lehnardt, S. (2012) An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. *Nat. Neurosci.* **15**, 827-835.
36. Bang, C. and Thum, T. (2012) Exosomes: new players in cell-cell communication. *Int. J. Biochem. Cell Biol.* **44**, 2060-2064.
37. Morelli, A. E., Larregina, A. T., Shufesky, W. J., Sullivan, M. L., Stolz, D. B., Papworth, G. D., Zahorchak, A. F., Logar, A. J., Wang, Z., Watkins, S. C., Falo, L. D., Jr. and Thomson, A. W. (2004) Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* **104**, 3257-3266.
38. Parolini, I., Federici, C., Raggi, C., Lugini, L., Palleschi, S., De Milito, A., Coscia, C., Iessi, E., Logozzi, M., Molinari, A., Colone, M., Tatti, M., Sargiacomo, M. and Fais, S. (2009) Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J. Biol. Chem.* **284**, 34211-34222.
39. Fitzner, D., Schnaars, M., van Rossum, D., Krishnamoorthy, G., Dibaj, P., Bakhti, M., Regen, T., Hanisch, U. K. and Simons, M. (2011) Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J. Cell Sci.* **124**, 447-458.
40. Ohno, S., Ishikawa, A. and Kuroda, M. (2013) Roles of exosomes and microvesicles in disease pathogenesis. *Adv. Drug Deliv. Rev.* **65**, 398-401.
41. Yuana, Y., Sturk, A. and Nieuwland, R. (2013) Extracellular vesicles in physiological and pathological conditions. *Blood Rev.* **27**, 31-39.
42. Sheldon, H., Heikamp, E., Turley, H., Dragovic, R., Thomas, P., Oon, C. E., Leek, R., Edelmann, M., Kessler, B., Sainson, R. C., Sargent, I., Li, J. L. and Harris, A. L. (2010) New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes. *Blood* **116**, 2385-2394.
43. Gross, J. C., Chaudhary, V., Bartscherer, K. and Boutros, M. (2012) Active Wnt proteins are secreted on exosomes. *Nat. Cell Biol.* **14**, 1036-1045.
44. Beckett, K., Monier, S., Palmer, L., Alexandre, C., Green, H., Bonneil, E., Raposo, G., Thibault, P., Le Borgne, R. and Vincent, J. P. (2013) Drosophila S2 cells secrete wingless on exosome-like vesicles but the wingless gradient forms independently of exosomes. *Traffic* **14**, 82-96.
45. Ratajczak, J., Miekus, K., Kucia, M., Zhang, J., Reca, R., Dvorak, P. and Ratajczak, M. Z. (2006) Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* **20**, 847-856.
46. Deregibus, M. C., Cantaluppi, V., Calogero, R., Lo Iacono, M., Tetta, C., Biancone, L., Bruno, S., Bussolati, B. and Camussi, G. (2007) Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* **110**, 2440-2448.
47. Ratajczak, M. Z., Kucia, M., Jadczyk, T., Greco, N. J., Wojakowski, W., Tendera, M. and Ratajczak, J. (2012) Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? *Leukemia* **26**, 1166-1173.
48. Kramer-Albers, E. M., Bretz, N., Tenzer, S., Winterstein, C., Mobius, W., Berger, H., Nave, K. A., Schild, H. and Trotter, J. (2007) Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics Clin. Appl.* **1**, 1446-1461.
49. Lachenal, G., Pernet-Gallay, K., Chivet, M., Hemming, F. J., Belly, A., Bodon, G., Blot, B., Haase, G., Goldberg, Y. and Sadoul, R. (2011) Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Mol. Cell Neurosci.* **46**, 409-418.
50. Chivet, M., Hemming, F., Pernet-Gallay, K., Fraboulet, S. and Sadoul, R. (2012) Emerging role of neuronal exosomes in the central nervous system. *Front. Physiol.* **3**, 145.
51. Bakhti, M., Winter, C. and Simons, M. (2011) Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. *J. Biol. Chem.* **286**, 787-796.
52. Wang, S., Cesca, F., Loers, G., Schweizer, M., Buck, F., Benfenati, F., Schachner, M. and Kleene, R. (2011) Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J. Neurosci.* **31**, 7275-7290.
53. Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W.,

Vidal, M., Laude, H. and Raposo, G. (2004) Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 9683-9688.
54. Gomes, C., Keller, S., Altevogt, P. and Costa, J. (2007) Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. *Neurosci. Lett.* **428**, 43-46.
55. Emmanouilidou, E., Melachroinou, K., Roumeliotis, T., Garbis, S. D., Ntzouni, M., Margaritis, L. H., Stefanis, L. and Vekrellis, K. (2010) Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *J. Neurosci.* **30**, 6838-6851.
56. Berckmans, R. J., Nieuwland, R., Kraan, M. C., Schaap, M. C., Pots, D., Smeets, T. J., Sturk, A. and Tak, P. P. (2005) Synovial microparticles from arthritic patients modulate chemokine and cytokine release by synoviocytes. *Arthritis Res. Ther.* **7**, R536-544.
57. Mause, S. F., von Hundelshausen, P., Zernecke, A., Koenen, R. R. and Weber, C. (2005) Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1512-1518.
58. Thery, C. (2011) Exosomes: secreted vesicles and intercellular communications. *F1000 Biol. Rep.* **3**, 15.
59. Clayton, A., Mitchell, J. P., Court, J., Mason, M. D. and Tabi, Z. (2007) Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2. *Cancer Res.* **67**, 7458-7466.
60. Hong, B. S., Cho, J. H., Kim, H., Choi, E. J., Rho, S., Kim, J., Kim, J. H., Choi, D. S., Kim, Y. K., Hwang, D. and Gho, Y. S. (2009) Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics* **10**, 556.
61. Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C. and Rak, J. (2009) Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 3794-3799.
62. Taraboletti, G., D'Ascenzo, S., Giusti, I., Marchetti, D., Borsotti, P., Millimaggi, D., Giavazzi, R., Pavan, A. and Dolo, V. (2006) Bioavailability of VEGF in tumor-shed vesicles depends on vesicle burst induced by acidic pH. *Neoplasia* **8**, 96-103.
63. Sunderkotter, C., Steinbrink, K., Goebeler, M., Bhardwaj, R. and Sorg, C. (1994) Macrophages and angiogenesis. *J. Leukoc. Biol.* **55**, 410-422.
64. Mostafa, L. K., Jones, D. B. and Wright, D. H. (1980) Mechanism of the induction of angiogenesis by human neoplastic lymphoid tissue: studies on the chorioallantoic membrane (CAM) of the chick embryo. *J. Pathol.* **132**, 191-205.
65. Stenzinger, W., Bruggen, J., Macher, E. and Sorg, C. (1983) Tumor angiogenic activity (TAA) production in vitro and growth in the nude mouse by human malignant melanoma. *Eur. J. Cancer Clin. Oncol.* **19**, 649-656.
66. Polverini, P. J. and Leibovich, S. J. (1984) Induction of neovascularization in vivo and endothelial proliferation in vitro by tumor-associated macrophages. *Lab. Invest.* **51**, 635-642.
67. Sunderkotter, C., Goebeler, M., Schulze-Osthoff, K., Bhardwaj, R. and Sorg, C. (1991) Macrophage-derived angiogenesis factors. *Pharmacol. Ther.* **51**, 195-216.
68. Pober, J. S. and Sessa, W. C. (2007) Evolving functions of endothelial cells in inflammation. *Nat. Rev. Immunol.* **7**, 803-815.
69. Valenti, R., Huber, V., Filipazzi, P., Pilla, L., Sovena, G., Villa, A., Corbelli, A., Fais, S., Parmiani, G. and Rivoltini, L. (2006) Human tumor-released microvesicles promote the differentiation of myeloid cells with transforming growth factor-beta-mediated suppressive activity on T lymphocytes. *Cancer Res.* **66**, 9290-9298.
70. Solinas, G., Schiarea, S., Liguori, M., Fabbri, M., Pesce, S., Zammataro, L., Pasqualini, F., Nebuloni, M., Chiabrando, C., Mantovani, A. and Allavena, P. (2010) Tumor-conditioned macrophages secrete migration-stimulating factor: a new marker for M2-polarization, influencing tumor cell motility. *J. Immunol.* **185**, 642-652.
71. Peinado, H., Aleckovic, M., Lavotshkin, S., Matei, I., Costa-Silva, B., Moreno-Bueno, G., Hergueta-Redondo, M., Williams, C., Garcia-Santos, G., Ghajar, C., Nitadori-Hoshino, A., Hoffman, C., Badal, K., Garcia, B. A., Callahan, M. K., Yuan, J., Martins, V. R., Skog, J., Kaplan, R. N., Brady, M. S., Wolchok, J. D., Chapman, P. B., Kang, Y., Bromberg, J. and Lyden, D. (2012) Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **18**, 883-891.
72. Kalluri, R. and Zeisberg, M. (2006) Fibroblasts in cancer. *Nat. Rev. Cancer* **6**, 392-401.
73. Vong, S. and Kalluri, R. (2011) The role of stromal myofibroblast and extracellular matrix in tumor angiogenesis. *Genes Cancer* **2**, 1139-1145.
74. Hinz, B., Celetta, G., Tomasek, J. J., Gabbiani, G. and Chaponnier, C. (2001) Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol. Biol. Cell* **12**, 2730-2741.
75. Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V. J., Richardson, A. L. and Weinberg, R. A. (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335-348.
76. Dong, J., Grunstein, J., Tejada, M., Peale, F., Frantz, G., Liang, W. C., Bai, W., Yu, L., Kowalski, J., Liang, X., Fuh, G., Gerber, H. P. and Ferrara, N. (2004) VEGF-null cells require PDGFR alpha signaling-mediated stromal fibroblast recruitment for tumorigenesis. *EMBO J.* **23**, 2800-2810.
77. Silzle, T., Kreutz, M., Dobler, M. A., Brockhoff, G., Knuechel, R. and Kunz-Schughart, L. A. (2003) Tumor-associated fibroblasts recruit blood monocytes into tumor tissue. *Eur. J. Immunol.* **33**, 1311-1320.
78. Webber, J., Steadman, R., Mason, M. D., Tabi, Z. and Clayton, A. (2010) Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res.* **70**, 9621-9630.
79. Luga, V., Zhang, L., Viloria-Petit, A. M., Ogunjimi, A. A., Inanlou, M. R., Chiu, E., Buchanan, M., Hosein, A. N., Basik, M. and Wrana, J. L. (2012) Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* **151**, 1542-1556.

80. Chen, H. Y., Yu, S. L., Chen, C. H., Chang, G. C., Chen, C. Y., Yuan, A., Cheng, C. L., Wang, C. H., Terng, H. J., Kao, S. F., Chan, W. K., Li, H. N., Liu, C. C., Singh, S., Chen, W. J., Chen, J. J. and Yang, P. C. (2007) A five-gene signature and clinical outcome in non-small-cell lung cancer. *N. Engl. J. Med.* **356**, 11-20.
81. Gonzales, P. A., Pisitkun, T., Hoffert, J. D., Tchapyjnikov, D., Star, R. A., Kleta, R., Wang, N. S. and Knepper, M. A. (2009) Large-scale proteomics and phosphoproteomics of urinary exosomes. *J. Am. Soc. Nephrol.* **20**, 363-379.
82. Tickner, J. A., Urquhart, A. J., Stephenson, S. A., Richard, D. J. and O'Byrne, K. J. (2014) Functions and therapeutic roles of exosomes in cancer. *Front. Oncol.* **4**, 127.
83. Keller, S., Ridinger, J., Rupp, A. K., Janssen, J. W. and Altevogt, P. (2011) Body fluid derived exosomes as a novel template for clinical diagnostics. *J. Transl. Med.* **9**, 86.
84. Iero, M., Valenti, R., Huber, V., Filipazzi, P., Parmiani, G., Fais, S. and Rivoltini, L. (2008) Tumour-released exosomes and their implications in cancer immunity. *Cell Death Differ.* **15**, 80-88.
85. Bobrie, A., Krumeich, S., Reyal, F., Recchi, C., Moita, L. F., Seabra, M. C., Ostrowski, M. and Thery, C. (2012) Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression. *Cancer Res.* **72**, 4920-4930.
86. Marleau, A. M., Chen, C. S., Joyce, J. A. and Tullis, R. H. (2012) Exosome removal as a therapeutic adjuvant in cancer. *J. Transl. Med.* **10**, 134.
87. Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F., Schwille, P., Brugger, B. and Simons, M. (2008) Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **319**, 1244-1247.
88. Nazarenko, I., Rana, S., Baumann, A., McAlear, J., Hellwig, A., Trendelenburg, M., Lochnit, G., Preissner, K. T. and Zoller, M. (2010) Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer Res.* **70**, 1668-1678.
89. Baietti, M. F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., Zimmermann, P. and David, G. (2012) Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat. Cell Biol.* **14**, 677-685.
90. Fais, S., Logozzi, M., Lugini, L., Federici, C., Azzarito, T., Zarovni, N. and Chiesi, A. (2013) Exosomes: the ideal nanovectors for biodelivery. *Biol. Chem.* **394**, 1-15.
91. Jang, S. C., Kim, O. Y., Yoon, C. M., Choi, D. S., Roh, T. Y., Park, J., Nilsson, J., Lotvall, J., Kim, Y. K. and Gho, Y. S. (2013) Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS Nano* **7**, 7698-7710.
92. Jang, S. C. and Gho, Y. S. (2014) Could bioengineered exosome-mimetic nanovesicles be an efficient strategy for the delivery of chemotherapeutics? *Nanomedicine (Lond)* **9**, 177-180.
93. Lee, T. H., D'Asti, E., Magnus, N., Al-Nedawi, K., Meehan, B. and Rak, J. (2011) Microvesicles as mediators of intercellular communication in cancer–the emerging science of cellular 'debris'. *Semin. Immunopathol.* **33**, 455-467.