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Review Article

Why do cells release vesicles?

Rienk Nieuwland *, Augueste Sturk

Department of Clinical Chemistry, Academic Medical Center, Amsterdam, The Netherlands

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ABSTRACT

Prokaryotic and eukaryotic cells release vesicles into their environment. To answer the question *why* eukaryotic cells release vesicles, we may learn from prokaryotes. Bacteria release outer membrane vesicles, resembling microparticles, which act as "multi-purpose carriers". They contain signalling molecules for other bacteria, deliver toxins to host cells and exchange DNA encoding virulence genes between bacteria. Similarly, cell-derived microparticles and exosomes from eukaryotic cells are multi-purpose carriers containing e.g. signalling molecules, cellular waste and functional genetic information. To illustrate our rapidly increasing knowledge on the multiple roles that cellular microparticles and exosomes play in disease progression, we focus on cancer, which is one of the best studied diseases in this aspect. The clinical applications of microparticles and exosomes, including diagnosis, prognosis and therapy, in cancer are discussed.

Contents

Introduction

Eukaryotic cells release vesicles into their environment. The most widely studied and best known types are microparticles and exosomes. Microparticles are budded from the cell membrane and are relatively large, diameter 100 nm - 1 μm. Exosomes, which are stored as intraluminal vesicles within multivesicular bodies, are smaller (30 nm -100 nm), and become released when multivesicular body membranes fuse with the cell membrane.

Sera and conditioned media from *in vitro* cultured cells, as well as body fluids such as blood or urine all contain substantial numbers of cell-derived vesicles. In body fluids, microparticles and exosomes from various cells coexist under physiological and pathological conditions. The numbers of these vesicles, their cellular origin, composition and function, however, can be disease state dependent. The detection and characterization of (individual) vesicles remains difficult due to their small size and heterogeneity, which has lead to confusing and sometimes even conflicting results between laboratories. At present, several attempts are being made, e.g. by dedicated flowcytometry, atomic force microscopy or dynamic light scattering combined with fluorescence, to gain a more detailed insight into the real concentrations, size distribution, cellular origin and composition of microparticles and exosomes.

For a long time, the functions of microparticles and exosomes were studied mainly by using purified preparations, which were and still are tested for their ability to initiate or propagate coagulation, inflammation and angiogenesis. On the one hand these studies have

* Corresponding author. Academic Medical Center, Laboratory of Experimental Clinical Chemistry, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Tel.: +31 20 5664851; fax: +31 20 6091222.
E-mail address: r.nieuwland@amc.nl (R. Nieuwland).

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provided a large insight into the potential biological significance of cell-derived vesicles, but on the other hand they have not answered the question what the true role of vesicles may be, in other words: *why* do cells release vesicles into their environment? In this review, a comparison is made between the physiological functions of outer membrane vesicles from prokaryotic cells and membrane vesicles from eukaryotic cells. In addition, the complex role(s) of eukaryotic membrane vesicles in disease will be illustrated by focusing on cancer, which is one of the best studied diseases in this aspect.

Lessons from Prokaryotes

To answer the question why eukaryotic cells release vesicles, we may learn from prokaryotes, which release outer membrane vesicles -resembling microparticles- into their environment.

Communication

Outer membrane vesicles from the bacterial pathogen *Pseudomonas aeruginosa* contain messages for other bacteria of the same species. In fact, these outer membrane vesicles facilitate the inter-bacterial communication [1].

Protection

Multiple bacterial virulence factors -such as cytolethal distending toxin- are packaged into (bacterial) outer membrane vesicles. When these outer membrane vesicles bind to host cells, they deliver their content directly into the cytoplasm of these host cells in a lipid-raft dependent mechanism [2,3]. Similarly, several fungal pathogenesis-related molecules are packaged into vesicles, and also the contents of these vesicles can be delivered to cells [4,5].

Exchange of genetic information

Bacterial vesicles contain DNA encoding virulence genes [6] and these genes can be transferred and expressed by recipient bacteria [7].

Taken together, membrane vesicles from prokaryotes act as "multi-purpose carriers" capable of (1) facilitating communication between bacteria, (2) protection and (3) exchange of functional genetic information.

Functions of membrane vesicles from eukaryotic cells

There is growing evidence that, similar to the outer membrane vesicles from prokaryotes, cell-derived vesicles from eukaryotic cells are important *inter*cellular "multi-purpose carriers" involved in communication, protection against intra- and extracellular stress and in the exchange of functional genetic information.

Communication

With regard to *inter*cellular communication, transport of functional receptors, cytokines and 2nd messengers to target cells by microparticles and exosomes has been demonstrated. A well known example of receptor transfer is tissue factor (TF), which can be transferred from the surface of leukocyte-derived microparticles in a lipid raft-dependent mechanism to the surface of activated platelets [8].

Protection

Platelets incubated with the complement C5b-9 complex release vesicles enriched in this complex, resulting in the protection of the platelets against complement-induced lysis [9]. However, the release of vesicles may not only protect cells against such "*external* stress", but also against "*internal* stress". With regard to the latter, vesicles seem to act as dust bins and play a crucial role in what may be called "cellular waste management". Examples of such waste management are the release of vesicles containing (compared to the parent cell) increased concentrations of e.g. chemotherapeutics, oxidized phospholipids, the redundant transferring receptor, or caspase 3. We and others have shown that microparticles and exosomes of viable cells contain caspase 3 [10,11]. Furthermore, we demonstrated that inhibition of the release of caspase 3-containing microparticles resulted in the intracellular accumulation of caspase 3 and subsequent apoptosis [12], strongly suggesting that the release of vesicles protects the cells against intracellular accumulation of dangerously high levels of caspase 3. Upon incubation of human endothelial cells with clinically relevant levels of simvastatin, the cells remained viable and seemingly unchanged, but a marked increase in caspase 3-containing vesicles was observed, at least suggesting that this increased release may help the cells to remain healthy and viable [13]. Taken together, the release of vesicles may protect cells from accumulation of dangerous or redundant compounds, and in this manner may contribute to cellular well being and even survival.

Exchange of genetic information

With regard to the exchange of functional genetic information and as already mentioned for versicles from prokaryotes, also eukaryotic vesicles were recently shown to contain mRNA and microRNAs. In a set of elegant experiments, Valadi et al. showed by proteomics approach that mRNA of mouse cell exosomes was transferred to and expressed by human mast cells [14]. In addition, microvesicles from murine embryonic stem cells supported self-renewal and expansion of adult stem cells by transfer of mRNA, and microvesicles from endothelial progenitor cells promoted angiogenesis by transfer of a specific subset of mRNA, including mRNA associated with the PI3K/AKT signalling pathway [15,16]. Thus, also eukaryotic vesicles are capable of intercellular exchange of genetic information.

Cancer: an example of microparticles in disease

The complex role of vesicles in disease can best be illustrated by one of the most studied diseases in this respect: cancer. As with other diseases, the major role of cancer cell-derived microparticles and exosomes seems to be that of modulating the disease progression rather than being the main cause of the disease itself. Microparticles and exosomes from cancer cells facilitate cancer progression and invasion in many different ways.

With regard to **communication**, the cancer cell-derived vesicles may suppress the immune system, e.g. by exposing the death-receptor ligand FasL (CD95L) [17]. In addition, the exchange of an oncogenic growth factor receptor between glioblastoma cells, i.e. a truncated form of the epidermal growth factor receptor EGFRvIII, is mediated by exosomes [18]. Furthermore, cancer cell-derived vesicles contain e.g. angiogenesis-promoting factors such as VEGF, which promotes angiogenesis and thus cancer growth [19].

With regard to **protection**, the cancer cell-derived vesicles (compared to the cells) contain increased levels of chemotherapeutics or metabolites thereof, thus contributing to drug resistance [20,21].

With regard to the **exchange of genetic information**, gliablastoma cells were shown to release mRNA-containing exosomes. This mRNA was taken up and expressed by microvascular endothelial cells, which then became prone to facilitate further tumour growth. Thus, also the intercellular exchange of genetic information by cancer cell-derived microvesicles may promote tumour growth [19].

Then, what are the clinical implications of our current knowledge on cancer cell-derived vesicles? The presence of unique cancer cell-derived microparticles and exosomes or messages enclosed in such vesicles offer novel *diagnostic possibilities*, e.g. cancer-specific microRNA has been observed in exosomes isolated from plasma samples of patients with ovarian cancer [22]. In several studies, the level of circulating microvesicles has been associated with the *prognosis* of survival of

cancer patients [23–25]. Another potentially interesting application of circulating vesicles, especially those exposing TF, in cancer patients is their association with venous thrombosis. Cancer patients have a hypercoagulable state compared to healthy subjects as reflected by elevated levels of plasma TF, activated coagulation factor VII, thrombin-antithrombin complexes and protrombin fragment F1+2 [26]. There is growing evidence that the plasma TF in cancer patients is associated –at least in part- with microparticles from tumour cells. Since the levels of these TF-exposing microparticles are higher in patients with cancer and venous thromboembolism (VTE) than in patients with cancer without VTE, the microparticles are thought to play a role in the pathogenesis of cancer-associated trombosis [23,27,28]. How these TF-exposing microparticles contribute to thrombosis, however, is unknown. Recently, Thomas and coworkers showed that TF-exposing microparticles from cancer cells can also expose P-selectin glycoprotein ligand 1 (PSGL-1). In mice developing a tumour, the endogenous cancer cell-derived microparticles exposing both TF and PSGL-1 accumulated at the site of injury, and the thrombotic state in these mice was inhibited by infusion of a blocking P-selectin antibody [29]. Thus, targeting microparticles may be an interesting clinical target to prevent thrombosis. To which extent VTE can also be predicted in cancer patients by studying the number and/or procoagulant properties of the TF-exposing microparticles is currently under investigation.

In a number of studies, autologous exosomes from antigen presenting (dendritic) cells have been administered as adjuvant *therapy* in phase I trials to patients with metastatic melanoma, advanced non-small cell lung cancer and colorectal cancer [30–32]. At present, this autologous anti-cancer immunotherapy is being optimized.

To which extent the release of vesicles from cancer cells is an interesting therapeutic target, however, is unclear. Membrane blebbing and formation of apoptotic bodies results from caspase-mediated cleavage of Rho-associated coiled-coil containing kinase (ROCK) 1 [33]. Inhibition of ROCK 1 by Y-27632 blocked migration and proliferation of glioblastoma cells in vitro, inhibition of migration and invasion of gliobastoma cells by danthron was paralleled by reduced mRNA expression of ROCK-1, and treatment of animals with lovostatin, which impairs the Rho/ROCK signalling, reduced glioma tumour mass in animals [34–36]. Whether or not these effects are due to inhibition of the release of vesicles, however, requires further investigation.

Taken together, there is a growing scientific and clinical interest in the various types of cell-derived vesicles. A better understanding of their biology and functions may provide new avenues of diagnostic, prognostic and therapeutic opportunities in various types of disease.

Conflict of interest statement

The authors state they have no conflict of interest.

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