Microvesiculation and Disease

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Abstract

The important roles of extracellular vesicles in the pathogenesis of various diseases are rapidly being elucidated. As important vehicles of intercellular communication, extracellular vesicles, which comprise microvesicles and exosomes, are revealing important roles in cancer tumorigenesis and metastases and in the spread of infectious disease. The September 2012 Focused Meeting ‘Microvesiculation and Disease’ brought together researchers working on extracellular vesicles. The papers in this issue of Biochemical Society Transactions review work in areas including HIV infection, kidney disease, hypoxia-mediated tumorigenesis and down-regulation of immune cell functions in acute myeloid leukaemia by tumour-derived exosomes. In all cases, microvesicles and exosomes have been demonstrated to be important factors leading to the pathophysiology of disease or indeed as therapeutic vehicles in possible new treatments. The aim was, having enhanced our molecular understanding of the contribution of microvesicles and exosomes to disease in vitro, to begin to apply this knowledge to in vivo models of disease.

Introduction

Ten years after a major review on exosomes [1] and many years after they were first described, 18 April 2012 was the official birth of a new area of cell biology, through the formation of the ISEV (International Society for Extracellular Vesicles). Just as haemopoietic stem cell isolation in the late 1980s stimulated a new area of stem cell biology or work with cell division cycle mutants in Xenopus [2] initiated an era of research into cell-cycle control, with applications in cancer, the foundation of ISEV clearly delineates a new era for EV (extracellular vesicle) research, so that we can finally dispel the earlier notion of ‘cellular dust’.

18 April 2012 marked the historic first meeting of the ISEV (http://www.isev.org) in Gothenburg, Sweden. In its role to educate and promote EV research, ISEV is presiding over important debates on EV terminology and methodology, but, broadly, EVs include ‘exosomes’ [3], of endocytic origin, and ‘microvesicles’, released from the plasma membrane [4].

Although basic methodology needs to be agreed, and biogenesis pathways fully elucidated, this very exciting burgeoning field is about to explode, as the role of EVs in intercellular communication becomes ever more apparent. Around the world, EVs are being studied in tumour biology and diagnostics and now in various disease models, as highlighted at ‘Microvesiculation and Disease,’ the Focused Meeting we hosted (http://www.biochemistry.org/tabid/379/MeetingNo/SA133/view/Conference/default.aspx) in London in September 2012 as potential therapeutic vectors for siRNAs (small interfering RNAs) in Alzheimer’s disease [5], as therapeutic agents against autoimmune and infectious disease and cancer, or even in models of spinal cord regeneration. EVs are ubiquitous, and their presence in food and water may raise important safety issues. All cells secrete them, including multicellular and protozoan parasites and bacteria (outer membrane vesicles), and, as we found, some intracellular pathogens are even taking advantage of microvesiculation (EV release) to infect host cells [6].

Extracellular vesicles: MVs (microvesicles) and exosomes

Microvesiculation is a mechanism occurring in all eukaryotic cells. Exocytosis is a process whereby surface proteins are taken by endocytosis to form an endosome, the contents of which may be degraded upon fusion with a lysosome or else undergo intraluminal budding to produce intraluminal bodies within a multivesicular body. Subsequent fusion with

Key words: cancer, exosome, extracellular vesicle, infectious disease, microvesicle.

Abbreviations used: EV, extracellular vesicle; ISEV, International Society for Extracellular Vesicles; MAC, membrane-attack complex; miRNA, microRNA; MMP, matrix metalloproteinase; MV, microvesicle; siRNA, small interfering RNA; TGFβ, transforming growth factor β1.

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the plasma membrane then causes release of its cargo of exosomes. Exosomes or exosome-like particles may also be directly released from the plasma membrane. Exosomes are small, approximately 50–100 nm in diameter [7], but the larger MVs are released either constitutively from the cell surface [8] or when cells become challenged by chemical or physical stress. These MVs range from 0.1 to 1 μm, carrying cytoplasmic and membrane (protein and lipid) constituents from the parent cell. They are released during early apoptosis and are smaller than apoptotic bodies which uniquely carry damaged DNA. The definition of MVs and exosomes remains challenging because of their diversity, and remains to be clearly established [9], especially as different subtypes with overlapping physical and biological properties start to be described. We highlighted recently [10] that MV and exosome isolation protocols and analysis parameters are still not standardized. Although we and others have attempted to improve the accuracy and reliability of such procedures [11], it remained apparent at the Microvesiculation and Disease meeting, as it had been at the earlier inaugural meeting of ISEV in Gothenburg, that this area of EV research still requires much attention.

EVs, as a whole, comprising MVs and exosomes, have various functions, and the multitude of cytokines and receptors that they carry reflect their cell of origin. One of the most important functions is that of intercellular communication [12]. Others include pro-coagulation [13], protein export [14], export of RNAs [15] and export of cellular products such as misfolded proteins commonly regarded as metabolic waste [16], as well as the export of peptide hormones [17]. EVs also carry RNAs including mRNA and miRNA (microRNA). Proteomics by MS is very much being used in diagnostics especially in cancers; for example, such analysis of urinary exosomes has been used successfully in identifying diagnostic markers of prostate cancer [18].

**EVs in disease**

The levels of EVs in blood are elevated in a number of diseases, including angina or myocardial infarction [19]. Type 2 diabetes mellitus, systemic lupus erythemaatosus where MVs inhibit the phagocytosis of apoptotic cells [20] and in diabetic nephropathy [21]. EVs have been shown to play a role in inflammation and in rheumatoid disease [22], multiple sclerosis and sickle cell anemia. They have been much studied in the cancer field where, for example, they have been shown to enhance the invasion and metastasis of various cancers [23,24], as well as to enhance angiogenesis.

**EV release and multidrug resistance in cancer**

Elevated levels of EVs, whether MVs or exosomes, have been reported in the blood of cancer patients for some time [25,26], and they have been suspected of playing a role, whether direct or indirect, in the pathogenesis of cancer [27,28]. For some time, EV shedding has been thought of as a way that cells rid themselves of toxic waste. Caspase 3, which has a central role in the establishment of apoptosis, is an example of a product that the cell could expel, in this case to avoid excessive accumulation and leading to the establishment of apoptosis. Besides caspase 3 being found in conditioned medium during cell culture [29,30], inhibition of MV release was shown to allow the accumulation of caspase 3, leading to apoptosis [31] and so this could explain one mechanism by which cancer cells could escape death. In a similar way, EV shedding could explain cancer cell insensitivity to chemotherapeutic drugs. This chemoresistance in cancer cells seems to coincide with the up-regulation of genes involved in EV release and indeed high levels of drugs, such as doxorubicin or cisplatin, can be detected at up to 2–3-fold higher levels in the EV released from resistant cells than from insensitive cells [32,33]. Such an escape mechanism from apoptosis induced by chemotherapeutic drugs could account for one mechanism leading to multidrug resistance.

**EV and immunosurveillance in the tumour environment**

Besides the concept that tumour cells express up-regulated levels of complement regulatory receptors [34], it has been known for some time that cells exposed to activated complement and the terminal MAC (membrane-attack complex) can survive by releasing deposited MAC through the release of EVs [35]. Not surprisingly, this mechanism of MAC removal through EV release is shared with cancer cells and is one of the ways they resist complement attack [36]. In addition, cancer cells, expressing high levels of the complement regulator membrane cofactor protein (CD46) release similarly endowed EVs which inactivate deposited complement protein C3b and C4b [37]. Thus EV release from cancer cells may protect them by both inactivating complement and releasing deposited MAC. Another trick of cancer cells is to escape immunosurveillance. This is aided by the ability of EVs to exchange surface integrins and lipids, thereby disguising the cells as non-cancerous. EVs are also able to remove FasL (Fas ligand) from cancer cells, with diminution of these immunomodulatory molecules thus weakening the function of the adaptive immune system [38].

**EVs in tumour growth, angiogenesis and metastasis**

EVs play an important role in supporting tumour invasiveness and metastasis because they are rich in MMP (matrix metalloproteinase)-2 and MMP-9, as well as uPA (urokinase plasminogen activator), all of which are capable of breaking down the basement membrane collagen and fibrin within the extracellular matrix, as was found in ovarian cancer where inhibition of these enzymes on EVs markedly reduced EV-mediated tumour invasiveness [39]. The way EVs aid angiogenesis is through the procoagulant fibrin they carry which protects the tumour from the effector mechanisms...
of the immune system and provides a supporting matrix [40]. EVs can also promote tumour growth through mRNA-coding growth factors they may harbour, such as VEGF (vascular endothelial growth factor) or HGF (hepatocyte growth factor) and which they could transfer between cells [41]. Non-cancerous cells such as fibroblasts and epithelial cells can also be transformed by receiving transglutaminase or fibronectin from cancer cells via EVs [42]. In human renal cell carcinoma, EVs released from cancer stem cells (CD105 +) were shown to stimulate angiogenic properties in endothelial cells [43], which upon transfer to a mouse model of renal carcinoma were able to stimulate angiogenesis through transmitted miRNAs and mRNAs and establishment of a pre-metastatic niche in lung.

**EVs released through hypoxia in tumours and stroke**

Low doses of hypoxia (1% O2) which are insufficient to induce apoptosis, but which can induce MV release in the human lung cancer cell line A549 [44], aid angiogenesis through chemotactic attraction of endothelial cells and fibroblasts as well as stimulating stromal cells to release angiogenesis-promoting cytokines. These experiments were corroborated in vivo. In similar experiments, this time with exosomes from hypoxic epidermoid carcinoma cells, A431 also enhanced angiogenesis in a chorioallantoic membrane assay as well as metastasis [45].

In acute ischaemic stroke resulting in cerebral hypoxia, it was found that high levels of platelet-derived MVs can persist for up to 6 months after the event [46]. Endothelial cell MVs are also elevated and these levels correlate with the pathology of the disease and outcome [47]. Some of the most promising therapeutic applications using EVs as drug-delivery vehicles have involved exosomes. In this approach, siRNA was delivered to microglia, neurons and oligodendrocytes [5] upon systemic delivery of exosomes.

**EVs and infection with intracellular pathogens**

There are numerous examples in the literature of intracellular pathogens, whether protozoan parasite, virus or bacteria as well as of host cell-derived EVs playing a role in infection [47]. Exosomes released from the plasma membrane are known to ‘hijack’ infectious particles such as HIV from the cytoplasm [48]. In addition to stimulating mammalian cells to secrete vesicles, parasites could themselves release MVs. In our recent work [6], we provide evidence that MVs isolated from monocytes, fuse with Trypanosoma cruzi and increase their invasion of Vero and HeLa cells by evading complement lysis. We suggested that TGFβ1 (transforming growth factor β1) played a role, having previously shown that monocyte-derived MVs carry a host of cytokines, including TGFβ1 on their surface, and others, especially those lacking a signal peptide, such as MIF (macrophage migration inhibitory factor), FGF-1 (fibroblast growth factor 1) and galectin-3 [49]. Furthermore, MVs isolated from the intestinal parasite Giardia intestinalis (causative agent of giardiasis and persistent non-bacterial diarrhoea) enhanced the parasite’s attachment to endothelial cells (I. Evans-Osses, E.A. Ansa-Addo, J.M. Inal and M.I. Ramirez, unpublished work). The attachment of G. intestinalis to the microvilli of endothelial cells is necessary for the parasite to effectively establish infection. In other preliminary observations from our laboratory in which Salmonella enterica serovar Typhimurium was used to infect Vero cells, it was found that, in the presence of host cell MVs, there was a significantly increased level of infection.

**Future directions**

Having linked elevated plasma EV levels with cancer, infectious disease and autoimmune disease, an obvious application is in disease diagnosis, an area that is proceeding apace. It is now also important to use this information to see how best to develop strategies to create novel therapies. It may be that a targeted modulation of microvesiculation may suffice in certain conditions. Using disease models in which microvesiculation has been diminished may also provide a useful tool to confirm its contribution to disease pathogenesis. Finally, as has already begun to occur, EVs, whether exosomes or MVs, could also be targeted as drug-delivery vehicles themselves and demonstrated in in vivo models of disease.

**Acknowledgements**

We are grateful to the Biochemical Society for their support of the Microvesiculation and Disease meeting, as well as all of our sponsors. We also thank the contributions of presenting authors to this issue of Biochemical Society Transactions.

**References**


