Review

CellPress

Extracellular vesicles: emerging targets for cancer therapy

Pieter Vader[1], Xandra O. Breakefield[2], and Matthew J.A. Wood[1]

[1] Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, OX1 3QX, UK
[2] Department of Neurology, Massachusetts General Hospital and Neuroscience Program, Harvard Medical School, Boston, MA 2129, USA

Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are released by almost all cell types, including tumour cells. Through transfer of their molecular contents, EVs are capable of altering the function of recipient cells. Increasing evidence suggests a key role for EV mediated intercellular communication in a variety of cellular processes involved in tumour development and progression, including immune suppression, angiogenesis, and metastasis. Aspects of EV biogenesis or function are therefore increasingly being considered as targets for anticancer therapy. Here, we summarise the current knowledge on the contributions of EVs to cancer pathogenesis and discuss novel therapeutic strategies to target EVs to prevent tumour growth and spread.

Extracellular vesicles: novel mediators of cell-to-cell communication

Intercellular communication is fundamental to survival and maintenance of homeostasis in all multicellular systems. By contrast, dysregulated pathways of communication appear to drive cancer development and progression. The development of successful anticancer treatments will therefore crucially depend on increasing our understanding of the complexity of interactions between tumour cells and other cells. Communication between cells takes place via direct cell-to-cell contact, for example, through adhesion molecules, gap junctions, and nanotubes, or via soluble communication signals such as cytokines, growth factors, and hormones secreted by both tumour and non-tumour cells. However, an additional novel mechanism that can operate over both short and long distances has recently emerged, based on the release and uptake of membrane-bound vesicles termed extracellular vesicles (EVs) [1]. The recent discovery that EVs are able to convey complex multimolecular biological messages between cells has the potential to revolutionise our understanding of the communication circuitry in cancer. Further, EV research is anticipated to directly advance various areas of clinical cancer science, including cancer diagnostics (Box 1) and therapy [2].

Biogenesis, composition, and function of extracellular vesicles

Over the past decade, research efforts into EV biology, function, and application have dramatically increased. It has now become clear that virtually all cell types release EVs, constitutively and/or upon activation (e.g., as a result of hypoxia or shear stress). EVs have been traditionally classified based on their cell or tissue of origin, for example, prostasomes are derived from prostate cells, and oncosomes are derived from tumour cells. More recently, however, different classifications of EVs are being used, based on intracellular origin or biogenesis mechanism. Using this approach, although there is currently little consensus in the field regarding nomenclature due to differences in classification criteria, three main classes of EVs can be distinguished: exosomes, microvesicles (also referred to as ectosomes or microparticles), and apoptotic bodies [3–5]. Exosomes have been defined as originating from multivesicular bodies (MVBs) and are secreted upon fusion of MVBs with the plasma membrane. Exosomes are believed to range between 40 and 150 nm in size with a buoyant density of 1.13–1.19 g/cm^3, and are often characterised using marker proteins such as ALG-2-interacting protein X (ALIX) and tumour susceptibility gene 101 (TSG101), which indicate an endocytic origin [6]. Microvesicles are shed from the plasma membrane through direct outward budding and are generally more heterogeneous in size (50–2000 nm). Apoptotic bodies are released upon fragmentation of cells undergoing apoptosis. They vary in size between 50 and 5000 nm and can contain DNA and histones. The biogenesis and characteristics of each type of EVs derived from tumour cells are summarised in Figure 1 and Table 1. A strict separation between the EV classes by size, density, markers, or morphology has however not yet been established [7]. Moreover, current isolation and detection techniques for EVs do not allow for a clear distinction between different vesicular subpopulations, therefore the term EV will be used throughout this review to include all classes of cell-derived EVs.

EVs are lipid bilayer limited vesicles and carry a broad repertoire of cargoes, including proteins (e.g., cytokines, membrane receptors, and receptor ligands), nucleic acids (e.g., DNA, mRNA, long and short noncoding RNA), and

Corresponding author: Wood, M.J.A. (matthew.wood@dpag.ox.ac.uk).
Keywords: extracellular vesicles; exosomes; microvesicles; cancer therapy; tumour microenvironment; metastasis.

1471-4914/
 http://dx.doi.org/10.1016/j.molmed.2014.03.002

Box 1. Extracellular vesicles: novel cancer biomarkers

The content of EVs found in bodily fluids is closely related to the nature and status of the cells from which the EVs are derived. Because tumour and stromal cell-derived EVs carry signatures and effectors of tumour development, EVs are increasingly considered as novel sources of biomarkers with diagnostic or prognostic value [2]. Additionally, they could be used to predict or monitor a patient's response to treatment. In comparison to monitoring via biopsy, which does not allow for frequent and longitudinal sampling, EVs offer noninvasive and almost continuous access to circulating information on the status of the tumour [1]. Depending on the tumour type and location, EVs can be isolated from plasma/serum, urine, cerebral spinal fluid, and even saliva.

Numerous reports have already aimed to characterise the components of EVs derived from a variety of cellular sources and bodily fluids, the results of which are often made available through community databases of high-throughput datasets of EV cargo [74,75]. These studies have shown that EVs derived directly from tumour cells or from the extracellular fluids of cancer patients have a distinct molecular signature on the protein [11,76–78], DNA [79], mRNA [8], and noncoding RNA [80,81] level, which could allow their potential use as biomarkers. However, technological challenges related to EV isolation, purification, and content analysis remain. For example, for multicentre validation studies, standardised isolation and characterisation methods are necessary, yet largely lacking [58]. To overcome some of these challenges, a novel platform has recently been described for rapid protein profiling of EV samples. EVs are introduced onto a microfluidic chip and labelled with target-specific magnetic nanoparticles, which allows for highly sensitive detection of antigens by micro-nuclear magnetic resonance (μ-NMR) [82]. In addition, novel and sensitive approaches based on BEAMing and droplet digital PCR have recently been described to reliably detect and quantify mutant transcripts in EVs, which may contribute to solve challenges related to the detection level of mutant RNA/DNA in tumour-derived EVs in a background of EVs from normal cells [83]. Further technological improvements may allow EV-based diagnostics to become routine clinical practice.

lipids. Although their content generally reflects the nature and status of the cell of origin, enrichment of specific proteins and nucleic acids suggests at least a degree of specific cellular sorting into EVs, although the mechanisms underlying this remain to be defined [8]. Release of EVs is thought to have various biological roles, including disposal of superfluous or harmful cellular contents [9]. A more recently discovered and probably important role, however, is to emit signalling and regulatory molecules that can be recognised by, or transferred to, other cells in a selective manner, thereby influencing the phenotype and function of the recipient cell.

EVs can interact with target cells via different mechanisms. For example, membrane proteins on the surface of EVs can interact directly with receptors on the target cell, thereby activating intracellular pathways. Alternatively, EVs can be internalised by target cells either via membrane fusion or via endocytosis/phagocytosis, with subsequent transfer and release of their cargo [10]. In this manner, EVs can shuttle functional membrane receptors from one cell to another, after which intracellular signalling via these receptors can take place in the recipient cell [11]. mRNAs present in EVs are also transferrable to recipient cells where they can be translated into functional protein [12,13]. Strikingly, even miRNAs have been shown to be shuttled between cells by EVs leading to the repression of mRNA translation in recipient cells [14,15], although recent evidence suggests that miRNAs can also be transported and delivered via other mechanisms [16]. Through this exchange of molecular information, EVs are thought to exhibit pleiotropic biological functions and increasing evidence supports their importance in a variety of fundamental physiological as well as pathological processes [3,5]. For instance, B lymphocyte-derived EVs present antigens and induce antigen-specific responses in T cells, suggesting a role in adaptive immune responses [6]. During pregnancy, placenta-derived EVs function to circumvent maternal immune surveillance by suppressing T cell activation [17]. EVs have also been implicated in neurodegenerative diseases such as Alzheimer's disease via the intercellular transfer of aberrant protein structures such as β-amyloid peptides [18]. Not surprisingly, numerous studies to date have also implicated EVs as critical contributors to tumour growth and spread.

Emerging roles of extracellular vesicles in tumour growth and spread

A variety of EVs can be readily isolated from bodily fluids of cancer patients, including from blood, lymph, urine, saliva, cerebrospinal fluid, and ascites. In fact, the number of circulating EVs in cancer patients seems to be higher than in healthy individuals and has been found to correlate with poor prognosis [19]. The vast majority of these circulating EVs seem not to be derived from tumour cells but arise from activated platelets (or megakaryocytes [20]), lymphocytes, macrophages, and erythrocytes [1]. Initially, EVs were thought to be mainly associated with venous thromboembolic events (VTEs) in cancer patients because of their ability to carry tissue factor (Tf) and to interact with components of the coagulation system. Several studies have linked increased incidence of VTEs to elevated Tf-bearing EV levels [21–23]. Currently, clinical trials are underway to test the possibility of either reducing the number of procoagulant EVs in cancer patients or to use them diagnostically as tools to predict a patient's risk of developing VTEs [24].

Recent large-scale proteomic and transcriptomic studies have revealed differences between the protein and nucleic acid content of EVs derived from cancer cells compared with those derived from normal cells (although some caution is required when interpreting these results because EV purification methods that are typically used are unable to completely purify EVs from non-EV contaminants and differ between laboratories). Many of the proteins and RNAs found in tumour-derived EVs are known for their roles in cancer development and progression. These include oncoproteins, oncogenes, chemokine receptors, as well as soluble factors and transcripts of proteins involved in angiogenesis or inflammation (reviewed in [9,25,26]). Because EVs are capable of transferring these molecules to other cells in the tumour microenvironment or at specific distant sites, they have increasingly become recognised as key players in a variety of cellular processes related to cancer pathogenesis (Figure 2). Important examples of the influence of EV-mediated signalling on tumour growth and spread are described in this review. It should be emphasised that much of our current understanding is based on data from *in vitro* experiments. Caution must be taken when correlating these results with the physiological