The role of microvesicles in cancer progression and drug resistance

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Abstract

Microvesicles are shed constitutively, or upon activation, from both normal and malignant cells. The process is dependent on an increase in cytosolic Ca2+, which activates different enzymes, resulting in depolymerization of the actin cytoskeleton and release of the vesicles. Drug resistance can be defined as the ability of cancer cells to survive exposure to a wide range of anti-cancer drugs, and anti-tumour chemotherapeutic treatments are often impaired by innate or acquired MDR (multidrug resistance). Microvesicles released upon chemotherapeutic agents prevent the drugs from reaching their targets and also mediate intercellular transport of MDR proteins.

Introduction

Cell communication has often been defined as the direct secretion and interaction of growth factors, small molecular mediators, enzymes and cytokines between cells. However, recent findings describe another mode of cell–cell communication that has probably existed throughout evolutionary time, involving the release of small cellular fragments termed MVs (microvesicles) [1]. MVs are small intact heterogeneous membrane vesicles (with a diameter from 0.1 to ≤1 μm) that contain elements involved in cell signalling and intercellular communication [2]. A variety of cell types, including platelets, neutrophils, reticulocytes, macrophages, megakaryocytes, monocytes, B- and T-cells, mast cells, endothelial cells and tumour cell lines, have all been described to release MVs either constitutively or upon stimulation with an extracellular stimulus and an increase in the concentration of intracellular Ca2+ [1]. MVs also vary in size, composition and biological effects. The process of microvesiculation allows a selective release of cell components to the surrounding microenvironment [2]. During shedding, MVs also engulf some cytoplasm and, as a result, contain proteins, mRNA (microRNA) and mRNA derived from the parental cell [3].

In addition to MVs, eukaryotic cells also release another type of extracellular vesicle called exosomes. Exosomes are smaller than MVs, ranging in size from 30 to 100 nm in diameter. Exosomes are coated with a lipid bilayer and a small diameter. Exosomes play a significant role in antigen presentation and in mediating death in target cells [5]. Specific exosomal marker proteins are important in distinguishing exosomes and other vesicles, and include MHC class I and II molecules as well as the tetraspanins (for example, CD9, CD63, CD81 and CD82), representing the most abundant protein family present in exosomes [6].

MDR (multidrug resistance) has been defined as the ability of cancer cells to survive treatment with various chemotherapeutic drugs and presents a major obstacle in cancer chemotherapy [7]. However, the mechanisms used by cancer cells to evade apoptosis induced by anti-cancer drugs remain unclear. The active efflux of a broad range of anti-cancer drugs via the cell membrane is aided by MDR proteins via ATP-dependent and -independent mechanisms in tumour cells [8]. These proteins are members of the ABC (ATP-binding cassette) transporter family including P-gp (P-glycoprotein) 1, MRP (MDR protein) and BCRP (breast cancer resistance protein) [9,10]; LRP (lung-resistance-related protein), however, is another protein involved in MDR not belonging to the ABC transporter family. These proteins are involved in uptake and efflux of substances from cancer cells [11].

Key words: cancer, microvesicle, multidrug resistance.

Abbreviations used: ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; CDK2, cyclin-dependent kinase 2; 5-FU, 5-fluorouracil; MDR, multidrug resistance; mRNA, microRNA; MMP, matrix metalloproteinase; MRP, MDR protein; MV, microvesicle; P-gp, P-glycoprotein; TF, tissue factor.

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Tumour-derived MVs contain growth factors, receptors, proteases, adhesion molecules, signalling molecules, as well as DNA, mRNA and miRNA sequences [3], and are capable of merging with recipient cells to transfer their cargo [12]. Many of the intracellular proteins, second messengers and genetic material which MVs contain are specifically sorted into MVs. As a consequence of sorting, the functional properties and biological role of MVs may differ from their parental cells. These contents can be transferred to non-transformed stromal cells and endothelial cells, and affect tumour invasion, angiogenesis, metastasis and drug resistance [15].

Role of MVs in cancer progression

It is thought that MVs might be involved in interactions between tumour cells and bone-marrow-derived cells where they may play a role in forming metastatic niches [16]. MVs are secreted from the primary tumour which then attracts haemopoietic cells. In the primary tumour, the microenvironment could then be developed by MVs secreted by platelets, thus promoting a tendency to malignancy by the tumour cells [17]. MVs from haemopoietic stem cells could then attract tumour cells to locations in the bone or other metastatic sites, thus initiating the development of secondary metastatic sites [18].

The involvement of MVs in escape from apoptosis

MV release removes intracellular stress and acts as a protective mechanism for cell survival [19]. Apoptosis or programmed cell death is a tightly regulated process which has a crucial role in development and tissue homoeostasis [20]. Caspase 3 is one of the main executioner enzymes of apoptosis and is found in MVs isolated from conditioned medium of viable cells, but not in the releasing cells [21]. This was confirmed further in another study when in caspase-3-deficient MCF-7 cells, inhibition of MV release was restored by transfecting cells with caspase 3. Various studies have documented that cancer cells prevent accumulation of intracellular caspase 3 by releasing caspase-3-bearing MVs [22]. This hypothesis was supported by evidence that inhibition of MV release results in accumulation of caspase 3 inside cells, leading to apoptosis. Release of caspase-3-containing MVs is thus assumed to contribute to cell survival [23].

Many investigations have demonstrated an association between MV release and MDR. To illustrate further the correlation between MV shedding and cancer cell survival, a study by Shedden et al. [24] showed that chemoinosensitive cancer cell lines express more membrane-shedding-related genes compared with chemo-sensitive cells. In addition, MVs released from chemoinosensitive cancer cells contained higher levels of the chemotherapeutic agent doxorubicin [24]. Safaei et al. [25] also demonstrated that exosomes from cisplatin-insensitive cancer cells contained 2–6-fold more of the chemotherapeutic drug cisplatin than MVs released from cisplatin-sensitive cells.

MVs and escape from the immune system

Complement activation induces the release of MVs [26]. Sims et al. [27] demonstrated that apoptosis was abrogated in human platelets which were co-cultured with a sublytic concentration of the MAC (membrane attack complex), C5b-9, because of the release of C5b-9-bearing MVs. This phenomenon, referred to as ‘complement resistance’, is a way by which MV release offers protection to most cells in response to exogenous stimuli [27]. The same mechanism aids cancer cells to escape from complement lysis. Cancer cells also shed MVs bearing the complement membrane cofactor protein CD46, which inactivates C4b and C3b and therefore results in reduction of inflammation in micrometastases [28]. To induce T-cell apoptosis and impair the adaptive immune system, cancer cells enhance their survival by expression of FasL (Fas ligand) (CD95L), a ligand of the death receptor Fas (CD95) [28].

Furthermore, cancer-cell-derived MVs fuse with the plasma membrane of monocytes and impair their differentiation to dendritic cells [4]. It has been demonstrated that in tumours developed in patients who suffer from Epstein–Barr virus infection, released MVs express LMP-1 (latent membrane protein-1), an immune suppressor transmembrane protein which restrains T-cell proliferation and therefore impairs immune system function [29]. MVs from tumour cells were also found to suppress T-lymphocyte activity in a TGFβl (transforming growth factor receptor β1)-mediated manner [30].

MVs contribute to metastasis and angiogenesis

MVs contain proteases such as MMP (matrix metalloprotease)-2 and MMP-9, which degrade collagens and facilitate tumour growth. Inhibition of MMP-2 and MMP-9 presented in MVs abolishes the ability of these MVs to support tumour invasiveness [31]. Tumour-derived MVs also carry uPA (urokinase-type plasminogen activator) which catalyses the conversion of plasminogen into plasmin. Plasmin degrades fibrin and, as a result, extracellular matrix is degraded which contributes to tumour growth [31]. Cancer cells are also coated with fibrin and escape from immune detection and attack, while the fibrin matrix simultaneously supports outgrowth of new blood vessels [32]. MVs in cancer patients contain TF, which contributes to thrombosis. The latter MVs are trapped by activated platelets and transfuse and compile their pro-coagulant TF at the site of damage [13].

Furthermore, these MVs may merge with platelets, leading to coagulation and emission of growth factors [21]. Cancer-cell-derived MVs also carry mRNA encoding growth factors such as VEGF (vascular endothelial growth factor), and these vesicles fuse with monocytes and transfer their nucleic acids and induce production of growth factor production [33]. Cancer-cell-derived MVs alter the functions of T-cells and show immunosuppressive activities [15], and suppression of the immune system by MVs might contribute...
to tumour cell metastasis and spread. This spread might be haematological through TF-bearing MVs originated from activated platelets which express and carry PSGL (P-selectin glycoprotein) [34] because cancer cells surrounded by these MVs are protected from immune system scrutiny. Moreover, pro-coagulant properties of TF-bearing MVs leads to intravascular formation of fibrin, so aiding the adherence of cancer cells to the vessel wall [23].

By contrast, cancer-cell-derived MVs transfer tumour antigens to antigen-presenting cells and facilitate immune attack. Antigen-presenting cells also release MVs and the latter vesicles suppress the growth of murine tumours [35]. Anti-cancer therapy may target the release of MVs, by counteracting the beneficial effects on tumour growth of MV release. ROCK (Rho-associated kinase) I and II are serine/threonine kinases and both affect cell morphology, migration, cell adherance and release of MVs [36].

The therapeutic role of dendritic-cell-derived MVs in metastatic melanoma, advanced non-small-cell lung cancer and colorectal cancer has been examined and may be promising for cancer therapy [32]. Inhibition of MV release could be a potential target in anti-cancer therapy because MV release is associated with many processes related to tumour growth. Another study illustrated that heat (42°C)-treated B-lymphoblastoid cells release exosomes of differing protein composition to control MVs, containing high quantities of heat-shock protein [37]. The efficacy of anti-cancer drugs can be examined through proteomic studies of cancer-cell-derived MVs which might indicate the effects of chemotherapy and could be considered as an early biomarker for assessment of drugs. Proteomics could also be used in bladder cancer where MVs were shown to have eight proteins at elevated levels compared with the control [6]. Cancer-specific mRNA or miRNA could be other markers to be considered for early detection of cancer [38]. Furthermore, MV quantification before administration of chemotherapeutic agents could be considered as a prognostic indicator of survival rate in cancer patients.

To examine the efficacy of anti-cancer drugs, the measurement of the protein composition of MVs could be an early biomarker to assess the effectiveness of anti-cancer therapy [12]. Furthermore, circulating MVs expose tumour-specific markers, which could be useful for early detection of cancer, and detection of cancer-specific mRNA and miRNA could be used in patients with a high risk of cancer [39].

Drug resistance

Drug resistance in solid tumours has been a hitherto unsolved pharmacological problem in cancer chemotherapy. There are two types of drug resistance: intrinsic (inherent) resistance, where resistance to chemotherapy already exists before starting a drug treatment programme, and acquired resistance, which develops during treatment [40]. In order to tackle this problem, new drugs that could specifically target cancer cells were developed. However, these drugs have not decreased the occurrence of drug resistance so far [41]. An alternative classification for drug resistance is either pharmacokinetic (marked intratumoral differences in drug exposure) or pharmacodynamic (failure to elicit cytotoxicity) [40]. Multiple pathways are affected and act synergistically to create resistance to anti-cancer drugs. Chemotherapy is one of the major therapeutic avenues in oncology especially after dissemination of cancer which is difficult to treat with radiation [11].

Cancer cells, in response to chemotherapy, display various pathways, which interact synergistically to confound the cytotoxicity of chemotherapeutic agents [42]. Anti-cancer drugs are designed to target cell proliferation through inhibition of specific steps of the DNA replication process, i.e. alteration of nucleoside biosynthesis, interaction with DNA and prevention of cell mitosis [43]. Chemotherapy results in changes in the biology of cancer cells, which consequently leads to chemoresistance. The transcription factor p53 is an essential regulator of cell stress response, which is activated by a broad range of stimuli such as DNA damage, hypoxia, loss of cell–cell contact and inappropriate oncogene activation [44]. An endogenous inhibitor, HDM2 (human double minute 2), promotes p53 degradation because p53 is a short-lived protein. Any cellular stress results in p53 stabilization leading to p53 post-translational modification and its interaction with DNA and co-operating factors [45].

Association of p53 in cancer drug resistance

The p53 gene is a tumour-suppressor gene located on chromosome 17 [46]. In the cell, p53 protein binds DNA, which in turn stimulates another gene to produce p21 that interacts with a cell-division-stimulating protein [CDK2 (cyclin-dependent kinase 2)]. When p21 is complexed with CDK2, the cell cannot pass through to the next stage of cell division. Consequently, a chain of events is initiated that reduces the effects of damage by up-regulation of numerous genes such as p21, which functions as a regulator of cell-cycle progression at G1-phase, resulting in suspension of the cell cycle. Cell-cycle suspension allows repair of DNA and cellular damage, thereby promoting cell viability and survival [44,47]. If DNA damage is irreparable, p53 induces the expression of apoptosis inducers (e.g. Fas and Bax) in order to halt replication of damaged DNA in daughter cells. This p53 functional duality demonstrates its critical role in cellular biology as a tumour-suppressor gene [45]. This crucial role is reflected by observations of approximately 53% of p53 mutations in cancers, this high degree of p53 mutations compromises the ability of p53 to regulate the cell cycle and promote apoptosis through the up-regulation of pro-apoptotic genes such as BAX, NOXA (NADPH oxidase activator), TRAIL-R2 (tumour-necrosis-factor-related apoptosis-inducing ligand receptor 2) (DR5) and FAS (CD95/Apo-1) [9] in response to cellular damage. Consequently, for p53, a striking role in dictating the effectiveness of genotoxic chemotherapeutic agents in cancer treatment is expected [44]. It has been shown for a number of
anti-cancer drugs such as 5-FU (5-fluorouracil) in colorectal cancer that loss of p53 function prevents the initiation of apoptosis following chemotherapeutic treatment, thereby conferring resistance [40].

As a result, gene therapy is being investigated for its potential to increase chemotherapeutic efficacy. However, many studies have failed to confirm the link between mutated p53 and anti-cancer drug resistance [7]. Moreover, different tissues vary in the way they use their repair pathways such that it is even more difficult to predict chemotherapeutic drug effectiveness [40]. Development of drug resistance is a result of several factors including increased drug efflux and decreased drug influx, drug inactivation, alterations in drug target, processing of drug-induced damage and evasion of apoptosis [42]. Numerous studies have indicated that dysfunctional p53 contributes to drug resistance (because of an inability to undergo apoptosis, the main target of chemotherapeutic drugs) in cells treated with 5-FU.

**Role of P-gp in cancer drug resistance**

P-gp and MRP are members of the ABC transporter protein family and are involved in drug efflux [7]. MRP1 is involved in the resistance of lung cancer cells [10], but the first glycoprotein discovered that was responsible for conferring drug resistance to cultured cells was P-gp. P-gp is an energy-dependent efflux transporter which pumps drug molecules out of cells [40].

P-gp is found in the epithelial cells of the intestine (enterocytes) along the apical (luminal) side of the cell. When a drug is taken orally, drug molecules have to pass through the enterocyte, but P-gp takes drug molecules from the cytoplasm transporting them back to the intestinal lumen for excretion. This action prevents drug molecules from reaching the systemic circulation, effectively limiting bioavailability [48]. Because P-gp is found throughout the intestinal tract, it affects the absorption of all susceptible oral drugs. P-gp is also present in the liver and kidney, where it acts to increase the excretion of drugs by transporting the molecules into the bile and urine respectively [49]. Overexpression of these proteins correlates directly to drug resistance and, specifically, MDR has been documented to be linked to overexpression of P-gp. Administration of P-gp inhibitors, however, such as cyclosporin and vaspodar has not been successful because of their cytotoxicity to other transporter proteins [11].

There are other members of the ABC superfamily of transporters which are associated with conferring resistance to chemotherapy, for example MRP1, which is involved in the resistance of lung cancer cell [50]. BCRP or mitoxantrone-resistance protein is isolated from cell lines resistant to mitoxantrone. Moreover, BCRP is an ABC half-transporter and translocates unmetabolized drugs. It confers resistance to a subset of drugs which is distinct from those transported by P-gp and MRP [51]. All of the transporters mentioned protect cancer cells from anti-cancer drugs and have a crucial role in drug resistance. ABCB1, ABCG1 and ABCG2 are the assigned systematic names for P-gp, MRP1 and BCRP respectively. They are expressed in various normal tissues specifically in the gastrointestinal tract, liver and blood–brain barrier with secretory/excretory function [51,52].

The first description of an acquired non-genetic mechanism involved in MDR was by Levchenko et al. [53]. They showed that co-culturing P-gp-positive cells with P-gp-negative cells results in direct cell–cell transfer of P-gp and expression of P-gp in recipient P-gp-negative cells [53]. An elegant study by Bebawy et al. in 2009 [54] showed the involvement of MVs in transferring P-gp from chemotherapy-resistant cells into chemosensitive leukaemic cells and conferring drug resistance in target leukaemic cells.

**Conclusions and future perspectives**

Drug-resistance mechanisms compromise a number of pharmacokinetic properties of drugs such as reduction of exposure of cellular targets to active drug species so that the tumour demonstrates a pharmacokinetic resistance to chemotherapeutic agents [40]. A significant proportion of cancer cells are located at a short distance from the nearest blood vessel. This factor has a crucial role in generating a local microenvironment and provides an acidic pH and hypoxia. In addition, the aberrant structure affects the intratumour pharmacokinetics of drugs leading to poor drug distribution and failure to expose the most distal regions to chemotherapy [55]. In many tumours, the lack of functional vessels prevents drugs from complete distribution. Following administration of chemotherapeutic agents, the plasma membrane of the cells comprising the tumour represents the first barrier, which impedes drug efficacy [7]. Consequently, there is a pressing need to develop new and more effective treatment strategies. MV trafficking is a significant process in tumorigenesis, so its alteration can contribute to MDR [16]. MVs carry and transfer adhesion molecules to target cells, which provide a platform for target cell binding [16]. The involvement of both P-gp and MRP1 as efflux transporters has been reported in MDR [10]. The significant role of MVs in transfer of P-gp and development of MDR requires consideration as a strategy to prevent MDR. One mechanism that contributes to cancer drug resistance is cancer cells’ ability to evade chemotherapeutic treatment by increasing active drug efflux. There is mounting evidence that the release of extracellular MVs is a highly important process in tumorigenesis [56]. Inhibition of MV release has the potential to be considered as a treatment strategy for circumvention of MDR. Finally, considering the newly recognized biological importance of cell-derived vesicles, there is therapeutic significance as they could potentially be used diagnostically to predict therapeutic response and guide individualized treatment strategies in cancer patients.

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