

EXOSOMES IN CANCER METASTASIS: NOVEL TARGETS FOR DIAGNOSIS AND THERAPY?

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Abstract

Tumour cells secrete small membrane vesicles (30-100nm) termed exosomes that are derived from late endosomal compartments of cells. Exosomes are increasingly being recognised for their role in intercellular communication in the tumour microenvironment. Exosomes contain an array of proteins, messenger RNA (mRNA) and micro-RNA (miRNA), which are transferred from donor to target cells, thereby mediating local and systemic cell communication. Here, we review the functional role tumour-derived exosomes play in controlling tumour progression and its metastasis, as well as the potential to use exosomes in the diagnosis and targeting of cancers.

The cross-talk between cancer cells and their surrounding stroma is essential in regulating tumour progression and systemic spread. While tumour cells are classically known to communicate via direct cell-to-cell contact and the secretion of soluble factors, alternative novel mechanisms have recently emerged. Evidence suggests that small membrane vesicles, termed exosomes, contribute to the intercellular cross-talk and subsequent reprogramming of the tumour microenvironment.¹ Exosomes are microvesicles of endocytic origin (from inside the cell) with a size of 30-100nm that are released under both physiological and pathological conditions. Originally described as a mechanism for the removal of redundant molecules from reticulocytes (immature red blood cells), it is now clear that exosomes have a much more significant biological role.¹

Exosomes are generated from secretory multivesicular bodies (MVB; late endosomes) that fuse with the plasma membrane for release into the extracellular environment. The release of exosomes has been observed in various cell types, including immune cells, epithelial cells, fibroblasts and various cancer cells.²⁻⁵ They contain lipids, proteins, messenger RNA (mRNA) and micro-RNA (miRNA), which are transferred from donor to target cells.^{1,6} In addition to canonical proteins commonly found in most exosomes, exosomes contain cell-type specific content in the form of biological molecules that can have significant impact on the phenotype of recipient cells (figure 1).⁷ Indeed, tumour-derived exosomes can transport various biological molecules that are postulated to assist cancer progression and epigenetic reprogramming to increase oncogenic potency of cancer cells.⁷ Importantly, the concentration of exosomes is often increased in the blood of cancer patients compared to normal controls,⁸⁻¹⁰ and while the mechanisms for this observation have not been fully elucidated, common microenvironmental cues in tumours, such as hypoxia and low pH, have been implicated in exosome secretion.^{11,12}

Functional role of exosomes in tumour progression

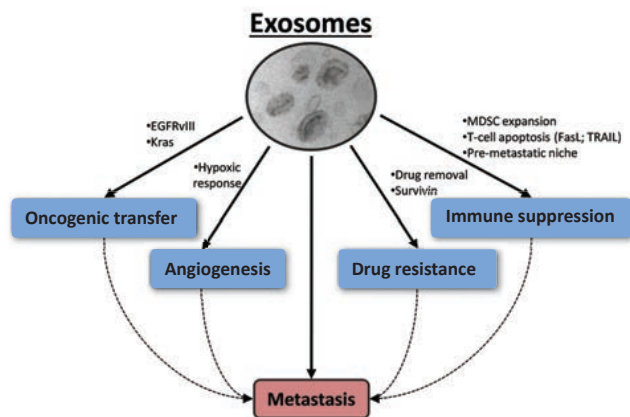
The tumour microenvironment is a complex system intimately linked by many cell types, including cancer cells, vascular cells, leukocytes, antigen-presenting cells and fibroblasts.¹³ The tissue milieu is integral in determining the functionality, physiology and spread (metastasis) of cancer. Progression of the primary tumour is often characterised by the accumulation of genetic change, vascular growth (neo-angiogenesis), increased proliferation and subsequent invasion/metastasis to distant organs. Increasing evidence suggests that the rich array of proteomic and genomic information carried by tumour-derived exosomes is a novel mechanism by which cancer cells modify surrounding stroma and malignant cell behaviour.⁷ Exosomes can affect signalling processes involved in neo-angiogenesis,¹⁴ and immune suppression,¹⁵ and induce drug resistance and oncogenic transfer.¹⁶⁻¹⁸ Moreover, the ability of exosomes to induce systemic changes is thought to promote metastatic dissemination, which accounts for a majority of patient deaths,¹⁹ as discussed later.

Oncogenic transfer

Recent findings indicate that exosomes shuttle both mRNA and miRNAs,²⁰⁻²² suggesting their involvement in the exchange of genetic information to recipient cells. This was first demonstrated by Valadi et al. showing that exosomes from mouse mast cells shuttle RNAs that can be transferred to human mast cells.²² Importantly, mRNA transcripts from donor exosomes may be translated into proteins in recipient mast cells, indicating that transferred mRNAs have functional consequences. Similarly, lung-derived exosomes can transfer lung-specific mRNA to marrow cells.²³ Analysis of the mRNA profile and proteome of marrow cells after internalisation of the lung-derived exosomes were vastly different compared to untreated cells, further suggesting that transferrable mRNAs are functional.²³ Moreover, endothelial cells exposed to

exosomes containing GFP mRNA transcripts subsequently produce GFP protein after their uptake, indicating the active translation of transcripts delivered by exosomes.

Figure 1: Exosomes are small vesicles released by cells into the extracellular milieu. Exosomes can carry signalling molecules that have diverse roles in promoting the growth and metastasis of tumours.



The transfer of oncogenic proteins by exosomes has also been reported (figure 1).¹⁷ Exosome transfer in glioma cells has recently been demonstrated to enhance tumorigenesis through delivery of a mutant epidermal growth factor receptor (EGFRvIII) isoform, resulting in increased expression of anti-apoptotic genes and enhanced proliferation.¹⁷ Similarly, colon cancer cells with a mutant form of KRAS are capable of enhancing the three-dimensional growth of wild-type KRAS colon cells via exosomal transfer of mutant KRAS to the wild-type cells. Additionally, non-metastatic melanoma cells can be induced to become more metastatic by the uptake of exosomes derived from a highly metastatic melanoma cell line.²⁴ However, whether this change in metastatic potential is permanent remains unclear.

Angiogenesis

Angiogenesis is a physiological process involving the growth of blood vessels. In a cancer context, neo-angiogenesis is necessary to overcome hypoxia and for continued tumour growth.²⁵ Indeed, a pro-angiogenic tumour profile is closely associated with poor patient survival.^{26,27} Recent studies have demonstrated that exosomes are a key mediator of hypoxia-dependent intercellular signalling between malignant and vascular cells to exert a pro-angiogenic response (figure 1).²⁸⁻³⁰ Exosomes derived from glioblastoma cells grown under hypoxic conditions significantly induced microvascular sprouting *ex vivo*, and enhanced vascularisation of tumours in xenograft models leading to accelerated tumour growth.¹⁴ Interestingly, these exosomes were enriched for several hypoxia-associated proteins, some of which are predictive of poor prognosis in glioma patients. Similarly, exosomes isolated from hypoxic squamous carcinoma cells enhanced angiogenic and metastatic potential by reducing blood vessel branching, and cell-extracellular matrix adhesion.²⁹ Furthermore,

tumour-derived exosome secretion can also be enhanced by low pH.¹² Given this, exosomes may serve as novel biomarkers in which to ascertain low oxygen tension, and therefore potential of disease progression in patients with solid malignancies.

Exosomes and immuno-suppressive mechanisms

Studies have shown that tumour-derived exosomes can suppress specific T-cell immunity and induce innate immune cells towards a pro-tumour phenotype.^{15,19} Exosomes derived from human colorectal and melanoma cells impair the differentiation of peripheral blood monocytes to functional dendritic cells, instead skewing them towards the phenotype of myeloid-derived suppressor cells (MDSCs).³¹ MDSCs are immature myeloid cells with various immunosuppressive functions, including the suppression of T-cell immune responses.³² Exosomes isolated from tumours of a breast carcinoma model promoted the accumulation of MDSCs via an exosomal prostaglandin E2 and TGF-beta mediated pathway,¹⁵ which enhanced tumour growth. This is important, as numerous studies have found that increases in MDSC populations correlate with poor prognosis and overall survival in cancer patients.³²

The immunosuppressive role of tumour-derived exosomes is not solely limited to prompting MDSC differentiation, but also the induction of apoptosis in recipient T cells. Various cancer cell types are described to secrete exosomes capable of inducing apoptosis in activated T cells by the transfer of the death ligands FasL and TRAIL.^{33,34} For example, exosomes isolated from sera of patients with ovarian cancer are enriched with FasL and can suppress the CD3-zeta chain of T-cells to induce T cell apoptosis.³⁴ Furthermore, the pre-treatment of mice with exosomes derived from mammary tumour cells was shown to accelerate tumour growth, an effect that was due to the suppression of IL-2-mediated activation of NK cells and their cytotoxic response to tumour cells.³⁵ Taken together, the data suggests exosomes are a major contributor in the immunosuppressive tumour microenvironment (figure 1).

Exosomes and resistance to anti-cancer therapies

Exosomes have a role in the development of drug resistance to current anti-cancer therapies. Multi-drug resistance pumps that are commonly associated with drug resistance of tumours *in vivo*, are capable of being transported between cells via exosomal communication.³⁶ Furthermore, various anti-cancer therapies, including radiation and certain cytotoxic drugs, enhance exosome secretion by cancer cells.^{37,38} Not only does the rate of exosome secretion increase, proteomic studies have also revealed that exosomes derived from cells exposed to anti-cancer therapies are enriched with anti-apoptotic proteins. For example, in response to radiation, HeLa cell exosomes are enriched in the protein Survivin,⁹ which is known to play a role in the suppression of cell death and regulation of mitosis. This suggests exosomes can be utilised by cancers as a form of self-protection. Exosomes may also be capable of directly reducing the efficacy of cytotoxic drugs by removing the drug from the cell before it has been capable of inducing any cytotoxicity.³⁹ This phenomena

has been shown in cisplatin-resistant cancer cells, where cisplatin is enriched within the exosome compared to the cytoplasm of parental cells.³⁹ Moreover, exosomes may be capable of sequestering targeted antibody therapeutics. For example, HER2-enriched exosomes, derived from HER2-overexpressing breast carcinoma cell lines, were shown to sequester and abolish the therapeutic activity of Trastuzumab.¹⁸ To this end, exosomes may be capable of carrying out a multi-faceted role in allowing cancer cells to evade the effects of current therapies (figure 1), and supports the concept that exosome secretion is an essential component enabling cancer cell survival under stressful conditions.

Functional role of exosomes in tumour metastasis and pre-metastatic niche establishment

Metastatic disease is responsible for over 90% of cancer-related deaths, and very few therapies have proven successful in the clinic for treating patients with metastatic deposits.⁴⁰ Therefore, we need to understand the underlying mechanisms of metastasis in order to generate novel, effective therapies for patients with advanced, aggressive cancers. The metastatic process comprises a number of sequential events that tumour cells are required to accomplish in order to successfully disseminate and implant in secondary organs. This complex multi-step process is known as the metastatic cascade, a series of systematic steps involving local invasion, survival and evasion of immune responses, intravasation into the circulation, extravasation at secondary organs, and finally proliferation of macroscopic metastatic tumour deposits.⁴¹ The seeding of cancer cells at secondary organs is not random, a concept originally recognised over 100 years ago with Paget's 'seed and soil' hypothesis.⁴¹ Accumulating evidence highlights that primary tumours can secrete factors capable of priming distant tissues for the arrival of cancer cells, thereby creating a supportive microenvironment termed a pre-metastatic niche.⁴²⁻⁴⁴

For both processes, the metastatic cascade and pre-metastatic niche formation, exosomes have been reported to be critical (figure 1).¹⁹ Enhanced aggressiveness due to exosomal communication has been demonstrated with prostate and lung cancer. Exosomes secreted from these cancer cells can modify the phenotype of stromal cells by up-regulating MMP-9, leading to increased angiogenesis, motility and enhanced resistance to apoptosis.⁴⁵ Furthermore, exosomes from melanoma cell lines are capable of increasing pre-metastatic niche formation and metastatic burden in mice.¹⁹

Exosome mediated promotion of metastasis is not solely limited to tumour-secreted exosomes. Stromal cells are also capable of enhancing metastasis, demonstrating the complex nature of the communication between normal and malignant cells. In vitro findings have shown that exosomes from untransformed stromal cells increase the motility and invasiveness of several cancer cell lines,⁴⁶ with mesenchymal stem cell exosomes shown to promote migration of MCF7 breast cancer cells through upregulation of WNT signalling.⁴⁷ Human primary cancer

associated fibroblasts also increase motility and metastasis of several breast cancer cell lines by secreting CD81 positive exosomes.⁴⁸ Moreover, exosomes from activated T-cells are capable of promoting invasion, and have been demonstrated to enhance the migration of murine B16 melanoma cells to the lung.⁴⁹

Relevance of exosomes in clinical diagnosis and cancer therapy

Exosomes as biomarkers in diagnosis

Currently, there is a large unmet need to develop non-invasive and informative diagnostic markers for a variety of solid malignancies. The proteomic and RNA information contained in tumour-derived exosomes has generated significant interest for the use of exosomes as a non-invasive diagnostic tool. As exosome isolation techniques are now well established, and because exosomes are stable in bodily fluids, including serum, urine and saliva, they demonstrate great potential as reliable biomarkers of disease progression.¹ Given that exosomes may provide molecular signatures of their cell of origin, proteomic and RNA analysis may also provide an efficient means to determine oncogenic mutations. Moreover, exosomes derived from patients may prove useful in understanding the progression and treatment options for the disease. This has already been demonstrated with exosomes isolated from melanoma patients, which exhibited high protein content and elevated expression of TYRP2, VLA 4 and HSP70, proteins that were enriched in patients with a poor prognosis.¹⁹ Not surprisingly, several commercial companies are developing exosome-based diagnostic tests to assist in determining diagnosis, drug response and prognosis in cancer patients, which are currently undergoing clinical validation.¹

Exosomes as therapeutic targets

Given that tumour-derived exosomes are capable of reprogramming tissue microenvironments to support tumour progression, it may be beneficial to selectively deplete exosomes in circulation. Conversely, some researchers believe exosomes may be used as a potential vaccine for cancer immunotherapy. For example, tumour-derived exosomes transduced to express MHC class II molecules possess enhanced capability of immune stimulation and can subsequently reduce tumour progression in immunised mice.⁵⁰ Using a similar approach, several studies have reported the potential use of dendritic cell-derived exosomes for cancer immunotherapy.^{51,52} Dendritic cell-derived exosomes were generated to contain functional MHC peptide complexes and further processed by the attachment of tumour antigens. These processed exosomes were shown in several phase I studies to be capable of inducing T-cell immune responses and tumour regression.⁵¹⁻⁵³ However, the efficacy of these approaches may be limited, given the immunosuppressive effects of tumour-derived exosomes in vitro and in vivo.

A more promising method may be to deplete exosome numbers with drugs such as dimethyl amiloride and its analog amiloride, which blocks H⁺/Na⁺ and Na⁺/Ca²⁺ channels associated with exosome secretion. Dimethyl

amiloride was shown to mitigate the immunosuppressive effect of exosomes and enhance the chemotherapeutic drug, cyclophosphamide.⁵⁴ This effect was mirrored in patients with colorectal cancer, where the administration of amiloride to treat hypertension also inhibited exosome formation, and reduced immune suppressive functions.⁵⁴ Exosome secretion can also be inhibited by sphingomyelinase inhibitors (e.g. GW4869), which prevent the formation of exosomes through depletion of ceramide.⁵⁵ This possibility is supported by work showing that GW4869 administered to Lewis Lung carcinoma bearing mice had significantly fewer lung metastases.⁵⁶ These approaches suggest that the further development of inhibitors specific for exosome secretion may be warranted.

Recently, novel approaches to remove circulating tumour-derived exosomes have been proposed. These involve the use of extracorporeal hemofiltration devices,⁵⁷ a process that would avoid the risk of drug toxicity associated with pharmacological approaches. Using this approach, patient blood is passed through porous fibres that contain affinity-capture moieties for exosomes, which are selectively absorbed and depleted from the blood. The safety and efficacy of these devices has already been demonstrated in other disease settings. For example, viral removal in patients infected with hepatitis C has demonstrated extracorporeal hemofiltration to be well tolerated and effective at reducing viral load.⁵⁷ These systems are currently being evaluated for their efficacy in capturing tumour-derived exosomes present in biological fluids of cancer patients. However, the biological impact of depleting exosomes derived from non-malignant cells remains unclear.

Future directions

Given that tumour-derived exosomes display unique proteomic and RNA content, together with established methods of recovery from a range of body fluids, they represent a class of novel targets for biomarker analysis and therapeutic intervention. Importantly, questions remain on how informative exosomes can be in detailing the aggressiveness and clinical response in patients undergoing therapy. The potential of exosome depletion as a therapeutic adjunct in cancer patients also poses exciting possibilities for future clinical therapies. However, these approaches are still in their infancy, and more research is required to fully understand the role exosomes play in tumour progression, and their potential efficacy in the diagnosis and treatment of cancer patients.

Acknowledgments

We would like to thank the members of the Tumour Microenvironment Laboratory at QIMR Berghofer for critical reading and feedback of the article. AM is supported by a fellowship of the National Breast Cancer Foundation Australia (ECF-11-09) and the laboratory is supported by grants from the National Health and Medical Research Council (APP1068510), Rio Tinto Ride To Conquer Cancer (QIMR Berghofer), Cancer Council Queensland (APP1045260) and the National Breast Cancer Foundation Australia (NC-13-26).

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CURRENT INSIGHTS INTO CLINICAL DORMANCY AND METASTASIS

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Abstract

Survival after breast cancer diagnosis and treatment has improved markedly, however recurrence is still a treatment challenge for clinicians. The underlying cause for late recurrence after a long disease-free period is still unknown. It is known that undetectable metastases exist in two states, with different mechanisms playing a role in each dormancy state. In tumour mass dormancy, extrinsic factors such as angiogenesis and immune surveillance are in equilibrium with the tumour cells to maintain dormancy. In tumour cell dormancy, mechanisms intrinsic to the isolated tumour cells can dictate a dormant cellular state. The process of senescence, or pathways leading to cell cycle arrest, may be the key to unlocking the mystery behind these tumour cell deposits. With greater knowledge of the mechanisms that control undetectable disseminated disease, we have the opportunity to target these pathways to enable therapeutic strategies against metastatic disease.

Survival after a cancer diagnosis has improved markedly with the advancement of modern therapies, leading to a shift in the paradigm of cancer epidemiology towards

chronic disease. With greater longevity, there is a new challenge in treating patients with metastatic recurrence long after successful treatment of the primary tumour.¹