Chapter 19

Cross-Talk Between Hypoxia and the Tumour via Exosomes

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Abstract

Cancer is one of the leading causes of death worldwide, and this is often attributed to the nonspecific symptoms. Additionally, delayed diagnosis and a lack of treatment options negatively impact prognosis. Recently, the role of extracellular vesicles in cancer progression, specifically, in metastasis and in the capacity of several tumours to invade and colonise specific organs has been established. Reduced oxygen tension due to imbalanced oxygen supply and consumption is termed hypoxia and is one of the most commonly observed features in solid tumours. This is often correlated with poor cancer prognosis. Several reports have established that low oxygen tension (i.e. hypoxia) is a common feature of the tumour microenvironment often enhancing the process of epithelial-to-mesenchymal transition (EMT) in cancer cells, thus promoting tumourigenesis and metastasis. Furthermore, hypoxia increases the number of extracellular vesicles released from cancer cells and also modifies their bioactivity and function. The aim of this chapter is to review the association between the tumour microenvironment and extracellular vesicles (EVs), focusing on a specific subpopulation of EVs of endocytic origin, termed exosomes.

Keywords: exosomes, metastasis, metastatic niche, tumourigenesis, cancer

1. Introduction

The global burden of cancer is on the rise and in 2012 around 14.1 million new cases were reported with 8.2 million deaths attributed to cancer [1]. Cancer can be subdivided into categories

depending on the area that is affected, including but not limited to lung cancer, pancreatic cancer and ovarian cancer [2, 3].

Consequently, the development of targeted treatments for a large population is difficult due to the heterogeneity of the tumours. Furthermore, in cases such as ovarian cancer, current treatments, which include the use of platinum-based cytotoxic chemotherapy, antiangiogenic drugs and poly (ADP-ribose) polymerase inhibitors, are only beneficial for patients with early stage disease [2]. However, in patients with more advanced stage disease, there is often recurrence of the disease after treatment due to the development of resistance [2]. Therefore, it is essential that diagnostic procedures be explored.

This paradigm shift from focusing on treatments to focusing on early diagnosis of cancer has brought exosomes to the forefront.

Exosomes are small membranous vesicles that are released following the fusion of multivesicular bodies (MVBs) with the cell membrane. They have multiple characteristics including a cup or spherical shape, maximum diameter of approximately 100 nm, a buoyant density of ~1.12 to ~1.19 g /mL on a sucrose gradient, endosomal origin and the enrichment of late endosomal membrane markers, including TSG101 and proteins from the tetraspanin family (e.g. CD63) [3, 4]. Exosomes are covered in a variety of cell surface receptors and contain several proteins such as cytoskeletal proteins, adhesion molecules and heat-shock proteins. Additionally, they encapsulate diverse miRNA and mRNA, which can impact the bioactivity and functionality of the target cells with which the exosomes interact.

While the role of exosomes during tumour progression remains to be fully established, we postulate that tumour cells release exosomes loaded with specific molecules in response to the microenvironment to prepare for and promote metastasis to specific organs.

2. Exosomes: a specific type of extracellular vesicle

Cells secrete a multitude of EVs of different origin, size, content and function. Recent reports have recognised a specific type of extracellular vesicle termed exosomes. Exosomes are believed to be tumour 'couriers', carrying signals and relocating packages of signalling molecules to initiate processes such as metastasis by preparing the metastatic niche [5, 6].

In contrast to other EVs, which are formed by an inward budding of the plasma membrane, exosomes are secreted through the intraluminal invagination of vesicles termed early endosomes [7]. This leads to the formation of multivesicular bodies (MVBs) which contain intraluminal vesicles (ILVs). These ILVs are then released by the cell through the fusion of the MVB with the cellular membrane. The released ILVs are termed exosomes [4, 5, 8]. Exosomes carry a common set of molecules along with cell-specific components. Therefore, exosomes contain proteins which are associated with the biogenesis of MVBs such as tetraspanins, Rab GTPases and Annexins [9]. The endosomal-sorting complex required for transport (ESCRT) pathway facilitates plasma membrane remodelling and is also believed to have a role in ILV formation

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[10]. Research has also shown that other pathways independent of the ESCRT complex also exist, as an MVB is also formed when the ESCRT complexes are repressed [11, 12, 14, 15].

Whilst the biogenesis of exosomes has been well understood and defined in recent literature, a consensus on the method to extract exosomes is yet to be established. However, a detailed discussion of the current methodological approaches is beyond the scope of this chapter [16, 17]. A NanoSight Tracking Analysis (NTA) comparison between exosomes and microvesicles is shown in **Figure 1**.

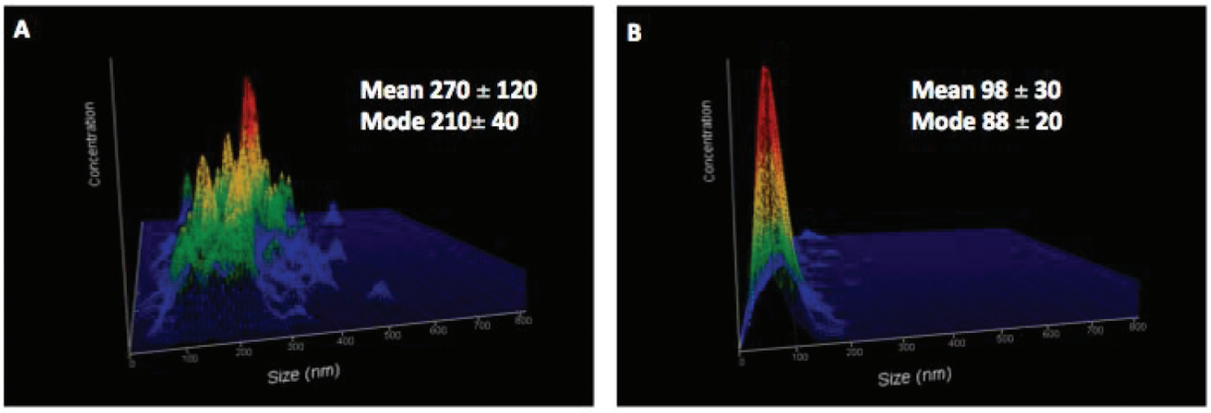

Figure 1. Nanoparticle-tracking analysis using the NanoSight. Representative image of the size distribution of 100,000 g pellet (A) and exosomes (B). The NanoSight instrument measured the rate of Brownian motion of nanoparticles and consists in a light-scattering system that provides a reproducible platform for specific and general nanoparticle characterization (NanoSight Ltd., Amesbury, UK).

Nonetheless, the requirement for a standard isolation procedure is essential as research moves towards examining exosomes as potential therapeutic agents in the context of several diseases such as cancer. Additionally, exosomes are being used to understand the characteristics of the solid tumour, circulating tumour cells and the tumour microenvironment, especially under conditions such as hypoxia.

3. The tumour microenvironment and hypoxia

Under normal conditions, the cellular microenvironment inhibits the development of cancerous cells through tumour interactions thus allowing the environment to annihilate the growth of cancerous cells. The tumour microenvironment comprises endothelial cells (ECs), fibroblasts, perivascular cells and inflammatory cells. These components tend to control the tumourigenic processes—that is, angiogenesis, desmoplasia, lymphangiogenesis and inflammation. Oxygen deficiency in tumour cells, also known as hypoxia, is among the major factors that trigger tumour development and hinder clinical diagnoses [13–15].

An imbalance between oxygen supply and demand causes hypoxic or anoxic conditions. The oxygen supply rate is equivalent to that of metabolic requirements in a normal cell or a tissue.

However, in developed solid tumours, the oxygen consumption rate may fluctuate to adjust for the insufficient oxygen supply, allowing the tissues to develop even in regions with low oxygen levels [15]. Accumulating evidence suggests that up to 60% of locally advanced tumours display hypoxic (≤1% O_2 compared to 2–9% O_2 or 40 mm Hg on average in most mammalian tissues) and/or anoxic (≤0.01% O_2 or undetectable oxygen) areas distributed heterogeneously throughout the tumour and that tumour hypoxia correlates with advanced stages of malignancy [16]. In cancer cells, respective mechanisms are activated to respond to changes in the availability of oxygen. The cells are subjected to lower levels of oxygen and must therefore modify their metabolism, respectively. In such conditions, the hypoxia-inducible factor-1 (HIF-1) transcription factor programme of gene expression changes. This change in expression is assumed to enable the cell to cope with the new environment [17–19].

HIF-1 is a heterodimer complex consisting of two bHLH transcription factors: HIF-1α and HIF-1β [20]. HIF-1α expression is significantly overexpressed in advanced ovarian tumours. O_2-dependent mechanisms primarily regulate HIF-1α degradation. Under normoxic conditions, the O_2-dependent hydroxylation of proline residues in HIF-1α by prolyl hydrolase-domain protein is recognised by the von Hippel-Lindau tumour-suppressor protein and ubiquitinated to be targeted for degradation. Under hypoxic conditions, HIF-1α stabilises and accumulates due to the inhibition of hydroxylation and von Hippel-Lindau protein-mediated ubiquitination, translocates to the nucleus and forms a complex with HIF-1β and a transcriptional co-activator CBP/p300 to activate the transcription of target genes by directly binding to their hypoxia-responsive elements [21].

During hypoxia, HIF-1 activates genes involved in proliferation, cell survival, angiogenesis, vascular tone, metal transport, glycolysis, mitochondrial function, cell growth and survival, and apoptosis and EMT, which all contribute to tumour progression. HIF-1-dependent expression of erythropoietin and angiogenic compounds further enhances the formation of blood vessels and thus facilitates the delivery of oxygenated blood to the hypoxic tissue through the induction of vascular endothelial growth factors (VEGFs). In vitro studies show that increasingly subjecting cancer cells to a hypoxic stimulus results in a gradual increase in VEGF mRNA levels and VEGF protein levels [22, 23].

The addition of HIF-1-induced glycolytic enzymes provides energy as a substrate for oxidative phosphorylation when mitochondria are starved of oxygen [17]. Moreover, due to lower levels of oxygen and nutrients, the ATP level decreases causing a deregulation in the actin cytoskeleton controlled by the down-regulation of Rho proteins. Rho kinase facilitates contractile force generation mediated by actin-myosin by phosphorylating a number of target proteins. Rho/Rho kinase plays a critical role in movement, penetration, cell-cell adhesion, smooth muscle contraction, cytokinesis, mitosis, multiplication, variation, apoptosis and oncogenic transformation within the cell [24–28].

Tumour hypoxia and HIF-1α overexpression have been demonstrated to induce EMT and metastatic phenotypes in cancer cells, yet the crosstalk between the HIF-signalling pathway and EMT is not completely understood [29]. Several studies propose potential molecular mechanisms, such as HIF-1-promoting EMT through the up-regulation of EMT transcriptional factors. Nonetheless, it is known that HIF-1 regulates TWIST expression by binding to their

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hypoxia-responsive elements. Thus, cells cultured under hypoxia or constitutive HIF-1α expression promoted EMT, whereas the repression of TWIST expression abolished the effect of HIF-1α, shifting the cells back to an epithelial phenotype from the mesenchymal phenotype [29]. HIF-1 expression induced by hypoxia represses E-cadherin-coding genes through SNAI1 and SNAI2 [30–32]. Along with transcriptional factors, hypoxia and HIF-1 activate EMT-associated signalling pathways. Hypoxia also activates the Wnt/β-catenin-signalling pathway by inhibiting GSK3β activation, preventing β-catenin phosphorylation and destruction to increase SNAI1 expression [33]. HIF-1 further interacts with the Notch intracellular domain to increase its transcriptional activity [34].

The Notch-targeted genes HES1 and HEY1 were increased under hypoxic conditions; however, a knockdown of HIF-1α abrogated the hypoxia-induced HES1 and HEY1 expression as well as the SNAI1 expression [35]. Furthermore, HIF-1α targeted lysyl oxidase and lysyl oxidase-like 2 and 3 enzymes, which promote tumour metastasis by mediating cells to matrix adhesion and stabilising SNAI1 activity to induce EMT [36, 37]. Under hypoxia, the consumption of glucose and GLUT1 expression in cancer cells increased as well [17].

It is also well established that bidirectional communication between cancer cells and their tumour microenvironment is essential for cancer progression. For example, most ovarian cancer patients present with ascites—excess fluid in the peritoneal cavity [38]. Ovarian cancer ascites contain molecular factors, including VEGF, cytokines, chemokines and TGF-β, to mediate cellular communication for effective tumourigenesis. Accumulating evidence suggests that cellular communication is not only limited to secretary molecules, but also includes EVs (such as exosomes) that mediate such communication [39]. The nomenclature of EVs is still a matter of debate due to the many terms used (e.g. microvesicles, nanovesicles, shedding vesicles and ectosomes), emphasising the range of EV populations secreted [9].

However, during tumourigenesis, hypoxia serves as a selective agent at various physiological levels. Under hypoxia, a number of transcriptional factors control the cell environment, including Nuclear Factor-kappaB (NF-κB), Activating Transcription Factors (ATFs) and p53s [40–42]. In NF-κB pathways activated by HIF during irregular hypoxia and re-oxygenation [41, 43] and ATF, anoxia drives signalling [44].

Moreover, carbonic anhydrase IX (CAIX) is among the genes in the hypoxic environment of solid tumours that increasingly express themselves. CAIX expression is perceived as causing bladder, ovarian, cervical, colorectal, oral, brain and breast cancers. It enables the balancing of intracellular pH through the extracellular hydration of CO_2 and the production of bicarbonate and protons. The bicarbonate goes back into the cell through bicarbonate transporters and balances the intracellular pH as alkaline, which is favourable for the cell's survival. The protons acidify the extracellular space, thus facilitating the tumour's migratory and invasive behaviour [45–47]. CAIX expression and activity also facilitates the production of Granulocyte-colony stimulating factor (G-CSF),which is in turn required for the transportation of granulocytic Myeloid-derived Suppressor Cells (MDSC) to the metastatic niche—an environment that promotes metastasis. CAIX expression is also required to stimulate NF-κB activity and G-CSF production mediated through hypoxia. The hypoxia-mediated NF-κB activity is triggered by a decrease in the pH level of the culture as well as hypoxia-induced glycolytic

activity in the cancer cells [46, 48–50]. The hypoxic areas of tumours usually have lower levels of extracellular pH due to increased metabolic activity [45]. It has been proven that the cells are hampered from acidifying the medium due to a smaller production rate of CAIX in a hypoxic environment [51]. Therefore, hypoxia and the tumour microenvironment are essential factors in regulating disease progression and metastasis.

4. Exosomes, the tumour microenvironment and hypoxia

An evaluation of cancer cells and their microenvironment plays a critical role in hypoxia. Tumour cells under hypoxia secrete molecules that modulate their microenvironment and facilitate tumour angiogenesis and metastasis. Hypoxia is a major hallmark of the tumour environment and is caused when there is a lack of blood supply. The lowered blood supply indicates a lower number of red blood cells being able to reach the tumour cells resulting in decreased oxygen delivery [52]. Moreover, hypoxic tumours have a greater ability to resist standard treatments and the tumour cells are often in a less differentiated or more stem cell-like state [53]. Emerging evidence has shown that exosomes are key membrane vesicles secreted by most cell types under hypoxia. It has also been shown that they have an ability to modulate the tumour microenvironment to ensure adequate nutrition and oxygen supply [54]. There has been an increasing interest in the role of exosomes as a mediator of cell-to-cell communication and its role in ultimately aiding cancer progression.

There have been several processes proposed regarding the release of exosomes into the tumour microenvironment. These processes involve several molecules such as proteins involved in fusion of the multivesicular bodies as well as plasma membrane proteins. Additionally, it has been shown that exosomes present in a cell's environment also regulate exosome release. Riches and colleagues showed that when exosomes were added to the culture medium of cells, the number of exosomes released by the cells decreased evidently [55]. Other proteins that may be involved in increased exosome secretion during hypoxia include the Rab family of proteins, specifically Rab27 as they regulate exosome secretion. The Rab27 protein has two isoforms: Rab27a and Rab27b. Ostrowski and colleagues noted that inhibiting Rab9a, Rab5a, Rab27a, Rab27b and Rab2b led to an inhibition in exosome release [35]. Furthermore, it has been previously shown that the presence of calcium (Ca^{2+}) ionophores can lead to an increase in the release of exosomes [56]. Therefore, although there are several hypotheses, the exact mechanism is still unclear. Thus, the mechanisms underlying exosome release under different tumour microenvironmental conditions such as hypoxia remain to be elucidated. Nonetheless, progress is being made.

The role of exosomes in tumour progression and invasion has been highlighted in literature with a clear correlation being found between the number of hypoxic exosomes released and the aggressiveness of the tumour [57, 58]. A significant increase in the number of exosomes released under hypoxia (1% oxygen) and severe anoxia (0.1% oxygen) was found in a study conducted on three breast cancer cell lines, in which the impact of hypoxia on tumour progression and the release of exosomes was investigated [58]. King and colleagues postulated that the enhancement of exosome release might be mediated by the hypoxia-inducible factor 1 oxygen-sensing pathway (detailed above). They tested their hypothesis by using the HIF

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hydroxylase inhibitor, Dimethyloxalylglycine (DMOG), to treat the breast cancer cell line, MDA-MB-231 [58]. The role of the DMOG was to trigger an HIF response. This led to a minor although significant rise in the number of exosomes secreted by the cells when quantified by nanoparticle-tracking analysis (NTA). Moreover, when the HIF-1α transcription factor was silenced using siRNA, the increase in exosomes in response to hypoxia was not seen. Therefore, it was concluded that the HIF pathway may have a significant role in the release of exosomes in response to hypoxia. Similar studies were carried out on different cell lines (e.g. leukaemia cell line, K562; human microvascular endothelial cells (HMEC-1); A431 squamous carcinoma; A549 non-small-cell lung (NSCL); H1299 NSCL and HFF-1 foreskin fibroblast cells) to investigate the level of exosomes released under hypoxia and normoxia [57]. The outcome was that the number of exosomes released under hypoxic conditions increased when compared to exosomes released by cells under normoxic conditions in the same amount of time. However, the pathways underlying the hypoxic enhancement of exosome release were unclear [15].

In addition, oncogenic miR-21 was identified at a significant level in exosome fractions [59, 64]. miR-21 is known to down-regulate programmed cell death 4 (PDCD4) expression by directly targeting its 3′-untranslated region. Moreover, it was found that exosomes isolated from peritoneal effusions (ovarian cancer) contained low PDCD4 expression, whereas oncogenic miR-21 was highly expressed compared to exosomes isolated from non-neoplastic peritoneal effusions [59]. The use of exosomal miR-21 as a biomarker for cancer diagnosis has been suggested in several studies as it exists in almost all bodily fluids, is stable and is protected from degradation [60]. Exosomal miR-21 has an effect on a number of signalling pathways which promote metastatic capacity and proliferation. It has been found that miR-21 suppresses phosphatase and tensin homolog expression and promotes the growth and migration of tumour cells [61]. miR-21 also regulates cellular functions by influencing signal transduction, proliferation, carcinogenesis, differentiation and immune response [62–64]. These observations provide key evidence that elevated exosome release under hypoxia is a critical factor affecting tumour proliferation.

Tumour-derived exosomes have the ability to transfer oncogenic activity among tumour cells. Human glioma cells can horizontally transfer an oncogenic form of epidermal growth factor variant III (EGFRvIII) to glioma cells lacking EGFRvIII [65]. The transfer results in an increased expression of the pro-survival gene and a reduction in the cell cycle inhibitor, increasing anchorage-independent growth capacity [65]. An interesting possibility that exosomes are key factors that affect the neighbouring cells is provided by these studies.

Exosomes facilitate communication among tumour cells and contribute to the development of a favourable microenvironment for tumour progression by enhancing processes such as angiogenesis. Angiogenesis is promoted by the activation of endothelial cells through tumour-derived exosomes, and is followed up by the activation of myofibroblasts, a source of matrix-remodelling protein [66, 67]. Tumour-derived exosomes trigger fibroblast to myofibroblast differentiation [68]. In addition to fibroblasts, exosomes can trigger conversion of mesenchymal stem cells from the tumour stroma and adipose tissue to myofibroblasts [69]. The exosomes also contribute to the formation of pre-metastatic niches by educating the bone marrow-derived cells (BMDC). BMDCs when combined with exosomes derived from highly and poorly

metastatic melanoma cells accelerated primary tumour growth and also increased the magnitude and number of metastases [6]. Additionally, evidence has shown that exosomes interact with immune cells to suppress antitumour responses and skew them towards the pro-tumourigenic phenotype [70]. Exosomes from hypoxic endothelial cells (EC) show up-regulation of collagen crosslinking activity by activation of lysyl oxidase-like 2 [71]. Lysyl oxidase-like 2 (LOXL2) has been linked to extracellular matrix (ECM) remodelling, angiogenesis, cell proliferation, migration, transcription regulation, fibroblast activation, EMT and metastatic niche formation through a number of processes [36, 72, 73]. The tumour cells can communicate with multiple different cell types via exosomes. Therefore, it is highly likely that this leads to a complex network of interactions.

The reaction of the target cells upon treatment with exosomes depends on the exosomal composition, which has been previously described as being diverse, and the transfer of encapsulated molecules [4, 67]. This ability of exosomes to protect and transfer molecules has led to the hypothesis that they could be used as potential tumour biomarkers or as a non-invasive tumour biopsy.

5. *In vivo* biodistribution of exosomes

Functional characterisation of exosomes often involves the use of an *in vivo* mouse model. Such experiments can give the biodistribution and pharmacokinetic parameters of the exosomes tested, which is important for understanding exosome trafficking and their physiological roles [74].

The starting point at which exosomes are to be isolated varies based on the experimental goals. In studies investigating the role of tumour-derived exosomes in cancer progression, exosomes were isolated from various cancer cell lines such as breast cancer, pancreatic cancer, gastric cancer and colorectal cancer [75]. Another area of interest is the potential use of exosomes as therapeutic carriers of antitumour microRNA or chemotherapy agents [76, 77]. This may allow for improved tissue targeting, increasing the potency of the delivered drug [78]. Exosomes are often isolated from cell-conditioned media with differential centrifugation being the most common method of enriching exosomes [75–77, 79, 80]. Most of the exosome isolation protocols involved low-speed centrifugation steps to remove cells and cell debris followed by high-speed centrifugation at 100,000 g and a washing step of the pellet with a final centrifugation. In a study by Alvarez-Erviti et al. [77], exosomes were derived from cultured dendritic cells, which was chosen based on data demonstrating that dendritic cell-derived exosomes contained immune-stimulating components such as major histocompatibility complex (MHC) class I and class II molecules in addition to T-cell-stimulating molecule, CD86 [81]. Studies also showed that isolated exosomes can be loaded with exogenous RNA or chemotherapy drugs by different methods, including electroporation and sonication [77, 78].

To enable the *in vivo* tracking of exosomes, they can be labelled post isolation with a lipophilic membrane dye such as Paul Karl Horan (PKH), DiOC18 (DIR) or DiIC18 (Dil) [75, 80, 82]. An alternative method of generating labelled exosomes is by transfecting donor cells with a

http://dx.doi.org/10.5772/65688

construct encoding for a fluorescence-membrane fusion protein. In this approach, a membrane-bound variant of bioluminescence reporter, Gaussia luciferase, is transfected into the donor cells, producing luciferase-labelled exosomes [82, 83]. A major difference between the two labelling approaches is the time and expertise required. The post-isolation membrane dye labelling is quick (~1 h), whereas the transfection of cells requires additional time (~2 weeks) and expertise in vector and viral cloning and transfection [79, 84]. Additionally, a study by Lai et al [83] reported quicker rates of clearance of transfected luciferase-labelled exosomes compared to the dye-labelled exosomes. The authors attributed this difference to the possibility of the highly stable dyes being an artefact instead of indicating intact exosomal presence.

Exosomes injected into mice are commonly quantified using the Coomassie dye (Bradford)-based method, or copper-based chemistry such as the Bicinchoninic Acid Assay (BCA) [75–77, 82–85]. The yield of exosomes obtained often ranges from 6 to 12 μg/10^6-cultured dendritic cells, 69.2 μg/2–5 × 10^7 of HEK293 and 2–4 ug/10^6 HEK cells [76, 77, 79]. There is some ambiguity in the quantification of exosomal protein concentration in these studies. Presumably, the exosomes were first lysed pre-quantification as without lysing the exosomes, only the membrane-bound proteins would be quantified. Another method of determining the required number of exosomes is to use the number of exosomes per gram of animal weight. Techniques such as NTA are used to quantify the number of exosomal particles and their size distribution [85, 90]. Importantly, in order to translate the use of exosomes into a clinical setting, standardising the dose of exosomes injected is critical. Given that isolated exosomes from current techniques such as ultracentrifugation are heterogeneous in size when observed using NTA [85], it is likely that the difference in size translates to differences in total protein concentration. Therefore, methods that quantify the total protein content within exosomes such as the Bradford/BCA assays are a better means of measuring the protein content and thus exosomal dose.

For biodistribution and tissue-uptake studies, the dose of injected exosomes ranged from 4 to 10 μg per mouse [75, 82]. Alternatively, a dosing range of 1.5 × 10^{10} particles/gram body weight (p/g), 1.0 × 10^{10} p/g and 0.25 × 10^{10} p/g was used [85]. In studies where exosomes were used as a potential therapeutic siRNA carrier, the dose of exosomes chosen was much higher, at 150 μg/mouse [77]. An explanation of a higher dose employed could be that systemically administered exosomes are rapidly cleared from the bloodstream, with evidence to suggest that macrophages play a role in exosome clearance [80]. Therefore, the higher dose was chosen to induce a measurable response.

Once the labelled exosomes are administered, the duration of monitoring ranged from 10 min to 6 h for biodistribution studies, which met the goal of tracking the localisation of exosomes over time [82, 83]. It was demonstrated that injected exosomes localised primarily in the liver and lungs [82, 85]. Moreover, it was shown recently that particular integrin expression on tumour-derived exosomes could be used to predict organ-specific metastasis [75]. In particular, exosomes expressing α6β4 and α6β1 were linked with lung metastasis, while exosomal integrin αvβ5 was associated with liver metastasis. For exosomes which carried modified cargo, such as siRNA targeting the abundant GAPDH, the effect induced by the cargo was

measured 3 days post injection [77]. This study showed the possibility of using exosome-mediated delivery of potentially therapeutic siRNA to induce a gene-specific knockdown.

In summary, *in vivo* characterisation is an important step in gaining an understanding of the physiological pathways that exosomes are involved in. Further research will strengthen the proposal of using exosomes as a therapeutic carrier and potential diagnostic tool.

6. New approaches to elucidate the role of exosomes in cancer

Identification of biomarkers to detect cancer during its early stages has the potential to improve patient outcomes significantly with exosomes currently being considered. As exosomes are released and circulate in the peripheral circulation, they can be collected from diverse bio-fluids through minimally invasive procedures from the blood and non-invasive procedures from saliva and urine. Through the isolation and purification process, exosomes are separated from highly abundant proteins present in bodily fluids [56]. Furthermore, cancer-derived exosomes can be specifically distinguished from exosomes originating from other cells by the expression of markers such as CD24 and EpCAM [86]. Storage of exosomes does not significantly affect their protein and miRNA contents thus highlighting their high stability [87]. Most importantly, the release and content of exosomes reflect the tumour state and their microenvironment [88].

Encapsulation of cellular proteins and RNA molecules into exosomes makes exosomes an enriched source of tumour markers, which provides an insight into the originating tumour cells. miRNAs are evolutionarily conserved regulating several cellular processes such as cell differentiation, proliferation and apoptosis [89]. These cellular processes are often altered in cancer-enhancing cellular transformation and tumourigenesis by impaired miRNA biogenesis; therefore, miRNA profiles can differentiate cancer tissues from benign tissues [90]. A complete miRNA-profiling study in epithelial ovarian cancer (EOC) has identified aberrantly expressed miRNA in different subtypes of EOC compared to normal ovaries [91]. Ovarian tumour-derived exosomes isolated from patient sera exhibited similar miRNA profiles to originating tumour cells and the exosome concentration was positively correlated with the progression of disease, highlighting the diagnostic potential of exosomal miRNA [92]. High exosomal miR-21, miR-23b and miR-29a expression of ovarian cancer patient effusion correlated with poor progression-free survival and poor overall survival was related to high expression of miR-21 suggesting their use as prognostic markers [93].

A recent study established the role of EOC-derived exosomes in mediating the activation of macrophages to a tumour-associated macrophage (TAM) state [94]. They also demonstrated that SKOV-3 cells when grown with conditioned media from the transformed macrophages were more likely to migrate and proliferate.

Additional studies have proposed the use of exosomes as both diagnostic biomarkers and therapeutic agents [95]. It has been proposed that exosomes be used to transport antitumour complexes such as drugs to the tumour cells, thus providing a form of targeted therapy.

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Furthermore, it has been shown that decreasing exosome production by blocking Rab27a (responsible for exosome release) can also reduce primary tumour growth [96].

Compared to the currently available detection methods, the use of exosomes as biomarkers will involve minimally invasive procedures and as the exosomal content reflects the originating cancer cells and their microenvironment, they will have greater specificity. This will decrease the need for surgical interventions and deaths from surgical complications as a result of false-positive results [97].

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