Exosomal miRNA in chemoresistance, immune evasion, metastasis and progression of cancer

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In the treatment of cancer, there are three significant limitations causing high mortality and recurrence rates among cancer patients. First, the escape of tumor cells from the immune system; second, the development of multi-drug resistance (MDR) to chemotherapeutic drugs; and, third, the noxious metastases of cancer cells. Exosomes are vesicular cargos involved in the transportation of miRNA, mRNA and proteins from one cell to another cell. This review details the current understanding of the exosomal transmission of miRNA and crosstalk with the downstream consequences, ultimately leading to the progression and metastasis of cancer. Further, this review also discusses how exosomal miRNA can provide promising novel targets for the treatment and detection of cancer.

Introduction

Cancer is the second-leading cause of death globally. Traditionally, chemotherapy is primarily used for the treatment of all cancers. However, recent clinical data concluded that the major cause of cancer progression is the development of resistance to standard chemotherapeutic agents. Immune evasion is another problem in cancer chemotherapy. Although substantial advances have been made in understanding how cancers evade destructive immunity, distinctive strategies based on the mechanism of immune evasion are required for targeting cancer immune surveillance [1]. Many biological interactions, including parallel protein signaling, miRNA–mRNA interactions, intercellular and intracellular signaling, and cellular and exosomal crosstalk, are responsible for cancer initiation, progression and development of resistance [2]. Along with active and passive transporter systems, there are many transportation systems through which cells interact with each other.

Exosomes are microvesicles (size: 50–100 nm) that play an important part in cell–cell communication. They are secreted in all body secretions such as a serum, saliva, urine, milk, sweat and tears [3]. It was found that the secretion of exosomes is drastically amplified in all cells in the tumor microenvironment (TM). Notably, the exosomes derived from different cell types have common proteins that can be used as cell surface markers *viz*. Alix, TSG101, hsp70 and hsp90, integrin and tetraspanins (CD63, CD9, CD81 and CD82), to name just a few [4]. Exosomes transfer the information to the neighboring target cells via ligand–receptor interaction, endocytosis and phagocytosis, as well as by fusion with the plasma membrane. This exosomal crosstalk involves the transport of RNA, proteins, miRNA and long noncoding RNA (lnc)RNA between donor cells and recipient neighboring cells [5]. Depending on the content of exosomes released by a particular cell, they can modulate neighboring cancer cells by changing their cellular fate or pathways, and hence support the initiation, progression or resistance in cancer [6].

Biogenesis of exosomes

Exosome biosynthesis takes place by initiation, endocytosis, multivesicular body (MVB) formation and exosome release, as shown in Fig. 1a. The endosomal sorting complexes required for transport

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GLOSSARY

miRNA micro RNA
HDL high-density lipoproteins
MSCs mesenchymal stem cells
VEGF vascular endothelial growth factor
CDKN1A cyclin-dependent kinase inhibitor 1A
CDKN1C cyclin-dependent kinase inhibitor 1C
CSC cancer stem cell
DDR DNA damage response
BCL-2 B cell lymphoma 2
Notch Notch homolog 1, translocation-associated
WNT wingless/integrated
BMI1 B-cell-specific Moloney virus integration site 1
MMP9 matrix metallopeptidase 9
CAF cancer-associated fibroblast
CAA cancer-associated adipocyte
APAF1 apoptotic protease activating factor 1
Snail1 zinc finger protein SNAI1
BRCA1 breast Cancer Type 1 Susceptibility Protein
ATG autophagy-related protein
NHEJ nonhomologous DNA end joining
APC antigen-presenting cell
NK natural killer
CTL cytotoxic T lymphocyte
TAA tumor-associated antigen
DC dendritic cell
Tregs regulatory T cells
TAM tumour-associated macrophage
RFXAP regulatory factor X associated protein
TLR Toll-like receptor
EGF epidermal growth factor
CCL2 cytokine chemokine (C-C motif) ligand 2
IS immunological synapse
MIIC major histocompatibility complex class II-enriched compartment
CEA carcinoembryonic antigen-containing
OKT3 muromonab-CD3
CD69 cluster of differentiation 69
ALDH1 aldehyde dehydrogenase 1
EZH2 enhancer of zeste homolog 2
TGF-β transforming growth factor beta
mTOR mammalian target of rapamycin
VPS4 vacuolar protein sorting protein 4
NPC nasopharyngeal carcinoma
CIML cytokine-induced memory-like
TM tumor microenvironment
GSCs glioma stem-like cell
DENND2D tumor suppressor gene
NFS normal fibroblasts
SHIP1 member of the inositol polyphosphate-5-phosphatase (INPP5) family

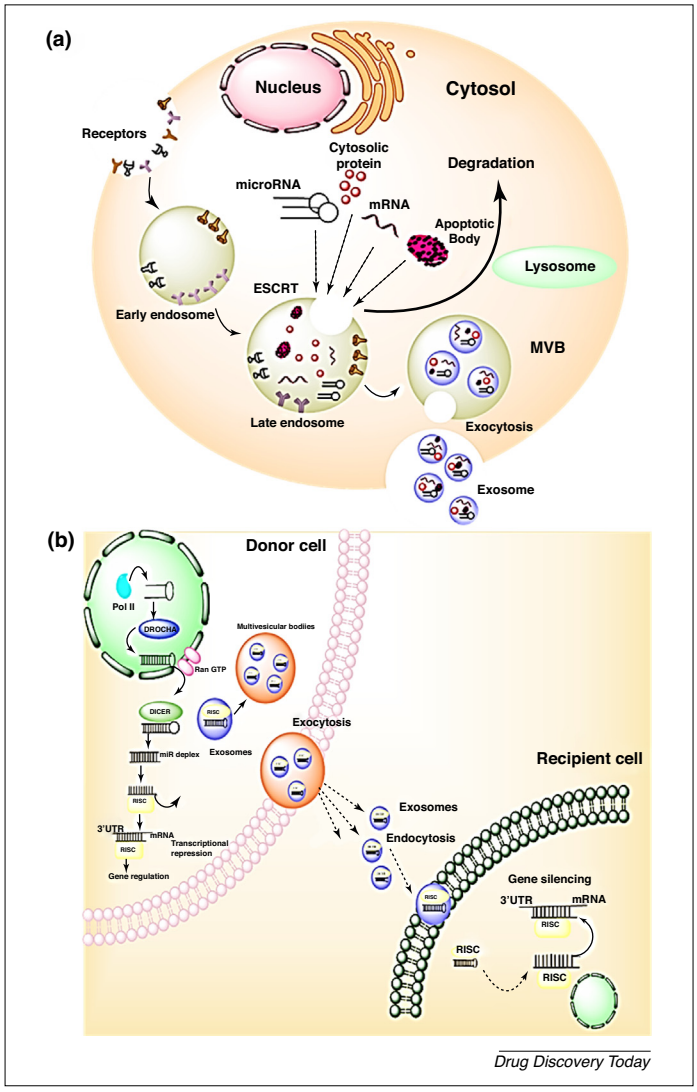
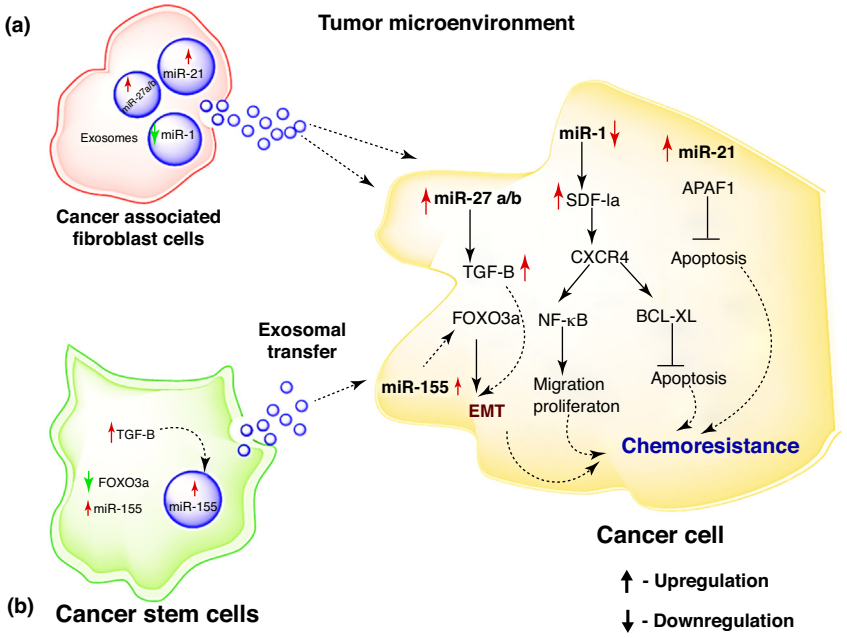


FIGURE 1

(a) Biogenesis of exosomes and their secretion. Exosomes are small, circular cell-secreted vesicular bodies; their biogenesis occurs in the cell via invert budding. Exosome secretion takes place through multivesicular bodies (MVBs), in that intracellular organelles (MVBs) fuse with the plasma membrane of the cell and expel their own contents outside the cell. The endosome arises in the early phase of biogenesis and is called an early endosome; endosomes in a late phase are called late endosomes, which are contained in molecules from the Golgi apparatus, cell surface receptors (e.g., Transferrin receptor), cytosolic proteins, apoptotic bodies, among others. Exosomes are compact bodies that contain RNA, DNA, proteins, lipids, among others. The lysozyme enzyme can degrade exosomes in the cell. **(b)** Biogenesis and exosomal transport of miRNA. This schematic demonstrates the biogenesis of miRNA in the cell along with its transport to another cell via exosomal crosstalk. miRNAs are transcribed by RNA polymerase II generating a primary miRNA (pri-miRNA) molecule, which is processed into a precursor miRNA (pre-miRNA) by the microprocessor complex comprising DGCR8 and Drosha. Pre-miRNAs are exported to the cytoplasm in a nucleocytoplasmic transporter containing Exportin 5 and Ran-GTP. Then, along with DICER, this pre-miRNA duplex in engulfed by the exosomes. When they are released from multivesicular bodies exosomes transfer miRNA to neighboring cells. Released microRNA complex acts on complementary mRNA and represses transcription.

(ESCRT) play a vital part in this biosynthesis process. They include ESCRT0, ESCRT1, ESCRT2, ESCRT3 and AAA-ATPase-Vps4 complex (a complex of ATPase associated with various cellular activities and vacuolar protein sorting protein 4) [7]. During the biosynthesis of exosomes, early endosomes mature into late endosomes, late endosomes then form the MVB, and ESCRT0 assigns the corresponding proteins for internalization. Further, ESCRT1 and ESCRT2 initiate inward budding of the endocytic membrane into the lumen of the cell to form intraluminal vesicles (ILVs). ESCRT2 causes de-ubiquitination of cargo proteins before construction of ILVs. Then ILVs fuse to form MVBs intracellularly. In the last step, invagination from the membrane plus separation is executed by ESCRT3 and, for this process, AAA ATPase-Vps4 supplies the required energy [8]. Finally, the exosomes are released by fusion of the plasma



Exosomal miRNA	Cancer	Drug causing miRNA transition	Nature of miRNA expression	Downstream target	Ref.
miRNA-21	Lung	Cisplatin	↑	TLR7 ,TLR8	[44]
miRNA-106a	Gastric	Cisplatin	↑	-	[84]
miRNA-21	Ovarian	Cisplatin	↑	NAV3	[30]
miRNA-155	Breast	Cisplatin	↑	-	[24]
miRNA-16	Breast	-	↑	VEGF	[15]
miRNA-23a	Breast	Adriamycin	↑	p27 , PTEN	[20]
miRNA-9	Ovarian	Cisplatin	↓	BRCA1	[34]

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FIGURE 2

(a) Schematic demonstrates the effect of cells present in the tumor microenvironment on the development of chemoresistance. Tumor microenvironment contains cancer stem cells (CSCs) and cancer-associated fibroblasts (CAFs). miRNA-155 gets deregulated in CSCs which are secreted in exosomes that are taken up by tumor cells that develop chemoresistance through induction of epithelial-to-mesenchymal transition (EMT) by acting on FOXO3a. CAF cells also secrete exosomal miRNA and prevent apoptosis of tumor cells with the aid of BCL-XL (antiapoptotic). This eventually leads to the survival of tumor cells through chemoresistance. **(b)** Table representing the nature of expression of dysregulated exosomal microRNA and its targets involved in chemoresistance of various types of cancer.

membrane with MVBs through exocytosis. Exosomes can be isolated by well-established methods including centrifugation, chromatography, filtration, polymer-based precipitation and immunological methods [9]. The protein, lipid and luminal contents, as well as sedimentation properties of exosomes from different sources, differ widely. If exosomes are to be isolated from cultured media, a very important consideration is whether to use serum-free media or exosome-free fetal bovine serum [10].

Exosomal miRNA and its dominance in the TM

Exosomes carry miRNA and mRNA, protecting them from enzyme ribonuclease in the TM. miRNAs are small, noncoding, single-stranded RNAs that are 20–22 nucleotides in length, and regulate the expression of a target gene [11,12]. The biogenesis of miRNA is succinctly shown in Fig. 1b [13]. miRNAs can act as oncogenes as well as tumor suppressor genes in cancer. miRNA regulation leads to changes in cell phenotype, cytokine expression and secretion, causing inflammation resulting in aggravated tumor performance [14].

In angiogenesis, exosomal miRNAs guide the interaction between mesenchymal stem cells (MSCs) and tumor cells. It was observed that the release of exosomal miRNA-16 suppresses angiogenesis via MSCs by decreasing expression of vascular endothelial growth factor (VEGF) in breast cancer [15]. This event is also accompanied by checking the inhibitors of the cell cycle (i.e., CDKN1A, CDKN1C and transcription factor E2Fs; see Glossary). A recent report suggests that, in chronic lymphocytic leukemia, exosomal miRNAs are transported to endothelial cells, altering the transcriptome of stromal cells causing the release of angiogenetic factors [16].

Roles of exosomal miRNA in chemoresistance

Chemoresistance is a state wherein cancerous cells maintain their growth and multiply in the presence of successive first-line cancer therapies [17]. Around 80% of patients attain chemoresistance when receiving standard chemotherapy [18]. Therefore, it is necessary to focus on various factors that are responsible for the induction of cancer [19]. The understanding of the underlying mechanism of

cellular components such as exosomes and their molecular pathways through which they participate in chemoresistance is imperative to understanding the whole problem and identifying a new target. Exosomes carry the miRNAs that modulate the response to cancer therapy and create chemoresistance (Fig. 2). Mao *et al.* reported that, in breast cancer, adriamycin resistance was acquired via exosomal miRNA (miR-23a, miR-24 and miR-222) by targeting p27 and phosphatase and tensin homolog (PTEN) expression [20].

Involvement of exosomal miRNA in recruiting cancer stem cells (CSCs) to mediate chemoresistance

CSCs express $CD44^+$ and $CD24^-$ markers on their cell surfaces and are formed during epithelial-to-mesenchymal transition (EMT) [21]. They have a high susceptibility for tumorigenic transformation. CSC-like cells are predominant in the chemoresistant phenotype and the CSC-associated exosomal miRNAs serve as novel biomarkers in cancer EMT (Fig. 2). Several exosomal miRNAs are involved in the chemoresistance through CSC self-renovation capability. Interestingly, the exosomes rich in oncogenic miRNA are highly concentrated in CSCs [22]. For instance, the exosomal miRNA let7 acts on CSCs to maintain their self-renewal capacity and is involved in intracellular communication of tumor cells with noncancerous cells [23]. A recent investigation revealed that miR-155 is found to be highly upregulated in serum exosomal profiling of breast cancer patients in the exosomes isolated from CSCs versus normal breast cells [24].

CSCs induce chemoresistance via the DNA damage response (DDR) pathway. Whenever there is drug-induced damage to the DNA, a DNA-repair signal acts on the cell cycle as checkpoints and signals to stop the cell cycle at G1/G2 phase [25]. Moreover, CSCs contain highly antiapoptotic proteins like B cell lymphoma 2 protein (BCL-2) and survivin, among others, which are involved in the efflux of drugs which decreasese the therapeutic ability of a drug [26]. A study suggests that the signaling pathways like Notch (Notch homolog 1, translocation-associated), Hedgehog and wingless/integrated (WNT) are involved in CSC-mediated chemoresistance [27]. Also, the downregulation of E-cadherin (EMT marker) and significantly raised expression levels of B-cell-specific Moloney virus integration site 1 (BMI1) and matrix metallopeptidase 9 (MMP9) in cisplatin-resistant cells confirms cellular transformation toward EMT [28].

Recent research reports that a stromal constituent of glioblastoma releases exosomal miR-1587 which stimulates the proliferation and clonogenicity of tumor-initiating glioma stem-like cells (GSCs). This is marked by the downregulation of the tumor-suppressive nuclear receptor co-repressor NCOR1, leading to an increment in tumorigenesis compared with untreated GSCs in orthotropic xenografts [29]. Hence, CSC-like cells significantly contribute to the development of chemoresistance in cancer via various exosomal miRNAs (Fig. 2).

Fibroblast-derived exosomal miRNA and role in chemoresistance

Fibroblasts are key players in chemoresistance and fibroblast-derived exosomal microRNAs are major players involved in tumor crosstalk (Fig. 2). Previously, fibroblast cells have been known to block drugs, preventing entry into epithelial cells. In ovarian carcinoma, exosomal transfer of miR-21 from cancer-associated fibroblast (CAF) to normal ovarian cells activates the chemoresistant phenotype through apoptotic protease activating factor 1 (APAF1) [30]. In pancreatic ductal adenocarcinoma, gemcitabine chemoresistance takes place through increased levels of exosomal miR-146a in CAFs via the target Snail1 [31].

Relationship between DDR, miRNA and chemoresistance

Chemotherapeutic agents directly or indirectly target cancer cells by inducing DNA damage. Upon recognizing DNA damage, cells initiate a variety of signaling pathways referred to as the DDR pathway [32]. The downregulation of exosomal miR-770-5p in ovarian cancer causes cisplatin chemoresistance via DDR [33]. Notably, exosomal miR-9 can re-sensitize cancer cells by targeting the BRCA1 gene through the regulation of DDR in ovarian cancer [34]. The targeted therapies that selectively inhibit the DDR can thus offer greater therapeutic effect.

Role of exosomal miRNA in autophagy-induced chemoresistance

Autophagy is activated in cells in response to stress and hypoxia to reduce the bioburden of cells. This process was found to be primarily regulated by ATG proteins (autophagy-related proteins) and miRNAs [35]. Autophagosomes engulf the impaired organelles, then fuse with a lysosome to form autophagolysosome-containing lytic enzymes, leading to their degradation [36]. In early-stage cancer, they act as a tumor suppressor by preventing alteration of DNA and genomic instability. Conversely, at the late stages, they promote growth and survival of the tumor by relieving the cellular stress through increased immune surveillance, increased survival under stress-induced hypoxia and chemoresistance. Autophagy supports the unresponsiveness of a tumor to chemotherapy; hence, the blockade of autophagy can resensitize a tumor to anticancer therapy [37].

A member of the miR-30 family, miR-30a is involved in the regulation of autophagy by acting on target genes *Beclin1* and *atg5*. In resistant ovarian cancer cells, the knockdown of *Beclin1* was found to decrease autophagy and re-sensitize cells to cisplatin chemotherapy [38]. In hepatocellular carcinoma (HCC), elevated exosomal miR-32-5p is transferred to the sensitive cell, which regulates autophagy, accelerates angiogenesis and develops resistance through the phosphoinositide 3 kinase (PI3K)/Akt pathway [39].

Exosomal miRNA and its role in modulation of the innate immune system

The immune system recognizes tumorogenic cells and further activates the immune response to provide protection to the body against cancer. Cancer cells express tumor-associated antigen (TAA) to recognize non-self-antigen [40]. They bear the capability to survive and evade the immune system to facilitate cancer progression (known as immune escape; Fig. 3). Exosomes released by cancerous cells carry damage-associated molecular patterns (DAMPs) which activate intracellular virus-sensing pathways via cyclic GMP-AMP synthase. These DAMPs act on interferon genes (cGAS-STING) and retinoic-acid-inducible gene I (RIG-I) and increase inflammatory cytokines like interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-8 and IL-1β [41].

Macrophages

Macrophages possess remarkable flexibility in the tumor environment and they are divided into two subclasses: M1 (classically activated macrophages) and M2 (nonclassical or alternatively

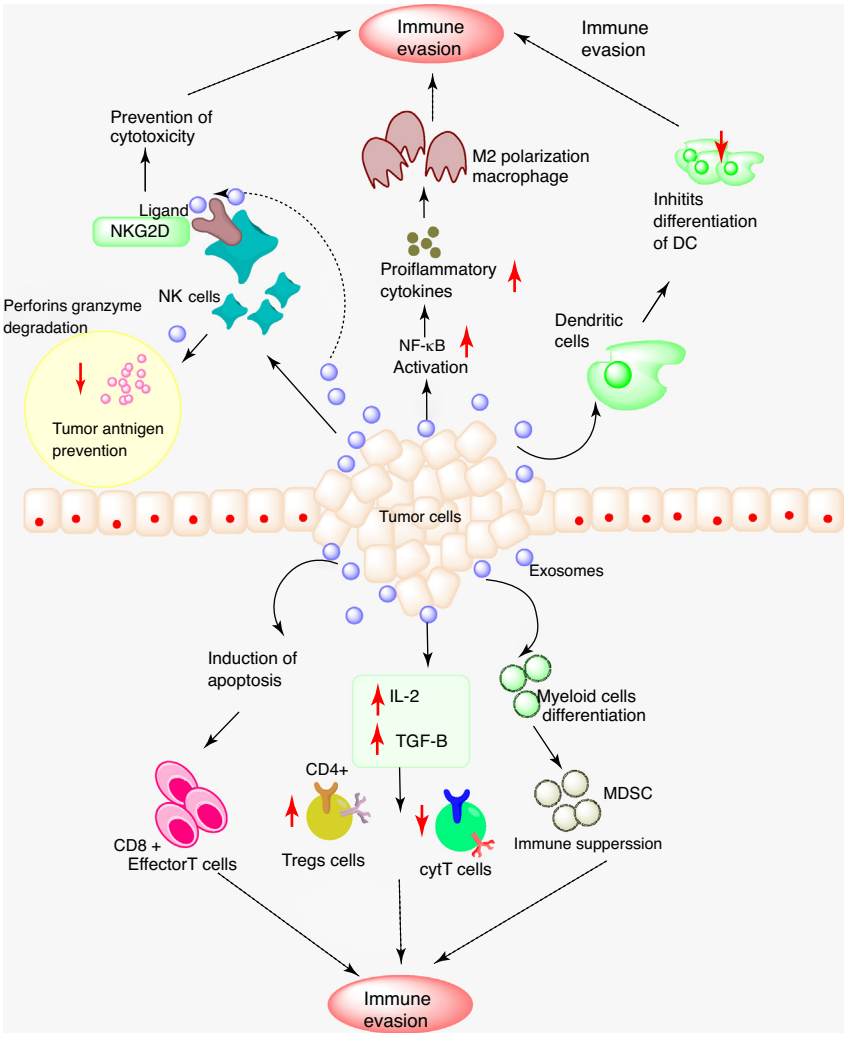

FIGURE 3

Schematic demonstrates the involvement of exosomal miRNA in evasion of the immune response. Various cells of the innate immune system and adaptive immune system. Tumor cells of epithelial lining secrete exosomes, exosomal miRNA acts on NF-κB pathway through which there is the release of proinflammatory cytokines which causes polarization of M2 macrophages that are pro-tumoral, also exosomes and derived miRNA act on dendritic cells and natural killer (NK) cells which prevents cytotoxicity of NK cells and degrades enzymes released by NK cells. There is degradation of $CD8^+$ effector T cells with the aid of exosomal miRNA. There is also decreasing levels of cytotoxic T cells and increasing differentiation of T regulatory (Treg) cells. Differentiation of myeloid cells leads to the production of MDSCs which causes immune suppression in the tumor microenvironment which further drives angiogenesis and thrombosis.

activated macrophages) [42]. M1 macrophages are tumor-suppressive being proinflammatory but M2 macrophages are tumor-favorable. Notably, M1 and M2 macrophages are interconvertible [43]. Classically activated tumour-associated macrophages (TAMs) subdue tumor growth but, when polarization occurs, TAMs are activated to mediate angiogenesis, immunosuppression and restructuring of the extracellular matrix (ECM) [44]. M2-TAM induces tumor proliferation by secreting various tumor growth factors, chemokines and cytokines, among others. Tumor-cell-derived exosomes majorly contribute macrophage polarization via miRNA. A recent study reports that M2-polarized macrophages are linked to increased levels of miR-21 via regulating the PTEN/PI3K/Akt pathway [45].

The same signaling pathway was found to be consequently responsible for preventing apoptosis in gastric cancer cells [46], wherein exosomes were found to activate the macrophage by releasing cytoskeleton centric protein [47]. In epithelial ovarian carcinoma, exosomal miR-222-3p induces M2-macrophage polarization via the SOCS3/STAT pathway [48]. Exosomal miR-25-3p and miR-92a-3p are linked with increased IL-6 secretion by M2-TAM in liposarcoma [49]. Tumour-derived exosomes are involved in activation of the nuclear factor (NF)-κB pathway in macrophages, leading to release of chemokines and cytokines which favors the TM. In lung cancer, exosomal miR-29a and miR-21 act on Toll-like receptors TLR7 and TLR8 inducing the NF-κB pathway, which leads to the secretion of TNF-α as well as IL-6 to induce metastasis [44]. Tumor exosomes highly express miR-155, which diminishes the proinflammatory effect of myeloid immune cells targeting SHIP1 [50].

Natural killer (NK) cells

NK cells are important players in the host rejection process of tumors. They contain granular enzymes and proteins in their cytoplasm – namely perforins and granzymes [51]. Upon release, perforins create pores in the cell membrane and, consequently, the granzymes and linked contents enter the cell to induce apoptosis (Fig. 3). NKG2D is an activating cytotoxicity receptor, the abnormal damage of which in cancer is responsible for immune escape and cancer progression. It has been reported that, in hypoxic conditions, exosomal transfer of miR-23a and TGF-β are accompanied by a decrease in NKG2D activator surface receptors in NK cells [52]. Notably, it was also found in prostate cancer – a tumor-derived exosome carries ligand for an NKG2D receptor on the cell surface that downregulates the receptor on NK cells and $CD8^+$ T cells [53].

It was further revealed that NK cells in cancer patients are responsible for a weaker antitumor response through the diminished expression of activating receptors like NKp30, NKp46, NKG2C and NKG2D. Tumor-cell-derived exosomes create disturbances in cell cytotoxicity of NK cells and decrease the expression profile of NK cells [54]. NK cells secrete exosomes that overexpress mutually typical NK markers and killer proteins (i.e., CD56, FASL and perforin). Decreased expression of activating receptors existing on NK cells encourages immune escape in the cancer cell. Exosomes bearing ligands of the activating receptor suppress the function of NK cells that ultimately protect cancer cells [55]. The *in vitro* stimulus of NK cells by IL-12, IL-15 and IL-18 gives them a memory-like behavior, characterized by greater effector responses when they are re-stimulated after a relaxation time. These pre-activated NK cells (also known as cytokine-induced memory-like; CIML) have numerous assets that make them a promising tool against cancer [56]. NK cells can obtain longstanding antitumor activity through pre-activation with the cytokines IL-12, IL-15 and IL-18. SMAD4 is responsible for immune-cell activation in the TM. The omission of SMAD4 in NK cells essentially leads to abridged tumor cell rejection and augmented tumor cell metastases, as well as hindered NK cell homeostasis and maturation [57].

Dendritic cells (DCs)

DCs are also a part of the exosomal miRNA-induced cellular crosstalk. These are antigen-presenting cells that ingest, process and present antigens and play a pivotal part in the commencement, regulation and maintenance of the immune response. Immature DCs are involved in inducing immune tolerance by downregulating expression of T cells, whereas developed DCs encourage immunity. To augment their functions, DCs interconnect with neighboring DCs through soluble intermediaries including exosomes. It is well known that shuttle miRNAs are incorporated in exosomes, to control the functions of DCs which suggests communication of DCs established with other neighboring cells is arbitrated via exosome-derived miRNAs [58]. Bone-marrow-derived exosomes containing miR-34a and miR-21 cause differentiation of hematopoietic precursor cells into myeloid DCs instead of monocytes. Exosomal-derived miR-125b-5p, miR-146a and miR-148 act on proinflammatory mRNA of DCs [58]. In pancreatic cancer, exosomal miR-212-3p from tumor exosomes inhibits the

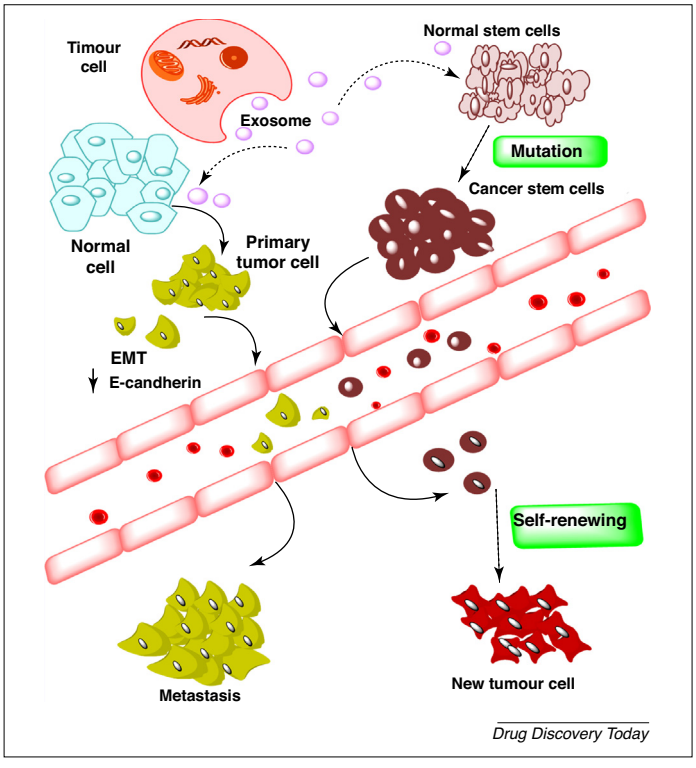

FIGURE 4

Exosomes encouraged mutation in normal stem cells, which will be converted into cancer stem cells possessing spear-forming ability in cells as well as being rich of epithelial-to-mesenchymal transition (EMT) character, giving cancer cells high mobility to migrate from one place to another place in the tumor microenvironment. Cancer-derived exosomes can enhance EMT in primary tumor cells; epithelial cells acquire mesenchymal- and fibroblast-cell-like properties, which will travel via intrastation or extravasation from one place to another.

function of the regulatory factor X-associated protein (RFXAP), which causes inhibition of major histocompatibility complex II (MHC-II) expression on DCs [59]. It was found that, in pancreatic cancer, released exosomal miR-203 acts on TLR-4 receptors and downstream targets TNF-α and IL-12 by inhibiting expression and consequently promoting immune escape [60].

Effect of exosomal miRNA on the adaptive immune response

The transfer of exosomal miRNA from melanoma tumor cells to cytotoxic T cells (CTLs) disturbs metabolism and immune response and is accompanied by an increase in cytokine production [61]. In nasopharyngeal carcinoma (NPC), cell-derived exosomes treated with OKT3 cells were shown to alter T cell proliferation *in vitro*. Upon further investigation, it was revealed that tumor-derived exosomes inhibit the differentiation of Th1 and Th17 cells but promote Treg expansion accompanying ERK, STAT1 and STAT3 expression, as well as leading to an amplification of cytokine production from $CD4^+$ and $CD8^+$ T cells [62]. Another study performed on NPC showed that exosomal miR24-3p is associated with T cell dysfunction via targeting FGF11 directly [63]. A tumor-derived exosome gives signals to cell-surface receptors that change mRNA profile in Tregs. Consequently, it decreases CD69 protein on $CD4^+$ T (conventional) cells, unlike B cells and monocytes which are taken up by exosomes to mediate miRNA transfer [64].

Impact of the exosomes in cancer metastasis

Exosomes enhance the growth of a tumor and metastasis. CSCs bear self-renewal capability with a significant expression of specific surface cell markers (CD44 and ALDH1) and less expression of CD24, which facilitated tumor recurrence and metastasis. The CSCs can ascend from epithelial cells undergoing EMT and, it is believed, by declining levels of protein E-cadherin through transcriptional repressors such as Snail and Slug (Fig. 4). These repressors act on enhancer box (E-box) of E-cadherin. E-box is a DNA response element that acts as a protein-binding site to regulate gene expression. It is a specific DNA sequence: CANNTG (where N can be any nucleotide), with a palindromic canonical sequence of CACGTG. It is known to be bound by transcription factors to initiate gene transcription, once the transcription factors bind to the promoters through the E-box, also other enzymes can facilitate transcription from DNA to mRNA through the binding on the promoter [65]. Snail and Slug are direct repressors of E-cadherin and act by binding to the specific E-boxes of the proximal promoter. These proceedings are complemented by an increase of stemness regarding transcription factors EZH2 and BMI1, which could initiate the evolution of epithelial cells into the mesenchymal state, carrying the capacity to spread to other tissues [24].

Tumor exosomes modulate the immune system to facilitate survival, growth and metastasis in the tumor [66]. For instance, exosomes released in squamous head and neck carcinoma cells after radiation alter the migration fate of neighboring cells. They enhance the Akt signaling pathway by regulating downstream mammalian target of rapamycin (mTOR). mTOR is the moderator of pro-migratory signals in head and neck cancer which is phosphorylated at ser2448 in response to stimuli that trigger the Akt24 pathway. The irradiated cells also secrete exosomes that were found to eventually facilitate the motile character in recipient cells escorted by enhanced activity of MMP enzymes. Akt triggers cellular motility by downregulating MMP2 and MMP9 targets. The increase in mTOR and MMP activity suggests that the irradiated cells secrete the exosomes to facilitate the Akt signaling pathway in neighboring cells [67].

Cell immigration is essentially linked with alterations in the actin cytoskeleton as well as advanced cell propagation. Extracellular vesicles that contain 14-3-3z and β-catenin proteins transfer to other nearby cells. β-catenin acts as a key effector protein of the established Wnt pathway functions in the nucleus with T cell factor/lymphoid enhancer factor (TCF/LEF) to activate the expression of Wnt target genes. Mainly, 14-3-3z and β-catenin form bleb-like structures and are secreted via extracellular vesicles to induce Wnt signaling in target cells. The cells interacting with these extravascular vesicles exhibit greater mobility and it is reasonable to assume the expression of 14-3-3z is involved in accepting cells and increases in cell relocation [68,69]. Certain exosomes possessing protein markers can be used as 'detective markers' in the case of organ-specific metastasis. For example, the analysis of muscle metastasis employs discoid in I-like domain 3 protein that are present in the urinary exosomes of bladder cancer patients [70].

Exosomal miRNA and cancer metastasis

During the process of cell migration, tumor cells interact with the new microenvironment. Early tumor cells make a pre-metastatic niche in different organs to assist the development of metastasis. Cancer cells possess plasticity, which provides the ability to adapt in particular environments [71]. There is a correlation between exosome-mediated miRNA and metastasis in various cancers. As discussed earlier, microRNAs perform a vital role between cell signaling, facilitating the expansion of pre-metastatic niches [72].

Jiang and Li reported that microRNA dysregulation could be assimilated to tumorigenesis and primary tumor progression. In some conditions, anomalous expression of miRNAs in mutation, downregulation and overexpression could affect expression levels of their target genes involved in cancer metastasis by inducing invasion, resistance and cell migration. miRNAs showed dual properties as oncogenics and therapeutics in specific conditions [73]. Exosomal miR-1246 enriched in the cancer cell is the oncogenic miRNA that produces the pre-metastatic phenotype. This event enhances the cell motility and invasion in the poorly metastatic cell line HOC313-P. miR-1246 directly binds to a 3′UTR segment of DENND2D (a tumor suppressor gene) and leads to an increase in migration and invasion by HOC313 cells [74]. In the TM, the exosomes communicate with corresponding proteins and miRNAs to mediate inflammation, which is essential for enrolling inflammatory CCR6þCD4þTh17þ to sponsor metastasis, angiogenesis and the proliferation of cancer cells [70]. Astrocyte-secreted exosomes facilitate the cell–cell transfer of miRNAs directing PTEN to metastatic malignant cells. Loss of PTEN in brain metastatic malignant tumor cells causes enhanced 'ooze' of cytokine chemokine (C-C motif) ligand 2 (CCL2). $Iba1^+$ myeloid cells mutually boost the development of brain metastatic tumor cells by improved propagation and condensed apoptosis [75]. Upregulation of miRNA-409 in normal fibroblasts (NFS) induced a CAF-like phenomenon and release of miRNA-409 from exosomes, which promotes the epithelial–mesenchymal transition pathway, as well as enhancing tumor proliferation [76].

Exosomal miRNA as emerging therapeutics for treating cancer

Exosomes activate T-helper ($CD4^+$) immune cells. They facilitate innate and adaptive immunity over other systems such as macrophages, carcinoembryonic-antigen-containing (CEA) tumor cells and mature DCs [77]. B cells acquire a MHC-II-enriched compartment, which is able to fuse in an exocytic fashion with the plasma membrane causing the discharge of small cell bodies like exosomes in culture media. B cells secreting exosomes possess the greater expression of functional MHC-II protein related to peptides and other accessory molecules like B7, ICAM-1 and LFA-3; giving them the ability to induce a stronger *in vitro*, MHC-II-restricted, antigen-specific, T helper response [78].

DCs secreting exosomes contain a number of proteins that are mainly involved in biogenesis, targeting and inducing the immune response [79]. Peptide-pulsed DCs secrete exosomes encompassing MHC-II–peptide complexes that are taken up by DCs possessing deficient MHC-II; this results in activation of T helper cells and initialization of an adaptive immune response [80]. Lindenbergh and Stoorvogel reported that exosomes derived from pathogen-infected macrophages induced $CD4^+$ and $CD8^+$ T cell responses *in vitro*, especially in the presence of DCs (which also occurred *in vivo*) [81]. miRNAs are selectively sorted in exosomes. For instance, the levels of miRNA-671-5p, miRNA-654-5p, miRNA-632 and miRNA-760 were observed to be predominantly higher in cancer-associated exosomes. miRNA-335 was derived only from exosomes of primary DCs. By contrast, the expression of miRNA-101, miRNA-32 and miRNA-21 occurs in DCs rather than in exosomes from dendritic cells. During the period of cognate immune interactions, there is a unidirectional flow of miRNA from T cells to antigen-presenting cells, leading to the generation of the immunological synapse (IS) driven by antigen [82].

In a recent report, Kalluri and colleagues showed that treatment with exosomes repressed cancer in mouse models of pancreatic cancer, improving the overall survival. In oncogenic KrasG12D, a mutation of pancreatic cancer, exosomes were retained, compared with liposomes, CD47-mediated exosomes were protected from phagocytosis. Exosomes released from normal fibroblast-like mesenchymal cells are specifically engineered to transport short interfering (si)RNA or short hairpin (sh)RNA specific to oncogenic KrasG12D. Matched to liposomes, the engineered exosomes can target cancer with superior improved effectiveness that is reliant on CD47 and is enabled by macropinocytosis [83]. Exosomal miRNA is dysregulated in the malignant condition; for example, in lung cancer miRNA17-3p, miRNA-21, miRNA-106a, miRNA-146, miRNA-155, miRNA-191, miRNA-192, miRNA-203, miRNA-205, miRNA-210, miRNA-212 and miRNA-214 are overexpressed compared with noncancerous lung cells [84].

Concluding remarks

Exosomes are secretory bodies of the cell that gets actively secreted in normal circumstances as well as in a pathophysiological condition such as cancer in the TM; but the secretion of exosomes by cells is increased in various cancers, which could be correlated with poor prognosis. Exosomes contain miRNAs, which play a vital part in various stages of cancer development. Tumor-cell-derived exosomal miRNA is dysregulated in cancer. A major obstacle in the treatment of cancer is rapidly growing resistance against chemotherapeutic drugs, which decreases anticancer efficacy of drugs by various mechanisms. The mediation of tumor-derived exosomal miRNAs acts at a post-transcriptional level to regulate target gene expression. More-detailed research is still warranted to delineate an exact mechanism by which exosomal miRNA hastily spreads chemoresistance in cancer cells.

Another aspect focused on in this review is the immune escape of tumor cells; exosomes and exosomal miRNA modulate host innate and adaptive immune responses by acting on various cells of the immune system. In tumor metastasis, exosomes derived from irradiated cells were found to increase motility of neighboring cells and, along with exosomal miRNA, are involved in regulating gene expression of adjacent cells via cellular crosstalk. Exosomal miRNAs act as potential biomarkers for diagnosis and prognosis in various types of cancer. The therapeutic applications of exosomal miRNAs form a promising approach to anticancer modality and could open new opportunities for therapeutic intervention or new biomarkers for the treatment of cancer; however, clinically these miRNAs have been less effective owing to poor target delivery systems toward tumor tissue.

Conflicts of interest

There are no conflicts of interest or disclosures associated with this manuscript.

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