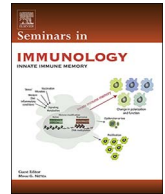




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Review

Exosome and mesenchymal stem cell cross-talk in the tumor microenvironment

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ABSTRACT

Mesenchymal stem cells (MSCs) are a major component of the tumor microenvironment (TME) and play a key role in promoting tumor progression. The tumor uses exosomes to co-opt MSCs and re-program their functional profile from normally trophic to pro-tumorigenic. These tumor-derived small vesicles called “TEX” carry and deliver a cargo rich in proteins and nucleic acids to MSCs. Upon interactions with surface receptors on MSCs and uptake of the exosome cargo by MSCs, molecular, transcriptional and translational changes occur that convert MSCs into producers of factors that are necessary for tumor growth and that also alter functions of non-tumor cells in the TME. The MSCs re-programmed by TEX become avid producers of their own exosomes that carry and deliver mRNA and miRNA species as well as molecular signals not only back to tumor cells, directly enhancing their growth, but also horizontally to fibroblasts, endothelial cells and immune cells in the TME, indirectly enhancing their pro-tumor functions. TEX-driven cross-talk of MSCs with immune cells blocks their anti-tumor activity and/or converts them into suppressor cells. MSCs re-programmed by TEX mediate pro-angiogenic activity and convert stromal cells into cancer-associated fibroblasts (CAFs). Although MSCs have a potential to exert anti-tumor activities, they largely provide service to the tumor using the multidirectional communication system established by exosomes in the TME. Future therapeutic options consider disruption of this complex vicious cycle by either molecular or gene-regulated silencing of pro-tumor effects mediated by MSCs in the TME.

1. Introduction

1.1. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are an important component of the tumor microenvironment (TME). MSCs are also referred to as “mesenchymal stromal cells.” This nomenclature, frequently used interchangeably, acknowledges the origin of MSCs from the stromal compartment and also implies that mesenchymal stromal cells have properties associated with stem cells. Indeed, MSCs are a population of adult multipotent cells capable of self-renewal and differentiation into osteoblasts, chondrocytes and adipocytes [1]. The most immature MSCs, i.e., true stem cells, can also differentiate into other embryonic lineages [2]. MSCs can be obtained from different tissues, e.g., bone marrow, adipose tissues, placenta or umbilical cord and can be propagated to give rise to cellular products that are used for therapy [3,4]. It is because of their self-renewal capacity, multi-potency and immunomodulatory properties that MSCs have become an especially attractive tool for regenerative medicine [5,6] and currently serve as a major cellular source for the replacement of damaged tissues or pathological lesions [7]. As MSCs do not express human leukocyte antigen

(HLA) class II molecules, they have low immunogenicity [6,8]. They are also able to suppress functions of various immune effector cells and promote activity of regulatory immune cells [8,9]. These features of MSCs underpin their role as therapeutic agents in a variety of diseases, including the graft versus host disease (GVHD) or myocardial infarction [3,10].

1.2. Mesenchymal stem cells in cancer

In cancer, MSCs play a crucial role in promoting tumor progression. On the one hand, they provide a framework for anchoring tumor cells in the form of tumor stroma and secrete factors that facilitate tumor growth [11]. On the other hand, MSCs present in the TME can trans-differentiate into M2-type macrophages, myeloid-derived suppressor cells (MDSC) or M2-type microphages under the influence of cytokines or chemokines [12–14].

As participants in re-programming of the TME, MSCs interact with the surrounding cells and communicate the messages for changes that are orchestrated by the tumor [11]. When the mechanisms responsible for MSCs interactions with other cells in the TME were searched for, it was discovered that MSCs secrete multiple bioactive factors with

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activities that can dramatically alter the key cellular functions of neighboring cells, such as survival, apoptosis, maturation and differentiation [15,16]. This secretome-based paracrine activity of MSCs is now widely recognized as a cell-free strategy mediated by soluble factors present in MSCs conditioned media (CM) [10]. However, more recent studies showed that in addition to the soluble secretome, these CMs also contained particulate fractions with biological activities associated with MSCs. This finding suggested that a novel cellular mechanism involving a particulate nano-communication system operated by MSCs may be responsible for dissemination of messages from MSCs to various recipient cells [17,18].

1.3. Exosomes as components of the tumor microenvironment

The objective of this review is to introduce extracellular vesicles (EVs) and, specifically, their smallest subset, exosomes, as the mediators of MSCs signaling that drives tissue re-programming in the TME. The focus of this review on exosomes, as opposed to all EVs, is deliberate. As discussed below, EVs represent a heterogeneous population of vesicles that may or may not share phenotypic and functional attributes. In the absence of firm criteria for the EVs' nomenclature, the International Society of Extracellular Vesicles has encouraged investigators to establish minimal requirements for the characterization of EVs, with strict attention to integrity, size, molecular cargo and functionality of the vesicle population being evaluated [19]. Focusing this narrative on exosomes as opposed to EVs narrows the scope of inquiry to the currently most widely studied and better characterized subset of EVs.

2. Extracellular vesicles (EVs) and exosomes

EVs are produced by all cells and come in several different vesicular formats, each with a different cellular origin, distinct size as well as a varied molecular content [20]. *Exosomes* are the smallest subset of EVs (30–150 nm in diameter) with a unique biogenesis. They originate from the endocytic compartment of the parent cell via a series of intraluminal invaginations taking place in the multivesicular bodies (MVBs). Consequently, their molecular content recapitulates, at least in part, the content of the parent cell [21]. Due to their endocytic origin exosomes are the only EVs that carry endosomal markers such as ALIX, TSG101 or syntenin-1 [21]. *Microvesicles* are larger than exosomes (500–1000 nm), are formed by “blebbing” or “pinching off” from the parent cell surface membrane and contain random assortments of cellular contents [22]. The largest EVs (1000–5000 nm) are *apoptotic bodies*, which represent remnants of cells undergoing apoptosis and contain a vast array of cellular debris [22].

2.1. Characteristics of exosomes

Among various EVs, exosomes are of great current interest, because their molecular/genetic profiles approximate those of the parent cells and their ubiquitous presence in all body fluids [21,23]. These traits qualify exosomes as potential candidates for circulating biomarkers expected to provide information about the molecular make-up and functions of the tissue-bound parent cells. If the parent cell is a tumor cell, for example, a proportion of exosomes present in plasma of cancer patients is likely to be tumor-derived and may be considered as a “liquid biopsy” of the tumor. Exosomes, due to their low immunogenicity, long half-life in the circulation and the ability to cross the blood-brain barrier, are also being tested as therapeutic nano-delivery systems for small interfering RNAs or other drugs [24].

Exosomes are enclosed by a protein-phospholipid bilayer membrane decorated by cell type-specific proteins, lipids and glycans. Exosome lumen is filled with various cellular proteins, nucleic acids, mRNA, miRNA and DNA, soluble factors, including cytokines and chemokines, enzymes and cofactors [25]. Components of the exosome cargo are

biologically active, as isolated exosomes mediate cellular-crosstalk when co-incubated with recipient cells or upon delivery to experimental animals *in vivo* [26,27]. Exosomes produced by different cell types carry distinct molecular and genetic components, and they may be “addressed” by the parent cell to reach a specific molecular address of the recipient cell. Upon contacting a local or distantly-located recipient cell, exosomes deliver signals that culminate in cellular re-programming [28,29]. The mechanisms responsible for delivery and processing of the exosome cargo in recipient cells are not entirely understood, but may include the initial ligand-receptor type of binding on the cell surface followed by endocytosis or phagocytosis of exosomes [30]. Whether exosomes signal via cognate receptors on their surface or are internalized, delivering their content of nucleic acids to the recipient cells, the exosome-recipient cell interaction results in a loss or gain of functions in the recipient cell [31]. Recent attention has been focused on transfer of miRNAs by exosomes as a major mechanism of the recipient cell modifications [31].

To date, much of what is known about exosomes comes from studies of cell line supernatants, where all vesicles are products of the cultured cell. In contrast, exosomes present in body fluids are heterogeneous mixtures of vesicles derived from various cells. Currently, methods are being developed to isolate and characterize not only total exosome fractions from human body fluids but also to separate subsets of exosomes released by e.g., T cells or tumor cells, based on specific markers, such as e.g., CD3 or a tumor-associated antigen carried by these exosomes. Isolation from body fluids and subtyping of exosomes is an evolving science [32,33]. Exosomal proteins, lipids and nucleic acids described in published studies have been listed in a data base, ExoCarta, which aims at the definition of specific molecular/genetic signatures of exosomes derived from different cell types [34]. It should be remembered, however, that almost all of the early studies were performed with exosomes derived from supernatants of cultured cell lines and the list of exosome components in the data base may not necessarily reflect the content of plasma-derived exosomes.

2.2. Tumor-derived exosomes (TEX)

Tumor cells are avid producers of exosomes, and tumor cell-derived exosomes, called “TEX” are ubiquitously present in the tumor milieu and in body fluids of all patients with cancer [27,35]. The ratios of TEX/normal cell-derived exosomes in the plasma of cancer patients varies, but generally TEX represent a substantial proportion of total exosomes recovered from plasma, especially in patients with advanced malignancies [36]. In the TME, TEX are major participants in inter-cellular cross-talk. Serving as information transfer vehicles, TEX carry messages from the parent tumor cell to other normal or malignant cells in the TME, including MSCs [37]. As Fig. 1 indicates, TEX can mediate autocrine, juxtacrine and paracrine signaling that the tumor cells establish and that is necessary for their survival [38]. Notably, TEX paracrine activities are not limited to the tumor site: TEX circulate and disseminate information to tissues and cells distant from the tumor.

2.3. MSC-derived exosomes

The tumor stroma is an essential component of the TME, with MSCs and their paracrine-based activity assuming a major role in the tumor-stroma cross-talk [11,16]. In the TME, MSCs remodel the extracellular matrix (EMT), participate in the epithelial-mesenchymal transition, promote establishment of distant metastases, inhibit anti-tumor immune responses and support survival of leukemic cells in the bone marrow, creating a leukemia niche [11,15,16]. Literature is replete with evidence for the role of MSCs in progression and, to a lesser degree, also suppression of tumor growth and metastasis [reviewed in 11]. A broad variety of functions attributed to MSCs in cancer has been shown to be mediated by soluble factors these cells produce, including cytokines, chemokines and growth factors [reviewed in 16]. With the

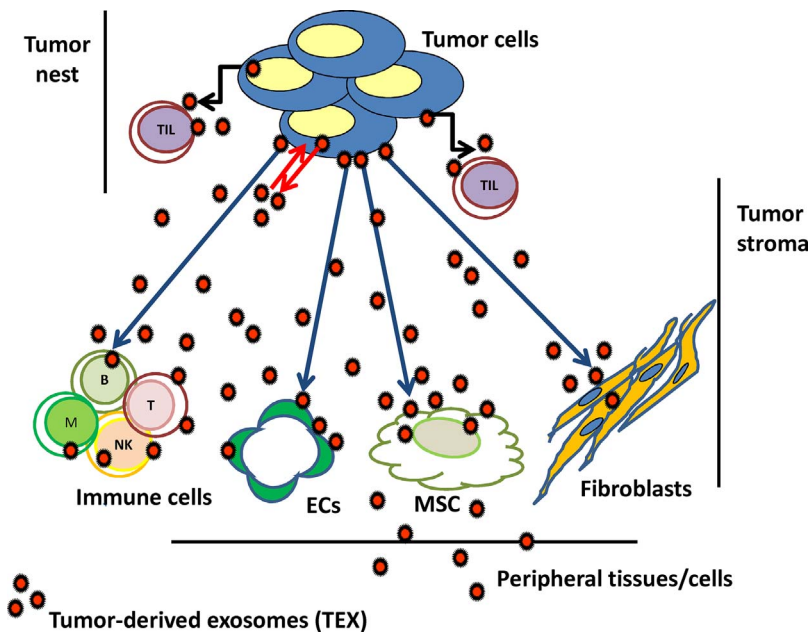


Fig. 1. In the TME, tumor-derived exosomes (TEX) communicate with the tumor cells producing TEX via autocrine interactions (red arrows). Tumor-infiltrating T cells (TILs) are also targeted by TEX via juxtacrine signaling (black arrows). Other cells in the TME: MSCs, fibroblasts, ECs, or immune cells are reprogrammed by TEX via paracrine interactions (blue arrows). TEX travel freely in tissues and body fluids and carry information to distantly located tissues and cells.

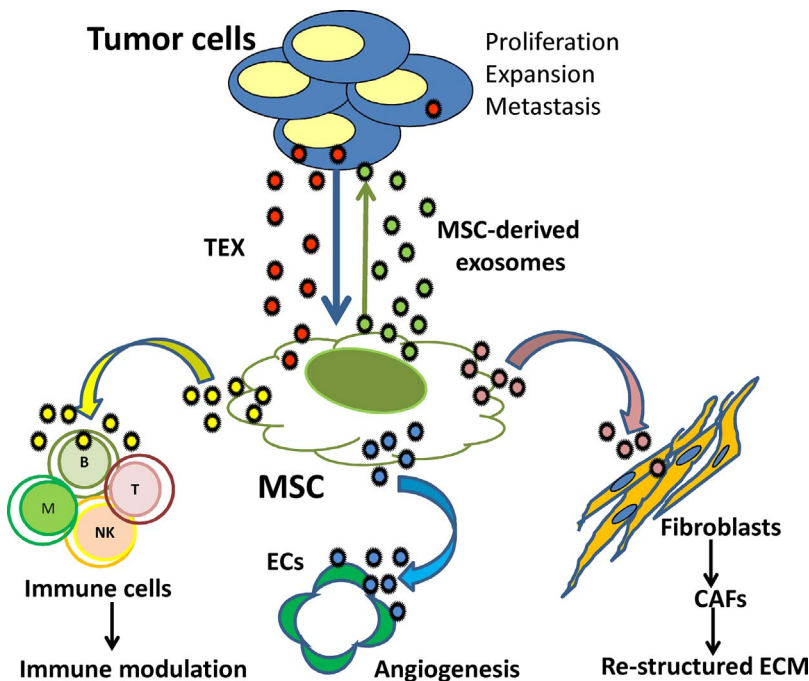


Fig. 2. TEX-mediated re-programming in the TME. TEX re-program MSCs, converting them into highly efficient producers of MSC-derived exosomes. MSCs cross talk with tumor cells and other cells in the TME using exosomes. Tumor to MSC cross-talk is bidirectional. MSCs re-programmed by TEX horizontally deliver exosomes carrying individual messages to fibroblasts, ECs or immune cells. MSCs play a central role in conveying pro-tumorigenic signals to these cells. It is not yet clear whether exosomes produced by the re-programmed parent MSCs are distinct subsets of vesicles each carrying an individually “addressed” message to recipient cells or are equipped with a broad cargo of molecular/genetic components to convey distinct messages to different recipient cells. In this context, recipient cells and the TME determine the outcome of exosome-mediated information transfer. The MSC plays a central role in conveying pro-tumor messages to cells present in the TME.

discovery of exosomes and realization that MSCs in the TME make and release an abundance of exosomes [39], the understanding of mechanisms underpinning interactions of MSCs with the tumor and *vice versa* has undergone a drastic change. MSCs are now viewed not only as recipients of signals emanating from the tumor but also as efficient producers of their own exosomes, which horizontally transfer information to neighboring cells and transform the cellular milieu into one supportive of the tumor survival (Fig. 2). In this context, it is important to recognize that the relationship between the MSCs and tumor cells is bidirectional (Fig. 2), and that the exosomes produced by MSCs that are re-programmed by TEX may exert profound effects on tumor growth [40]. This aspect of the MSCs biology is of intense interest, because of the potential for future therapeutic interventions it offers for cancer control via MSCs-derived exosomes [24].

3. MSCs re-programming by TEX

In the TME, the tumor-MSCs interactions begin at the inception of tumor growth, when MSCs are first attracted to the tumor and participate in the formation of the “pre-metastatic niche.” The recruitment of MSCs to tumor and their re-programming by TEX result in dramatic changes in the MSCs phenotype and functions. The coopted MSCs become pro-tumorigenic and convert into producers of factors promoting tumor progression. MSCs-derived exosomes mediating pro-tumorigenic effects maintain the cross-talk with the other cells in the TME, including tumor cells (Fig. 2).

The ability of TEX to orchestrate re-programming of MSCs and effectively recruit their help in re-modeling of the TME has been the subject of intense recent studies. It is known that molecular signatures of TEX are distinct from those of exosomes derived from normal cells

Table 1
Molecular/genetic contents of tumor-derived exosomes (TEX).

Molecular/genetic content ^a	Selected examples
Exosome Surface	
Signal transduction receptor/ligands	G-proteins
Inhibitory	Fas/FasL, PD-1/PDL-1 TRAIL/TRAIL-R, TGF- β /TGFR
Stimulatory	CD80, CD86, OX40/OX40L
MHC molecules	Class I; class II
Adhesion molecules	EPCAM, ICAM, CD44
Integrins	α v β 5, α v β 6, α v β 1, α 6 β 4
Tetraspanins	CD9, CD63, CD81, CD82
Membrane fusion proteins	Rab25, Rab27b, Annexins
Tumor-associated antigens	MAAs, LAAs ^b
Chaperones	Hsp70, Hsp90
APM components	TAP-1, TAP-2
Transmembrane proteins	LAMPs, CXCR4
Enzymes	CD39, CD73, CD26
Lipids	Cholesterol, ceramides, sphingomyelins, phospholipids
Exosome Lumen	
ESCRT-associated components	ALIX, TSG-101, syntenin-1, dynamin
Nucleic acids	mRNAs, miRNAs, DNA
Cytosolic proteins	Histones, nucleoproteins, ribosomal proteins
Proteases	GTP-ases, PL-ases, MMPs
Growth factors	EGF, FGF
Suppressive factors	PGE ₂ , adenosine

^a The exosome components listed above were selected from a list of many other molecules as reported in the ExoCarta based on the frequency of literature reports describing their presence in TEX.

^b MAA = melanoma-associated antigens; LAA = leukemia associated antigens.

[27]. It has also been shown that TEX released by different types of tumor cells carry distinct molecular signatures [33]. TEX signatures contain molecular and genetic signals that are able to induce modifications in recipient MSCs [27,41]. Valadi et al. were the first to report that exosomes mediated transfer of mRNAs and miRNAs between various cells [42].

3.1. The molecular/genetic cargos of TEX

Table 1 indicates that the TEX surface is enriched in numerous biologically active proteins. The cargo of TEX isolated from plasma of patients with cancer is enriched in immunosuppressive receptor/ligands, including PD-1/PD-L1, Fas/FasL, TRAIL/TRAILR [27]. However, immunostimulatory molecules, such as CD80, OX40, OX40L, or CD70, are also present, as are the major histocompatibility complex (MHC) molecules and various tumor associated antigens (TAAs). The TEX content suggests that TEX are equipped to supply immune-inhibitory as well as immune-stimulatory proteins to recipient cells. TEX are also enriched in adhesion molecules, presumably to facilitate their surface interaction with recipient cells and subsequent entry into the cytosol to deliver nucleic acids carried in the TEX lumen [43]. TEX also carry oncogenic DNA (including KRAS, HRAS, BCR-ABL); oncogenic microRNAs (miR125b,130b,155) and mRNAs (e.g., BrR-ABL1) and oncogenic proteins (e.g., EGFRVIII). TEX were shown to elicit tumorigenic conversion of immortalized rodent fibroblasts, epithelial cells or stem cell lines [44]. TEX carry tetraspanins (CD9, CD63, CD81), which are often used as “exosome markers,” although they are not selective components of exosomes. While the TEX content varies depending on their cellular origin, TEX are well equipped to induce modifications in MSCs functions, as they carry most if not all of the factors previously reported to mediate MSCs differentiation [45]. For example, it is well known that VEGF and TGF- β 1 are involved in angiogenesis as well as tumor metastasis [46,47]. Both these factors are carried by TEX produced by various solid tumors or by leukemia cells [48], and these TEX have been shown to trigger MSCs differentiation into pro-angiogenic and pro-invasive myofibroblasts [49].

3.2. TEX induce phenotypic and functional changes in MSCs

Emerging evidence for TEX serving as inducers of changes in MSCs that have been previously ascribed to tumor cells is largely derived from *in vitro* experiments, where isolated MSCs are co-incubated with TEX produced by cultured tumor cells [50]. Direct contact between cells in transwell-type experiments appears not to be required for re-programming of MSCs. While soluble factors or chemokines and cytokines produced by the tumor are likely to be partly responsible for the induced modifications as suggested [16], newer evidence indicates the involvement of another mode of communication. First, *in vivo* experiments with TEX in experimental animals have shown that TEX freely circulate and induce cell alterations in distantly-located cells and at distant tissue sites, suggesting delivery of messages protected from enzymatic degradation during transit [51]. Second, re-programming of MSCs by TEX appears to be a continuous and relentless process designed to introduce additional changes in MSCs to fulfill better the needs of tumor cells as they expand and metastasize. An epigenetic type of modifications mediated by genetic elements introduced by exosomes to recipient MSCs appears to be involved [52]

3.3. Mechanisms underpinning tumor to MSCs cross talk

The available data indicate that MSCs acquire the ability to perform a new and diverse set of functions upon exposure to TEX (Table 2). The cellular, molecular and genetic mechanisms responsible for re-programming of MSCs by TEX are under intense scrutiny. Cooperation of some sort between TEX and MSCs appears to be taking place, each partner contributing to the mechanism driving information transfer. The initial contact may be in the form of receptor-ligand signaling to which TEX contributes ligands decorating its membrane and MSCs contribute cognate receptors on their cell surface, leading to activation of one or many intracellular activation pathways in recipient cells [53]. This receptor-ligand type of interaction has been reported for exosomes carrying FasL, TRAIL, PD-L1, Wnt3a or TGF- β [38]. TEX carry numerous cell adhesion molecules (CAMs) and thus can readily fuse with adhesion receptors on MSCs allowing for the protein/gene transfer to the MSCs cytosol (Table 1). TEX carry integrins that determine tissue tropism of the vesicles. Thus, TEX-associated integrins α 6 β 4 and α 6 β 1 were reported to associate with lung metastasis, while exosomal integrin α v β 5 was linked to liver metastasis [54]. Phagocytosis of TEX carrying opsonins or complement components by MSCs expressing Fc receptors (FcRs) and/or complement receptors (CRs) as well as endocytosis, to which TEX contribute opsonins or ligands and MSCs the clathrin-coated pits, provide other means of delivery of proteins and/or genes to MSCs [55]. In each instance, the protein/gene transfer can lead to a cellular response or to lysosomal degradation of the transferred materials [55]. It remains unclear whether the protein transfer alone is sufficient for re-programming of MSCs by TEX or whether the transfer of transcription factors and nucleic acids is obligatory. In this context, it

Table 2
TEX re-program MSCs in the TME to mediate pro-tumorigenic functions.

Migration of MSC to the tumor site [68,69]
Production of pro-inflammatory cytokines [71]
Conversion of primary tumor site to the “pro-metastatic niche” [86,88]
Activation of intracellular molecular pathways [39,53]
Promotion of tumor growth <i>in vivo</i> [105]
Induction of epithelial-to-mesenchymal transition (EMT) [75,76]
Migration of tumor cells to metastatic sites in various tissues [69]
Recruitment and implantation of tumor cells in the bone marrow [79]
Alteration of the extracellular matrix [63]
Promotion of angiogenesis [104–106]
Immunomodulatory functions:
Suppression of immune effector cells [39,97–101]
Expansion of immune regulatory cells [94,97,100]
Increased resistance to cancer therapies [116,118]

is important to remember that some cells, e.g., T lymphocytes, do not readily internalize exosomes [29] and may depend on the protein–protein signaling for information transfer.

3.4. Nucleic acids transfer by TEX

Transfer of mRNA transcripts or of various miRNAs to MSCs by TEX is currently considered to be the major mechanism responsible for information transfer between the tumor and MSCs [reviewed in [56]]. In particular, non-coding RNAs (ncRNAs), including miRNAs and long ncRNAs, have been featured as candidates responsible for the phenotypic/functional changes induced by TEX in recipient cells [57]. miRNAs act on RNA by silencing or post-transcriptionally regulating gene expression, while long ncRNAs participate in imprinting and gene dosage regulation, including histone modification and formation of ribonucleoprotein complexes [58]. More recent evidence indicates that exosomes may also transfer DNA, and thus can modify gene expression in recipient cells [59]. Huan et al. reported that AML cell-derived exosomes labeled with the PKH26 dye were readily taken up by bone marrow stromal cells. These exosomes carried RNA transcripts relevant to leukemia pathogenesis such as FLT3, NPM1, CXCR4, MMP9 or IGF-1R [60]. Leukemia-derived exosomes also carried miR-150, which targets CXCR4, a cognate receptor for SDF-1 α , decreasing its surface expression levels in recipient cells as well as migration toward SDF-1 α . In addition, transcriptional activity of miR150 disturbed the CXCR4-CXCL12 axis, which is necessary for the retention of hematopoietic progenitor cells (HPCs) in the bone marrow and their normal differentiation. This study suggests that leukemia-derived exosomes promote leukemia growth by interfering with CXCR4-CXCL12 signaling [60]. Studies by Paggetti et al. showed that CLL-derived exosomes taken up by MSCs *ex vivo* and *in vivo* transfer miRNAs and proteins, inducing an inflammatory phenotype and transforming MSCs into cancer-associated fibroblasts (CAFs) [61]. Among miRNAs known to be abundant in TEX produced by various tumor types and shown to exert priming effects on MSCs upon transfer are the miR17-92 cluster and miR21. The miR17-92 cluster includes seven different miRNAs that have pro-angiogenic activity and also target the E2F transcription factor family- a critical regulator of the cell cycle and apoptosis in recipient cells. The miR17-92 cluster can regulate angiogenesis by directly targeting anti-angiogenic factors such as thrombospondin and connective tissue growth factor [62]. This dual role of miR17-92 in angiogenesis may be regulated at the level of the TEX cellular origin or the nature of recipient cell. TEX may also be highly enriched in miR21, which is considered to be an oncogene, as it promotes cell proliferation, migration and invasiveness by targeting a number of tumor suppressor genes, including p53, PTEN, and antagonists of the RAS pathway [63]. It also enhances expression of VEGF and thus promotes angiogenesis [64]. A recent study investigated the cargo composition of human (h) MSC-derived exosomes by next-generation sequencing (NGS) and proteomic analysis [65]. These exosomes carried a large number of proteins known to support tumor growth (e.g., PDGFR- β , TIMP-1 and TIMP-2 and miRNAs with pro-tumorigenic functions (e.g., miR-21 and miR-34a) when delivered to MCF-7 breast cancer cells. Numerous other miRNA species are carried and transferred by exosomes from the tumor to MSCs and from re-programmed MSCs to other cells in the TME (reviewed in [56]). Dissecting this complex network of gene-regulating elements is rapidly becoming the new frontier of cancer research [31].

4. MSC re-programmed by TEX cross-talk with tumor cells

MSCs service to tumors has been extensively studied (reviewed in [15]) and consists of different mechanisms, some involving direct transfer of growth factors to the tumor and others indirectly benefiting the tumor through re-structuring of its environment via modifications of non-tumor cells in the TME. Furthermore, the bulk of these direct as well as indirect interactions between cells appears to be contact-

independent and is executed by exosomes. As new details of exosome-mediated information transfer emerge, the functional relevance of TEX to cancer progression is becoming increasingly clear: aside from utilizing the autocrine feed-back mechanism (Fig. 1), TEX re-program the MSCs and engage them in pro-tumor activities. Such re-programmed MSCs become a rich source of exosomes that carry and deliver to the tumor factors necessary for its proliferation, differentiation, survival, invasion and metastasis [66]. Thus, exosome-mediated information transfer between the tumor and MSCs is a bidirectional process (Fig. 2).

4.1. Functional profiles of MSC re-programmed by TEX

Table 2 lists various pro-tumor functions mediated by the MSCs that were re-programmed by TEX. Remarkably, the modifications induced by TEX in MSCs mimic those reported previously to be induced directly by the tumor cells [40]. Lindoso et al. reported, for example, that TEX produced by renal cancer stem cells promote migration of MSCs to the tumor and induce expression of the pro-tumorigenic phenotype in these MSCs [67]. These changes in MSCs correlated with over-expression of genes involved in cell migration (CXCR4 and CXCR7), in the matrix remodeling (*collagen type IV alpha 3 chain*) and in angiogenesis or tumor growth (IL-8, OPN and myeloperoxidase) [67]. TEX in other tumor types were also reported to promote MSCs migration to the tumor site [68,69]. Exosomes produced by primary or metastatic colorectal cancers (CRCs) were shown to reprogram colonic MSCs, inducing morphological and functional alterations which favored tumor growth and its metastasis [70]. TEX derived from prostate cancer, breast cancer or CLL cells were shown to induce MSCs differentiation into myelofibroblasts overexpressing alpha smooth muscle actin (α SMA) [71]. TEX produced by lung cancer cell line, A549, stimulated production and secretion of inflammatory cytokines in MSCs, including IL-6, IL-8 and MCP-1, via the NF- κ B-TLR signaling pathway [72]. Further, this priming of MSCs was shown to involve activation of TLR 2/ NF- κ B signaling by interaction of HSP70 on the surface of TEX with the recipient cells. In several other studies examining TEX interactions with MSCs, enhanced secretion of IL-6 or IL-8 in recipient cells which, in turn, promoted cancer expansion [73,74] and the epithelial to mesenchymal transition (EMT) [75,76]. MSC-derived exosomes carrying growth factors and IL6 were shown to promote proliferation of multiple myeloma cells *in vitro* and *in vivo* [77]. Recent report provides evidence that AML blast-derived exosomes remodel MSCs in the bone marrow niche into leukemia-growth permissive cells and suppress normal hematopoiesis *in vivo* [78]. Either *in vivo* engrafted AML cells or AML-derived exosomes enhanced numbers and functions of MSCs. Further, AML exosomes induced broad downregulation of factors supporting normal hematopoiesis, such as CXCL12, KITL, IL-7 and IGF1, in MSCs. Disruption of exosome secretion in AML cells through targeting Rab27a, which is responsible for regulation of exosome release, significantly delayed leukemia development in mice [78]. In these experiments, TEX-MSCs cross-talk has first led to changes in the recipient cell phenotype that then enabled re-programmed MSCs to participate in cancer progression. The overall conclusion of these recent experiments is that exosomes released by the MSCs re-programmed by TEX are essential for the promotion of tumor progression.

5. Cross-talk of the re-programmed MSCs with other cells in the TME

Under normal physiological conditions, MSCs are a rich source of exosomes [79], which modulate functions of various neighboring tissue cells. At various tissue sites, MSCs establish an intimate relationship with stromal cells, HPCs, immune cells or endothelial cells in the vascular compartment [80]. Such a relationship is beneficial for normal physiological functions of these cells. The critical roles of MSCs in normal hematopoiesis, tissue repair and vascularization as well as regulation of immune cell functions have been extensively evaluated

Table 3
Mechanisms involved in TEX-driven re-programming of MSCs in the tumor microenvironment.

Mechanism	Changes induced in re-programmed MSCs	References
Genetic	↑ Proliferation	[30,55,62]
Uptake & transfer of miRNA species	↑ Cellular activity via upregulation of common signaling pathways (TGF- β , AKT, β -catenin, Wnt, Notch) ↑ Production of growth factors and cytokines necessary for TME re-modeling ↑ Pro-angiogenic activity ↑ Immunoregulatory gene alterations	[55,56,60,72] [50,55,56,60] [59,62,64] [49,61]
Proteomic		
Surface receptor signaling	↑↓Major molecular pathways	[37,55,63]
Uptake of TEX cargo	↑↓Major molecular pathways	[29,49,67]
Transfer of: Enzymes	↑Immunosuppressive phenotype	[27,41]
Cytokines/growth factors	↑Stromal remodeling ↑Immune modulation	[39,40,48,66]
Survival proteins	↑Resistance to apoptosis	[71,74]
Immunosuppressive molecules	↓Anti-tumor immune functions	[36,38]
MHC molecules	Altered interactions with T lymphocytes	[25,36,37]
Metabolic	↑ Hypoxia	[118,119]
Changes in cellular metabolism	Switch from oxidative phosphorylation to glycolysis	

[40]. Importantly, it is becoming evident that MSC-derived exosomes are responsible for many functions generally attributed to MSCs [81]. In the TME, this physiological exosome-driven communication network becomes deranged, and the normal reconstructive functions of MSCs become replaced by a new repertoire of pro-tumor activities mediated by the exosomes produced by the MSCs re-programmed by TEX. These MSC-derived exosomes have the capacity to interact with multiple cell types in the TME and to ensure they adequately support tumor growth. They carry a complex cargo of molecules and genes comprising > 850 unique gene products and > 150 different miRNAs [82,83] and thus have a potential to elicit diverse cellular responses in a broad variety of cells (Table 3).

5.1. Re-programmed MSCs vs tumor stroma

The tumor-stroma interaction is critical for cancer progression, and it too is mediated via exosomes. A key function of MSC-derived exosomes in the TME is to ensure that stromal support is provided for the tumor. It has been shown that interaction of MSCs with cancer cells leads them to acquire the CAF-like phenotype characterized by expression of CXCL12, α -SMA and fibroblast surface protein (FSP) [84,85]. In fact, conversion of fibroblasts and MSCs into CAFs is accomplished by TEX, and exosomes secreted by CAFs promote tumor growth [86]. TEX from primary CLL cells re-programmed MSCs to the CAF phenotype characterized by increased NF- κ B signaling and

elevated secretion of cytokines and chemokines, which enhanced tumor cell survival *in vitro* and *in vivo* [61]. The well-known regenerative functions of MSCs are also mediated by exosomes as per recently reported evidence that CD63+ exosomes produced by BM-MSCs carry Wnt3a protein on their surface and are able to engage recipient cells expressing the cognate receptor (Frizzled) and activate the downstream Wnt pathway, leading to stimulation of fibroblast proliferation as well as up-regulation of endothelial cell activities [87]. It is likely that many of the above described effects can be attributed to miRNAs carried by exosomes, which are implicated in stroma-restructuring, angiogenesis and metastasis, including miR-200c, miR-146, miR-92, miR-301, miR-7 g and miR-130b [88].

5.2. Re-programmed MSCs vs immune cells

An increasing number of studies report that MSC-derived exosomes mimic the well-known capability of MSCs to modulate activities of immune effector cells, including T and B lymphocytes, NK cells, monocytes/macrophages and dendritic cells (DC) [39,89,90]. The word “modulation” is frequently used to describe the effects of MSCs or exosomes they produce on immune cells. Presumably, this implies that MSCs and MSC-derived exosomes can inhibit as well as stimulate immune responses. The dual ability of TEX to mediate suppression and activation of immune responder cells is well known [91] and it has been shown that TEX carry and deliver both immunosuppressive and

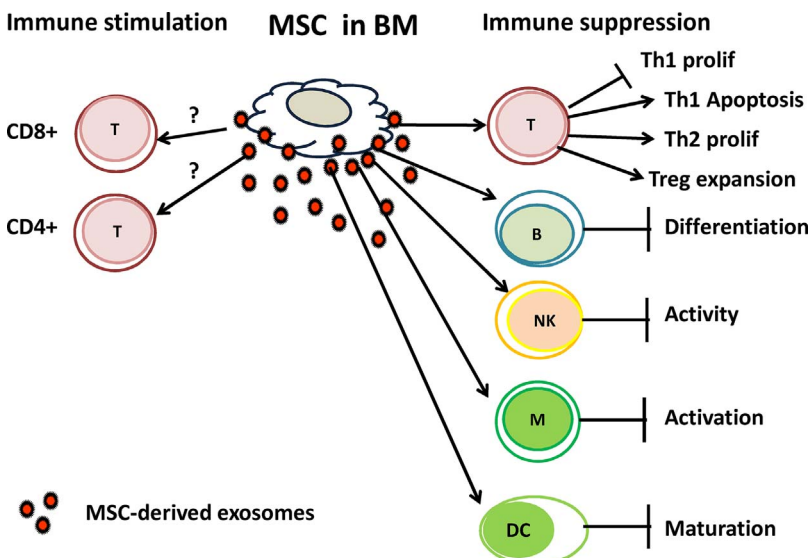


Fig. 3. Immune modulation by exosomes produced by MSCs isolated from normal human or murine bone marrow. These exosomes co-incubated with normal human immune cells induce immune suppression. No convincing evidence is currently available to support immunostimulatory effects of MSC-derived exosomes. The potential mechanisms of immune suppression mediated by MSC-derived exosomes are shown in Table 4.

Table 4
Immune modulation by MSC-derived exosomes^a.

MSCs source	Immune function modulated	Effector mechanism	Reference
Normal human BM-MSC	↓Lymphocyte proliferation	TGF-β	[101,102]
Normal human BM-MSC	↓Survival ↓T cell activation	TGF-β	[101]
BM-derived MSC	↑Treg ↑Th2 TH1 TH17 ↓CD3+ T cell proliferation	IDO IL-10	[98]
Normal human BM-MSC	↑Apoptosis ↑Treg/Teff ratio	IL-10 TGF-β	[100]
Murine BM-MSC	↓B cell proliferation ↓T cell proliferation	PT-L1, TGF-β	[97]
Human ESC → MSC	↓T cell proliferation ↑Treg (CD4 + CD25 + FOXP3 +) Re-programmed THP-1	↑IL-10, TGF-β ↓IL-1β, IL-6, TNFα	[39]
Normal human BM-MSC	↑Survival of allogeneic skin graft <i>in vivo</i> ↓M0 activation	↓TLR signaling	[99]

^a MSC-derived exosomes used in co-incubation experiments with various immune cells were obtained from cultures of BM-MSC.

immunostimulatory signals to recipient cells [37]. It is less clear at present whether exosomes derived from non-transformed cells have the same ability for dual modulation of immune cells. In the context of the MSCs, which are known to be able to produce a vast array of immunostimulatory and immunoinhibitory cytokines [92], “modulation” seems to be an appropriate functional designation. Interestingly, recent studies show that MSC-derived exosomes mediate immune immunosuppression rather than immune stimulation (Fig. 3). Only infrequent reports mention immunopotentiating capabilities of MSCs [93], although none refer to MSC-derived exosomes. Further, not only re-programmed MSCs in the TME but also exosomes produced by MSCs isolated from the normal human or murine bone marrows (BM) are immunoinhibitory (Table 4). MSC-derived exosomes carry CD39 and CD73, ectonucleotidases catalyzing adenosine production [94] and, in parallel to MSCs, a plethora of other immunosuppressive factors, including indoleamine 2,3-dioxygenase (IDO), TGF-β, IL-6, PGE₂, PD-1, galectin -1 and HLA-G5 [95–97].

The inhibitory effects of MSC-derived exosomes on functions of immune cells were demonstrated in several recent studies [81,94,98–100]. In co-cultures of PBMCs with exosomes isolated from MSCs, T-cell activation tended to be suppressed, and the fraction of regulatory T cells (Treg) was enlarged [101]. The authors of this study also reported that exposing CD4+ T cells to MSC-derived exosomes favored expansion of Th2 cells, while limiting proliferation of Th1 and Th17 cell subsets. The observed effects were associated with a shift in a cytokine profile of PBMC from pro-inflammatory IL-1β and TNF-α to anti-inflammatory TGF-β [101]. These results suggest that exosomes produced by normal MSCs can suppress T-cell activation and help maintain immune homeostasis. Perhaps, the immunoinhibitory effects of exosomes produced by normal MSC could be looked upon not as overt immune suppression but rather as down-regulation of T-cell activation, which is designed to prevent destructive inflammatory responses and thus protect tissue cells from damage by activated immune cells.

Several other studies show that MSC-derived exosomes lower the immune system activation through induction of anti-inflammatory cytokines and regulatory immune cells [39,100,101]. MSC-derived exosomes can activate monocytes by the TLR signaling pathway [39,100,101]. When monocytes are stimulated by MSC-derived exosomes they differentiate into macrophages which secrete IL-10, leading to Treg expansion [39] similar to effects induced by MSCs [102]. In the TME, these anti-inflammatory effects of MSCs are amplified, designating exosomes produced by MSCs as the major mechanism responsible for expansion of Treg and MDSCs [39]. Thus, MSC-derived exosomes contribute to maintaining of an immunosuppressive climate

throughout the TME. However, in the normal tissue milieu, MSC-derived exosomes might protect tissue cells from an immune-mediated damage.

5.3. Re-programmed MSC vs endothelial cells

One of the tumor-promoting functions of MSCs is to support angiogenesis in the TME. In a seminal paper Skog et al. demonstrated that glioblastoma-derived EVs carrying mRNA for Gluc were internalized by human brain microvascular endothelial cells (HBMVEC), which translated it to the Gluc protein and activity. Further, HBMVEC co-incubated with these EVs in the presence of angiogenic growth factors developed into tubular structures in matrigels [103]. This was the first demonstration that EVs in the TME transport RNAs and proteins promoting angiogenesis in the recipient cells. Since then the role of tumor-derived as well as MSC-derived exosomes in vessel growth has been confirmed in numerous studies [104–107]. Co-culture of tumor cells with MSCs or subcutaneous injections of MSC-tumor cell mixtures into nude mice promoted tumor growth associated with expansion in the tumor vasculature [105]. Further, MSC-derived exosomes upregulated VEGF expression in tumor cells by activating the extracellular signal-regulated kinase1/2 (ERK1/2) pathway. Inhibition of ERK1/2 activation or the pre-treatment of exosomes with RNase abrogated these effects, confirming the crucial involvement of mRNAs delivered by exosomes in the promotion of angiogenesis [105]. MSC-derived exosomes also carried transcripts and proteins related to angiogenesis and proliferation, including VEGF, TGF-β, fibroblast growth factor (FGF) and cytokines, including IL-6, IL-8 and IL-1β [94,95]. MSC-derived exosomes transfer miRNAs to endothelial cells (ECs). For example, Gong et al. showed that MSC-derived exosomes carrying a pro-angiogenic miR-30b promoted tube formation with HUVEC, increased migration of EC and increased blood flow in tubes formed in matrigel plugs, following their implantation into mice [104]. The available data confirm the pro-angiogenic potency of MSC-derived exosomes in the TME and indicate that transfer by exosomes of mRNAs and relevant proteins to ECs is the mechanism accounting for EC reprogramming.

6. Anti- tumor effects of MSC-derived exosomes

While exosomes produced by MSCs in the TME largely mediate pro-tumor effects, there is also evidence for anti-tumor activity of MSC-derived EVs. Bruno et al. reported that exosomes derived from normal human BM-MSCs inhibited growth and survival of various human tumor cell lines [108]. *In vivo* inhibition of tumor growth by these exosomes was also reported in NOD/SCID mice [108,109]. Studies from

several other groups confirmed the tumor-inhibitory potential of MSC-derived exosomes on breast cancer or hepatocellular carcinoma cells [110–112]. However, the sources of EVs used in these experiments differed widely as did methods for EV isolation. It may be that exosomes and MVs mediate quite different or overlapping functions [113]. It may also be that exosomes from normal MSCs could be tumor inhibitory perhaps by up-regulating immune effector cell functions *in vitro* or in experimental animals, as suggested [109,111], or by protecting tumor cell progenitors from destruction by e.g., radiation [114]. Interestingly, enrichment of the exosome cargo in certain pro-angiogenic miRNAs, e.g., miR-100, and the ability of these exosomes to suppress VEGF production and thus angiogenesis in breast cancer cells through modulating the mTOR/HIF-1 α signaling axis is directly related to anti-tumor effects they mediate [112]. Growth of glioma was reported to be inhibited by stromal cell-derived exosomes rich in miR-146b [115], and MSC-derived exosomes appear to be enriched in miR-23b which promotes breast cancer cell dormancy [116]. Thus, modulation by miRNAs carried in MSC-derived exosomes of vascular responses and/or tumor growth may be responsible for their anti-tumor activity. Nevertheless, exosomes produced in the TME by TEX-conditioned MSCs carry immunosuppressive cargos, inhibit anti-tumor immune responses *in vitro* or *in vivo* and promote tumor growth [77]. At the same time, it should be remembered that exosomes carry rich and diverse cargos and that not all MSC-derived exosomes are equal [45]. It is also known that their interactions with immune cells could lead to immune response inhibition or to immune stimulation [38]. The dichotomy of exosome-mediated immune-modulatory functions has been previously discussed [91] and remains a subject of great interest. Similar dichotomy, albeit less frequently discussed, seems to exist in respect to other pro-tumor activities modulated by MSC-derived exosomes.

7. Conclusions

Exosomes produced by MSCs in the TME emerge as a new communication system operating between cells engaged in supporting tumor progression. MSCs, due to their abilities to produce a broad spectrum of cytokines, chemokines and growth factors, have been considered to be a key cell in the promotion of tumor growth (reviewed in [40,56]). This is not surprising, as MSCs are well known for their salutary or rescue effects [117]. It has been shown that tumor cells are highly effective in altering the MSC functional phenotype from physiological to pathological activities, and that this alteration is associated with the expression of a new set of cytokines and chemokines in MSCs. However, the molecular/genetic underpinning of the transition has remained obscure. Rapidly emerging data focus on exosomes as a remarkably versatile and effective transport and delivery system of nucleic acids and proteins between cells engaged in the biological cross-talk in the TME. MSC-derived exosomes function largely via horizontal transfer of mRNAs, miRNAs and proteins, which upon delivery to targeted recipient cells alter the phenotypic and functional profiles of these cells. In the TME, these exosome-mediated alterations are largely pro-tumorigenic, although it appears that MSCs can also exert anti-tumor activities. These immunosuppressive inhibitory activities appear to be mediated by miRNAs which may be of interest as future cancer therapeutics.

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Conflict of interest statement

The author indicates no potential conflicts of interest.

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