



# The Potential Biomarkers and Immunological Effects of Tumor-Derived Exosomes in Lung Cancer

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Lung cancer remains the leading cause of cancer-related deaths worldwide. Despite considerable achievements in lung cancer diagnosis and treatment, the global control of the disease remains problematic. In this respect, greater understanding of the disease pathology is crucially needed for earlier diagnosis and more successful treatment to be achieved. Exosomes are nano-sized particles secreted from most cells, which allow cross talk between cells and their surrounding environment *via* transferring their cargo. Tumor cells, just like normal cells, also secrete exosomes that are termed Tumor-Derived Exosome or tumor-derived exosome (TEX). TEXs have gained attention for their immuno-modulatory activities, which strongly affect the tumor microenvironment and antitumor immune responses. The immunological activity of TEX influences both the innate and adaptive immune systems including natural killer cell activity and regulatory T-cell maturation as well as numerous anti-inflammatory responses. In the context of lung cancer, TEXs have been studied in order to better understand the mechanisms underlying tumor metastasis and progression. As such, TEX has the potential to act both as a biomarker for lung cancer diagnosis as well as the response to therapy.

**Keywords:** tumor-derived exosome, lung tumor, exosomes, microRNA, NSCLC

## INTRODUCTION

Lung cancer is one of the most common cancers and the leading cause of cancer-related death worldwide. The two histological subtypes of lung cancers are non-small cell lung cancer (NSCLC) that encompasses >80% of lung cancers, including adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma and small cell lung cancer, which accounts for the remaining 20% of cases (1).

Exosomes are small vesicles (30–100 nm in size) that originate from most cells and are released into biological fluids, such as saliva, plasma, urine, and breast milk. Exosomes enable cell-to-cell communication by transferring their contents including RNA (mRNA and non-coding RNA), DNA (mtDNA, ssDNA and dsDNA), proteins, and lipids (2). This communication influences physiological

process of the recipient cell and may be involved in pathological conditions such as cancer (3).

The exosomes derived from tumor cells are called tumor-derived exosomes (TEX) (4) (**Figure 1**). TEXs are the main mechanism of intracellular communication between tumor and host cells and enable cancer cells to modulate their surroundings to favor an optimal microenvironment for tumor initiation and progression. TEX contains a variety of different immunostimulatory and immuno-inhibitory factors that support the cellular reprogramming of the recipient cells. For example, exosomes are involved in promoting cancer growth by transfer of oncoproteins such as K-RAS and MET or oncogenic miRNAs to otherwise healthy cells (5). TEX may also drive metastasis by creating a pre-metastatic niche and directing the disseminated tumor cells to future metastatic sites (6, 7). Interestingly, this does not occur randomly and is regulated by integrin expression on the TEX (8). In contrast, TEX can also induce an antitumor immune response by modulating killer cell lectin-like receptor K1 (KLRK1 or NKG2D) expression on natural killer cells (NKs) and thereby affecting their function (9, 10).

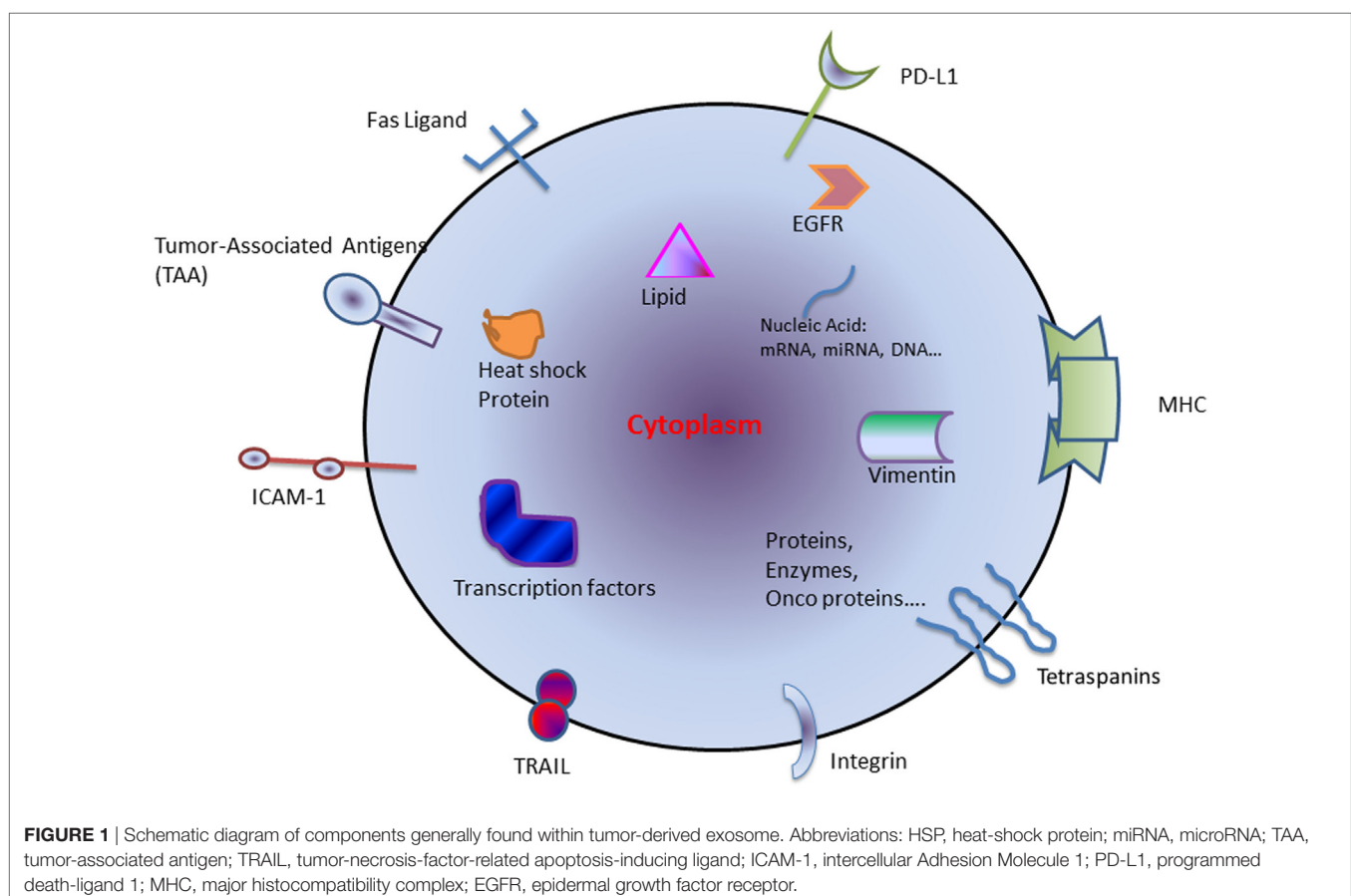
In this article, we review the immunological effects and function of TEX in cancers with an emphasis on lung cancers development and metastasis. Since NSCLC encompasses >80% of lung cancers, so we summarized recent research preferentially for this type of lung cancer. In addition, we evaluate the potential of these exosomes to act as a diagnostic biomarker in lung cancer.

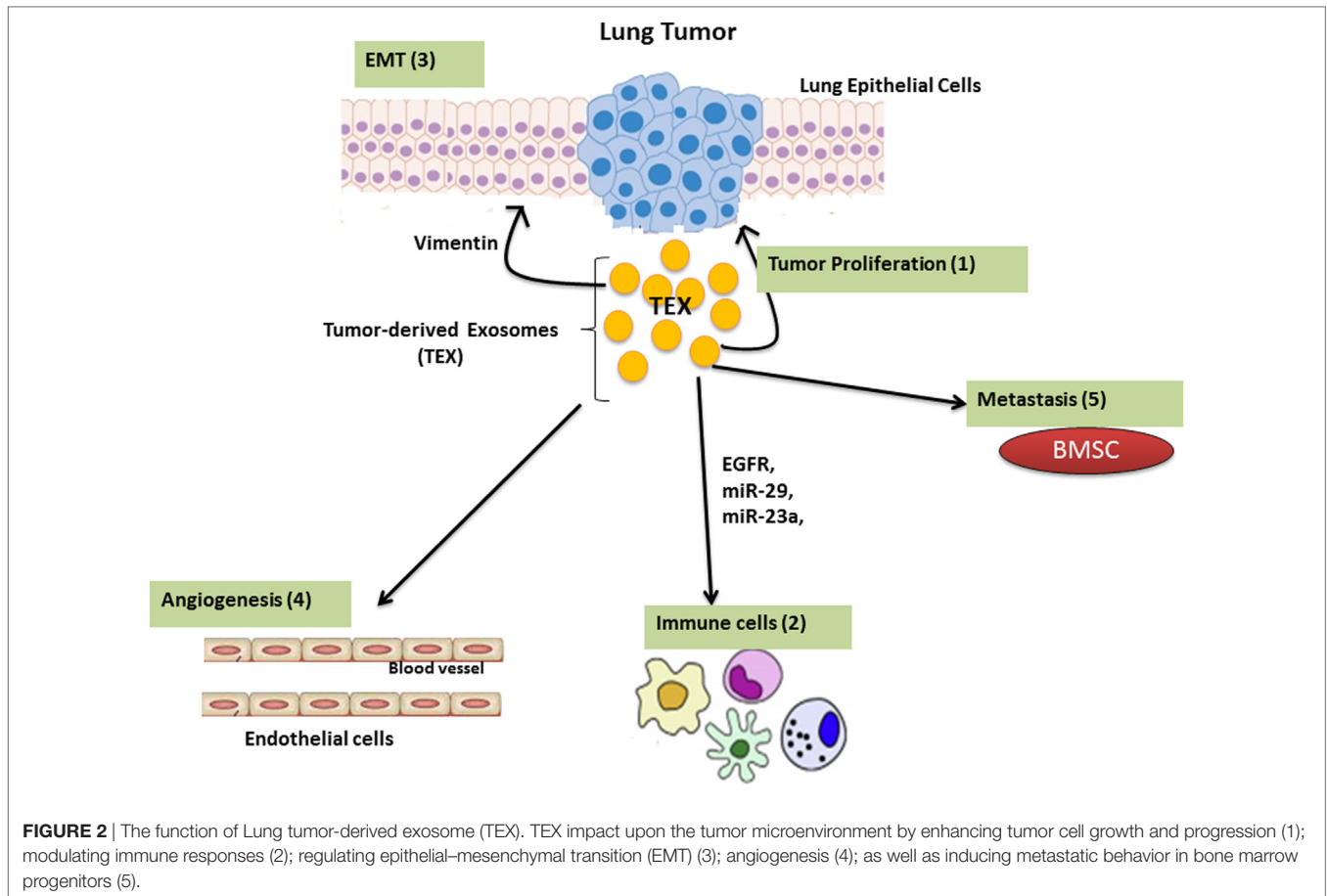
## THE ROLE OF TEX IN THE LUNG TUMOR MICROENVIRONMENT

The tumor microenvironment consists of different components with various properties based on the tumor's origin. The most abundant components in the tumor microenvironment are: carcinoma cells, immune cells, extracellular matrix (ECM), and stromal tissues (11, 12). The molecular and cellular nature of the tumor microenvironment determines malignancy by modulating local immune responses (13). TEX contain stimulatory and inhibitory components that, when delivered to the recipient cells, enable crosstalk between tumor cells and its surrounding environment. TEXs are involved in modulating the immune response, regulating epithelial–mesenchymal transition (EMT) and cancer-associated fibroblast function as well as playing a key role in angiogenesis (**Figure 2**).

### Tumor-Derived Exosomes and the Immune Response

The immune system has a significant impact on cancer outcomes (14). The immune system acts like a double-edged sword in cancer by destroying cancer cells and suppressing tumor growth as well as supporting the chronic inflammation and suppressing antitumor immunity which leads to tumor progression (15) (**Figure 3**).





### Natural Killer Cells

Lung cancer cell-associated TEX contain miR-21 and -29a which can both bind to intracellular toll-like receptors (TLRs) on immune cells including NKs, and thereby trigger a pro-metastatic inflammatory response due to activation of NF- $\kappa$ B eventually resulting in metastasis and tumor growth (16).

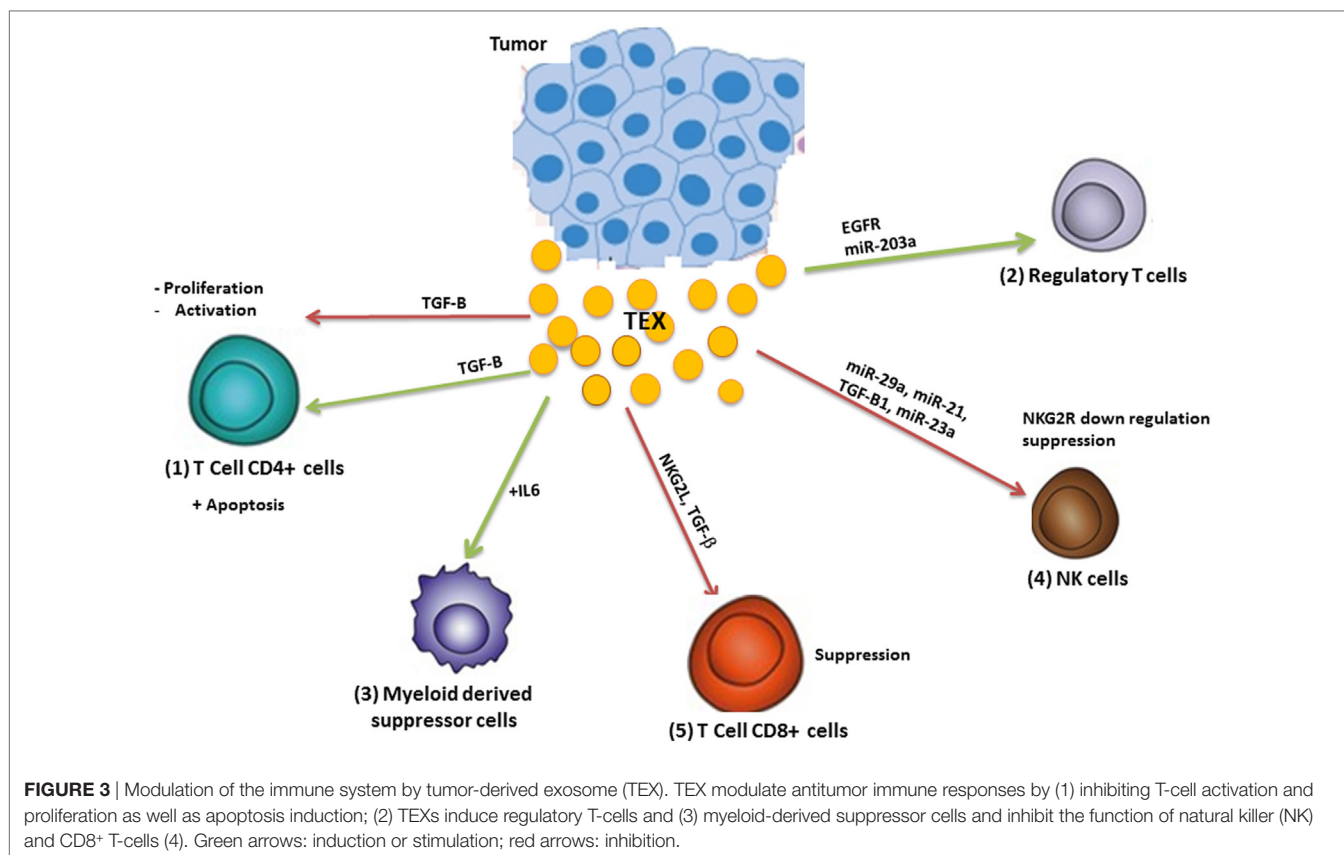
In NKs, the C-type lectin-like receptor NKG2D serves as an activating receptor to trigger cytotoxicity toward cancerous cells that express its ligand (17). TEX originating from hypoxic tumor cells inhibit NK function by delivering transforming growth factor (TGF)- $\beta$ 1 to NKs and subsequently reducing NKG2D expression (18). In a mouse model, TEX reduced the percentage of NKs found within the lungs (19). The downregulation of cell-surface receptors particularly that of NKG2D, may account for the reduced activity of NKs seen in lung cancer patients (20). miR-23a derived from TEX may directly target CD107a, a molecule that protects NKs from granule-mediated degradation (21).

Tumor-derived exosome can also downregulate NKG2D expression on NKs by shedding the NKG2D ligand on tumor cells. This will result in receptor desensitization and internalization and lower activity of NKs (22–25). TEXs may also attenuate NK activity *via* other mechanisms including the down-modulation of interleukin (IL)-2-mediated pathways (26), suppressing perforin or cyclin D3 production (19) and janus kinase (Jak)3 activation resulting in a failure of NK-mediated cytotoxicity (19).

### Dendritic Cells (DC) and Myeloid-Derived Suppressor Cells (MDSCs)

It is well-known that tumor microenvironment educate DCs to promote tumorigenicity. TEXs have important roles in this context by shuttling signaling molecules and tumor antigens and developing cell-to-cell communication (27).

Approximately 80% of the exosomes isolated from lung cancer biopsies contain epidermal growth factor receptor (EGFR) which has the potential to induce tolerogenic DC and regulatory T-cells, ultimately leading to the suppression of tumor antigen-specific CD8<sup>+</sup> cells (28). In pancreatic cancer, TEX contain miR-203a, that decrease the expression of TLR4 on DCs and subsequently leads to a reduced production of downstream cytokines including tumor necrosis factor (TNF)- $\alpha$  and IL-12 (28, 29) which result in dysfunction of DC and cellular immunity (29). TEXs can also prevent DC maturation and function. In a murine delayed-type hypersensitivity (DTH) model, administration of TEXs loaded with ovalbumin result in suppression of DTH responses by inhibiting DC maturation *via* TGF- $\beta$ 1. This result highlights the roles of TEXs in the promoting tumor antigen-specific immunosuppression, possibly by modulating the function of DCs (30). In melanoma and colon cancer, TEXs promote the differentiation of CD14<sup>+</sup> monocytes to MDSCs rather than to DCs (31). MDSCs are an immature population of myeloid cells identified in humans and mice that expand in cancer and have strong immunosuppressive



effects on the antitumor T-cell response (32). TEX interaction with monocytes, results in a monocyte phenotype that is characterized by a failure to upregulate co-stimulatory molecules (29, 33) and decreased human leukocyte antigen-DR expression (34, 35) with unchanged CD14 surface expression (35). Collectively, TEXs alter monocyte differentiation to DCs and promote the maintenance of an immature monocyte status. These cells spontaneously secrete immune inhibitory cytokines such as TGF- $\beta$  and prostaglandin E2 which inhibit T-cell proliferation and antitumor functions (31).

However, the overall effect *in vivo* is likely to be complex. Intravenous injection of TEXs into mice resulted in the accumulation of MDSCs and a marked increase in the production of inflammatory mediators, including IL-6 and vascular endothelial growth factor (VEGF) (36). On the other hand, the accumulation of MDSCs increased the production of immunosuppressive factors, such as nitric oxide and reactive oxygen species, which cause T-cell apoptosis (31). Both of these processes lead to tumor progression.

The presence of heat-shock protein 72 (HSP72) on the surface of TEXs, could trigger the activation of STAT3 and autocrine IL-6 production in MDSCs in a TLR2/MyD88-dependent manner which promotes the suppressive activity of MDSCs (37–39). Treatment of mice with TEX significantly increased tumor metastasis along with the recruitment of MDSCs into the lung. These effects were mediated by MyD88 which is a cytoplasmic adaptor molecule needed for the integration and transduction of TLR signaling (24).

### Tumor-Associated Macrophages (TAMs)

Tumor-associated macrophages are the major modulators of the tumor microenvironment that regulate angiogenesis, invasion, metastasis, as well as immunosuppression in tumor stroma (40). During tumor progression, circulating monocytes and other inflammatory lymphocytes are recruited into tumor tissue and alter tumor microenvironment. Monocytes are the precursors of TAMs that can get a continuous survival subsist in the inflammatory tumor microenvironment and generate TAMs (41). TEXs have a pivotal role in monocyte survival and in TAM generation within the tumor inflammatory niche. TEXs trigger the mitogen-activated protein kinase (MAPK) pathway in monocytes through delivery of functional receptor tyrosine kinase, which in turn leads to inhibition of apoptosis-related caspases (42).

Hsp72 and palmitoylated proteins on the TEX surface also modulate TLR signaling and the function of TAMs, which have a critical role in reinforcing tumor metastasis and invasion. Thus, TEX, acting through TLR2 and triggering the NF- $\kappa$ B signal pathway can promote the secretion of pro-inflammatory cytokines by macrophages (43). The induction of breast cancer invasion and metastasis by TAMs requires the upregulation of Wnt5 $\alpha$  in macrophages that leads, in turn, to the activation of  $\beta$ -Catenin-independent Wnt signaling in tumor cells. TEX mediate the crosstalk between tumor cells and TAMs and TAM-derived exosomes have a reciprocal supportive role in providing factors that activate  $\beta$ -Catenin-independent Wnt signaling in the breast cancer cells (44). This reciprocal interaction of TEX and TAM-derived exosomes may maintain TAM survival within the

inflammatory niche (45). In lung cancers, interaction between TAMs and tumor cells results in tumor progression *via* STAT3 activation and TAM-derived IL-6 (46). This may simply be a result of the nature of exosomes in cell–cell communication and in shuttling signaling molecules.

Exosome released from TAMs also can be important on cancer progression. Comprehensive proteomic analysis showed TAM-derived exosomes have different proteomic signature and higher proteolytic activity (47). In epithelial ovarian cancer (EOC), TAMs derived exosomes inhibit the migration endothelial cells (ECs) by targeting the miR-146b-5p/TRAF6/NF- $\kappa$ B/MMP2 pathway. On the other hand, EOC-derived exosomes reverse this effect on ECs by transferring long non-coding RNAs (48). Exosomes derived from TAMs also are involved in induction of cisplatin resistance in gastric cancer by transferring miR-21 (49). Overall, the existence of different mechanisms for several cancer cells exosome types in tumor microenvironment enforces the role of exosomes as the major player in cancer progression.

### T-Regulatory (Treg) and B-Regulatory (Breg) Cells

In contrast to immune cells such as NKs, B-cells, and monocytes that internalize TEX, TEX induce a Ca<sup>2+</sup> influx in T-cells in the absence of exosome internalization. Plasma TEX from cancer patients cause a strong and sustained increase in inosine production in Treg cells which suggests a functional consequence of TEX signaling on these recipient cells (50). TEXs also enhance Treg and Breg proliferation *via* TGF- $\beta$  and IL-10-dependent mechanisms and thereby increase their resistance to apoptosis (20, 31).

The level and suppressor activity of Tregs are higher in the peripheral blood of patients with cancer compared to healthy subjects (51). This may reflect the ability of TEXs to stimulate Treg expansion, increase their resistance to apoptosis and enhance their suppressor activity (52, 53).

Tumor-derived exosomes induce the conversion of CD4<sup>+</sup>CD25<sup>neg</sup> T cells into CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup> Treg cells. On the other hand, incubation of Treg with TEXs, increased the expression of FasL, IL-10, TGF- $\beta$ 1, cytotoxic T-lymphocyte associated protein 4 (CTLA-4), granzyme B and perforin as well as Smad2/3 and STAT3 phosphorylation in Tregs (52).

Tumor-derived exosomes stimulate the expression of CD39 and adenosine production in Treg *via* modulation of related genes in the adenosine pathway (4). Treg produce adenosine *via* ATP hydrolysis by both CD39 (ATP-hydrolase) and CD73 (5'-nucleotidase) on their surface. Adenosine is an immunosuppressive factor that suppress T cell function by binding to its receptors A1, A2A, A2B, and A3. TEX contain surface CD39 and CD73, directly deliver membrane-tethered CD73 to CD39<sup>+</sup> cells and negatively modulate T cells function by production of extracellular adenosine and thus decrease the local immunity (54). These TEX-mediated mechanisms are important in regulating tolerance of tumor and can promote tumor invasion in cancers. TEXs also induce loss of CD69 on the surface of conventional CD4<sup>+</sup>T (Tconv), which leads to their functional decline (4).

Regulatory B cells (Breg) are a subset of B cells with immunosuppressive properties that mediate immunological tolerance. Breg produce molecules, such as IL-10, IL-35, TGF- $\beta$ , programmed death-ligand 1 (PD-L1), and IL-21, and induce the production of

Treg and thereby prevent immunopathologic events by inhibition of pro-inflammatory lymphocytes (55). Elevated levels of regulatory Bregs are reported in PBMCs of invasive carcinoma of breast cancer patients (56).

It was shown that exosomes released from mycoplasma-infected tumor cells preferentially activate IL-10-producing B cells which in turn inhibit T cell activity (57). Exosomes released from the esophageal cancer cells also induce Breg production. These microvesicles carried LAMP1 and matrix metalloproteinase (MMP)9 and induce differentiation naive B cells into TGF- $\beta$ -producing regulatory B cells which subsequently suppress CD8<sup>+</sup> T-cell activities (58). These information highlight the importance of TEXs in tumor immunity by the mechanisms involved in modulation of Tregs and Bregs.

## THE ROLE OF TEX IN EMT IN LUNG CANCER

Epithelial–mesenchymal transition is a process by which epithelial cells acquire mesenchymal cell properties. In this process, the epithelial cells lose their cell polarity and adhesion properties and gain a motile trait, which gives them an invasive character (59). This enables the epithelial cell to migrate to distant sites allowing metastasis and tumor progression (60). EMT is also important in providing the stemness characteristics of cancer cells by supporting the correct microenvironment (60). The importance of EMT in cancer, particularly lung cancer, has been highlighted (60–62). TEX isolated from the serum of late stage lung cancer patients, like highly metastatic lung cancer cells, contain high levels of vimentin and the TEX can induce EMT in recipient human bronchial epithelial cells (63). Vimentin, a member of the type III intermediate filament protein family, is normally expressed in mesenchymal cells and is widely used as a marker for EMT (64). The association of vimentin expression with increased metastasis and invasion ability has been reported for many cancers including lung (63, 65, 66), prostate (67, 68), and gastric cancers (69). In lung cancer, vimentin changes cancer cell adhesion by regulating the VAV2–Rac1 pathway and modifying focal adhesion kinase activity (65). EMT induction in epithelial adenocarcinoma A549 cells by TGF- $\beta$  leads to the production of exosomes with a different cargo (70). Exosomes from mesenchymal-like A549 cells contain high levels of  $\beta$ -catenin, vimentin, and E-cadherin, as well as miR-23a in comparison to those from epithelial-like A549 cells. miR-23a mediates TGF- $\beta$ -induced A549 cell EMT by targeting E-cadherin in a smad-dependent manner (71). Interestingly, autologous treatment of A549 cells with these exosomes induced overexpression of  $\beta$ -catenin indicating the potential for autocrine signaling by TEX (71).

## ANGIOGENESIS ENHANCEMENT BY TEX IN LUNG CANCER

Angiogenesis or the formation of a vasculature network is essential for tumor growth and metastasis. This process is regulated by different mechanisms and angiogenic factors, including VEGF, TGF- $\beta$ , and fibroblast growth factor. Exosomes have a crucial role

in vascular tube formation and the observed effect is dependent upon the site of exosome origin (72). Hypoxia is a hallmark of the tumor microenvironment and is reported to lead to an increase in TEX production by tumor cells and a change in their content. The change in TEX cargo under the hypoxic conditions enables them to alleviate the stress conditions in the tumor microenvironment by induction of angiogenesis (73, 74). In CL1-5 lung adenocarcinoma cells, TEX production and the level of TEX miR-23a was enhanced during hypoxia-induced angiogenesis. Uptake of TEX-associated miR-23a by ECs enabled targeting of prolyl hydroxylase 1 and 2 (PHD1 and 2) leading to the accumulation of hypoxia-inducible factor (HIF)-1 $\alpha$  and the enhancement of angiogenesis (75).

Tissue inhibitor of metalloproteinases (TIMP)-1 is a factor that strongly supports lung cancer progression (76–79) and its expression is elevated in all stages and types of lung cancer particularly in adenocarcinoma (73). Overexpression of TIMP-1 induces the expression of the tumorigenic miR-210 in lung adenocarcinoma cells and within their derived exosomes under the control of the PI3K/Akt/HIF-1 pathway. In turn, TEX released from these cells downregulate Ephrin A3 in ECs and promote angiogenesis (73). The expression of TEX miR-210 in the serum of lung cancer patients is increased compared to non-cancerous control subjects (80, 81).

Tumor-derived exosome from lung tumor cells contain EGFR and uptake of TEX by ECs can trigger EGFR-dependent responses which are accompanied by the autocrine activation of VEGF receptor 2 (VEGFR-2) and elevated VEGF expression promoting angiogenesis (82). Furthermore, administration of TEX from a lung cancer patient into a rat critical limb ischemia model markedly augmented the expression of VEGFR-2, increased angiogenesis and improved blood flow (83). Together, these observations indicate the important role of TEX in the upregulation of tumor angiogenesis.

## LUNG CANCER METASTASIS AND TEX

The primary step required for metastasis is the formation of a pre-metastatic niche: a supportive microenvironment in a secondary organ that enables its colonization by circulating tumor cells (CTCs) (84). The site of metastasis is not random but is selected following modification by tumor cells before the initiation of metastasis (85). In contrast, the metastatic niche is initiated and formed upon CTC arrival (86). The formation of the pre-metastatic niche is initiated through a variety of mechanisms that promote a sequence of events that begins with vascular leakage. In the lung cancer vascular permeability increases upon upregulation of angiotensin 2 (Angpt2), MMP3, and MMP10 in the pre-metastatic stage (87). Exosomes can increase vascular permeability at lung pre-metastatic sites by reprogramming bone marrow (BM) progenitors within the niche toward a provascular phenotype *via* the MET receptors. Finally, vascular leakiness facilitate extravasation and attraction of CTCs to the pre-metastatic site (88).

It is now evident that TEX has important roles as mediators in the formation of pre-metastatic niches and the resultant metastasis (89, 90). The role of exosomes in lung metastasis was first demonstrated by Janowska-Wieczorek et al. in 2005 (91). The

authors showed that microvesicles derived from activated platelets (PMV) induce tumor progression, metastasis, and angiogenesis in lung cancer. Intravenous injection of pmv-covered Lewis Lung Cancer cell line (LLC) enhanced lung metastasis. These vesicles transferred the integrin  $\alpha 2\beta$  (CD41) to lung cancer cell lines and subsequently promote proliferation and tumor progression in mice (91). In addition, renal cancer stem cells trigger an angiogenic switch and tumor progression and play important role in lung pre-metastatic niche formation (92).

The small RNA content of lung TEX promotes the formation of a pre-metastatic niche by selectively targeting and activating TLR3 in lung epithelial cells. This results in enhanced chemokine secretion and subsequent neutrophil recruitment to the lung which together promotes pre-metastatic niche formation (93).

Melanoma-derived TEXs are important in the primary tumor formation and lung metastasis. Intravenous injection of labeled TEX into a naïve mouse, demonstrated lung residency within 24 h associated with an increased permeability of lung ECs at the TEX-induced pre-metastatic niche. Upregulation of pre-metastatic niche effector molecules such as S100A8 and S100A9 as well as the vascular permeability factor TNF- $\alpha$  was also observed at the site of TEX injection (94). In addition, TEX administration caused an upregulation of inflammatory and ECM-related genes (95). Importantly, TEX obtained from highly metastatic melanomas had a greater burden on the lung compared to those obtained from poorly metastatic melanomas. It is proposed that these melanoma TEXs promote pre-metastatic niche formation and tumor growth by overexpressing the oncogene MET within BM-derived DCs to obtain a pro-vasculogenic phenotype. In support of this, TEX re-program BM progenitors to increase the pro-angiogenic c-Kit<sup>+</sup>Tie2<sup>+</sup> cell population in the lung pre-metastatic niche. In addition, TEX could also transfer the oncogene MET from melanoma cells to BM progenitor cells and thereby promote metastasis (95).

Exosome target cells selection is determined by their surface adhesion molecules such as integrin. Specific integrin profiles on the surface of tumor-derived exosomes direct them to a specific organ, so driving metastatic organotropism (8). For example  $\alpha 6\alpha 4$  integrin heterodimer target exosomes to lung PMNs. Lung fibroblasts with upregulated s100 genes, are the main cells that uptake these exosomes and drive PMN formation (8). Exosomes derived from the 4175-LuT breast cancer cells have  $\alpha 6\beta 4$  and  $\alpha 6\beta 1$  integrins on their surface and localize in regions of the lung which are rich in laminin and promote lung metastasis (8).

Bone is the common metastatic site for NSCLC which result in osteolytic lesions (96). In NSCLC, EGFR is upregulated (97) and amphiregulin (AREG), an EGFR ligand, is packaged in exosomes derived from lung cancer cells (98, 99). NSCLC-exosomes containing AREG, active the EGFR pathway in pre-osteoclasts which leads to an increase in the expression of RANKL and proteolytic enzymes in turn, triggering a vicious cycle driving osteolytic bone metastasis (100). Conversely, extracellular vesicles released from the highly metastatic bone tumors are localized preferentially to lung and can derive metastatic behavior (101).

The lung is the common target for many metastatic primary tumors (102–104) but the precise molecular mechanism behind

this tissue-specific metastasis is not completely understood. It is illustrated that lung microenvironment promote the formation of PMN and possibly TEXs play the key roles in this process. Further studies will more clear that the mechanism of specific exosomes effect on tumor microenvironment and promoting lung invasion along with that of other organs.

## THE ROLE OF TEX AS A BIOMARKER AND THEIR THERAPEUTIC IMPLICATIONS IN LUNG CANCER

Despite considerable achievement in both diagnostics and treatment, the global control of lung cancer remains problematic (105). This lack of success is attributed to a failure of early disease detection due to an absence of reliable biomarkers (106). Biomarkers serve as indicators of a particular physiological or biological state in the body and are important in medicine to distinguish a normal or pathogenic condition or/and a response to a therapy (107). In the context of cancer, biomarkers can be prognostic and predictive markers for the risk of progression, recurrence or the effectiveness of a therapeutic intervention (108). Due to their contents reflecting abnormalities in the parent cells and their stability in most biological fluids, exosomes have potential to serve as a promising “liquid biopsy” biomarkers of lung cancer (106, 109). Importantly, in comparison to tissue biopsy that requires surgery, exosomes-based biomarkers would provide a non-invasive diagnostic approach (106).

Exosomal markers such as proteins and non-coding RNAs have been measured in lung cancer. The analysis of 49 proteins attached to the membrane of plasma exosomes of 276 NSCLC patients indicated that some of these proteins including NY-ESO-1 had a significant correlation with survival (110). Microarray-based analysis of serum exosomal miRNAs in NSCLC patients showed a significant upregulation of miR-21 and miR-4257 in patients with a recurrence of the disease (111). In addition, TEX from NSCLC patients has increased EGFR presence (112). These EGFR-contained TEX activate MAPK and Akt/protein kinase B pathways in recipient ECs resulting in VEGF overexpression and increased tumor vascularity (113).

The exosomal expression of two miRNAs associated with Tumor suppression, namely miR-51 and miR-373, was decreased in lung cancer patients and this reduction was associated with poor prognosis (114). Other exosomal miRNAs have been reported as markers of therapeutic response in lung cancer. For example, miR-208a and miR-1246 bind to p21 and DR5 mRNAs, respectively, to promote tumor growth and resistance to radiotherapy (113).

TEX-based markers may provide higher sensitivity and specificity in cancer diagnostics over conventional biopsy methods which require surgery. However, the lack of standardized methods for isolating pure exosome populations and the heterogeneity in cancer-derived exosomes present problems (108). Despite these concerns, there is much interest in TEX-based miRNAs in lung cancer with efforts made to combine purified TEX with next generation sequencing or proteomic analysis to achieve greater insight into TEX-based lung cancer diagnosis. Exosomal

miRNA studies report miR-378a, miR-379, miR-139-5p and miR-200b-5p (115), miR-21 (80, 111, 116), miR-155 (116), miR-23b, miR-10b-5p (80), and miR-4257 (111) that vary in the expression level in lung cancer patient in compare to healthy subjects.

However, recently a method of the using Surface-Enhanced Raman Spectroscopy (SERS) combined with principle component analysis (PCA) was suggested for classification of exosomes based on their specific surface pattern of protein and lipids. Lipid and membrane proteins results in a specific Raman spectra; thus, the tumor-derived exosomes and normal cell-derived exosomes vary in their Raman spectral patterns. In this study, lung cancer cell-derived exosomes were differentiated from those from normal cells by 95.3% sensitivity and 97.3% specificity (117). Current challenges in exosome biology using conventional methods include the need for large amounts of highly concentrated sample and the presence of heterogeneity in cancer-derived exosomes. This approach combining SERS with PCA analysis may be good choice to be translated in clinical practice (117).

In another study by Ueda et al., the mass spectrometric quantification of 1,369 exosomal proteins in 46 serum samples of patient with advanced stage of NSCLC demonstrated CD91 as a lung adenocarcinoma specific antigen on exosomes surface (118). Jakobsen et al. also identified a profile of serum exosomal protein in NSCLC patient with advanced stage of disease. In this study, the authors performed a multivariate extracellular vesicle array (EV Array) approach to phenotype plasma exosomes and the results identified a panel of 30 exosomal surface protein marker including CD91, CD317, and EGFR which could distinguish 75% of the patients correctly. This result suggests that EV Array analysis as a potential complementary method in diagnosing NSCLC (119). Exosomal proteins were also investigated in body fluids to survey exosomal biomarkers. Proteomic mass spectrometry showed that leucine rich alpha-2-glycoprotein 1 (LRG1) was highly expressed in urinary exosomes and also in cancer tissues from NSCLC patient in compare to healthy subjects (120). CD171 and CD151 and tetra-spanin 8 was also suggested as potential diagnosis biomarker for NSCLC (121).

Besides diagnostic approaches, exosomes have been considered as suitable vehicles for drug and nucleic acid delivery to target organs. It was demonstrated that bEND.3 (brain Endothelial Cell Line)-derived exosomes can pass through the blood-brain barrier and reduce VEGF levels *in vivo* by delivering drug to a brain tumor. This, in turn, results to a significant decrease in the tumor size (122). In a murine lung cancer model, cow milk exosomes were subjected for drug delivery for lung cancer. After injection of exosomes loaded with aferin-A, a tumor inhibitory effect was observed at doses lower in compare to unencapsulated drug (123).

The biological properties of exosomes give them with a valuable potential in medical research including cancer therapy. For example, since exosomes shuttle tumor-specific antigens, can be also attractive as anticancer vaccines (109). Given that uptake of TEXs is organotropism and performed through integrin-mediated signaling (8), thus blocking integrins through decoy peptides can be a good strategy to inhibit exosome fusion and uptake, subsequently result in blocking of tumor progression (124).

One of the recent immunotherapy method in treatment of lung cancer rely on blocking negative regulators of T-cell activation such as PD-1 and PD-L1 and inflammatory signals in the tumor microenvironment which can be mediated and reinforced by exosomes (110, 125). Another approaches such as blocking exosomal release or inhibition of the exosome-mediated cellular crosstalk in the tumor environment may be appropriate in suppress the development of a favorable tumor microenvironment (109). On the other hand, exosomes may modulate anti-inflammatory signals within the tumor microenvironment which may effectively enhance the efficacy of immunotherapy in lung cancer (126).

Overall, exosomes are starting to be considered in medical research especially in cancer diagnosis and treatment. Because of their unique biological properties, such as specific targeting, small size, shuttling signaling, and biological molecules, as well as the ability to cross biological barriers; exosomes can have a range of applications from diagnosis biomarkers to drug delivery and tumor immunotherapy. Despite some limitations in exosome usage, such as inconvenient nature of their isolation and purification methods, it is anticipated that exosomes will be utilized in cancer therapy in the near future. However, further more

sophisticated clinical studies that address these current limitations in exosome biology is needed for translation of exosome-based technologies to clinical application.

## CONCLUSION

Exosomes mediate cross talk between the cells and their surrounding environment in normal and pathological conditions. TEXs are emerging as the major mechanism for communication between cancerous cells and the tumor microenvironment, which has a significant effect in tumor progression and metastasis. The data obtained to date using analytes within TEX as potential markers for the diagnosis and outcomes of lung cancer has provided much insight although further research is still required. The clinical use of TEX will open a new window to lung cancer management and treatment in the near future.

## AUTHOR CONTRIBUTIONS

SA wrote first draft. EM and MV revised the manuscript. MM, AK, JG, and IA has revised final version and added extra information.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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