

# Exosome-mediated communication between tumor cells and tumor-associated macrophages: implications for tumor microenvironment

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## ABSTRACT

Exosomes are extracellular vesicles released from numerous types of cells that are involved in multiple tumors development. Exosomes contribute to the modulation of tumor microenvironment (TME) through intercellular communication. As essential immune stromal cells in the TME, tumor-associated macrophages (TAMs) participate in tumor development by mediating angiogenesis, metastasis, chemoresistance, and immune escape. Due to communication with multiple cells in the TME, they exhibit plasticity and heterogeneity during the progress of polarization from monocytes to macrophages. Previous studies suggest that targeting TAMs is a promising therapeutic strategy; however, the detailed mechanism by which TAMs regulate tumor development still remains unclear. In this review, we provide an overview of the roles of exosomes as messengers in the communication between tumor cells and polarization of TAMs; we also describe the effects of their interaction on tumor development. Finally, we comprehensively discussed the potential application of exosomes as the promising tumor immunotherapy strategy.

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## 1. Introduction

The malignant phenotypes of tumors are determined by both the cancer cells themselves and the surrounding tumor microenvironment (TME).<sup>1,2</sup> In addition to tumor cells, the TME includes multiple stromal cells, including fibroblasts, lymphocytes, inflammatory cells, endothelial cells, and mesenchymal stem cells;<sup>3</sup> these cells are always evolving together with the tumor cells. Cellular communication in the TME significantly influences tumor occurrence and development, as well as invasion, metastasis, and other malignant biological behaviors.<sup>4</sup> However, the molecular mechanisms involved in communication between cancer and immune cells have not been fully elucidated. Previous studies have focused on the heterotypic interactions between tumor cells and immune cells through direct contact and secretion of signaling molecules, such as soluble cytokines and chemokines.<sup>5</sup> The discovery of exosomes, regulatory agents in the intercellular communication of cancer, provides a new perspective for the in-depth study of tumor immunity. Increased infiltration of tumor-associated macrophages (TAMs) is observed in most human cancer tissues compared with control para-cancer or normal tissue,<sup>6–9</sup> suggesting an essential role of TAMs in tumor development. Multiple studies have shown that TAMs interact with multiple cell types that reprogram and induce macrophage polarization<sup>10,11</sup>. TAMs lose their killing ability and subsequently acquire an inhibitory phenotype, which promotes tumor development. In this process, exosomes derived from tumor cells play important roles in macrophage polarization. Simultaneously, TAMs release exosomes that contribute to cancer progression. This review focuses on the effects of

exosomes on the communication between cancer cells and TAMs, and mainly focuses on the emerging role of engineered exosome-mediated immune therapy. Further, potential applications for exosomes in targeted therapy are addressed with the aim of providing novel options.

## 2. TAMs

As a vital plastic and heterogeneous population of cells in the TME, TAMs account for 30%–50% of infiltrating immune cells.<sup>12</sup> Circulating monocytes extravasate from nearby blood vessels and enter tumor tissue, contributing to their polarization into different phenotypes.<sup>13,14</sup>

Generally, macrophages differentiate into two main phenotypes: classically activated (M1) and alternatively activated (M2).<sup>15,16</sup> In detailed, M2 macrophages are further subdivided into M2a, M2b, M2c, and M2d phenotypes depending on the polarizing stimuli and gene expression profiles.<sup>17,18</sup> Classical and alternative types of TAMs occupy two extremes of a phenotypic continuum in which macrophages respond to secreted factors that evoke distinct functional responses. Previous reviews<sup>19,20</sup> have addressed the influential factors of M1 and M2 polarization, as well as the associated phenotypic changes; thus, these topics are not reviewed in detail here. Due to the heterogeneity of macrophages, the dichotomous M1/M2 distinction is insufficient to explain macrophage polarization in the TME.<sup>21,22</sup> Recently, with the development of single-cell technology and the emergence of multi-omics, researchers could have a further glimpse into TAM phenotypic diversity and the dynamics of TAM polarization ecosystems.<sup>22,23</sup>

Increasing evidence has confirmed that TAMs are composed not only of a homogeneous population, but also a mixed population of macrophages in malignant solid tumor tissues, including in hepatocellular carcinoma (HCC),<sup>24</sup> breast cancer,<sup>25</sup> human renal cell carcinoma,<sup>22</sup> and gastric cancer (GC).<sup>26</sup> In these cases, apart from M1 and M2 properties, other macrophage clusters have been explored, especially those in the subset of PD-L1 positive TAMs and CD163<sup>+</sup> TAMs, which can significantly promote tumor progression. Thus, the coexistence of M1 and M2 signatures indicates that TAMs exhibit more complexity than that simply described by the classical M1 and M2 models.

The mechanisms related to macrophage polarization and communication between TAMs and tumor cells in the TME is rather intricate. Previous studies have demonstrated the dependent role of the TME in TAMs phenotype acquisition, which depends on sophisticated intercellular communication via direct cell-to-cell contact or classical paracrine signaling loops involving cytokines or growth factors.<sup>27–29</sup> Emerging reports suggests that exosomes are important as one of essential regulators of macrophage polarization in the TME.<sup>30–33</sup>

### 3. Exosomes

Exosomes are extracellular vesicles (EVs) with diameters ranging between 30 and 150 nm, have an endosomal origin, and are present in the blood, urine, and other cerebrospinal fluid.<sup>34</sup> Depending on the cell of origin, exosomes contain multiple cellular components as cargos, including DNA (mitochondrial and genomic), lipids (membranes), proteins, transposable elements, and RNAs (coding and non-coding).<sup>35</sup> These cargos are elaborately sorted and packaged into endosomes. When endosomes mature, they transform into multivesicular bodies (MVBs) and transported to the plasma membrane, and released as exosomes. Notably, several studies have identified that sorting mechanisms of MVBs contribute to the selective enrichment of exosomes with specific proteins and RNA molecules. These mechanisms suggest that molecular sorting into exosomes is a regulated process rather than a pathological resemblance.<sup>36,37</sup> Posttranslational modifications have been involved in protein sorting into exosomes. For example, ubiquitination is responsible for sorting the major histocompatibility complex II (MHC-II)<sup>38</sup> and the oncogenic protein epidermal growth factor receptor (EGFR).<sup>39</sup> In addition, RNA-induced silencing complex (RISC) proteins, including AGO2, GW182,<sup>40</sup> and RNA binding proteins (RBPs), which contain the GAGG sequence motif,<sup>41</sup> have been confirmed to be necessary for the sorting of particular RNAs into exosomes. Exosomes are internalized by recipient cells through receptor-mediated endocytosis, pinocytosis, phagocytosis, or fusion with the cell membrane, resulting in direct release of their cargo into the cytoplasm. Transmission of molecular signals by exosomes has been shown to change the function and phenotypes of recipient cells. The targeted accumulation of specific cellular components in exosomes suggests that they play essential roles in regulating intercellular communication.<sup>42,43</sup>

Exosomes participate in tumor pathogenesis and progression by modulating the TME. Similarly, exosome-mediated

intercellular communication also exists between tumor cells and TAMs, which can regulate the biological characteristics of tumor cells and influence the polarization of the TAMs, thus promoting or impeding tumor progression.<sup>44,45</sup> The functions of exosomes have primarily been characterized by their origin and content.<sup>46,47</sup> Furthermore, other studies have shown that exosome-induced cellular communication is specialized in long-distance interaction,<sup>48,49</sup> facilitating the transfer of proteins<sup>50</sup> and functional mRNA and microRNAs (miRNAs) for subsequent protein expression in target cells.<sup>51</sup> This communication mechanism is a highly efficient, robust, and economical way of exchanging information among cells. Therefore, an in-depth understanding of the molecular mechanisms by which exosomes facilitate communication between TAMs and tumor cells has the potential to contribute to the development of new anti-tumor therapies that reverse tumor suppressor immune response.

## 4. Tumor-derived exosomes modulate macrophage polarization

Tumor-derived exosomes also play crucial roles in immune modulation. Several studies have shown that they can remodel the tumor immune microenvironment (TIME), favoring a tumor-promoting status that supports tumorigenesis, invasion, angiogenesis, premetastatic niche formation, and metastatic dissemination.<sup>52–54</sup> Based on various molecular constituents, exosomes can switch macrophages either to the M1 or M2 phenotype.<sup>55</sup>

### 4.1 miRNAs

miRNAs are small, non-coding RNAs that are usually 20–25 nucleotides in length. They bind to particular mRNA sequences and inhibit target-gene translation.<sup>56</sup> One of the most distinctive hallmarks of cancer-derived exosomes is a cargo consisting of multiple miRNAs, with mature miRNAs accounting for 41.7% of all RNAs.<sup>57</sup> Multiple miRNAs have been suggested to be critical bioactive components of exosomes. Exosomal microRNAs are able to regulate TAM function by affecting macrophage phenotype, cytokine secretion, T cell-related immune exhaustion and hypoxic microenvironment.

Increasing evidence has revealed that exosomal miRNAs exert important physiological functions in TAMs infiltration and polarization by regulating target molecules. Zhao et al. demonstrated elevated expression of miR-934 in areas with abundant CD163<sup>+</sup> TAM infiltration in colorectal cancer (CRC) liver metastasis tissue; moreover, exosomal miR-934 induced M2 macrophage polarization via downregulation of phosphatase and tensin homolog deleted on chromosome ten (PTEN) expression and activation of the PI3K/AKT signaling pathway.<sup>58</sup> Many essential transcription factors, such as PPARs, STAT3, and STAT6 are involved in macrophage polarization in the TME. For example, Ying et al.<sup>59</sup> showed that epithelial ovarian cancer (EOC)-derived exosomal miR-222-3p induced a TAM-like macrophage phenotype and polarization into the M2 phenotype via the SOCS3/STAT3 pathway.

Recent studies have shown that abnormal chemokines secreted by tumor cells recruit macrophages to infiltrate tumors and affect macrophage phenotype.<sup>60,61</sup> For example, activation of the chemokine receptor CXCR4/CXCL12 signaling has been implicated in TAM recruitment by melanomas.<sup>62</sup> Moreover, upon CXCL12/CXCR4 axis activation, CRC cells transferred several miRNAs, including miR-25-3p, miR-130b-3p, and miR-425-5p, to TAMs via exosomes. These exosomal miRNAs elicited the M2-polarized macrophage phenotype by suppressing the common target gene PTEN and activating the PI3K/AKT pathway.<sup>63</sup>

Exosomes affect macrophage polarization and influence the interactions between them with other immune cells, especially T cells that participate in TIME regulation. For example, endoplasmic reticulum (ER) stress induces HCC cells to release exosomal miR-23a-3p, which upregulates PD-L1 expression in macrophages by activating the STAT3 signaling pathway, attenuating the anti-HCC immune response.<sup>64</sup> Exosomal miR-21 and miR-29a from cancer cells trigger human TLR8 – and murine TLR7-mediated nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation in macrophages to produce IL-6 and TNF- $\alpha$ , which promote melanoma lung metastasis and lung cancer progression.<sup>65</sup>

Besides immunosuppression, another crucial characteristic of TME is hypoxia. In the TME, hypoxic tumor cells extensively communicate with immune cells to establish conditions favorable for cancer cell immune escape. Several studies have demonstrated that hypoxia promotes exosome secretion, leading to an aggressive phenotype of cancer cells and angiogenesis.<sup>66–68</sup> Numerous studies have demonstrated that exosomes from hypoxia-induced tumors contain various non-coding RNAs, especially miRNAs involved in tumor progression. These exosomes also promote M2 macrophage polarization and microRNA-mediated macrophage metabolic shifts. Chen et al.<sup>69</sup> demonstrated that miR-21-3p, miR-125b-5p, and miR-181-5p were enriched with hypoxic exosomes derived from EOC. These miRNAs were endocytosed by the original macrophages and promoted TAM polarization by activating the SOCS4/5/STAT3/HIF-1 $\alpha$  pathway.

Hypoxia creates an indispensable environment for the progression of epithelial-mesenchymal transition (EMT) by activating numerous transcriptional factors such as Snail and Twist.<sup>70</sup> In Snail-overexpressing head and neck squamous cell carcinoma (HNSCC) cells, Snail can enhance miR-21 transcription by binding directly, followed by sorting into exosomes. After being untaken by macrophages, exosomal miR-21 downregulated PDCD4 and IL-12A expression and mediated M2 polarization.<sup>71</sup> In addition to miRNAs, immunomodulatory proteins and chemokines, including CSF-1, CCL12, FTH, FTL, and TGF- $\beta$ , have been found in exosomes derived from hypoxic tumor cells. These proteins, together with miRNAs, influence macrophage recruitment and promoted M2 polarization.<sup>72</sup>

Summarizing our current understanding for above studies, exosomal miRNAs derived from tumor cells have critical influence on TAM polarization in the TME. These miRNAs can be used as tumor biomarkers to monitor tumor progression or can be utilized as important drug design targets or drug carriers, which will be discussed in a later section.

## 4.2 Long non-coding RNAs (lncRNAs)

lncRNAs provide essential transcriptomic information for transcripts longer than 200 nucleotides but have no/little protein-coding capacity.<sup>73</sup> lncRNAs exhibit low sequence identity (~50-60%) and low abundance; their median expression level is ~10-fold lower than that of mRNAs.<sup>74</sup> Circulating lncRNAs are unstable in the presence of high amounts of RNase in peripheral blood, while they are relatively stable when packaged into multiple types of EVs which prevent RNase digestion for lncRNAs.<sup>75</sup>

Kogure et al. reported that lncRNA TUC339 is highly expressed in HCC-derived exosomes. It can transfer from one to another among HCC cells to promote tumor growth and metastasis.<sup>76</sup> Furthermore, the exosomal lncRNA TUC339 can transfer into neighborhood macrophages and induce macrophage polarization to the M2 phenotype, which dampens the anti-tumor immune response. Microarrays revealed that Toll-like receptor (TLR) signaling and FcR-mediated phagocytosis pathways were downregulated in macrophages by exosomal TUC339, with TUC339 knockdown increasing their phagocytic activity. TUC339 is also involved in cytokine and chemokine receptor signaling pathways, although the underlying mechanisms need further elucidation.<sup>44</sup> Similarly, exosomal lncRNA BCRT1 derived from breast cancer cells was internalized by macrophages to promote M2 polarization. It conferred increased migration and chemotaxis abilities to cancer cells, accelerating breast cancer progression.<sup>77</sup>

Many lncRNAs, which are mainly located in the cytoplasm, serve as competing endogenous RNA (ceRNA) sponges to modulate the expression and biological functions of miRNAs; importantly, they are involved in tumor progression.<sup>78–80</sup> Cytoplasmic lncRNAs can be sorted into exosomes and absorbed by TAMs, but whether they regulate TAM polarization via sponge adsorption is yet to be further studied. Moreover, other non-coding RNAs, such as circular RNAs (circRNAs), have been found in cancer cell-derived exosomes.<sup>81–83</sup> These exosomal circRNAs can affect cellular communication in the TME. For example, HCC-derived exosomal circUHRF1 induces NK cell exhaustion by targeting miR-449 c-5p/TIM-3, causing resistance to anti-PD-L1 therapy.<sup>84</sup> Whether exosomal circRNAs affect macrophage polarization remains to be investigated.

## 4.3 Proteins

In addition to RNAs, plasma-membrane proteins are selectively sorted into the extracellular space through ectodomain shedding and microvesicle release. In contrast to vesicles released by plasma membrane budding,<sup>85</sup> exosomes are generated in multivesicular bodies through endocytic pathways, then are removed when these bodies fuse with the plasma membrane.<sup>86</sup> Peinado et al. have identified an oncoprotein transported via exosomes from melanoma cells, the receptor tyrosine kinase MET, which initiates long-distance inflammation to chemotactically attract circulating cancer cells.<sup>87</sup> Oncoproteins packaged into exosomes can maintain their activity after exosome uptake by recipient cells.<sup>88,89</sup> Chen et al.<sup>90</sup> developed a SILAC-based mass spectrometry (MS) strategy to characterize the proteome functionally transported from

CRC cells to bone marrow-derived macrophages (BMMs) via exosomes. Results showed that among the 1092 differentially expressed proteins, cytoskeleton-centric proteins were the most abundant functional class. Cytoskeletal rearrangement is a primary phenotype of monocyte/macrophage activation and maturation.<sup>91,92</sup> Exosomal vimentin enhanced F-actin cytoskeleton rearrangement in macrophages, as indicated by the shape index and polarization. Similarly, Gabrusiewicz et al. reported that exosomal STAT3 derived from glioblastoma stem cells caused reorganization of the actin cytoskeleton and induced monocytes to shift toward the immune-suppressive M2 phenotype.<sup>93</sup> Exosomes derived from metastatic osteosarcoma cells could induce M2 polarization, while decreasing phagocytosis, efferocytosis, and macrophage-mediated cytotoxicity. These effects are modulated by the overexpression of immunosuppressive TGF- $\beta$ 2.<sup>94</sup>

Emerging evidence indicates that tumor-derived exosomal proteins alter the phenotypes of monocyte-derived macrophages. Liang et al.<sup>95</sup> found that ribonuclease P RNA component H1 (RPPH1), binds to  $\beta$ -III tubulin (TUBB3) to prevent its ubiquitination and, then induces EMT to promote metastasis. Furthermore, RPPH1 was transferred by exosomes to macrophages to mediate macrophage M2 polarization. After treatment with exosomes derived from CRC cells, monocyte-derived macrophages (MDMs) exhibited a CD206<sup>high</sup>/HLA-DR<sup>low</sup> phenotype, with a stretched and elongated M2 cellular morphology. Similarly, Ham et al.<sup>96</sup> revealed that the IL-6 receptor gp130 was present in breast cancer cell-derived exosomes, where it stimulated IL6/STAT3 signaling in bone marrow-derived macrophages (BMDMs). After activation, phosphorylated STAT3 translocated to the nucleus and induced the transcription of target genes, including macrophage polarization-associated genes, IL-6, IL-10, CXCR4, and CCL2. This activation subsequently promoted BMDM survival and induced the switching of BMDMs toward a cancer-promoting phenotype. Comparing with studies of RNA in exosomes, the function and mechanism of exosomal proteins in macrophage and TME need more further research to elucidate.

#### 4.4 Lipids

Some fusogenic membrane lipids have been identified in exosomes and play essential roles in fusing exosomes with the plasma membrane. Arachidonic acid (AA) is the primary polyunsaturated fatty acid precursor for the production of pro-inflammatory lipid mediators. It destabilizes membranes by converting closely associated lipid bilayers into a hexagonal structure to promote annexin2-mediated membrane fusion.<sup>97–99</sup> Exosomes derived from pancreatic cancer (PC) cells AsPC-1 contain higher AA levels than the recipient cells, facilitating their fusion with THP-1-derived macrophages. AA elevated the secretion of PGE2, increased the levels of the M2 phenotype (CD14<sup>hi</sup>CD163<sup>hi</sup>CD206<sup>hi</sup>), and enhanced the secretion of protumoral bioactive molecules, including VEGF, MCP-1, IL-6, IL-1 $\beta$ , MMP-9, and TNF- $\alpha$ , which contribute to tumor progression.<sup>100</sup>

A recent study demonstrated that metabolic reprogramming of macrophages plays a predominant role in regulating

their phenotype and plasticity.<sup>101</sup> LPS (+IFN)-activated inflammatory macrophages convert arginine into nitric oxide (NO) through inducible NO synthase (iNOS) activity.<sup>17,102</sup> Macrophages also increased the accumulation of succinate, thus stabilizing HIF-1 $\alpha$  and induction of IL-1 $\beta$ .<sup>103</sup> Conversely, arginine is metabolized by arginase-1 in IL-4-induced alternatively activated macrophages (M2).<sup>104,105</sup> However, it is unclear whether tumor cell-derived exosomes are involved in metabolic reprogramming of TAMs. Thus, this question needs more attention to settle.

The above studies focused on exosomal induction of TAM polarization toward the acquisition of the M2-phenotype. However, researchers should not ignore the possibility of exosome-mediated induction of macrophage polarization into the M1-phenotype. Xiao et al. confirmed this hypothesis and found that exosomal THBS1 derived from OSCC significantly upregulated the expression of M1-related genes, including TNF $\alpha$ , IL-1 $\beta$ , and IL-6 in macrophages. Furthermore, the p38, Akt, and SAPK/JNK signaling pathways activated by exosomes were found to be involved in M1 phenotype polarization.<sup>106</sup> Similarly, miR-125b-5p, which increases in primary melanoma, is delivered to macrophages by exosomes and can reinforce the activation of M1 macrophages through the induction of CCL1, CCL2, and IL-1 $\beta$ .<sup>107</sup> These findings further demonstrate the complexity of exosomal function in regulating macrophage polarization; further studies are needed to elucidate the detailed mechanisms. In summary, these studies describe the critical effectors of macrophage polarization in TME induced by tumor-derived exosomes, suggesting that most signal molecules are loaded toward immune tolerance, which benefits tumor progression<sup>77,108</sup> (Figure 1). A summary of the molecules involved in macrophage polarization is provided in Table 1.

Notably, existing studies have focused on the effects of exosomes on macrophages polarization into M1 or M2-type states. However, as mentioned above, this dichotomous classification is far from sufficient to account for the complexity of TAMs, because polarization is a dynamic and reversible process in the TME; thus, further research is needed to understand how exosomes determine macrophage polarization to various TAM phenotypes. It is necessary to establish model systems to study different subtypes of macrophages and to search for more accurate molecular markers to characterize TAMs. It is also very crucial to point out that, in recent studies, endocytosis has been considered as the primary mechanism for exosome to deliver its contents to TAMs. Furthermore, the interaction between exosome surface molecules and the surface molecules of target cells that do not take up exosomes is still capable of signals of target cells.<sup>109,110</sup> Whether this process plays a role in the polarization of TAMs, and whether it is different from the effects of endocytosis, is worth further investigation.

## 5. Macrophage-derived exosomes are involved in tumor progression

Most TAMs lack the ability to phagocytize tumor cells while facilitate tumor cell escape from killing to enable their metastases to distant tissues. Reciprocal exosome exchanges between TAMs and cancer cells also modulate cancer progression.

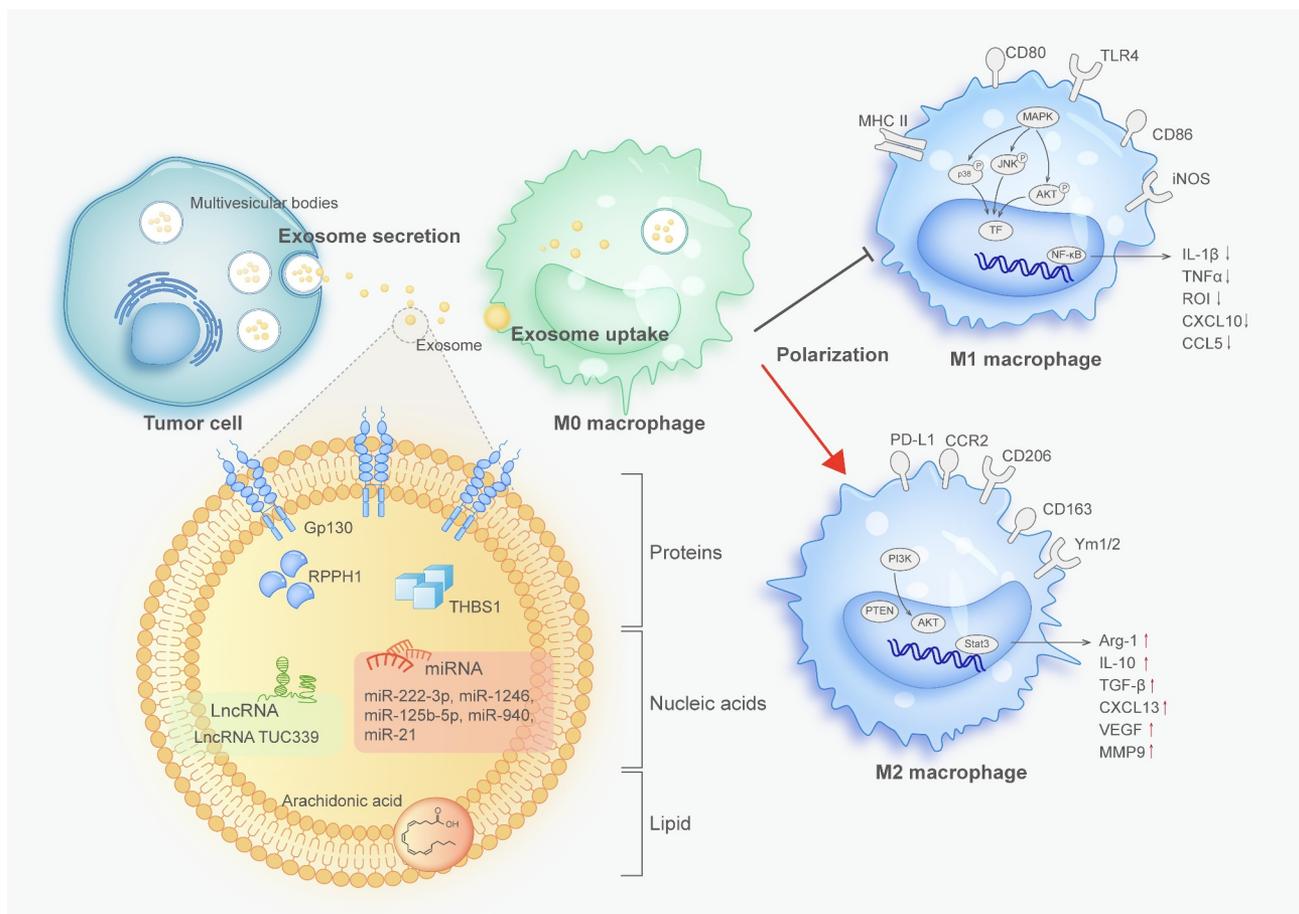
### 5.1 Regulating cancer proliferation, migration and invasion

Increasing evidence has revealed the essential roles of TAM-derived exosomes in fostering the pace of cancer initiation and progression. Song et al. reported that macrophages transfer miRNAs via exosomes to breast cancer cells to promote migration and invasion. miR-223 is a macrophage-dominant miRNA that is enriched in exosomes derived from IL-4-activated macrophages. After incubation with breast cancer cells, exosomal miR-223 was internalized more efficiently and activated the miR-223/Mef2c/ $\beta$ -catenin pathway to promote the invasiveness of breast cancer cells.<sup>111</sup> Similarly, miR-21-5p and miR-155-5p derived from macrophages are critical for colon cancer cell migration and invasion.<sup>112</sup> In contrast, other exosomal miRNAs from TAMs inhibit tumor progression. For example, exosomal miR-7 was found to be transferred from TAMs to EOC cells, in which it suppressed metastasis by reducing the activity of the EGFR/AKT/ERK1/2 pathway.<sup>113</sup> Exosomal miR-142-3p and miR-223 from macrophages functionally inhibited the proliferation of HCC cells by decreasing the expression levels of stathmin-1 and insulin-like growth factor-1 receptor (IGF1R).<sup>114</sup>

LncRNAs in macrophage-derived exosomes also participate in tumor development. LncRNA SBF2-AS1 is enriched in the exosomes from M2 macrophages. It was taken up by pancreatic cancer cells PANC-1 and promoted proliferation, invasion, and migration. Mechanistically, exosomal lncRNA SBF2-AS1 increased miR-122-5p expression and restrained X-linked inhibitor of apoptosis protein (XIAP) from PC progression, suggesting that the lncRNA SBF2-AS1/miR-122-5p/XIAP axis is involved in communication between macrophages and cancer cells.<sup>115</sup>

Arginase-1, an enzyme critical for converting arginine to ornithine and urea, is mainly expressed on the macrophage surface. Juliana et al. observed that arginase-1 levels were increased in TAM-derived exosomes, which is critical for promoting glioblastoma cell proliferation.<sup>116</sup> Tumor cells leave the primary site, enter the blood circulation, and attach to vascular endothelial cells, creating a critical link in the metastatic process.

The migratory potential of invasive cells is dependent on matrix metalloproteinases (MMPs) anchored on the cell surface via the integrin family. Wu et al.<sup>117</sup> found that CD11b/CD18, an integrin derived from M2 macrophage exosomes,



**Figure 1.** Tumor-derived exosomes modulate macrophage polarization. Exosomes derived from tumor cells transfer multiple molecules, including miRNAs, lncRNAs, oncoproteins, and lipids. These bioactive molecules are transported into macrophages, regulate the activity of multiple signaling pathways, and induce macrophage polarization toward the M1 or M2 subtypes. M1 macrophages can be marked with iNOS, CD80/86, and MHC II; they secrete numerous cytokines and chemokines, including IL-1, TNF- $\alpha$ , CXCL10, and CCL5. In contrast, M2 macrophages can be marked with CD163, CCR2 and CD163, which secrete alternative cytokines and chemokines, including IL-10, TGF- $\beta$ , CXCL13, and VEG. In the TME, M2 macrophages are privileged, helping to maintain the immunosuppressive microenvironment. AKT, protein kinase B; JNK, c-Jun NH2 terminal kinase; MAPK, mitogen activated protein kinase; MHC, major histocompatibility complex; NF- $\kappa$ B, nuclear factor kappa B; PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; Stat3, signal transducer and activator of transcription 3; TF, transcription factors; VEGF, vascular endothelial growth factor.

**Table 1.** Tumor cells – derived exosomes involve in macrophages polarization.

cargos		Donor cancer cell type	biological function on macrophage	Target molecules	Ref
miRNA	miR-934	CRC	M2 polarization	PTEN-PI3K/AKT	58
	miR-222-3p	EOC	M2 polarization	SOCS3/STAT3	59
	miR-25-3p, miR-130b-3p, miR-425-5p	CRC	M2 polarization	PTEN-PI3K/AKT	63
	miR-23a-3p	HCC	Upregulate PD-L1 in macrophages	STAT3	64
	miR-21, miR-29a	melanoma	TLR-mediated immune escape	TLR7/8/NF-kB	65
	miR-21-3p, miR-125b-5p, miR-181d-5p	Hypoxic EOC	M2 polarization	SOCS4/5/STAT3/HIF-1 $\alpha$	69
	miR-21	HNSCC	M2 polarization	PDCD4/IL-12A	71
	miR-125b-5p	melanoma	M1 polarization	CCL1, CCL2, IL-1 $\beta$	107
LncRNA	TUC339	HCC	M2 polarization	TLR	44
	BCRT1	Breast cancer	M2 polarization		77
Proteins	vimentin	CRC	M2 polarization	F-actin cytoskeleton	90
	STAT3	Glioblastoma	M2 polarization	actin	93
	TGF- $\beta$ 2	Metastatic osteosarcoma	M2 polarization		94
	RPPH1	CRC	M2 polarization	TUBB3	95
	gp130	breast cancer	M2 polarization	STAT3	96
	THBS1	OSCC	M1 polarization	P38, Akt, SAPK/JNK	106
Lipids	arachidonic acid	ascites of PDAC	M2 polarization	PGE2	100

Note: CRC, colorectal cancer; EOC, Epithelial ovarian cancer; HCC, Hepatocellular carcinoma; HNSCC, Head and neck squamous cell carcinoma; OSCC, Oral squamous cell carcinoma; PDAC, Pancreatic ductal adenocarcinoma.

expedited HCC cell potential and metastasis by activating MMP-9, but did not affect cancer cell proliferation. Apolipoprotein E (ApoE), a major protein component of very-low-density and high-density lipoproteins, induces macrophages to convert the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype due to its antiatherogenic activity.<sup>118</sup> Zheng et al. revealed that ApoE, a particular protein in M2 macrophage-derived exosomes, mediates the intercellular transfer of the ApoE-activating PI3K/AKT pathway in recipient GC cells and enhances metastatic potential.<sup>119</sup> These results highlight the importance of macrophage exosome-derived proteins in the crosstalk between TAMs and tumor cells during tumor progression.

## 5.2 Promoting angiogenesis

Tumor growth and metastasis largely depend on angiogenesis, the process by which new blood vessels grow from existing vessels surrounded by a growing tumor mass. Some studies have shown that the number of TAMs infiltrating tissue is closely related to the vascular density in tumors.<sup>120,121</sup> Large aggregates of TAMs were found in anoxic areas, especially in necrotic tissues.<sup>122</sup> TAMs are regarded as significant producers of proangiogenic factors in malignant tumors because they release IL-1 $\beta$ , VEGF, TNF- $\alpha$ , and other cytokines.<sup>123,124</sup> Studies have recently shown that exosomes play an important role in cancer invasiveness by harboring various cargos to accelerate angiogenesis. El-Arabey et al. found that the transcription factor GATA3 was released in abundance from TAM-derived exosomes, in which it played a critical role in the interactions between the TAMs and mutant TP53-expressing high-grade serous ovarian carcinoma (HGSOC) to promote angiogenesis and EMT through epigenetic regulation.<sup>125</sup> Similarly, M2 macrophage-derived exosomal miR-501-3p enhanced the tube-formation ability of pancreatic ductal adenocarcinoma (PDAC) cells by increasing their expression of angiogenesis-related proteins VEGF-A

and VEGFR-2, suggesting that exosomal miR-501-3p is essential for the angiogenesis in PDAC.<sup>126</sup>

## 5.3 Generating drug resistance

Drug therapy is the primary therapeutic strategy for controlling tumor progression. However, drug resistance is a significant obstacle in devising and implementing such therapies. Recent studies have indicated that both tumor and stromal cells can secrete exosomes containing drug resistance-related factors and transfer them to the TME, where they interact with each other, thus increasing drug resistance.<sup>84,127–129</sup>

Cisplatin-based chemotherapy is mostly used to treat advanced GC; however, drug resistance still occurs frequently. Zheng et al.<sup>130</sup> showed that exosomes derived from M2 macrophages are involved in mediating cisplatin resistance. Using miRNA profiles, researchers identified significantly higher levels of miR-21 in exosomes and cell lysates of polarized M2 macrophage. Exosomal miR-21 can be directly transferred from macrophages to GC cells, suppressing apoptosis, and enhancing the activation of the PI3K/AKT signaling pathway by downregulating PTEN. Similarly, the activated PTEN/PI3K/AKT pathway has also been associated with drug resistance in EOC cells induced by TAM-derived exosomes containing miR-223.<sup>45</sup>

Gemcitabine is a cytidine analog that inhibits cell division by arresting DNA replication. PDAC is known for its development of gemcitabine resistance within weeks of therapy initiation. Macrophages transmit molecular signals to cancer cells by shuttling exosomes that are selectively internalized by PDAC cells. The transfer of exosomal miR-365 among macrophages and PDAC cells inhibits the effect of gemcitabine by upregulating pyrimidine metabolism and increasing triphosphate-nucleotide (NTP) levels in cancer cells.<sup>131</sup> These studies suggest that macrophage-derived exosomes mediate drug resistance primarily through the horizontal transfer of molecules that confer drug resistance to otherwise sensitive cells.

#### 5.4 Promoting immune escape of tumor cells

The responses of T lymphocytes are critical for anti-tumor immunity. Several studies have demonstrated that TAM-derived exosomes can affect the immune response of different T cell subtypes in the TME, and therefore promote tumor cell escape from immune recognition and eradication. CD8<sup>+</sup> T cells are cytotoxic T cells, which are also effector cells that play vital roles in anti-tumor effects. TAMs suppress the CD8<sup>+</sup> T cell response to cancer by secreting immunosuppressive factors, including CCL5, CCL17, and CCL22.<sup>132</sup> In addition to these chemokines, Yang et al.<sup>133</sup> found that M2-BMDM-derived exosomes (BMDM-Exos) shuttle miR-21 to promote migration, proliferation, and invasion, and inhibit the apoptosis of glioma cells by reducing the expression of paternally expressed gene 3 (PEG3). More importantly, this work provided evidence that BMDM-Exos can reduce the percentage of CD8<sup>+</sup> T cells and inhibit the extent of cell cytotoxicity by accelerating the immune escape of gliomas and enhancing their progression. Tregs and T helper 17 (Th17) cells are two subsets of CD4<sup>+</sup> T cells that play opposing immune regulatory roles. Treg/Th17 cell imbalance has been observed in several tumors and is associated with worse patient outcomes. Zhou et al.<sup>134</sup> showed that TAM-derived exosomes transferred miR-29a-3p and miR-21-5p to synergistically induce the Treg/Th17 cell imbalance by targeting STAT3 in CD4<sup>+</sup> T cells, driving EOC progression and metastasis.

#### 5.5 Reprogramming tumor metabolism

Metabolic reprogramming is a hallmark of malignant tumors.<sup>135</sup> Initially, Warburg found that the rate of glycolysis in cancer cells was much higher than oxidative phosphorylation (OXPHOS), even under sufficient oxygen known as the Warburg effect.<sup>136</sup> Since then, a series of studies have demonstrated that, except for glucose, abnormal metabolites involved in glucose, amino acid, and lipid metabolism pathways also induce tumor-related metabolic reprogramming, giving rise to the field of immunometabolism research.<sup>137–139</sup> These molecular metabolites are transferred into the TME via exosomal pathways and influence the metabolism of recipient cells to favor cancer progression. For example, Wang et al. demonstrated that the metabolic enzymes ALDOA and ALDH3A1, containing in exosomes from irradiated lung cancer cells, enhanced the migration and invasiveness of non-irradiated lung cancer cells by accelerating glycolysis.<sup>140</sup>

A recent study revealed that HIF-1 $\alpha$ -stabilizing long non-coding RNA (HISLA), an EV-packaged lncRNA, enhanced aerobic glycolysis and apoptotic resistance of breast cancer cells via EV transmission from TAMs to tumor cells. Mechanistically, HISLA interacted with PHD2 to inhibit the hydroxylation and degradation of HIF-1 $\alpha$ . Moreover, lactate released from tumor cells upregulated HISLA in macrophages, constituting a feed-forward loop between TAMs and tumor cells.<sup>141</sup> These results suggest that exosome transfer is a useful method for cellular communication among multiple cell types in the TME. In addition to glycolysis, tumor metabolic reprogramming also includes lipid, amino acid, and tricarboxylic acid cycle (TCA) metabolism. Exosomes might also take part in

these metabolic processes.<sup>142</sup> For example, cancer-associated fibroblasts (CAFs) secrete exosomal miR-522 to inhibit ferroptosis in GC cells by targeting arachidonate lipoxygenase 15 (ALOX15) and blocking lipid-ROS accumulation to support tumor progression and drug resistance.<sup>129</sup> However, whether TAM-derived exosomes are involved in these processes is unknown and requires in-depth study.

As discussed in this review, the latest evidence confirms that TAM-derived exosomes govern the cancer-promoting immune response in the TME. Therefore, we speculate that targeting the regulation of exosome-related mechanisms may be an attractive strategy to overcome chemical resistance by the immune transfer of miRNA to primary tumors via macrophages (Figure 2). The molecules identified in TAM-derived exosomes are summarized in Table 2.

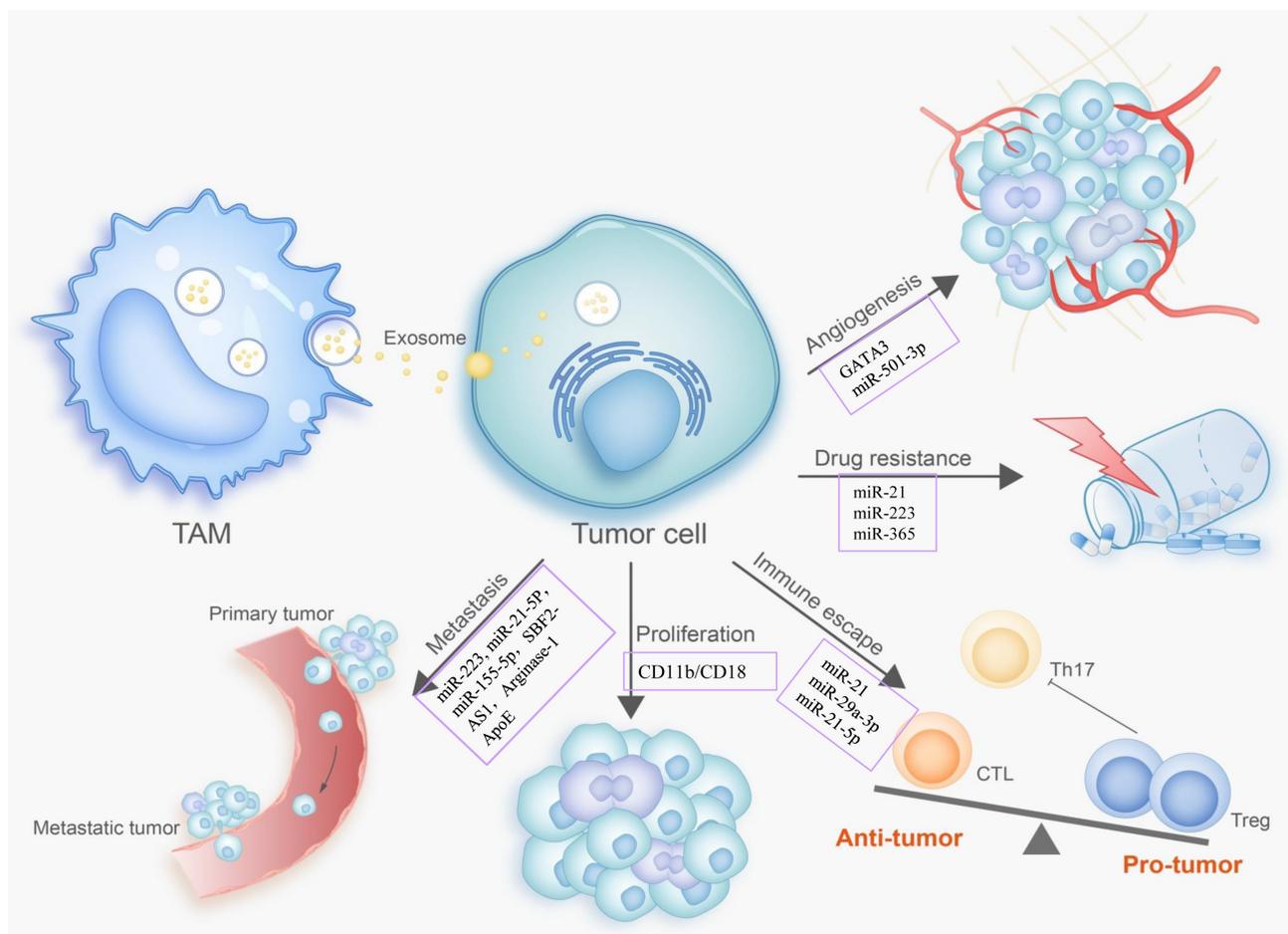
### 6 Exosomal function in targeted TAM-related immunotherapy

As the enrichment of TAMs is closely associated with poor prognosis for patients,<sup>143,144</sup> TAMs are promising candidate targets for immuno-therapeutics. As described above, one of the critical characteristics of macrophages is plasticity, which enables them to acquire different phenotypes depending on the TME. The M1 phenotype imbues macrophages with the ability to eliminate and destroy tumor cells. Therefore, reprogramming TAMs to an anti-tumor phenotype is a very promising therapeutic strategy. A new avenue for developing interventions aims to potentiate the switch of M2 macrophages into M1 macrophages and block their immunosuppressive effects.<sup>145,146</sup> Traditional methods usually use nanoparticles and liposomes to deliver targeted therapy.<sup>147</sup> However, the benefits of such methods are limited due to poor bioavailability, histocompatibility, and tumor selectivity.<sup>120,148,149</sup>

Fortunately, exosomes can be considered a competent vehicle for the delivery of therapeutics, as they have several unique advantages. For example, exosomes have a unique lipid bilayer structure, which protects their contents from being degraded by ribonuclease and other enzymes, thus providing exosomal contents the biological stability. Moreover, comparing with traditional liposome carriers, exosomes have lower toxicity, as well as better tissue tolerance and biocompatibility. Thus, it is possible to design and modify engineered exosomes for immunological therapeutic strategies.<sup>150</sup> Taken together, the role of exosomes in macrophage polarization has prompted the design of therapeutic exosomes to trigger reprogramming from M2 to M1.

#### 6.1 Modification of exosomes derived from tumor cells to promote TAM polarization

As explained above, miRNAs in exosomes regulate TAM polarization into the M2 phenotype; thus, alteration in their levels may mediate macrophage repolarization toward the M1 phenotype. Trivedi et al.<sup>151</sup> established a platform for manipulating exosomal content using dual-targeted hyaluronic acid-based nanoparticles. After transfecting wt-p53 and miR-125b into SK-LU-1 human lung adenocarcinoma cells, the exo-miRNA profile of the SK-LU-1 cells was reprogrammed.



**Figure 2.** TAM-derived exosomes modulate tumor progression. Exosomes derived from TAMs deliver various molecules into tumor cells, which assist tumor malignant characteristics. Exosomal CD11b/CD18 promotes tumor cell proliferation. Exosomal miR-223, miR-21-5p, and Arginase-1 promote the metastasis of tumor cells from the primary tumor to distal organs. Exosomal GATA3 and miR-501-3p promote the angiogenesis of tumors, which helps provide the nutrients needed for the tumor. Exosomal miR-21, miR-223, and miR-365 help tumor cells develop resistance to chemotherapy. Exosomal miR-21 reduces CTL infiltration and inhibits the extent of cell cytotoxicity by accelerating the immune escape of tumor cells. miR-29a-3p and miR-21-5p synergistically induce the Treg/Th17 cell imbalance, which enhances Treg infiltration and immunosuppression in the tumor. CTL: cytotoxic T lymphocyte

Moreover, TAMs were polarized to the M1 phenotype, suggesting that exosome modification may be a viable therapeutic strategy for affecting TME. Similarly, Su et al.<sup>152</sup> used hyaluronic acid-poly (ethylene imine)/hyaluronic acid-poly (ethylene glycol) (HA-PEI/HA-PEG) self-assembling nanoparticles to transfect miR-155 and miR-125b2 into PANC-1 cells. They found that the PANC-1-derived exosomes had high levels of miR-155 and miR-125b2, which triggered the repolarization of J774A.1 macrophages from the M2 to the M1 type by increasing IL-1 $\beta$ /Arg1 and iNOS/Arg1 ratios. These experiments demonstrate the feasibility of modulating tumor cell-derived exosome cargo to achieve anti-tumor effects through M1 macrophage reprogramming.

Construction of engineered exosomes has become a strategy in the design of anti-tumor drugs. Nie et al. constructed pH-responsive M1 exosome nano-bio-conjugates. Exosomes were conjugated with aCD47 and aSIRP $\alpha$  through acid-cleavable benzoic-imine bonds. These exosomes efficiently accumulated in tumor tissues and released the antibodies. Consequently, M1 exosomes reeducated pro-tumoral M2 macrophages driving their switch to M1 macrophages, and enhancing their phagocytic capacity.<sup>153</sup> This engineering strategy provides an

exosomal platform that can be loaded with modified ligands that recognize and/or eradicate tumor cells.

Recently, researchers presented the results of a preclinical trial on engineered exosomes targeting macrophages,<sup>154</sup> in which they confirmed that the engExTM Platform program (exoASO), incorporating an antisense oligonucleotide (ASO), could selectively reprogram M2 macrophages into the M1 phenotype and promote targeted anti-tumor activity. Mechanistically, exoASO inhibited STAT6 and C/EBP $\beta$  expression and increased the level of TNF- $\alpha$  in macrophages. This study opens up new possibilities for engineered exosomes to regulate TAM polarization in tumor therapy.

In addition to the direct modification of exosomes, some anti-tumor drugs are involved in the regulation of macrophage polarization. Epigallocatechin gallate (EGCG), a polyphenol present in green tea, has been shown to inhibit tumorigenesis by inhibiting tumor cell proliferation, invasion, metastasis, and angiogenesis.<sup>155</sup> Studies have shown that in murine breast-cancer cells, EGCG upregulates miR-16, which was transferred to TAMs via exosomes, in which it inhibited TAM infiltration and M2 polarization.<sup>156</sup> This type of modification might provide a new strategy for exploring anti-tumor effects by

**Table 2.** Exosomes derived from TAMs modulate tumor progression.

Cargos	Recipient cancer cell type	Biological Function	Target	Ref.
miR-223	Breast cancer	invasion	Mef2c/ $\beta$ -catenin	111
miR-21-5p	Colon cancer	migration		112
miR-155-5p		invasion		
miR-7	EOC	migration	EGFR/AKT/ERK1/2	113
miR-142-3p	HCC	proliferation	Stathmin-1/ ILGF1R	114
miR-223				
LncRNA SBF2-AS1	PC	invasion, migration	miR-122-5p/XIAP	115
Arginase-1	Glioblastoma	proliferation	–	116
CD11b/CD18	HCC	metastasis	MMP-9	117
ApoE	GC	migration, EMT	PI3K/AKT	119
GATA3	HGSOC	Angiogenesis, EMT	–	125
miR-501-3p	PDAC migration, invasion, angiogenesis		VEGFA,VEGFR2	126
miR-21	GC	Cisplatin resistance	PTEN/PI3K /AKT	130
miR-223	EOC	drug resistance	PTEN/PI3K /AKT	45
miR-365	PDAC	gemcitabine resistance	pyrimidine metabolism, 131	
miR-21	Glioma	Inhibit CD8 + T cytotoxic activity	PEG3 133	
miR-29a-3p, miR-21-5p	EOC	Treg/Th17 imbalance	STAT3 134	
LINC HISLA	Breast cancer	aerobic glycolysis, apoptotic resistance	PHD2 141	

Note: CRC, colorectal cancer; EOC, epithelial ovarian cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HGSOC, high-grade serous ovarian carcinoma; PC, pancreatic cancer; PDAC: pancreatic ductal adenocarcinoma.

manipulating TAMs and the TME. Recently, preclinical evidence showed that the STAT3 inhibitor pacritinib mediated a reduction in oncomiR-21-5p-enriched exosomes secreted by glioblastomas, targeting the tumor suppressor PDCD4. Treatment with a combination of pacritinib and temozolomide appeared to reduce tumorigenesis and retard M2 polarization.<sup>157</sup>

### 6.2 Modified macrophage exosomes for the delivery of chemotherapeutic drugs

Classically activated M1 macrophages suppress tumor growth by releasing pro-inflammatory factors. M1 macrophage-derived exosomes propagate pro-inflammatory signals and establish a local immunostimulatory microenvironment. Wang et al. indicated that M1-exosomes (M1-Exos) act as carriers to deliver paclitaxel into tumors, where they enhance anti-tumor effects by increasing the activation of caspase-3 induced apoptosis and the production of pro-inflammatory cytokines.<sup>158</sup> Similarly, cisplatin-loaded M1 macrophage-secreted exosomes (DDP-M1-Exos) significantly inhibited cell proliferation and induced apoptosis by upregulating Bax and Caspase-3 in lung cancer. Furthermore, an *in vivo* study showed that M1-Exos encapsulated DDP and enhanced its anti-lung cancer activity.<sup>159</sup>

### 6.3 Exosomes derived from M1 cells can be combined with other immunotherapies to treat cancer

It is well known that most tumor-associated antigens (TAAs) have weak immunogenicity, allowing them to quickly escape the immune surveillance. Thus, it is necessary to provide appropriate biological adjuvants to enhance antigen presentation and improve the efficacy of tumor vaccines. Vaccine adjuvants enhance the body's adaptive immune responses to

antigens and/or change the type of immune response when used simultaneously with or before antigen administration.<sup>160</sup> Many molecules and complex formulations have been used as vaccine adjuvants, including aluminum hydroxide,<sup>161</sup> lipopolysaccharide,<sup>162</sup> and mycobacteria.<sup>163</sup>

Exosomal membrane lipids may possess adjuvant activity. Cheng et al. found that exosomes from M1 macrophages, but not M2 macrophages, display tropism toward lymph nodes, which are enriched with naïve macrophages and dendritic cells (DCs). Exosomes improved the activity of lipid calcium phosphate (LCP) nanoparticle-encapsulated Trp2 vaccine and enhanced the antigen-specific cytotoxic T cell response.<sup>164</sup> These data suggest that exosomes derived from M1 macrophages have potential as vaccine adjuvants.

The application of immune-checkpoint inhibitors is an important milestone in anti-tumor immunotherapy. The application of anti-PD-1 and anti-PD-L1 has been shown to improve the recognition and killing ability of T cells that recognize and eradicate tumor cells.<sup>165,166</sup> However, their curative effect is limited in some solid tumors, where many TAMs infiltrate the TME to induce T cell exhaustion, decreasing immunotherapeutic efficacy.<sup>167</sup> Therefore, a synergistic immunotherapy scheme based on a combination of targeting TAMs and T cells may enhance anti-tumor therapies. Yeon et al.<sup>168</sup> demonstrated that exosome-mimetic nanovesicles derived from M1 macrophages (M1NVs) polarized M2 macrophages to acquire the M1 phenotype by changing miRNA and mRNA expression profiles. The repolarization of M1 macrophages resulted in the secretion of pro-inflammatory cytokines, which potentiated the anti-tumor efficacy of PD-L1. Injection of M1NVs and PD-L1 into tumor-bearing mice induced the repolarization of M2 TAMs into M1 macrophages, leading to a significant inhibitory effect on tumor growth.

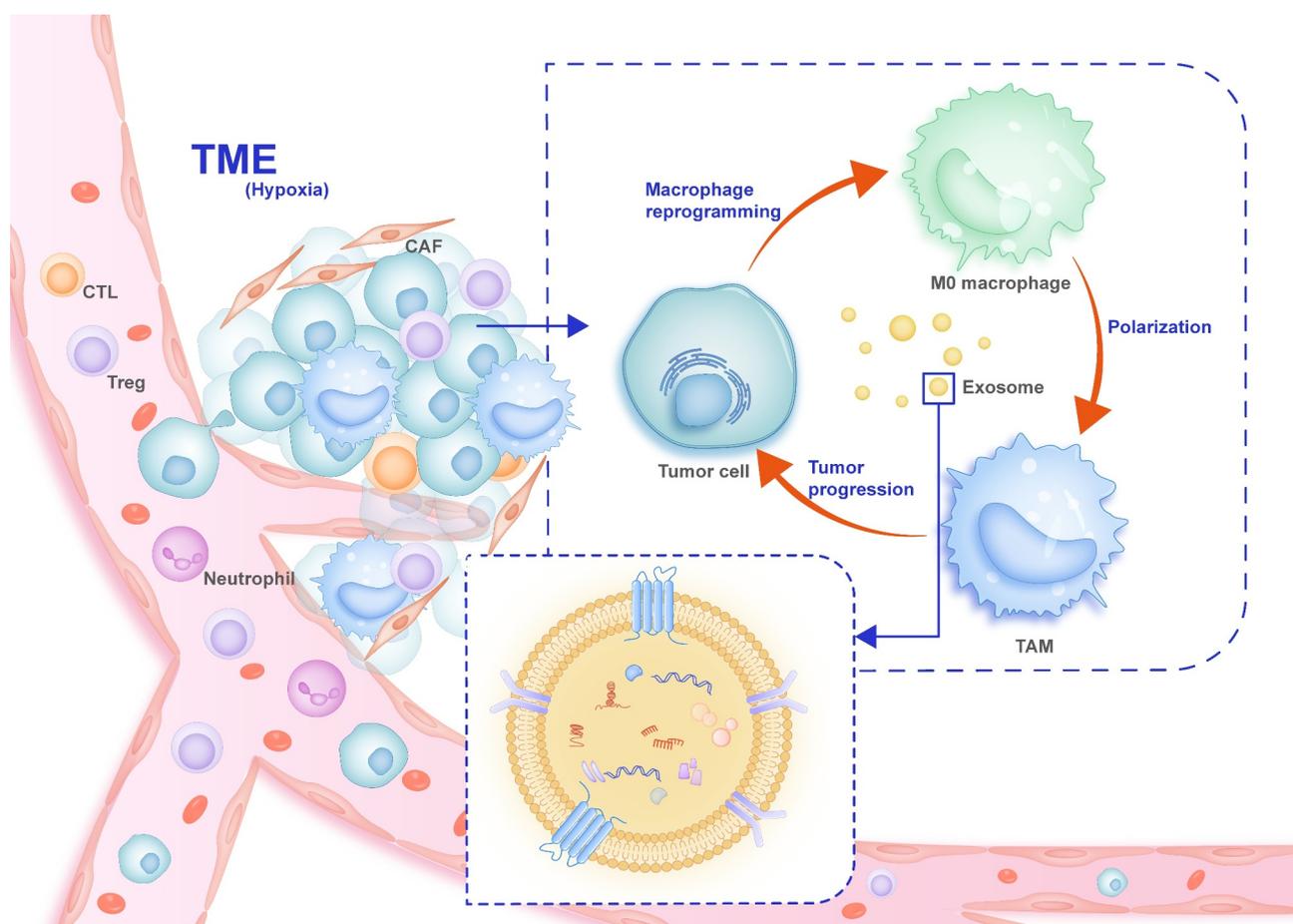
These data suggest that exosomes derived from M1 macrophages can be used as immune regulators in the

TME to potentiate checkpoint inhibitor therapies for cancer (Figure 3).

## 7. Perspective

Multiple studies have inexorably demonstrated that exosomes reflect their cellular origin and disease stage through the bioactive cargos they transport, making them useful as potential biomarkers for diagnosing and predicting clinical prognosis. By adjusting the body's immune defense, tumor immunotherapy can transform immune cells or use multiple types of immuno-active substances to balance the immune system and tumors. As crucial members of the TME, macrophages are attractive targets for immunotherapy drug development because of their characteristics. Using molecularly drugs targeted to treat tumors has led to breakthroughs through in-depth research into cancer molecular biology.

Additional in-depth studies of the unique biological activities of exosomes are still necessary. The heterogeneity of macrophages has been described for a variety of solid tumors; however, it is not clear whether, in addition to their effects on M1 and M2 macrophages, exosomes are involved in the phenotypic polarization of other macrophages or whether particular macrophage subsets produce unique exosomes that regulate the TME. Exosomes as biomarkers can be used to explore new TAM cell subtypes, as well as phenotypes and key regulatory molecules related to tumors. Moreover, they can reverse TAM-mediated immune escape, regulate the TME, and enhance tumor cell recognition and killing. This may be an essential treatment strategy in addition to immune checkpoint inhibitor treatment. However, it is worth noting that even though engineered exosomes perform well *in vitro* experiments, other factors must be considered for the complexity of TME in cancer patients to ensure that sufficient exosomes



**Figure 3.** Overview of exosomes in the communication between tumor cells and TAMs in the tumor microenvironment (TME). TME plays a fundamental role in tumor behavior. Newly formed blood vessels provide nutrition and oxygen to the tumor, contributing to its growth and development. Under hypoxic conditions, tumors exhibit abundant macrophage infiltration. Tumor-derived exosomes switch macrophage polarization to the TAM phenotype, resulting in less phagocytosis and more potency. Meanwhile, exosomes derived from TAMs further promote tumor progression. Thus the communication between tumor cells and macrophages through exosomes forms a positive feedback loop and helps create a protumoral TME.

can reliably access target cells *in vivo*. Recent research ongoing *in vivo*, *in vitro*, and preclinical studies, as well as clinical trials on TAMs, are expected to yield encouraging results.

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## Declaration of competing interest

All authors have declared that no conflict of interest exists.

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