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Review

Function and mechanism of exosomes derived from different cells as communication mediators in colorectal cancer metastasis

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SUMMARY

Colorectal cancer (CRC) ranks as the second leading cause of cancer-related mortality, with metastasis being the primary determinant of poor prognosis in patients. Investigating the molecular mechanisms underlying CRC metastasis is currently a prominent and challenging area of research. Exosomes, as crucial intercellular communication mediators, facilitate the transfer of metabolic and genetic information from cells of origin to recipient cells. Their roles in mediating information exchange between CRC cells and immune cells, fibroblasts, and other cell types are pivotal in reshaping the tumor microenvironment, regulating key biological processes such as invasion, migration, and formation of pre-metastatic niche. This article comprehensively examines the communication function and mechanism of exosomes derived from different cells in cancer metastasis, while also presenting an outlook on current research advancements and future application prospects. The aim is to offer a distinctive perspective that contributes to accurate diagnosis and rational treatment strategies for CRC.

INTRODUCTION

Colorectal cancer (CRC) ranks as the second leading cause of cancer-related mortality globally, contributing to an estimated annual death toll of approximately 910,000 individuals.[1] Upon diagnosis of CRC, approximately 70.4% of patients develop metastasis, primarily to the lymph nodes, while 20–25% of patients experience distant metastasis, with liver metastasis being the most prevalent.[2,3] Tumor metastasis serves as the primary determinant of an unfavorable prognosis in patients, with a 5-year survival rate of 71% observed for CRC patients exhibiting peripheral metastasis and merely 14% for those presenting distant organ metastasis.[2] Despite significant advancements in surgical techniques and targeted therapies that have improved long-term survival rates for patients, the prognosis for CRC patients with metastasis remains unfavorable.[4,5] Therefore, the current focus of research on CRC is primarily directed toward the exploration of more effective and safer diagnostic and therapeutic approaches.

The metastasis of CRC is a complex and dynamic biological process involving multiple genes and links.[6] Currently, the underlying mechanisms of tumor metastasis remain poorly understood. Metastasis of tumor requires a series of sequential steps known as the metastatic cascade.[7] This sequence includes the initiation of invasion by tumor cells into the surrounding tissues of the primary tumor. Subsequently, these cells enter the bloodstream and manage to survive in circulation. They then adhere to vessel walls and extravasate into the parenchyma of distant tissues. Micrometastatic colonies form within the parenchyma, followed by reactivation of proliferation, leading to clinically detectable overt metastatic lesions. This last process being termed colonization.[7,8] Importantly, interactions between tumor cells and non-tumor stromal cells play a crucial role throughout the invasion-metastasis cascade. Exosome-mediated intercellular communication plays a crucial role in various critical steps of metastatic colorectal cancer (mCRC) development and progression, including enhancing tumor cell invasion and migration capacity, altering vascular permeability, facilitating immune escape, and promoting pre-metastatic niche formation.[9–11] Exosomes also act as triggers or drivers for tumor metastasis occurrence when interacting with other factors.[6,12]

During the progression of the disease, intercellular communication occurs not only among cancer cells within the tumor but also between diverse cell types in the tumor microenvironment (TME).[13,14] TME is a complex network of diverse cell types embedded within the extracellular matrix (ECM), including stromal cells such as fibroblasts, pericytes, and adipocytes, as well as immune cells such as T lymphocytes, B lymphocytes, natural killer cells (NKs), and tumor-associated macrophages (TAMs).[15,16] Cellular communication encompasses diverse

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mechanisms, including direct cell-to-cell interactions and indirect regulation mediated by secreted bioactive molecule or extracellular vesicle (EV), thereby reshaping TME and exerting multifaceted influences on tumor development, encompassing cancer cell proliferation, invasion, metastasis, immune escape, angiogenesis, and chemotherapy resistance.[17,18]

In recent years, extensive researches on EVs have revealed their pivotal role in mediating and regulating intercellular communication associated with physiological and pathological processes.[19–21] Consequently, they have garnered significant attention in the biomedical field for their potential applications in cancer diagnosis and treatment. EV is a collective term, encompassing a diverse range of cell-released subtypes of membrane structures.[22] Exosome, a specific subtype of EV, possesses a diameter ranging from approximately 40 to 160 nm (with an average size of about 100 nm). They consist of various membrane-associated higher-order oligomeric protein complexes, nucleic acids, lipids, ECM components, transcription factors, intracellular and extracellular receptors, enzymes as well as other protein complexes.[23,24] The biogenesis and release of exosomes constitute a series of intricate, multi-step processes. Intracellular endosomes undergo continuous invagination of the plasma membrane, leading to the formation of multivesicular bodies. These dynamic structures can engage in interactions with other intracellular vesicles and organelles before ultimately fusing with the plasma membrane for secretion into the extracellular space.[25] Exosomes can be secreted by a wide range of cell types, encompassing tumor cells, macrophages, T lymphocytes, B lymphocytes, fibroblasts, dendritic cells (DCs), astrocytes, and mesenchymal stem cells (MSCs). These exosomes exhibit stability in diverse bodily fluids such as blood, urine, saliva, tears and cerebrospinal fluid.[24,26] In the humoral milieu, exosomes exist as distinct subpopulations characterized by variations in size, morphology, composition, and biogenesis mechanisms. Primarily functioning as intercellular communicators, exosomes facilitate the exchange of molecular cargo between cells, thereby modulating the biological attributes of recipient cells.[27] The intercellular communication by exosomes can be summarized as follows: (1) Exosomal membrane proteins directly engage recipient cells, initiating intracellular signaling cascades; (2) Their cargo to recipient cells through fusion with the cellular membrane; (3) Recipient cells internalize exosomes and release their signaling molecules following lysosomal degradation.[24] Furthermore, mediators facilitating interactions between exosomes and recipient cells have been identified, encompassing tetraspanins (i.e., CD81), integrins, lipids, lectins, heparan sulfate proteoglycans, and ECM constituents. The interplay between integrins and ECM proteins (primarily fibronectin and laminin) has demonstrated its indispensable role in the binding of exosomes to recipient cells.[28,29] Within the TME, diverse cellular components, including exosomes and other communication mediators, coordinate a range of biological effects to facilitate tumor cell invasion and metastasis. These effects encompass migratory potential acquisition from the primary tumor, promotion of angiogenesis, evasion of immune surveillance, organ-specific metastasis patterns, establishment of pre-metastatic niches, and growth initiation at secondary sites.[18,30] Tumor-derived exosomes (TDEs) play a pivotal role in creating a conducive microenvironment at future metastatic sites while also mediating non-random patterns of metastasis by selectively targeting organs such as lung or liver based on their integrin composition.[28] Other cells within the TME, including cancer-associated fibroblasts (CAFs) and macrophages, also play crucial roles in different stages of cancer progression and metastasis. LINC00659, originating from CAFs in the TME, is transferred to CRC cells through exosomes, thereby promoting cancer cell proliferation, invasion, and migration. Additionally, it facilitates epithelial-mesenchymal transition (EMT).[31] Exosomes derived from M2 macrophages, carrying miR-21-5p and miR-155-5p, exerted a downregulatory effect on the expression of Brahma-related gene 1 (BRG1) receptor in CRC cells, thereby enhancing their metastatic potential.[32] Exosomes derived from tumor cells or other cells play a crucial role in various stages of cancer development by delivering proteins, metabolites, or nucleic acids to recipient cells, thereby modulating the transcriptome and/or cell phenotype of the recipients. In CRC, this phenomenon manifests as biological processes that either promote or inhibit biological processes such as tumor proliferation, invasion, migration and metastasis.[30]

Elucidation of the regulatory mechanism underlying intercellular communication mediated by exosomes in metastasis of CRC is pivotal for gaining deeper insights into tumorigenesis and development, as well as for future clinical applications.[30] Exosomes enable the transmission of biological signals between different types of cells or tissues, and their regulation of intracellular signaling pathways enhances their potential diagnostic value and therapeutic efficacy in various diseases, including cancer.[18,23] Exosomes can be detected in a diverse range of human bodily fluids, and their isolation through liquid biopsies provides a convenient approach to acquire comprehensive genetic and metabolic information using various detection methodologies.[23] The primary potential of exosome-based liquid biopsy lies in its capability for disease status determination and stage diagnosis.[33] Furthermore, comprehensive analysis of exosomes enables determination of disease progression and treatment response.[23] The application of exosomes lies not only in their diagnostic value but also in their great potential in the therapeutic management of tumors and other diseases.[34] The lipid and protein composition of exosomes can influence the pharmacokinetic properties of drugs while their inherent components may contribute to enhanced bioavailability and reduced adverse reactions. Exosomes can be engineered to serve as efficient carriers for delivering a diverse range of therapeutic drugs, such as short interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), chemotherapy drugs, and immunomodulators, effectively targeting specific sites.[23] Especially in the field of nanomedicine, EVs have broad application prospects as a new type of drug carrier.[35,36] This review critically examines the functions and underlying mechanisms of exosomes derived from diverse cellular sources, encompassing tumor cells, fibroblasts, immune cells, and other cell types, as pivotal mediators in mCRC. Simultaneously, this article elucidates the potential applications of exosomes in CRC diagnosis and treatment along with their future development prospects to offer valuable insights for rational disease management.

COLORECTAL CANCER-DERIVED EXOSOMES

Exosomes are actively secreted by tumor cells, serving as carriers of genetic and metabolic information between cells of origin and neighboring healthy or abnormal cells, thus playing a crucial role in the TME. Notably, compared to the healthy population, tumor patients exhibit increased production and release of exosomes with distinct sources and molecular contents. For instance, significant differences at the

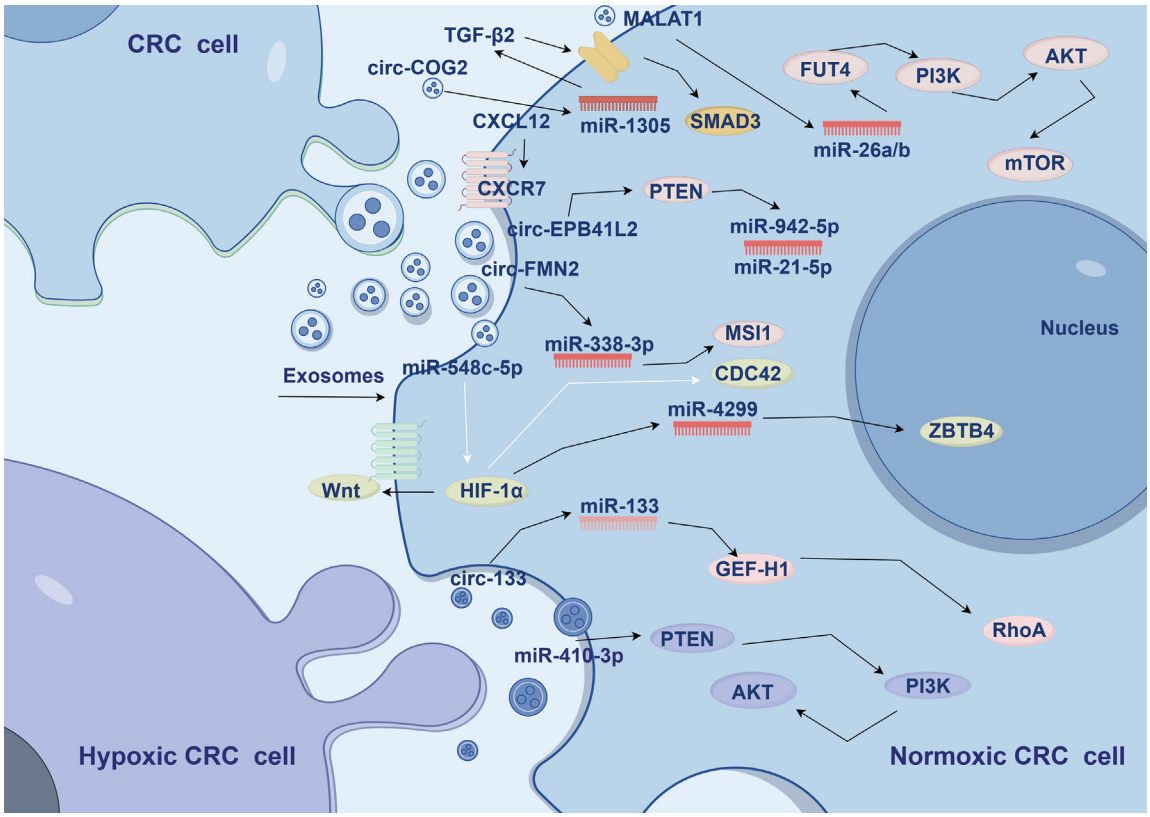

Figure 1. Exosomes derived from CRC promote tumor invasion and migration

For instance, exosomal circCOG2 released by CRC cells in normoxic conditions regulated the invasion and migration abilities of tumor cells by activating the miR-1305/TGF-β2/SMAD3 signaling pathway.

protein level have been observed among exosomes isolated from plasma samples of healthy controls, patients with primary CRC, and those with colorectal cancer liver metastasis (CRLM).[37] Additionally, previous research has revealed a higher proportion of microRNA(miRNA) in exosomes compared to parent cells.[38] Exosomes derived from primary or metastatic cancer cells exert regulatory control over cancer cell metastasis through diverse signaling mechanisms, including the reprogramming of protein composition and secretion via autocrine or paracrine pathways. This phenomenon enhances the migratory capacity of primary tumors, promotes angiogenesis, facilitates evasion from the immune system, supports the establishment of pre-metastatic niches, and further fosters the growth of metastatic tumors at secondary sites.[39] Tumor cells regulate metastasis of cancer by orchestrating intercellular communication through the secretion of exosomes, which facilitate the exchange of critical information and coordinate diverse aspects of the metastatic cascade.

Invasion and migration of tumor cells

The initial step in the formation of cancer metastasis entails the local dissemination of cancer cells derived from the primary tumor, which is instigated by modulating the TME.[9] Tumor cells enhance their invasive and migratory capabilities by secreting diverse exosomes, thereby facilitating the EMT and ECM remodeling, ultimately promoting tumor metastasis. Exosomal cargo, including RNA, proteins, lipids, or metabolites, mediates autocrine or paracrine signaling to augment invasion and migration of tumor cells (Figure 1).[30]

The genetic information or metabolic substances carried by exosomes derived from CRC cells can modulate the invasion and migration of tumor cells through diverse mechanisms upon transfer to recipient cells.[40] MiRNAs are evolutionarily conserved, short, non-coding RNAs (ncRNAs) that play a pivotal role in the post-transcriptional regulation of gene expression.[41] As the most biologically active molecules found within exosomes, miRNAs have garnered increasing attention due to their crucial involvement in regulating the expression of genes associated with metastasis of CRC.[42] A previous study demonstrated a significant elevation of exosomal miR-106b-3p in the plasma of patients with mCRC, which was associated with advanced TNM staging and larger tumor volume. Additionally, both CRC cells and exosomes derived from CRC cell supernatant exhibited significantly increased expression levels of miR-106bp compared to NCM 460 cells. Furthermore, it was discovered that exosomal miR-106b-3p promotes EMT by inhibiting dynein light chain 1 (DLC-1), thereby enhancing the invasive and metastatic potential of CRC cells.[43] Bigagli et al. discovered exosomes derived from HCT-8 cells carrying miR-210 are implicated in anoikis resistance and EMT markers, thereby preserving the conducive microenvironment for local cancer growth and guiding metastatic cells toward new dissemination sites.[44] Moreover, exosomal miR-193a derived from CRC cells exhibited inhibitory effects on tumor cell invasiveness and

proliferation, while let-7g demonstrates contrasting effects. By assessing the expression levels of exosomal miR-193a and let-7g in the plasma of patients with CRC, their functions as diagnostic and prognostic markers for this disease were confirmed.[45] Exosomal miR-548c-5p derived from CRC cells exerted inhibitory effects on the proliferation and invasion of CRC cells *in vitro*, as well as prevents the development of CRC *in vivo* in nude mice. Furthermore, it demonstrated that exosomal miR-548c-5p derived from CRC cells modulates CRC cell growth, migration, and invasion through the hypoxia-inducible factor 1 alpha (HIF-1α)/cell division control protein 42 (CDC42) axis.[46] Rezaei et al. demonstrated that the efficient delivery of miR-375 mimics by TDEs *in vitro* resulted in down-regulation of β-catenin, vimentin, zinc finger E-box binding homeobox 1 (ZEB1), and snail, while significantly reducing the expression of E-cadherin. Furthermore, TDEs inhibited the migratory and invasive capabilities of HT-29 and SW480 cells and induced an upregulation in CD44 and CD133 expression during EMT.[47]

Recent studies have demonstrated that circular RNAs (circRNAs) are highly enriched in exosomes and play a pivotal role in various biological processes within tumors, particularly by functioning as molecular sponges for miRNAs.[48–50] CircRNAs are characterized by their closed structure and single-stranded nature, lacking poly (A) tails or 5′–3′ ends.[9] Due to this unique feature, they exhibit enhanced stability and prolonged lifespan within cells as they can withstand degradation caused by exonucleases.[51] Yu et al. demonstrated the upregulation of circFMN2 in both serum and exosomes from CRC patients, as well as CRC cells. Additionally, they discovered that exosomal circFMN2 promotes CRC cell proliferation and metastasis while inhibiting apoptosis through the miR-338-3p/Musashi1 (MSI1) axis. Moreover, overexpression of MSI1 counteracts the suppressive effect of miR-338-p on CRC progression, suggesting that targeting exosomal circFMN2 could be a potential therapeutic strategy for treating CRC.[52] In another study, exosomal circEPB41L2 derived from CRC cells inhibited the progression of CRC by sponging miR-21-5p and miR-942-5p and regulating the phosphatase and tensin homolog (PTEN)/AKT signaling pathway.[49] Furthermore, it has been demonstrated that circ-ABCC1, carried by exosomes derived from CD133+ CRC cells, facilitated cell stemness and metastasis in CRC via activation of the Wnt/β-catenin pathway.[53] Gao et al. reported that exosomes derived from CRC cells containing circCOG2 facilitate the proliferation, migration, and invasion of CRC by activating the miR-1305/transforming growth factor β2 (TGF-β2)/SMAD3 pathway. Moreover, this effect can be transferred from high metastatic potential CRC cells to low metastatic potential CRC cells through exosomal communication.[54] Chen et al. proposed a novel mechanism for the secretion of circRNAs in exosomes, whereby circRHOBTB3 is specifically sorted into exosomes through interaction between ESCRT-II complex member SNF8 and its own specific element, resulting in extracellular release via the "tumor exosome escape mechanism." Additionally, based on regulatory elements for circularization and exosomal secretion, ASOs were designed and synthesized to increase expression of circRHOBTB3 while blocking its exosomal secretion. It was found that these ASOs inhibited growth and metastasis of CRC both *in vitro* and *in vivo*.[55] In a previous study, aberrant upregulation of circ-PABPC1 expression was observed in both CRC tissues and exosomes. *In vitro* and *in vivo* functional experiments provided evidence supporting the oncogene of exosomal circPABPC1. Mechanistically, circPABPC1 recruits KDM4C to the HMGA2 promoter, resulting in reduced H3K9me3 modification and subsequent initiation of transcription within the nucleus. Moreover, cytoplasmic circPABPC1 promoted CRC metastasis by safeguarding A disintegrin and metalloproteinase 19 (ADAM19) and bone morphogenetic protein 4 (BMP4) from degradation mediated by miR-874/miR-1292.[56]

Exosomal long non-coding RNA (lncRNA) regulates intercellular communication between tumor cells and their microenvironment, playing a pivotal role in the intricate process of tumor metastasis. The lncRNA refers to an RNA molecule that exceeds 200 nt in length and lacks the ability to encode proteins itself.[57] In recent years, numerous studies have elucidated the functions and underlying mechanisms of exosomal lncRNA in facilitating metastasis of CRC. Exosomal lncRNA PCAT1 derived from CRC cells regulates the activity of miR-329-3p/Netrin-1-CD146 complex to facilitate EMT and liver metastasis in CRC through the mediation of circulating tumor cells.[58] A previous study demonstrated that the expression level of UCA1 in serum exosomes from CRC patients was upregulated, thereby augmenting the proliferation and migration abilities of CRC of the miR-143/MYO6 axis. Furthermore, UCA1 was found to be overexpressed in CRC tissues and acted as a competitive endogenous RNA for miR-143, consequently regulating CRC cell proliferation and metastasis.[59] Xu et al. discovered that exosomal MALAT1 from CRC engulfs miR-26a/26b, thereby regulating fucosyltransferase 4 (FUT4) and activating phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway, ultimately promoting malignant behavior in CRC cells.[60]

The precise role of exosomal proteins, particularly membrane-bound proteins, remains incompletely understood. Previous studies have elucidated the pivotal role and underlying mechanisms of exosome-associated proteins derived from CRC cells in facilitating tumor invasion, migration, and metastasis.[30] Among the ADAM protein family, A disintegrin and metalloproteinase 17 (ADAM 17) is a membrane protein. Exosome-derived ADAM17 levels are elevated in both mCRC cells and serum from mCRC patients. Additionally, it has been discovered that exosomal ADAM the migration ability of CRC cells and contributes-metastatic niche formation by disrupting E-cadherin connections. Furthermore, the potential of circulating exosomal ADAM17 as a biomarker for predicting tumor metastasis in CRC patients has been convincingly demonstrated.[61] Moreover, exosomes derived from CRC cells with upregulated Ca2+-dependent secretion activator protein 1 (CAPS1) exhibited the ability to augment the migratory potential of normal colonic epithelial FHC cells, while inhibition of exosome release using GW4869 can impede FHC cell migration. Overexpression of CAPS1 induces alterations in the expression profile of exosomal proteins implicated in cellular migration, bone morphogenetic protein 4 potentially playing a crucial role in CAPS1-mediated cell migration.[62]

Exosomes derived from diverse malignant tumor cells induce the reprogramming of MSCs within the TME, resulting in their differentiation into CAFs.[63–65] The reciprocal communication between tumor cells and CAFs, as well as the biomechanical remodeling of the ECM by CAFs, significantly contributed to the migratory and invasive properties of tumor cells.[66] In a study investigating the regulation of fibroblast phenotype in the TME by exosomes derived from early-stage (SW480) and late-stage (SW620) CRC cells, both CRC cell lines induced quiescent fibroblasts with normal characteristics (α-SMA-, CAV+, FAP+, VIM+) to differentiate into CAFs-like cells (CAFs-like; α-SMA+, CAV-, FAP+, VIM+). However, the activated CAFs exhibited diverse biological functions. Early CRC cells induced fibroblast activation with potent

proliferation-promoting and-promoting capabilities, accompanied by elevated expression of angiogenesis-promoting proteins (IL8, RAB10, NDRG1) and proliferation-promoting proteins (SA1008, FFPS). In contrast, late-stage CRC cells triggered fibroblast activation that displayed remarkable invasive potential within the ECM through upregulation of pre-invasive regulatory factors involved in membrane protrusions (PDLIM1, MYO1B) and matrix remodeling proteins (MMP11, EMMPRIN, ADAM10). The conserved features of fibroblast activation mediated by exosomes encompass augmented ECM secretion, including collagen type I α-1 and Tenascin-C/X, as well as oncogenic transformation and metabolic reprogramming involving the downregulation of caveolin-1 and upregulation of glycogen metabolism (GAA), amino acid biosynthesis (SHMT2, IDH2), and membrane transporters for glucose (GLUT1), lactate (MCT4), and amino acids (SLC1A5/3A5).[67] Zhou et al. discovered that LINC01915 facilitates the conversion of normal fibroblasts (NFs) into CAFs via the miR-92a-3p/KLF4/CH25H axis. Additionally, LINC01915 impeded the uptake of EVs from CRC cells by NFs through the miR-92a-3p/KLF4/CH25H axis, thereby inhibiting angiogenesis and preventing the transformation of NFs into CAFs to suppress tumor growth.[68] Bhome et al. investigated the impact of EMT status on fibroblast phenotypes and the regulation of EVs as mediators of intercellular communication in CRC. Epithelial CRC-derived EVs suppressed TGF-β-induced myoblast differentiation, while mesenchymal CRC-derived EVs do not exhibit this effect, with miR-200 (miR-200 a/b/c, −141) playing a crucial role in driving these responses.[69] In another study, exosomal miR-146a-5p and miR-155-5p facilitated CXCL12/CXCR7-induced metastasis in CRC through intricate interplay with CAFs. Their study unraveled that exosomes derived from CRC cells overexpressing CXCR7 can be internalized by CAFs, thereby promoting their activation via JAK2-STAT3/NF-κB signaling pathway by targeting cytokine signal transduction inhibitor 1 (SOCS1) and zinc finger and BTB structure domain 2 (ZBTB2). The activated CAFs exhibited a substantial elevation in the levels of pro-inflammatory cytokines interleukin-6, tumor necrosis factor-alpha, transforming growth factor β, as well as CXCL12, which collectively instigate EMT in CRC.[70]

Angiogenesis and changes in vascular permeability

Tumor angiogenesis, the process of neovascularization from pre-existing vessels, facilitates the provision of oxygen and nutrients to tumor cells, thereby promoting their active proliferation. The distinctive characteristics of primary tumor vasculature and the TME contribute to the enhanced permeability and retention (EPR) effect. Augmented vascular permeability is a hallmark feature of tumor blood vessels and plays a pivotal role in driving the EPR effect.[10] Emerging evidence suggests that tumor cell-derived exosomes facilitate the transfer of bioactive substances to endothelial cells (ECs) or modulate angiogenesis alterations through intermediary cells such as fibroblasts and immune cells, thereby promoting tumor metastasis (Figure 2).[71,72]

Angiogenesis plays a pivotal role in all stages of cancer development, exerting profound influence on tumor progression. Exosomes derived from CRC encompass a diverse array of bioactive molecules that intricately modulate angiogenic processes and permeability alterations, thereby potentiating the invasive and metastatic potential of cancer cells.[73] Hu et al. discovered a significant elevation in miR-1229 levels within serum exosomes derived from CRC patients, which exhibited a strong correlation with tumor size, lymph node metastasis, TNM stage, and unfavorable prognosis. Furthermore, their investigation revealed that CRC cell-derived exosomal miR-1229 facilitated angiogenesis by targeting HIPK2 and activating the VEGF pathway. Inhibition of miR-1229 using anti-miR-1229 effectively suppressed tumor growth and angiogenesis in a xenograft mouse model.[74] Hong et al. discovered that EVs derived from SW480 CRC cells exhibited an enrichment of cell cycle-related mRNAs known to stimulate endothelial cell proliferation, thereby implicating cancer cell-derived EVs in the facilitation of tumor growth and metastasis of angiogenesis.[75] In another study, Exosomal circTUBGCP4 facilitated the formation of tip cells and promoted metastasis in colon cancer by suppressing miR-146b-3p and activating the AKT signaling pathway in endothelial cells.[76] Growth/differentiation factor 15 (GDF15), a member of the TGF-β/bone morphogenetic protein (BMP) superfamily, exhibits expression in diverse tissue types. Exosomes derived from colon cancer cells resistant to 5-fluorouracil are enriched with GDF15 and possess the ability to promote angiogenesis through activation of the Smad/POSTN axis.[77] Moreover, EVs derived from CRC cells were found to induce Egr-1 activation and facilitate migration of endothelial cells as angiogenesis via the ERK1/2 and JNK signaling pathways.[78] In a separate investigation, exosomal B7-H3 derived from CRC facilitated tumor angiogenesis and metastasis by activating the AKT1/mTOR/VEGFA signaling pathway.[79] Moreover, fibroblasts activated by exosomes derived from SW480, exhibited augmented proliferation and angiogenesis-promoting functions, characterized by elevated expression levels of angiogenic factors (IL8, RAB10, NDRG1) and proteins associated with cellular proliferation (SA1008, FFPS).[67]

Tight junctions and adherens junctions are present endothelial cells, and disruption of tight junctions results in increased vascular permeability.[80] The tight junctions consist of two proteins, Zonula occludens-1 (ZO-1) and Claudin-5. Exosome-mediated delivery of miR-29a from tumor cells regulates the expression of ZO-1, Claudin-5, and occludin by targeting Kruppel-like factor 4 (KLF4). *In vivo* experiments confirmed that exosomal miR-29a promoted liver metastasis in CRC mice.[72] In a separate study, exosomal miR-25-3p derived from CRC effectively modulated the expression of VEGFR2, ZO-1, occludin, and Claudin 5 in endothelial cells, thereby facilitating vascular angiogenesis and permeability. Meanwhile, exosomal miR-25-3p originating from CRC cells significantly induced hepatic and pulmonary vascular leakage while promoting metastasis in CRC.[71]

Hypoxia, a crucial driver of tumor progression, regulates angiogenesis, invasion, metabolism, and genetic instability.[81] Huang et al. discovered that CRC cells under hypoxic conditions promote the proliferation and migration of endothelial cells through activation of the Wnt/β-catenin signaling pathway mediated by EVs.[82] Furthermore, they observed that these exosomes rich in Wnt4 are dependent on HIF-1α and can be inhibited by the ICG-001 inhibitor. Moreover, knockdown of RAB27a impeded CRC cell-induced proliferation and migration of endothelial cells mediated by EVs.[82] Cellular prion protein (PrPC) is a cell surface glycoprotein that has been found to play an important role in tumor metastasis in recent years. Yun et al. demonstrated that the hypoxic TME induces an up-regulation of exosomal PrPC expression in CRC, thereby modulating the biological behavior of CRC cells and promoting tumor metastasis, including enhanced endothelial permeability,

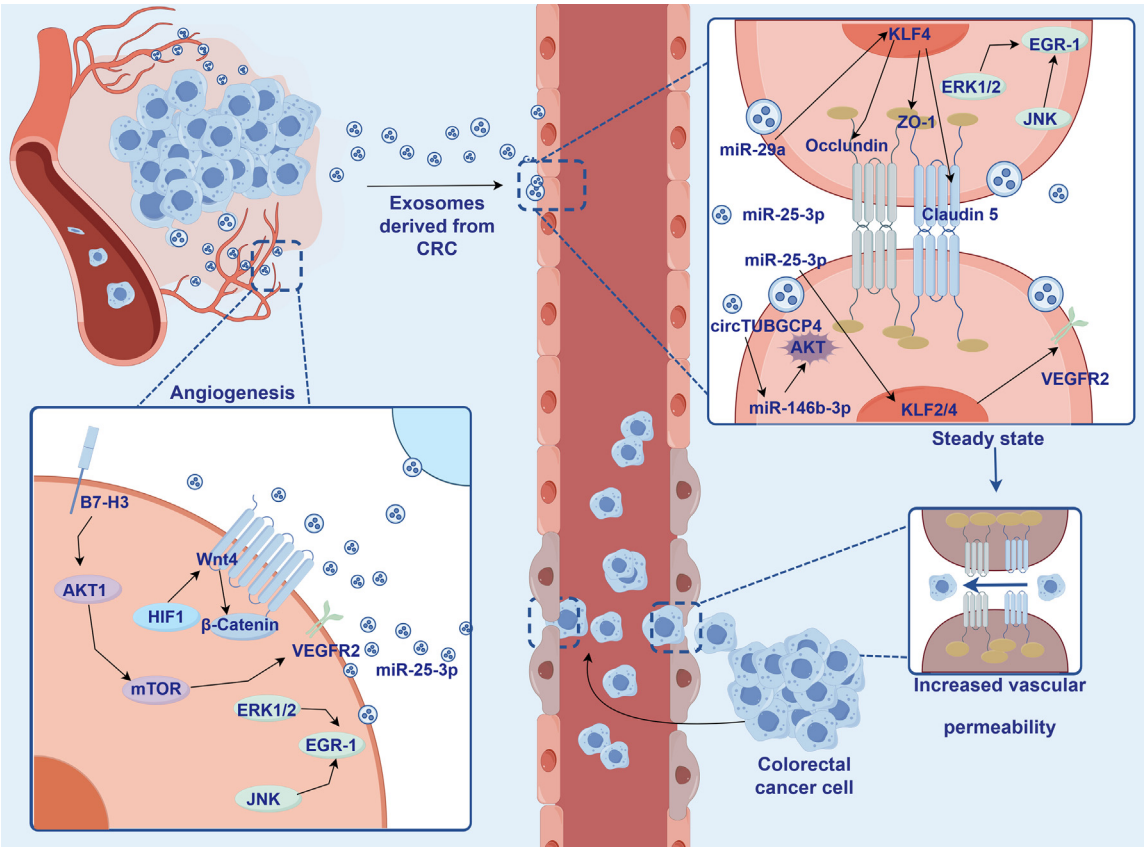

Figure 2. Exosomes derived from CRC promote angiogenesis and alter vascular permeability

For instance, by targeting KLF4, the exosomal miR-29a from CRC cells modulated the expression of ZO-1, Claudin-5, and occludin to disrupt the integrity of the vascular endothelial barrier and facilitate metastasis in CRC.

migration, invasion, and secretion of angiogenic factors. Notably, the progression of CRC in xenografted mouse models was effectively inhibited by anti-PrPC antibody.[83]

Immune escape

The involvement of exosomes in the dynamic interactions between tumor cells and the immune microenvironment enables them to regulate multiple processes associated with tumor metastasis. In most solid tumors, macrophages constitute the predominant component of the tumor stroma, with their diversity often characterized two opposing polarization states: pro-inflammatory M1 and anti-inflammatory M2 macrophages.[84,85] TAMs, primarily derived from peripheral blood monocytes recruited to tumor masses, constitute distinct subpopulations of macrophages that support tumor growth and are classified as M2 macrophages.[86,87] In addition to macrophages, the metastasis of tumor cells is also regulated by various immune cells including neutrophils, NKs, and T cells. Cancer cells infiltrate the circulatory system with the assistance of immune cells and employ multiple mechanisms to evade recognition by cytotoxic lymphocytes and phagocytes.[88,89] Upon arrest distant capillaries, cancer cells extravasate into the parenchyma of target organs and initiate colonization. Subsequently, established cancer cells must acquire resistance against immune responses and other host tissue defenses in order to ensure survival (Figure 3).[9]

Exosomes derived from tumors can induce macrophage polarization, leading to the activation of either anti-inflammatory M2 immune cells that promote tumor growth or pro-inflammatory M1 immune cells tumor growth, thereby modulating the M1/M2 ratio in the TME and regulating CRC progression.[42,86,90] Exosomal miRNAs derived from CRC cells have been extensively investigated in previous studies on immune evasion during metastasis. Zhao et al. revealed that exosomal miR-934 derived from CRC cells induces polarization of M2 macrophages through PTEN downregulation and activation of the PI3K/AKT signaling pathway.[91] In addition, reprogramming of macrophages into tumor-supportive M2 macrophages was facilitated by mutant p53 exosome-mediated miR-1246.[87] In another study, exosome derived fragments of cytoskeleton proteins from CRC cells were found to have the potential to induce phenotypic transitions between M1 and M2 states.[92,93] Takano et al. reported that exosomal miR-203 derived from CRC patients can modulate TAMs implicated in CRC metastasis.[94] Exosomal miR-203a-3p from CRC cells induced M2 macrophage polarization by controlling PTEN and activating the PI3K/AKT signaling pathway. M2 polarized macrophages secreted CXCL12, which promoted tumor metastasis through the CXCL12/CXCR4/NF-κB signaling pathway.[95] Sun et al. demonstrated that exosomes derived from CRC stem cells delivered miR-17-5p to HCT116 cells, thereby suppressing

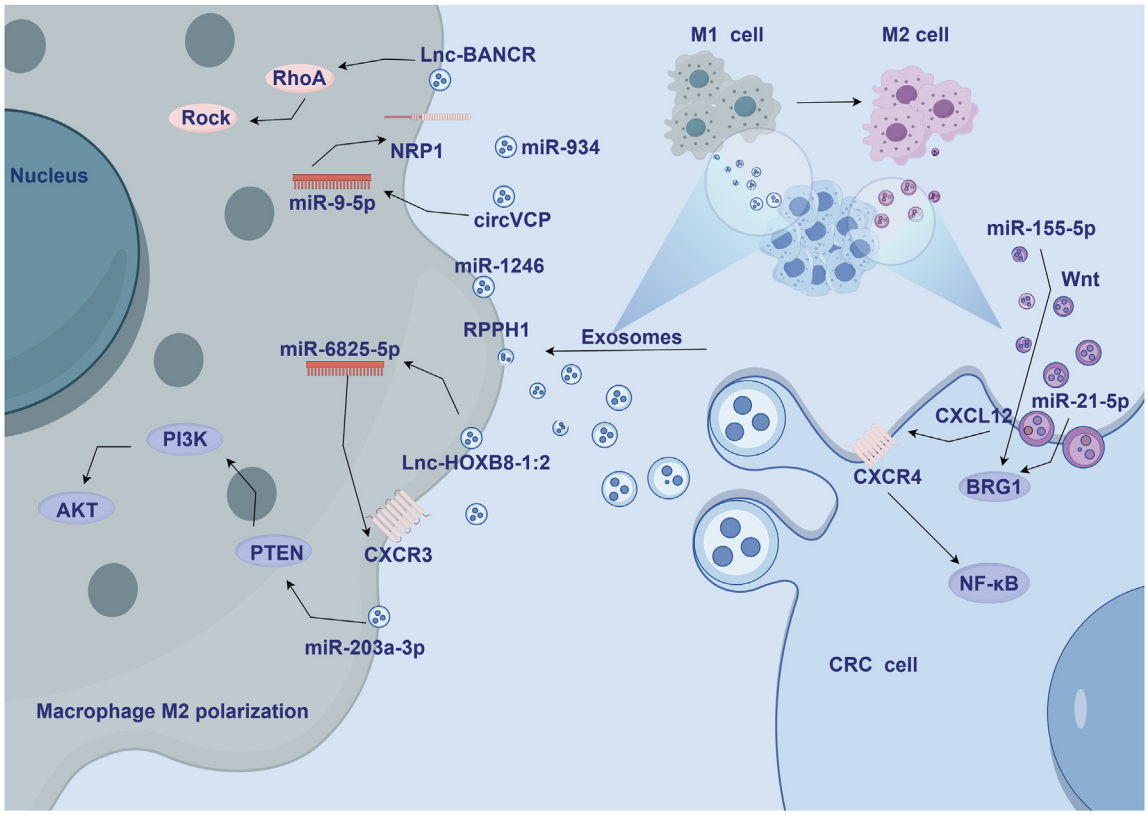

Figure 3. Exosomes derived from CRC are involved in tumor immune escape

For example, the exosomal miR-203a-3p derived from CRC induced macrophage M2 polarization by modulating PTEN and activating the PI3K/AKT signaling pathway, thereby exerting its role in facilitating tumor metastasis.

anti-tumor immunity and promoting their malignant behavior. Conversely, the overexpression of speckle-type POZ protein (SPOP), an E3 ubiquitin ligase adaptor with tumor suppressor properties, exhibited an opposing effect. Upregulated miR-17-5p enhances tumor cell growth by upregulating programmed death ligand 1 (PD-L1) to inhibit SPOP expression and impairs anti-tumor immunity in CRC.[96] Yang et al. discovered that EMT-associated CRC cells facilitated M2-like polarization of macrophages through direct exosomal transfer, leading to a significant elevation in miR-106b-5p levels within macrophages. Mechanistically, the upregulated miR-106b-5p activated the PI3Kγ/AKT/mTOR signaling cascade by directly suppressing programmed cell death 4 (PDCD4) at the post-transcriptional level, thereby promoting M2 polarization of macrophages.[97] Hypoxia-induced exosomal miR-135a-5p is associated with the development, clinical severity, and prognosis of CRLM. Mechanistically, Kupffer cells phagocytize exosomes containing highly expressed miR-135a-5p from the bloodstream and transport them to the liver. Exosomal miR-135a-5p activates the large tumor suppressor kinase 2 (LATS2)-yes-associated protein (YAP)-matrix metalloproteinase 7 (MMP7) axis, thereby promoting CRLM. Moreover, the intricate process is associated with immunosuppression signaling mediated by cluster of differentiation 30-TNF receptor-associated factor 2 (TRAF2)-p65.[98]

In addition to exosomal miRNAs, other genetic or metabolic molecules present in tumor-derived exosomes, such as lncRNAs, circRNAs, and others, exert regulatory effects on the metastasis of CRC through various mechanisms including immune evasion.[18,99] The plasma level of exosomal lncRPPH1 was elevated in untreated patients with CRC and significantly decreased following tumor resection. Exosomes derived from CRC cells facilitated the transfer of lncRPPH1 into macrophages, leading to M2 polarization and consequently promoting CRC cells metastasis and proliferation. Moreover, both *in vitro* and *in vivo* experiments demonstrated that lncRPPH1 played a crucial role in driving CRC metastasis. Mechanistically, lncRPPH1 interacted with tubulinβ-III (TUBB3) to prevent its ubiquitination, thereby inducing metastasis of CRC cells.[100] Additionally, lncKCNQ1OT1, originating from CRC cells, modulated M2 macrophage polarization by regulating the miR-30a-5p/ubiquitin-specific protease 22 (USP22) signaling axis, thereby ubiquitinating and promoting immune evasion of CRC. Furthermore, cancer cell-derived exosomes promote remodeling of the lymphatic system and trigger sentinel lymph node metastasis.[57]Exosomes harboring lnc-HOXB8-1:2 derived from neuroendocrine-differentiated CRC cells competitively sequestered hsa-miR-6825-5p, resulting in the upregulation of CXCR3 expression and subsequent recruitment of TAMs with M2 polarization, thereby facilitating the progression of neuroendocrine-differentiated CRC.[101] In a separate investigation, exosomal lncBANCR derived from SW620 and HCT-15 cells facilitated EV-mediated polarization of M2 macrophages and enhanced CRC metastasis through modulation of the RhoA/ROCK signaling pathway.[102]

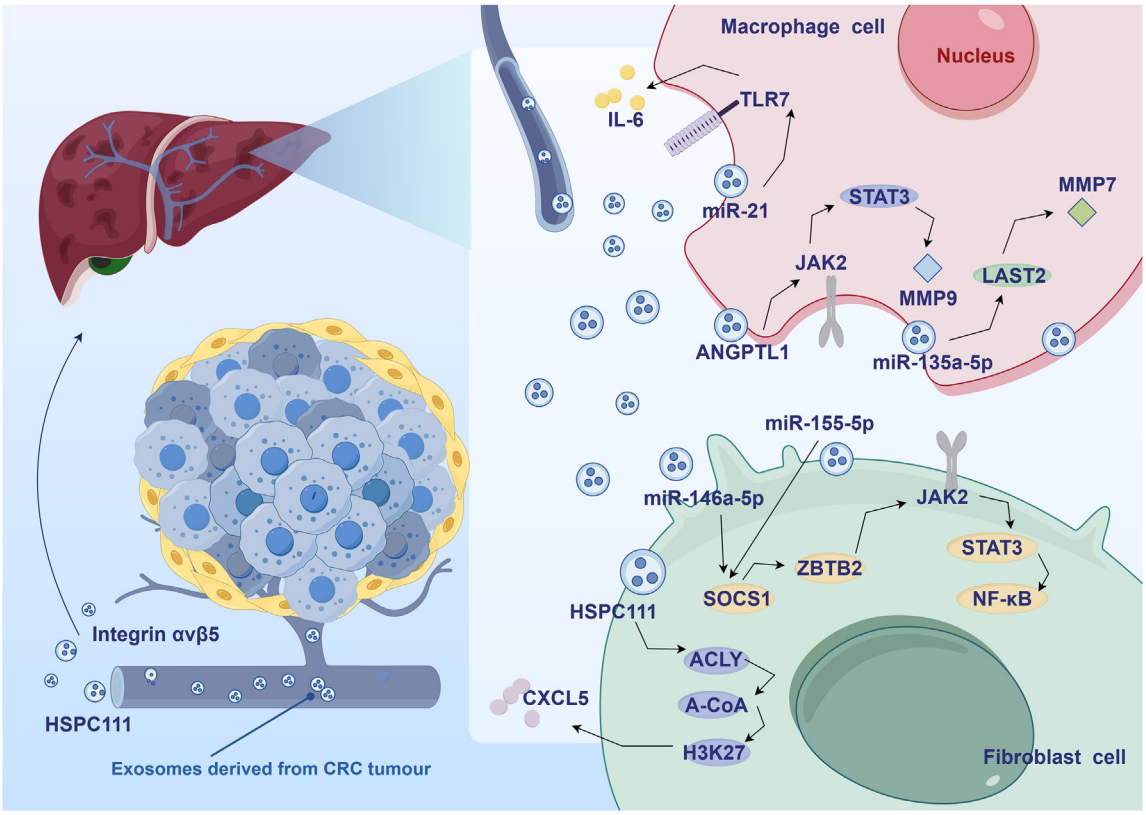

Figure 4. Exosomes derived from CRC are involved in the formation of pre-metastatic niches

The exosomal HSPC 111 from CRC induced changes in the lipid metabolism of CAFs through phosphorylation of ACLY, resulting in an up-regulation of A-CoA levels. This process promoted the formation of a pre-metastatic niche and facilitates CRLM.

Moreover, the upregulation of exosomal circVCP can potentially enhance M1/M2 macrophage polarization through modulation of the miR-9-5p/NRP1 axis, thereby contributing to the progression of CRC.[103] CRC cells induced T cell apoptosis *in vitro* and *in vivo* by releasing EVs carrying Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand.[104]

Formation of pre-metastatic niches

Tumors instigate the establishment of microenvironments in distant organs through diverse communication mediators prior to metastasis, thereby facilitating the survival and proliferation of tumor cells upon their arrival at these sites. These predetermined microenvironments are commonly referred to as pre-metastatic niches.[11,105] Pre-metastatic niches are established through systemic signals emanating from the primary tumor prior to the arrival of cancer cells, which recruit supportive stromal cells and other cellular constituents. The perivascular microenvironment facilitates the extravasation of cancer cells and their dissemination along the basement membrane of capillaries. Specialized niches are orchestrated by secretory products released by cancer cells, serving as an autocrine or paracrine source for recruiting supportive signals from stromal components. Cancer cells infiltrate host tissue's endogenous stem cell niches, thereby directly occupying favorable microenvironments (Figure 4).[9]

Exosomes derived from CRC cells have the ability to disseminate through body fluids over considerable distances, thereby facilitating the establishment of pre-metastatic niches and subsequent tumor colonization.[106] Exosomes can modulate the ECM to facilitate adhesion of circulating tumor cells, such as by augmenting fibronectin deposition in hepatic tissue. In a previous study, exosomes derived from CRC cells carrying miR-21 have the ability to induce an inflammatory pre-metastatic niche in macrophages through activation of the miR-21/Toll-like receptor 7/IL-6 axis.[107] In a murine model of mCRC, exosomal miR-25-3p selectively targeted transcription factors Krüppel-like factors 2 and 4, leading to downregulation of VEGFR2 expression as well as tight junction components occludin and claudin-5 in the liver and lungs, thereby facilitating extravasation and enhancing vascular permeability.[71] In another study, it was reported that circulating Kupffer cells (KCs) in CRC patients could phagocytose exosomes containing highly expressed miR-135a-5p from the bloodstream and promote the formation of pre-metastatic niches, thereby contributing to the development and clinical severity of CRLM.[98] The expression of miR-221/222 consistently exhibited upregulation in serum exosomal samples obtained from patients with CRLM. Exosomes derived from CRC cells, which carry miR-221/222, play a pivotal role in the establishment of the pre-metastatic niche by suppressing SPINT1 expression and

activating hepatocyte growth factor (HGF), thereby facilitating CRC colonization.[108] Exosomal miR-10a derived from CRC cells suppressed the proliferation and migration activity of normal human lung fibroblasts (NHLFs), as well as downregulates the expression levels of IL-6, IL-8, and IL-1β in NHLFs. Their study provided valuable insights into the role of exosomal miR-10a derived from CRC cells in inducing phenotypic alterations in NHLFs and enhanced our understanding of potential mechanisms underlying CRC lung metastasis.[109] The CXCL10, belonging to the CXC chemokine family, binds to the CXCR3 receptor and exerts its biological effects. Exosomal CXCL10 RNA has the potential to serve as a novel biomarker for liver metastasis from mCRC and could be targeted for prevention and treatment of these patients.[110] In another study, compared with paired normal tissues, the level of ANGPTL1 protein in exosomes derived from CRC is significantly downregulated. Exosomal ANGPTL1, which is mainly taken up by Kupffer cells (KCs) and downregulates intracellular MMP9 levels by inhibiting the JAK2-STAT3 signaling pathway, regulated CRLM and prevented vascular leakage in liver PMNs.[111] Zhang et al. demonstrated that exosomal HSPC111 can modulate the lipid metabolism of CAFs by phosphorylating ATP-citrate lyase, promoting the formation of pre-metastatic niches and distant metastases. Consistently, patients with CRC who have developed liver metastasis exhibited higher levels of HSPC111 in serum exosomes, primary tumors, and CAFs within the liver metastases compared to those without liver metastasis.[112] A study identified that CRC-derived exosomal ADAM17 is involved in the formation of pre-metastatic niches and promotes the migration ability of CRC cells by cleaving E-cadherin. Moreover, overexpression of exosomal ADAM17 in CRC highlighted its function as a tumor-promoting factor, both *in vitro* and *in vivo*.[61]

FIBROBLAST-DERIVED EXOSOMES

CAFs, present in the TME adjacent to cancer cells, possess the ability to remodel the ECM, secrete soluble factor and EV, and coordinate their biological functions for the regulation of tumor development. Fibroblasts typically remain quiescent in normal tissue but can undergo activation upon tissue injury.[17,113] Although the exact definition of CAFs is still controversial, cells that are negative for epithelial, endothelial, and leukocyte markers with an elongated morphology and lacking the mutations found within cancer cells, are generally considered as CAFs.[114] CAFs exhibit significant heterogeneity and can be categorized into distinct subtypes based on the differential expression of specific biomarkers. These diverse subtypes possess varying functions, with certain subtypes demonstrating anti-cancer properties while others exhibiting pro-cancer characteristics.[113] In most instances, CAFs exhibit pro-carcinogenic functions. CAFs play a pivotal role in ECM remodeling through the secretion of ECM components and enzymes. Moreover, CAFs not only serve as physical barriers but also orchestrate the regulation of various cell types within the TME by releasing soluble, EVs, and ECM constituents, thereby promoting cancer cell proliferation, invasion, migration, angiogenesis, and therapy resistance. Additionally, CAFs possess the ability to modulate cancer cell metabolism and facilitate immune evasion. Exosomes derived from CAFs serve as crucial mediators of intercellular communication, involving intercellular communication and exerting regulatory effects on various aspects of the TME in CRC. Notably, their predominant influence lies in promoting cancer cell invasion and migration, and thus facilitate tumor metastasis.[17]

Fibroblasts within the TME undergo activation and transformation into CAFs in response to regulatory exosomes released by tumor cells. Subsequently, CAFs release exosomes that promote cell proliferation, migration, and invasion, while exosomes derived from NFs do not significantly impact the tumor biology of CRC cells.[115] Cyclin-dependent kinase inhibitor 1A (CDKN1A) serves as a potent suppressor of cyclin-dependent kinases. Shi et al. discovered that miR-345-5p exhibited significant upregulation in exosomes derived from CAFs, compared to exosomes from normal fibroblasts (NFs). This elevated expression facilitated the progression and metastasis of CRC by interacting with CDKN1A.[115] Zhang et al. isolated and characterized exosomes derived from CAFs and NFs, revealing that CAFs-derived exosomes exhibited elevated expression levels of miR-17-5p compared to NFs-derived exosomes. In addition, these CAFs-derived exosomes delivered miR-17-5 to CRC cells by directly targeting of runt-related transcription factor 3 (RUNX3) to activate transforming growth factor β (TGF-β) signaling pathway. The interaction between RUNX3 and the oncogene MYC, along with their binding to the promoter region of TGF-β1, ultimately led to the activation of the TGF-β pathway and facilitated tumor progression. Moreover, the RUNX3/MYC/TGF-β1 signaling cascade sustains autocrine TGF-β1 to activate CAFs, inducing increased release of exosomal miR-17-5p into CRC cells, thus establishing a positive feedback loop that promotes CRC progression.[116] Exosomal transfer of miR-92a-3p derived from CAFs regulated CRC cells, resulting in a significant miR-92a-3p levels within CRC cells. Mechanistically, enhanced expression of miR-92a-3p activated the Wnt/β-catenin pathway in CRC cells, thereby promoting cell stemness, EMT, metastasis, and resistance to 5-fluil/oxaliplatin (5-FU/L-OHP) through direct inhibition of FBXW7 and MOAP1-mediated mitochondrial apoptosis. Additionally, the expression of miR-92a-3p in CRC tissues is significantly upregulated and exhibits a negative correlation with the expression levels of FBXW7 and MOAP1. Moreover, elevated serum levels of miR-92a-3p are strongly associated with metastasis and resistance to chemotherapy in patients with CRC.[117] Jiang et al. demonstrated that exosomes derived from CAFs co-cultured with CRC cells induce the upregulation of miR-181b-3p, which in turn promotes the malignant progression of CRC by targeting SNX2.[118] Zhang et al. revealed that CAFs-Exo facilitated the delivery of miR-625-3p to CRC cells, thereby, invasion, EMT, and chemoresistance through inhibition of the CELF2/WWOX pathway.[119] Additionally, miR-17-5p facilitated the metastasis of CRC through modulation of the RUNX/TGF-β1 signaling axis. Prolonged autocrine TGF-β1 stimulation activated CAFs and enhanced the release of exosomal miR-17-5p into CRC cells, thus establishing a positive feedback loop that drives CRC progression.[116] Hu et al. demonstrated that exosomes derived from CAFs mediated EMT in CRC cells by transferring miR-92a-3p. Mechanistically, upregulated expression of exosomal miR-92a-3p activated the Wnt/β-catenin pathway in CRC cells, thereby promoting cancer progression and chemoresistance by directly inhibiting FBXW7 and MOAP1-mediated mitochondrial apoptosis. Clinically, there is a negative correlation between the expression levels of FBXW7 and MOAP1, while high levels of exosomal miR-92a-3p in serum are closely associated with metastasis and chemotherapy resistance in CRC patients.[117] Furthermore, miR-625-3p

derived from CAFs-Exo may enhance the migratory potential, invasiveness, EMT, and chemoresistance of CRC cells through the inhibition of the CELF2/WWOX signaling pathway.[119] Upregulation of exosomal miR-200b-3p counteracted the stimulatory effect exert hypoxic CAFs on CRC cell proliferation *in vitro* and *in vivo*. Moreover, administration of miR-200b-3p agomir enhanced the sensitivity of SW480 cells to 5-FU through downregulation of ZEB1 and E2F3 expression, thus suppressing colon cancer cell migration, invasion, and stemness.[120] In a previous investigation, CAFs were identified as mediators of circN4BP2L2 delivery to CRC cells via exosomes, modulating cell proliferation and migration through the miR-664b-3p/HMGB3 signaling pathway.[121] Hypoxia induced the secretion of cyclin EIF3K from CAFs, and knockdown of circEIF3K in exosomes derived from CAFs significantly attenuated the proliferation, invasion, and angiogenesis of HCT116 and SW620 cells. Furthermore, their study elucidated that the circEIF3K/miR-214/PD-L1 axis mediated hypoxia-induced CRC progression through exosomes derived from CAFs.[122] LINC00659, which is significantly upregulated in exosomes derived from CAFs, facilitated the transfer of exosomal LINC00659 to CRC cells. It interacted with miR-342-3p and enhanced the expression of annexin A2 (ANXA2), promoting CRC cell proliferation, invasion, and migration.[31] Additionally, lncCCAL is transferred from CAFs to cancer cells via exosomes and directly interacts with the mRNA stabilizing protein HuR, thus activating β-catenin pathway. It confers chemoresistance and inhibits apoptosis in CRC cells.[123] LncRNAH19, a maternally imprinted gene, plays a crucial role in embryonic development and growth control. Exosomes derived from CAFs were found to be enriched with lncRNAH19. By transferring exosomal lncRNAH19, CAFs can activate the β-catenin pathway in CRC cells and act as competitive endogenous RNA sponges for miR-141 in CRC, regulating tumor development and chemotherapy resistance.[124] Furthermore, CAFs activated by exosomes derived from SW480 had highly pro-proliferative and pro-angiogenic functions, and showed high expression of proteins promoting angiogenesis (IL8, RAB10, NDRG1) and proliferation (SA1008, FFPS).[67]

IMMUNE CELL-DERIVED EXOSOMES

The immune system of cancer patients is frequently found to be in a state of suppression. Mounting evidence suggested that exosomes play a pivotal role in the immune microenvironment, facilitating tumor development by coordinating interactions between tumor cells and immune cells.[125] Exosomes derived from immune cells modulate crucial processes within the immune system, including antigen presentation, immune signal transduction, and intercellular communication among immune cells. These exosomes exert their effects by modifying the functionality of recipient cells, thereby establishing a conducive microenvironment for tumor progression and metastasis.[126] The TME harbors a diverse array of immune cells, including DCs, macrophages, neutrophils, NKs, and myeloid-derived suppressor cells (MDSCs), which collectively exert regulatory control over the growth, invasion, and metastasis of tumor cells through the secretion of exosomes.[85] In CRC, particular attention has been directed toward investigating the immunomodulatory role played by exosomes derived from TAMs.[97] Further investigation is warranted to elucidate the functions and mechanisms of immune cell-derived exosomes in mCRC, with the aim of identifying more effective, rational, and safe treatment strategies. It includes exploring the correlation between exosomes derived from immune-suppressed tumors and responses to immune therapy, which may enhance the efficacy of immune checkpoint inhibitors.

Exosomes derived from CRC cells reprograms macrophages, establishing a tumor-promoting microenvironment that facilitates the progression of tumor cells through a series of biological events and ultimately promotes metastasis.[97,127] In a recent study, analysis of exosomes derived from the plasma of CRC patients revealed that the integrin alpha M (ITGAM) subpopulation of macrophage-derived exosomes exhibited an inhibitory effect on development of CRC. Furthermore, this study also identified the potential value of integrin subunit beta 3 (ITGB3) and ITGAM subpopulation of exosomes as biomarkers and therapeutic targets for early diagnosis of CRC.[37] The Switch/Sucrose Non-Fermentable (SWI/SNF) complex plays a crucial role in early embryonic development, inflammation, and immune response. Numerous studies have consistently reported a significant decrease in BRG1 expression, which serves as a core motor of the SWI/SNF complex, within cancerous tissues. In the TME, exosomes derived from M2 macrophages actively downregulated BRG1 through miR-21-5p and miR-155-5p mechanisms to facilitate migration and invasion of cells. Furthermore, M2 macrophages secrete exosomes containing WNT that induce CRC stem cell activity involved in metastasis development.[32] In addition to macrophages, exosomes derived from neutrophils within the TME also exert crucial regulatory functions in the growth, and progression of CRC. Overexpression of miR-4780 from N2-like neutrophil-derived exosomes promoted EMT and angiogenesis. The aberrant expression of miR-4780 in exosomes derived from N2-like neutrophils has been demonstrated to play a pivotal role in the metastasis and progression of tumors in CRC models featuring liver metastasis. Moreover, miR-4780 exhibited regulatory effects on its target gene SOX11, thereby EMT and angiogenesis in CRC.[128]

OTHER CELLS-DERIVED EXOSOMES

The establishment of TME and the progression and metastasis of tumors rely not only on the interplay among tumor cells, but also on the cross-talk between tumor cells and diverse cell.[30] In the context of CRC, exosomes derived from diverse cellular sources within the TME, including tumor cells, fibroblasts, immune cells, as well as MSCs, hepatocytes, and Schwann cells, collectively orchestrate various processes involved in tumor cell metastasis.[66] These processes encompass augmented migratory capacity of primary tumors, facilitation of EMT, suppression of immune response, establishment of pre-metastatic niches, and sustenance of growth at secondary metastatic sites.

MSCs exist in many tissues and have the ability to trigger immune response, tissue healing, cell proliferation, and tumor progression control. As regulators of the TME, MSCs release exosomes and their involvement in tumor occurrence and metastasis is controlled by many growth factor receptors, including EGFR and PDGFR.[129] Elevated ITGA6 expression is correlated with unfavorable overall survival in patients with CRC. Exosomes derived from MSCs carrying miR-3940-5p target ITGA6 and subsequently deactivate TGF-β1, thereby inhibiting invasion and EMT of CRC cells, as well as suppressing tumor growth and metastasis.[130] Jahangiri et al. discovered that exosomes derived from MSCs

possess the capability to impede the proliferation, migration, invasion, and metastasis of CRC cells through modulation of the miR-100/mTOR/miR-143 axis, while concurrently inducing apoptosis. Furthermore, it was proposed that exosomal miR-100 derived from MSCs could be regarded as a promising therapeutic strategy for CRC.[131] Exosomes derived from human umbilical cord MSCs (hUC-MSCs) carrying miR-1827 suppressed M2 macrophage polarization by downregulating SUCNR1 expression, thus inhibiting the proliferation, migration, and invasion of CRC cells. Moreover, *in vivo* experiments demonstrated that exosomes derived from hUC-MSCs carrying miR-1827 effectively block CRLM. These findings provided a theoretical foundation for comprehending the mechanism underlying exosome-based targeted therapy for CRC.[132] Previous studies have demonstrated that exosomes derived from hUC-MSCs and Human bone marrow MSCs (hBM-MSCs) containing tumor-suppressive miR-3940-5p can inhibit the proliferation, migration, and invasion of CRC cells by regulating the ITGA6/TGF-β1 signaling pathway.[133] In a separate investigation, miR-203a-3p derived from EVs originating from hepatocytes played a pivotal role in the hepatic colonization of CRC cells. Mechanistically, miR-203a-3p derived from hepatocyte-derived EVs upregulated E-cadherin expression in CRC cells, downregulated Src expression and facilitated MET in CRC cells.[134] Wang et al. demonstrated that EVs derived from hepatocytes in fatty liver enhanced the progression of CRLM by promoting oncogenic YAP signaling and creating an immune-suppressive microenvironment.[135] The involvement of the peripheral nervous system (PNS) in TME components has been documented in relation to the progression and metastasis of diverse cancer types.[136] Schwann cells, the principal glial cells in the PNS, have been demonstrated to actively contribute to the dissemination and metastasis of lung and pancreatic cancer through direct cellular interaction or paracrine signaling.[137,138] In a recent study, it was discovered that the intercellular communication between Schwann cells and colon cancer cells played a crucial role in promoting the proliferation and metastasis of colon cancer. Furthermore, nerve growth factor (NGF) and miR-21-5p from EVs had been identified as potential therapeutic targets for combating colon cancer.[139]

APPLICATION OF EXOSOMES IN THE DIAGNOSIS AND TREATMENT

Exosomes, emerging liquid biopsy options and being stable information carriers, hold great promise as biomarkers for the diagnosis or treatment of metastasis of CRC.[33] With many advantages, including good stability and the ability to carry different bioactive molecules, exosomes participate in intercellular communication and regulate the proliferation, invasion and metastasis of tumor cells.[18] In addition, exosomes are significant nanomaterials due to their excellent biocompatibility and long-term stability in the bloodstream, making them ideal targeted drug carriers for cancer treatment.[23,140] As a result, exosomes possess immense potential and value in tumor diagnosis, differentiation, and therapy.

Exosomes exhibit remarkable stability and are rich in bioactive molecules such as ncRNAs and proteins that play a key role in multiple steps of tumor metastasis.[18] Additionally, exosomes can be readily detected in various bodily fluids including blood, urine, cerebrospinal fluid, and saliva, thereby exhibiting immense potential as diagnostic markers for metastasis of CRC.[23] The diagnostic significance of exosome-related biomarkers in the metastasis of CRC had been reported by several previous studies. In a recent study, liquid biopsy based on a panel of exosomal miRNAs (miR-181b, miR-193b, miR-195, and miR-411) robustly identified T1 CRC patients at risk for lymph node metastasis in the preoperative setting (AUC, 0.84; 95%CI 0.70–0.98).[141] In another study, the accuracy of epithelial growth factor (EGFR) and ITGB3 in plasma exosomes for distinguishing CRC from mCRC was (AUC, 0.91; 95%CI 0.76–1.00) and (AUC, 0.87; 95%CI 0.72–1.00).[37] Zeng et al. found that the expression level of miR-25-3p from circulating exosomes was significantly higher in CRC patients with metastasis than those without metastasis and could be used as a blood biomarker for CRC metastasis.[71] In a study, it was discovered that tumor-secreted exosomal miR-208b promoted the expansion of regulatory T cells by targeting programmed cell death factor 4 (PDCD4), potentially leading to reduced sensitivity to oxaliplatin-based chemotherapy in CRC. Additionally, exosomal miR-208b was identified as a potential predictive biomarker for response to oxaliplatin therapy, offering a novel target for immunotherapy.[142] Moreover, Shao et al. evaluated the level of immune cell infiltration in the microenvironment by constructing a risk model of exosome related genes, resulting in a high degree of accuracy. This approach offered a novel perspective for evaluating immunotherapy efficacy in patients with CRC.[143]

The treatment of metastatic malignant tumors using exosome-based therapies has witnessed a gradual increase in recent years, primarily encompassing drug delivery vectors and targeted therapy specifically designed for exosomes.[144–146] Considering the intricate TME surrounding CRC, achieving safe and efficacious drug delivery to CRC cells currently presents one of the most significant challenges. In recent decades, significant advancements have been achieved in the field of targeted therapies for CRC through the utilization of drug delivery systems based on synthetic nanomaterials.[12,36,147–149] Compared to traditional nanoparticles, EVs or exosomes offered significant advantages in terms of biocompatibility, phagocytic clearance evasion, and intrinsic homing ability. As such, they have the potential to become a new generation of drug delivery systems.[12,150,151] hBM-MSCs exhibit remarkable proficiency in large-scale exosome production, thereby offering a promising avenue for the delivery of miRNAs in cancer therapy. In a previous study, exosomal miR-99b-5p secreted by hBM-MSCs exerted inhibitory effects on the proliferation, invasion, and migration of CRC cells through targeted regulation of FGFR3.[152] Additionally, Liang et al. used engineered exosomes to simultaneously deliver anticancer drug 5-FU and miR-21 inhibitor oligonucleotide (Mir-21i) to CRC cells expressing Her2, and showed good therapeutic potential.[153] Additionally, Huang et al. developed a novel engineered exosome delivery system based on primary cells, enabling simultaneous delivery of siRNAs targeting Coiled-coil domain-containing protein 80 (CCDC80) and enhanced sensitivity to chemotherapy. Their findings demonstrated the remarkable anti-tumor metastasis efficacy of this exosome in a mice model of CRLM.[140] The findings of a recent study demonstrated that exosome circTUBGCP4, produced by CRC cells, stimulated vascular endothelial cell tilt to enhance angiogenesis and promote tumor metastasis through the activation of the Akt signaling pathway. These results offered novel insights for future applications of engineered exosomes in the treatment of mCRC.[76] Van et al.'s isolated EVs from CT26 colon cancer cells and 4T1 mouse breast cancer cells, followed by electroporation-mediated loading

with doxorubicin (DOX). The findings demonstrated that the vector exhibited excellent biocompatibility, high drug-loading capacity, controllable drug release kinetics, and remarkable selectivity toward CRC cells. Notably, it significantly impeded tumor growth in BALB/c mice bearing colorectal tumor.[150] Reprogramming TAMs to adopt a pro-inflammatory M1 phenotype represents an innovative strategy for tumor immunotherapy, while the M2 phenotype is regulated by crucial transcription factors such as signal transducers and transcriptional activator 6 (STAT 6).[154] The natural killer group 2 member D (NKG2D) receptor facilitates the interaction between anti-tumor EVs derived from NK cells and spheroids from CRC cells.[155] CRC cells exhibit elevated expression levels of the NKG2D ligand MICA/B, and the susceptibility of tumor tissue to NK cell-derived EVs is associated with differential expression of the NKG2D ligand MICA/B, which can be inhibited by anti-NKG2D antibodies. Their study demonstrated the potential of engineered exosomes targeting NK cells in cancer immunotherapy.[155] Programmed cell death ligand-1 (PD-L1) antibodies bind to and are consumed by the exosome PD-L1 in peripheral blood.[156] One study found that sulindac down-regulated PD-L1 by blocking the NF-κB signaling pathway, thereby reducing the amount of exosome PD-L1 secreted by tumor cells and enhancing the efficacy of PD-L1 immunotherapy in patients with pMMR phenotype CRC.[157] Exosomes present an appealing therapeutic target for malignancies with metastatic disease. Various drugs have been identified to inhibit tumor-derived exosomes by targeting their formation, suggesting the possibility of repurposing them for metastatic anticancer therapy. For instance, apatinib used in the treatment of mCRC may exhibit a potential anti-tumor mechanism in CRC through multiple pathways, including inhibition of tumor-promoting exosome secretion.[145] These novel approaches for anti-tumor activity offered a fresh perspective on the treatment of exosomes in CRC.

CURRENT CHALLENGES AND FUTURE PROSPECTS

Metastasis is the primary determinant of unfavorable prognosis in patients with CRC.[5] Exosomes play a pivotal role in facilitating bidirectional communication between CRC cells and recipient cells, as well as orchestrating the establishment of a dynamic network within the TME.[30] This regulation is crucial for controlling pivotal processes such as tumor cell proliferation, invasion and migration, EMT, remodeling of ECM leading to physical alterations and metastasis angiogenesis, modulation of vascular permeability, along with immunosuppressive effects.[17,30] (Table 1). In recent years, there has been an increasing use of exosome-based liquid biopsy and drug delivery systems in clinical diagnosis and treatment, indicating the broad application potential of exosomes in the medical field.[23,140] It provides a novel perspective for future diagnosis, treatment, and prognostic assessment of mCRC. However, there remain numerous challenges associated with utilizing exosomes for the diagnosis and treatment of CRC.

Exosomes, as crucial mediators of communication within the circulatory system, exert distinct regulatory roles in various events impacting tumor cell metastasis.[23] Exosomes derived from diverse cellular sources exhibit unique molecular compositions and respond to intricate genetic information, encompassing the promotion of tumor cell invasion and migration, facilitation of angiogenesis, modulation of tumor immunity, and facilitation of pre-metastatic niche formation.[17,18] Exosomes play a pivotal role in either promoting or inhibiting tumor metastasis, thereby indicating their potential utility as valuable diagnostic and prognostic markers for alterations in the TME and mCRC.[18] Enhanced understanding of the underlying molecular mechanisms governing tumor metastasis and intercellular communication after various treatments will facilitate the refinement of therapeutic strategies aimed at eradicating residual tumors.[18,33] Despite notable progress made in understanding the functions and mechanisms of exosomes in metastasis of CRC, several pivotal questions persist unanswered for further investigation. Specifically, a comprehensive comprehension of exosome communication within the TME throughout metastatic progression is lacking, encompassing aspects such as their recognition, binding dynamics, and functional mechanisms across various recipient cells.[30] Although the current evidence substantiates the pivotal role of exosomes in facilitating tumor cell dissemination through EMT induction, their involvement in metastatic growth, such as reactivation of metastasis or MET, remains largely unexplored.[30] Moreover, future investigations on exosomes in cancer should prioritize exploring the heterogeneity of exosomes derived from tumor patients and elucidating their specificity in carrying biological information.[37,158,160] Additionally, the underlying mechanisms behind the selective packaging of cellular components into exosomes remain largely unknown. Similarly, there is limited understanding regarding the impact of exosomes derived from metastatic cells on primary tumors, as well as the precise quantity and timing required for functional effects on recipient cells during the metastatic process. Furthermore, the mechanisms underlying pre-metastatic niche formation are still poorly understood. The 'seed/soil' hypothesis provided the framework for explaining the phenomenon of tumor metastasis.[161] Addressing these inquiries will enhance our comprehension of exosome involvement in metastasis of CRC and offer novel insights for developing exosome-based anti-metastatic therapies.

Currently, extensive research has elucidated the crucial role of exosomes as mediators of intercellular communication by investigating their structure, biological origin, secretion, and function.[23,29] Furthermore, their significant potential in the diagnosis and treatment of mCRC has been gradually unveiled. Previous preclinical and clinical evidence had demonstrated immense promise for utilizing exosomes as diagnostic biomarkers in liquid biopsies and as effective therapeutic agents for various diseases, including tumors and autoimmune disorders.[48,140] Particularly, the noteworthy value is their efficacy as efficient biological carriers for targeted drug delivery.[36,161] In contrast to conventional nanoparticles, exosomes of endogenous origin exhibit superior biocompatibility, evade phagocytosis, and have significantly reduced immunogenicity.[36,162,163] Specifically, exosomes can easily infiltrate the ECM of tumor tissues and are less affected by TME, thus overcoming the challenges of cell therapy.[164] The research and application of certain nanomaterials in tumor-related diseases concurrently offer novel insights for the advancement of exosomes as drug carriers.[147,148,163] However, in order to translate exosomes from the laboratory to the clinic, several challenges must be overcome. Firstly, liquid biopsies need to meet multiple criteria including minimal sample requirements, simplicity, efficiency, affordability, high sensitivity and specificity as well as reproducibility for blood, urine and other samples. The technology of microfluidic separation and detection holds promising potential for clinical application. It is crucial to establish standardized

Table 1. Function and mechanism of exosomes derived from different cells as communication mediators in metastasis of CRC

Origin	Exosomal cargos	Regulation	Mechanism	Biological function	Reference
CRC	miR-106b-3p	Up-regulated	Inhibit the expression of DLC-1	Invasion and migration	Liu et al.[43]
CRC	LINC01915	Down-regulated	Inhibit miR-92a-3p/KLF4/CH25H axis	Invasion and migration	Zhou et al.[68]
CRC	circFMN2	Up-regulated	Activate miR-338- 3 p/MSI1 pathway	Invasion and migration	Yu et al.[52]
CRC	miR-146a-5p	Up-regulated	Promote the expression of Cxcl12/Cxcr7 and regulate JAK2-STAT3/NF-κB pathway	Invasion and migration	Wang et al.[70]
CRC	miR-155-5p	Up-regulated	Promote the experssion of Cxcl12/Cxcr7 and regulate JAK2-STAT3/NF-κB pathway	Invasion and migration	Wang et al.[70]
CRC	circ-ABCC1	Up-regulated	Activate Wnt/β-catenin pathway	Invasion and migration	Zhao et al.[53]
CRC	ADAM17	Up-regulated	Block the E-cadherin connections	Invasion and migration	Sun et al.[61]
CRC	PCAT1	Up-regulated	Downregulate miR-329-3p and upregulate Netrin-1 and CD146	Invasion and migration	Fang et al.[58]
CRC	circCOG2	Up-regulated	Inhibit miR-1305/TGF-β2/SMAD3 pathway	Invasion and migration	Gao et al.[54]
CRC	circEPB41L2	Down-regulated	Regulate PTEN/AKT pathway and sponge miR-21-5p and miR-942-5p	Invasion and migration	Jiang et al.[49]
CRC	miR-548c-5p	Down-regulated	Regulate HIF-1α/CDC42 pathway	Invasion and migration	Yan et al.[46]
CRC	UCA1	Up-regulated	Inhibit miR-143/MYO6 axis	Invasion and migration	Luan et al.[59]
CRC	MALAT1	Up-regulated	Activate the PI3K/AKT/mTOR pathway to sponge miR-26a/26b and regulate FUT4	Invasion and migration	Xu et al.[60]
CRC	miR-1229	Up-regulated	Target HIPK2 and activate the VEGF pathway	Angiogenesis	Hu et al.[74]
CRC	circTUBGCP4	Up-regulated	Upregulate PDK2 to activate Akt signaling pathway by sponging miR-146b-3p	Angiogenesis	Chen et al.[76]
CRC	B7-H3	Up-regulated	Activate the AKT1/mTOR/VEGFA pathway	Angiogenesis	Wu et al.[79]
CRC	GDF15	Up-regulated	Inhibit the Smad signaling pathway and increase POSTN levels	Angiogenesis	Zheng et al.[77]
CRC	miR-25-3p	Up-regulated	Regulates the expression of VEGFR2, ZO-1, Occludin and Claudin5 in endothelial cells by targeting KLF2 and KLF4	Angiogenesis	Zeng et al.[71]
CRC	miR-17-5p	Up-regulated	Promote the expression of PD-L1 to inhibit SPOP	Immune escape	Sun et al.[96]
CRC	circVCP	Up-regulated	Regulate the miR-9-5p/NRP1 axis	Immune escape	Tang et al.[103]
CRC	lncRNA BANCR	Up-regulated	Activate RhoA/Rock pathway by recruiting IGF2BP2	Immune escape	Ding et al.[102]
CRC	miR-203a-3p	Up-regulated	Regulate PTEN and activate the PI3K/Akt pathway.	Immune escape	Pei et al.[95]
CRC	lncRNA RPPH1	Up-regulated	Interacte with TUBB3 to prevent its ubiquitination	Immune escape	Liang et al.[100]

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Table 1. *Continued*

Origin	Exosomal cargos	Regulation	Mechanism	Biological function	Reference
CRC	miR-106b-5p	Up-regulated	Activate the PI3Kγ/AKT/mTOR signaling cascade by directly suppressing PDCD4	Immune escape	Yang et al.[97]
CRC	LncRNA KCNQ1OT1	Up-regulated	Modulate the miR-30a-5p/USP22 axis and regulate PD-1 ubiquitination	Immune escape	Xian et al.[57]
CRC	miR-135a-5 p	Up-regulated	Activate the large tumor suppressor kinase 2-yes-associated protein-matrix metalloproteinase 7 axis	Formation of pre-metastatic niches	Sun et al.[98]
CRC	miR-21	Up-regulated	Activate miR-21/Toll-like receptor 7/IL-6 axis	Formation of pre-metastatic niches	Liang et al.[153]
CRC	circPABPC1	Up-regulated	Protect ADAM19 and BMP4 from miR-874-/miR-1292-mediated degradation.	Formation of pre-metastatic niches	Li et al.[56]
CRC	ANGPTL1	Down-regulated	Downregulates intracellular MMP9 levels by inhibiting the JAK2-STAT3 signaling pathway	Formation of pre-metastatic niches	Jiang et al.[111]
CRC	miR-221/222	Up-regulated	Activate HGF by inhibiting SPINT1 expression	Formation of pre-metastatic niches	Tian et al.[108]
CRC	miR-10a	Up-regulated	Reduces the proliferation and migration activity of NHLFs and the expression levels of IL-6, IL-8, and IL-1β in NHLFs	Formation of pre-metastatic niches	Wang et al.[109]
CAFs	miR-200b-3p	Up-regulated	Inhibit colon cancer cell migration, invasion, and stemness by downregulating the expression of ZEB1 and E2F3	Invasion and migration	Gong et al.[120]
CAFs	miR-345-5p	Up-regulated	Promote CRC progression and metastasis by interacting with CDKN1A	Invasion and migration	Shi et al.[115]
CAFs	miR-92a-3p	Up-regulated	Activate the Wnt/β-catenin pathway in CRC cells and promotes cell stemness, EMT, metastasis, and resistance to 5-FU/L-OHP by directly inhibiting FBXW7 and MOAP1-mediated mitochondrial apoptosis.	Invasion and migration	Zhou et al.[68]
CAFs	miR-181b-3p	Up-regulated	Promote malignant progression of CRC by targeting SNX2	Invasion and migration	Jiang et al.[118]
CAFs	miR-625-3p	Up-regulated	Promote EMT and drug resistance in CRC by inhibiting the CELF2/WWOX pathway	Invasion and migration	Zhang et al.[119]
CAFs	miR-17-5p	Up-regulated	Promotes colorectal cancer metastasis by regulating the RUNX3/MYC/TGF-β1 signaling axis	Invasion and migration	Zhang et al.[116]
CAFs	circN4BP2L2	Up-regulated	Regulate cell proliferation and migration through the miR-664b-3p/HMGB3 pathway	Invasion and migration	Yang et al.[121]
CAFs	LINC00659	Up-regulated	Bind with miR-342-3p, and increase the expression of ANXA2	Invasion and migration	Zhou et al.[31]

(*Continued on next page*)

Table 1. *Continued*

Origin	Exosomal cargos	Regulation	Mechanism	Biological function	Reference
CAFs	lncCCAL	Up-regulated	Interact with mRNA stabilizing protein HuR and activates the β-catenin pathway	Invasion and migration	Deng et al.[123]
CAFs	LncRNAH19	Up-regulated	Activate the β-catenin pathway and act as a competitive endogenous RNA sponge for miR-141	Invasion and migration	Ren et al.[124]
CAFs	circEIF3K	Up-regulated	Mediate hypoxia-induced CRC progression via circEIF3K/miR-214/PD-L1	Invasion and migration	Yang et al.[122]
CAFs	miR-200b-3p	Up-regulated	Inhibit colon cancer cell migration, invasion, and stemness by downregulating the expression of ZEB1 and E2F3	Invasion and migration	Gong et al.[120]
M2 macrophages	BRG1	Down-regulated	Downregulate BRG1 through miR-21-5p and miR-155-5p to regulate the expression of SWI/SNF complex	Invasion and migration	Lan et al.[32]
N2-like neutrophil	miR-4780	Up-regulated	Target gene SOX11 to influence EMT and angiogenesis	Invasion, migration and angiogenesis	Wang et al.[128]
MSC	miR-3940-5p	Up-regulated	Target ITGA6 and inactivate TGF-β1 to inhibit the invasion and EMT	Invasion and migration	Li et al.[130]
MSC	miR-100	Up-regulated	Regulate the miR-100/mTOR/miR-143 axis	Invasion and migration	Jahangiri et al.[131]
hUC-MSC	miR-1827	Up-regulated	Inhibit M2 macrophage polarization by downregulating SUCNR1 expression	Immune escape	Chen et al.[132]
hUC-MSC	miR-3940-5p	Down-regulated	Active ITGA6/TGF-β1 pathway	Invasion and migration	Li et al.[130]
hepatocytes	miR-203a-3p	Up-regulated	Increase the expression of E-cadherin and inhibit the expression of Src to promote MET	Formation of pre-metastatic niches	Xu et al.[134]
hepatocytes in fatty liver	Rab27a	Up-regulated	Enhance the progression of CRC liver metastasis by promoting oncogenicYAP signaling and immune-suppressive microenvironment.	Formation of pre-metastatic niches and immun escape	Huang and Feng[82]

CRC, Colorectal cancer; CRLM, Colorectal cancer liver metastasis; TME, Tumor microenvironment; ECM, Extracellular matrix; NKs, Natural killer cells; TAMs, Tumor-associated macrophages; EV, Extracellular vesicle; DCs, Dendritic cells; ECs, Endothelial cells; KCs, Kupffer cells; NHLFs, Normal human lung fibroblasts; MSCs, Mesenchymal stem cells; TDEs, Tumor-derived exosomes; BRG1, Brahma-related gene 1; MALAT1, Metastasis-associated lung adenocarcinoma transcript 1; EMT, Epithelial-mesenchymal transition; TUBB3, Tubulinβ-III; PDCD4: Programmed cell death 4; USP22, Ubiquitin-specific protease 22; IFN-2, Interferon-regulated factor 2; DLC-1, Dynein light chain 1; HIF-1α, Hypoxia-inducible factor 1 alpha; CDC42, Cell division control protein 42; ZEB1, zinc finger E-Box binding homeobox 1; SPOP, Speckle-type POZ protein; PD-L1, programmed death ligand 1; MSI1, Musashi1; PTEN, phosphatase and tensin homolog; ADAM 17, A disintegrin and metalloproteinase 17; BMP4, bone morphogenetic protein 4; GDF15, Growth/differentiation factor 15; ZO-1, Zonula occlusion 1; PAD4, Peptidyl arginine deiminase 4; CDKN1A, Cyclin-dependent kinase inhibitor 1A; NFs, Normal fibroblasts; SNF, Sucrose Non-Fermentable; RUNX3, runt-related transcription factor 3; ANXA2, annexin A2; YAP, Yes-associated protein; hUC-MSCs: human umbilical cord mesenchymal stem cells; hBM-MSCs, human bone marrow mesenchymal stem cells; MDSCs, Myeloid-derived suppressor cells; PNS, peripheral nervous system; NGF, nerve growth factor; PBA, Proximity-dependent barcoding assay; MET, mesenchymal-epithelial transition; EPR, enhanced permeability and retention; HGF, hepatocyte growth factor.

procedures for exosome isolation, purification and characterization while considering sample handling protocols. Meanwhile, developing high-throughput exosome analysis platforms that integrate various techniques such as nanoparticle tracking analysis (NTA), electron microscopy (EM) and flow cytometry can lead to improved exosome characterization.[165] Secondly, although exosomes hold great promise as novel biomarkers for cancer diagnosis and prognosis; enhancing their specificity and functional relevance remains a challenge. Proximity dependent barcode analysis (PBA), as a new high-throughput method for single exosome analysis, can distinguish different exosome subgroups by identifying specific combinations of exosome membrane proteins.[159] Third, the sources and composition of naturally occurring exosomes are diverse and intricate, rendering the sorting mechanism of exosomes not fully comprehended. Rigorous biosafety verification is imperative when employing natural or modified exosomes as drug delivery vehicles. Accurate identification of exosomal composition holds great potential for enhancing their therapeutic effectiveness while minimizing side effects. Lastly, addressing the challenge of mass production of engineered exosomes for therapeutic purposes emerges as a crucial task in future clinical translational processes.

Conclusions

Exosomes, serving as intercellular communication mediators, play a pivotal role in the progression of tumors. Gaining an in-depth understanding of the function and mechanism of exosomes derived from various cell types in mCRC contributes to a more profound comprehension of this disease. In recent years, research on tumor exosomes has explored their potential as biomarkers and drug delivery vehicles, thereby advancing precision cancer medicine. However, the complex intercellular communication mediated by exosomes and the engineered production of exosomes still pose numerous challenges. Overall, exosomes are anticipated to enhance our comprehension of the biology and oncology associated with CRC metastases, as well as facilitate the development of novel strategies for tumor diagnosis and treatment.

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AUTHOR CONTRIBUTIONS

Conceptualization, Chunzhao Yu, Yimin E, and Chen Lu; writing—original draft preparation, Yimin E, Pengcheng Ji, Minjie Meng and Kuixuan Zhu; writing—review and editing, Chunzhao Yu, Jing Sun, and Zhengxia Liu; supervision, Chunzhao Yu and Wenyuan Li. The Figures in this manuscript were drawn by Figdraw, Yimin E. Yimin E, Chen Lu and Kuixuan Zhu contributed equally to this work and should be considered as co-first authors. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors have declared that no competing interest exists.

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