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Understanding pre-metastatic niche formation: implications for colorectal cancer liver metastasis

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Abstract
The liver is the most commonly metastasized organ in colorectal cancer (CRC), and distant metastasis is the primary cause of mortality from CRC. In recent years, researchers have discovered that tumor cells create a "pre-metastatic niche (PMN)" favorable to metastasis before reaching the metastatic location. This review discusses the many processes and mechanisms that lead to PMN formation in CRC, including gut microbiota, stem cell stimulation, immunocyte interactions, and the induction of extracellular vesicles that carry important information. It examines research methods and diagnostic and therapeutic approaches for treating metastatic CRC with PMN. The crucial significance of PMN formation in metastatic CRC is also highlighted.

Introduction

Colorectal cancer (CRC), with clinical features of blood in stool, change in bowel habits, abnormal stool shape, abdominal pain, anemia and malaise [1], stands as the predominant malignant tumor affecting the digestive tract. According to the latest epidemiological data, CRC ranked third globally in incidence and second in mortality [2]. The occurrence of colorectal liver metastases (CRCLM) is a primary factor influencing the prognosis of CRC patients and leading to mortality. During the progression of colorectal cancer, the liver is the most susceptible target organ for hematogenous metastasis of tumor cells. Around 50% of individuals diagnosed with CRC either exhibit liver metastasis at the initial diagnosis or develop such metastases within five years of the diagnosis [3]. Therefore, liver metastasis prevails as the primary form of metastasis in CRC, with peritoneal metastasis following closely [2, 4] and bone metastasis ranking next [5].

The standard treatment for hepatic metastases confined to the liver from colorectal cancer is surgery [6]. Only 10-20% of patients can achieve curative resection. Additionally, Despite the implementation of resection surgery, there is a potential for the promotion of tumor growth and the initiation of metastasis. Surgery can induce the release of cancer cells into the circulation, and the site of surgical trauma becomes a favored location for metastasis. This phenomenon may be associated with post-surgical hypoxia, inflammation, and angiogenesis [7]. Furthermore, systemic chemotherapy, radiotherapy, and targeted therapy are commonly employed modalities in the treatment of CRCLM. Nevertheless, their efficacy is constrained by drug resistance, notable adverse effects, and other factors [1, 8, 9]. Presently, the most extensively researched immunotherapeutic approach for treating various cancer types involves directly obstructing immunological checkpoints to impede immune escape. The

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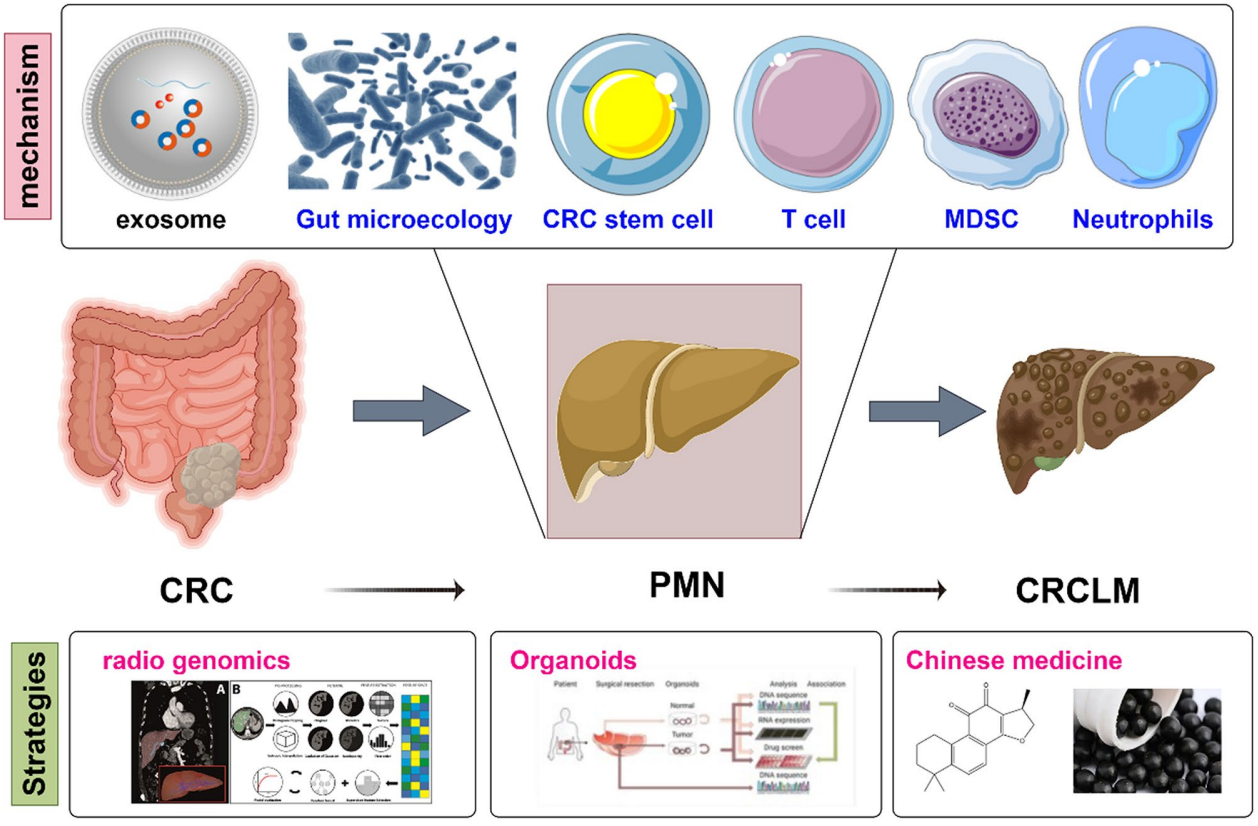
Graphical abstract



Keywords Colorectal cancer, Pre-metastatic niche, Liver metastasis

response of individuals with CRC to immune checkpoint inhibitors (ICIs) is influenced by genetic sensitivity. ICIs demonstrate heightened effectiveness in CRCLM-deficient patients with DNA microsatellite instability (MSI) or defective mismatch repair status (MMR). Conversely, individuals with CRCLM characterized by microsatellite stability and/or proficient mismatch correction show modest results when treated with ICIs, either alone or in combination [9, 10]. Consequently, there is an urgent need for more potent strategies to impede the progression of metastatic disease and enhance the life expectancy of patients in cases of CRCLM.

In 1989, PAGET [11] proposed the Seed and Soil Hypothesis regarding tumor metastasis. According to this hypothesis, a specific tumor cell (seed) is assigned to a specific organ (soil), and metastasis is favored when the microenvironment of the organ, or the soil, is suitable for the seed to grow. In 2005, KAPLAN et al. [12] presented the concept of "pre-metastatic niche (PMN)," referring to the microenvironment created by the primary tumor site that favors metastasis at the secondary metastatic site. The "seed and soil theory" unveils the intimate link between tumor cells ("seeds") and the pre - metastatic microenvironment ("soil"). Tumor cells selectively migrate to favorable organs and secrete cytokines to reshape the microenvironment, boosting angiogenesis. The suitable microenvironment not only enables tumor cells to survive, proliferate and spread, helping them evade immunity, but may also restrict their metastasis due to nutrient deficiency or over - activated immune cells [12, 13]. The primary tumor releases circulating tumor cells (CTCs) into the vascular system, initiating colonization of distant organs and establishing metastasis. There is increasing evidence that primary tumors can create a favorable microenvironment in secondary organs for CTC colonization by secreting cytokines and other factors [14]. This complex process involves multiple elements, such as inflammatory factors, tumor-associated cells, and immunity [15]. These factors can influence the invasion and metastatic ability of tumor cells, regulate the immune response of the tumor microenvironment (TME), and impact the drug resistance of tumor cells. Recently, increased focus has been directed toward understanding the role of PMN in

CRCLM, aiming to find new therapeutic strategies and predictive indicators. Cao et al. [16] proposed six features of the pre-metastatic ecological niche: immunosuppression, inflammation, angiogenesis/vascular permeability, lymphangiogenesis, organ growth, and reprogramming. These features collectively facilitate the colonization of tumor cells and contribute to the promotion of metastasis. A deeper understanding of CRCLM PMN in immune evasion, matrix remodeling, and liver-specific immune responses, is crucial for the development of novel therapeutic strategies.

This study summarized and discussed the mechanism of PMN in CRC invasion and metastasis. Key elements of this process encompass exosomes, gut microbiota, stem cells, immunosuppression, and additional factors such as alcohol, lactic acid, and stem growth factors. Additionally, a compilation of viable strategies for CRC treatment was assembled, including nano-agents, radio genomics, organoid platforms, herbal compounds, and probiotics, among others. A thorough exploration of the metastatic mechanisms in CRCLM not only aids in the early detection of liver metastasis but also provides insights for the development of new targeted therapeutic approaches. This endeavor holds substantial significance in augmenting therapeutic efficacy and overall survival rates for CRC patients, which is of great significance in preventing and treating the disease.

The role of exosomes in PMN formation

The exosome, characterized by a vesicular structure with a lipid bilayer membrane, is secreted by diverse living cells and encompasses various components such as proteins and RNAs [17]. It is widely present in diverse body fluids, serving as a carrier and transmitter for critical signaling molecules that influence the physiological state of cells. The exosome is closely correlated with the development and progression of various diseases. Specific tumor-derived exosomes (TDEs) play crucial roles in the pre-metastatic ecological niche [18]. Exosome exerts a key role in CRC initiation, promotion of anti-apoptotic signaling pathways, regulation of the TME, enhancement of tumorigenicity, facilitation of angiogenesis, proliferation of stem cells, endothelial cell migration, establishment of immunosuppressive environments, formation of pre-metastatic ecological niches and metastasis [19, 20].

The role of Exo-RNA in PMN formation

Exosomes contain various types of non-coding RNAs (ncRNAs), including miRNAs, lncRNAs, and circRNAs [21]. These ncRNAs play critical roles in exosome-mediated intercellular communication, tumor metastasis, immune regulation, and other biological processes. Exosome-derived ncRNAs serve as initiating factors for the onset of epithelial-mesenchymal transition (EMT) at the primary site of colorectal cancer (CRC) [22]. This process establishes a metastasis-favorable pre-metastatic niche (PMN) for CRC, characterized by the development of an inflammatory microenvironment, immune suppression, angiogenesis promotion, and extracellular matrix remodeling in distant organs [23]. Additionally, Exo-ncRNAs can be selectively encapsulated into exosomes and transported from donor cells to recipient cells, modulating the behavior of recipient cells. Therefore, Exo-ncRNAs contribute to the modulation of the tumor microenvironment (TME), facilitating the formation of pre-metastatic ecological niches and inducing drug resistance through intercellular communication (Table 1). These attributes position ncRNAs as potential biomarkers for diagnosing CRC, predicting prognosis, and monitoring therapeutic responses [24].

Yuan et al. found that aberrant expression of miRNAs is a central mediator of metabolic changes in tumor cells [25]. Exosome-derived miRNAs can regulate various cell types in hepatic tissue, with macrophages playing a crucial role in colorectal cancer (CRC) liver metastasis (CRCLM). CRC cell-derived miR-934 and miR-203a-3p can induce polarization of M2 macrophages by targeting PTEN expression and activating the PI3K/AKT signaling pathway. This induction leads to the formation of the pre-metastatic niche (PMN) and promotes colorectal cancer liver metastasis (CRCLM) [26, 27]. Circulating exosomal miR-203 promotes CRCLM by facilitating the differentiation of monocytes (THP-1) into M2-TAMs and the formation of PMNs in CRC. In contrast, serum miR-203 facilitates CRC progression and is associated with poor prognosis by acting as a messenger between tumor and host cells [28].

Additionally, CRC exosome-derived miR-221/222 can translocate to hepatic stromal cells, inhibiting SPINT1 expression to induce HGF secretion and form PMN, thereby promoting CRCLM [29]. The hypoxic environment promotes exosome secretion, where Kupffer cells (KCs) can take up miR-135a-5p, which then enters the liver through blood circulation, initiating the LATS2-YAP-MMP7 axis (LATS2, large tumor suppressor kinase 2). This mechanism improves cellular adhesion and promotes CRCLM through CD30-TRAF2-p65-mediated immunosuppressive signaling [30]. Rapamycin (RAPA) treatment induces a considerable upregulation of miR-6127, miR-6746-5p, and miR-6787-5p in metastatic CRC cell lines, potentially serving as an epigenetic mechanism to regulate the pre-metastatic ecological niche in post-transplant CRC induced by RAPA treatment [31].

Furthermore, exosome-derived miRNAs have been found to promote the formation of PMN in CRCLM by regulating endothelial cell function, promoting angiogenesis, and influencing vascular permeability. Hu et al. reported that circulating exosomal miR-1229 inhibited

Table 1 Exosome-derived NcRNAs in PMN formation in CRC

NcRNAs	acting target genes	source	References
miR-92a-3p	FBXW7 and MOAP1	CAFs secreted exosomes	HU J L et al. [40]
miR-146a-5p	zinc finger and BTB domain containing 2 (ZBTB2)	serum exosomal	WANG D et al. [41]
miR-155-5p	suppressor of cytokine signaling 1 (SOCS1)	serum exosomal	WANG D et al. [41]
microRNA-106b-5p(miR-106b)	programmed cell death 4 (PDCD4)	EMT-CRC cells exosomal	YANG C et al. [42]
miR-25、miR-130b、miR-425	PTEN/PI3K/Akt pathway CXCL12/CXCR4	serum exosomal	WANG D et al. [43]
miR-27b-3p	p120 and VE-Cad	EMT-CRC cells	DOU R et al. [44]
miR-1229	HIPK2	Circulating exosome	Hui-Ying Hu et al. [32]
miR-25-3p	KLF2 and KLF4	CRC exosome	Zeng et al. [33]
circ_001422	miR-195-5p	CRC exosome	Ghafouri et al. [36]
circRHOBTB3	metabolic enzymes ENO1 and ENO2	CRC serum exosomes	CHEN C et al. [45]
circEZH2	CREB1 mRNA	CRC tissues	Yao B et al. [46]
circALG1	miR-342-5p/PGF signalling pathway	peripheral blood and tumour tissues of patients with CRC	Lin C et al. [47]
circPACRGL	miR-142-3p/miR-506-3p- TGF-β1 axis	CRC-derived exosomal	Shang A et al. [48]
circLPAR1	METTL3-eIF3h	plasma exosomal	Zheng R et al. [49]
LncRNA PWAR6	NRF2-Keep1, SLC38A2	myCAF exosome	Fang, H. et al. [37]
lncRNA RPPH1	β-III tubulin (TUBB3)	CRC cell-derived exosomes	LIANG Z X et al. [50]

the protein expression of HIPK2, thereby activating the VEGF pathway and promoting tubule formation in HUVECs, which facilitates the formation of PMN [32]. Zeng et al. found that exosomal miR-25-3p was involved in PMN formation by regulating the expression of VEGFR2, ZO-1, occludin, and Claudin5 in endothelial cells through targeting KLF2 and KLF4, consequently promoting vascular permeability and angiogenesis [33].

Some negative regulatory miRNAs have also been discovered. MiR-214, identified as a negative regulator of CRCLM, may play a potential role in determining the metastatic ecological niche, given its expression in primary CRC (pCRC) [34]. MiR-214-5p promotes the antitumor activity of NK cells by regulating the USP27X/Bim pathway, thereby inhibiting colorectal cancer (CRC) liver metastasis [35].

In addition, exosome-derived circular RNAs and long coding RNAs have been identified to influence the PMN, thereby promoting liver metastasis in CRC. Exosome-derived circ_001422 from HCT-116 cells was found to enhance the proliferation and migration of endothelial cell by inhibiting miR-195-5p activity, thereby activating KDR and mTOR signaling to promote angiogenesis [36]. In CRCLM, LncRNA PWAR6 derived from myofibroblast-derived cancer-associated fibroblast (myCAF) exosomes inhibits NRF2 degradation by competitively binding to Keap1, leading to the upregulation of SLC38A2 expression. This enhanced the glutamine uptake in CRC cells while depleting glutamine availability for natural killer (NK) cells [37]. Moreover, LncRNAs such as CRNDE, H19, UCA1, and HOTAIR have been identified to promote liver metastasis in colorectal cancer (CRC) through exosomal transfer [38, 39].

The role of Exo-protein in PMN formation

The protein fractions within the exosome play a role in CRCLM. The CRC cell-derived protein, HBV pre-S2 trans-regulated protein 3 (HSPC111), increases the level of acetyl coenzyme A, thereby altering lipid metabolism in cancer-associated fibroblasts (CAFs). Acetyl coenzyme A further promotes CXCL5 expression and secretion by enhancing H3K27 acetylation in CAFs. Subsequently, CXCL5 enhances HSPC111 excretion, promoting metastatic PMN formation and CRCLM [51]. Yanyuchen et al. [52] found an upregulation of 36 proteins and a downregulation of 22 proteins in serum-purified exosomes (SPEs) in individuals with CRC. Among the upregulated proteins, S100A8 and S100A9 were identified as contributors to the modulation of the pre-metastatic TME. Their involvement in activating the Wnt/β-catenin pathway facilitates the recruitment of leukocytes, leading to inflammation. The study suggests that SPEs derived from CRC patients play a crucial role in enhancing tumor invasiveness, with minimal impact on potential alterations in tumor survival or proliferation. A disintegrin and metalloproteinase 17 (ADAM17), alternatively recognized as tumor necrosis factor-alpha (TNF-alpha)-converting enzyme (TACE), is a membrane protein belonging to the ADAM protein family [53]. Elevated levels of exosome-derived ADAM17 are observed in the serum and metastatic CRC cells of patients with metastatic CRC. ADAM17, through the cleavage of E-cadherin, amplifies the migratory capacity of CRC cells, upregulates

mesenchymal expression of pro-CRC EMTs, participates in pre-metastatic ecotone formation, and promotes CRC progression [54]. Recently, ADAM17 derived from the circulating exosomes of patients with CRC was reported to mediate the formation of a PMN in nude mice by inducing vascular leakage and enhancing vascular permeability by influencing vascular endothelial cadherin cell membrane localization [55]. Interestingly, Jiang et al. reported that Angiopoietin-like protein 1 (ANGPTL1) from exosome could regulate Kupffer cell secretion pattern and impede MMP9 induced vascular leakiness through inhibiting the JAK2-STAT3 signaling pathway, therefore attenuate the formation of PMN and CRCLM [56]. Understanding the function and mechanism of exosomes in CRCLM may provide new ideas for its diagnosis and treatment in clinical settings.

Exosomes play a crucial role in the pre-metastatic niche (PMN) of colorectal cancer (CRC) liver metastasis (CRCLM). By carrying various molecules, such as miRNAs, lncRNAs, and proteins. In clinical practice, exosomes and their cargo hold significant promise as biomarkers for early diagnosis, metastasis monitoring, and prognosis evaluation in CRC(Fig. 1). For instance, exosome-derived miRNAs and lncRNAs can be detected in bodily fluids like blood, providing real-time information on metastasis and treatment efficacy [57]. Furthermore, exosomes are emerging as novel targets for therapeutic interventions aimed at blocking metastasis. By inhibiting exosome secretion or targeting key signaling molecules they carry, it is possible to suppress the formation of the pre-metastatic niche and reduce tumor metastasis [58].

Cancer stem cells (CSCs)

CSCs maintain tumor proliferation, resulting in resistance to various cancer treatments, including conventional, targeted, and immunotherapeutic approaches. They contribute to cancer progression through CSC-intrinsic molecular mechanisms. Increasing evidence suggests that metastasis is triggered by specific tumor cells with CSC properties [59, 60]. CSCs exhibit a robust association with heightened tumorigenicity, resistance to chemotherapy or radiotherapy, and the propensity for metastasis and recurrence, particularly evident in CRC. Originating from the crypts, colorectal CSCs demonstrate increased self-renewal, proliferation, and tumorigenic potential relative to normal CRC cells [61]. CSCs play a pivotal role in promoting angiogenesis, local invasion, distant metastasis, and resistance to apoptosis. The progression of liver metastasis entails alterations in tumor cell metabolism and EMT. This process is influenced by inflammatory cytokines, miRNAs, hypoxia, and pH, all of which contribute to CSC dissemination. Upon attachment and formation, CSCs establish a pre-metastatic ecological niche [62].

CRC stem cells (CCSCs) are characterized by tumor initiation, self-renewal, and acquired multidrug resistance [63]. The characteristics and behaviors of CCSCs are regulated by various factors, including TME and the gut microbiota, which synergistically influence CCSC characteristics and drive CRC progression [64]. CCSCs assume a crucial role in the immune adaptation and modulation of TME. They evade immune surveillance by eluding recognition by the innate immune system and actively shape the TME through the release of exosomes, cytokines, and chemokines. These mechanisms collectively contribute to the generation of immunosuppressive ecological niches, promoting cancer progression [65]. CCSCs are involved in CRC genesis and metastasis, and 5-HT drives CCSC self-renewal and tumorigenesis. HTR1B/1D/1F, a 5-HT receptor, is highly expressed in CCSCs and initiates Wnt/β-catenin signaling by binding to 5-HT. Blocking 5-HT signaling in mice impedes CCSC self-renewal and suppresses CRC tumorigenesis and metastasis [66]. CUI G found that both CSCs and IL-8 expression are enhanced in adenoma and CRC epithelial tissues. IL-8 directly affects the biological behavior of CSCs mediated by its receptors IL-8RA and IL-8RB. The activation of the IL-8 network within the CSC microenvironment progresses from precancerous adenomas to the CRC stage. This activation is likely triggered by IL-1β in CRC cells [67].

CCSCs express Lgr5, whereas most disseminated cells in CRC are $Lgr5^{-}$. The presence of Lgr5 CSCs is associated with the formation of distant metastasis [68]. Specifically, Lgr5 CSCs have been directly linked to metastatic processes, and the elimination of Lgr5 CSCs impedes hepatic colonization and leads to regression of identified metastases. Additionally, the ablation of DCLK1 cells, which co-express Lgr5, CD44, and CD133, results in polyp regression without notable toxicity to healthy tissues. This highlights DCLK1 as a CSC-specific marker in $Apc^{Min/+}$ polyps [69].

The origin of CSCs is intricately connected to the EMT process, where EMT can also confer stem cell characteristics [70]. The FBXW7-ZEB2 axis regulates several important cancer cell characteristics in vitro and in animal metastasis models. This axis serves as a link between EMT and the TME to promote CCSCs and chemoresistance [71].

ZHANG investigated the impact of $CD133^{+}$ HUHPCs on the growth and metastasis of four CRC cell lines by intercellular co-culture. The human umbilical hematopoietic progenitor cells ($CD133^{+}$ HUHPCs) promote the proliferation and invasion of CRC cells in vitro, as well as the growth and metastasis of tumors in vivo. The observed effects indicate that $CD133^{+}$ HUHPCs may induce the proliferation or metastasis of CRC cells by

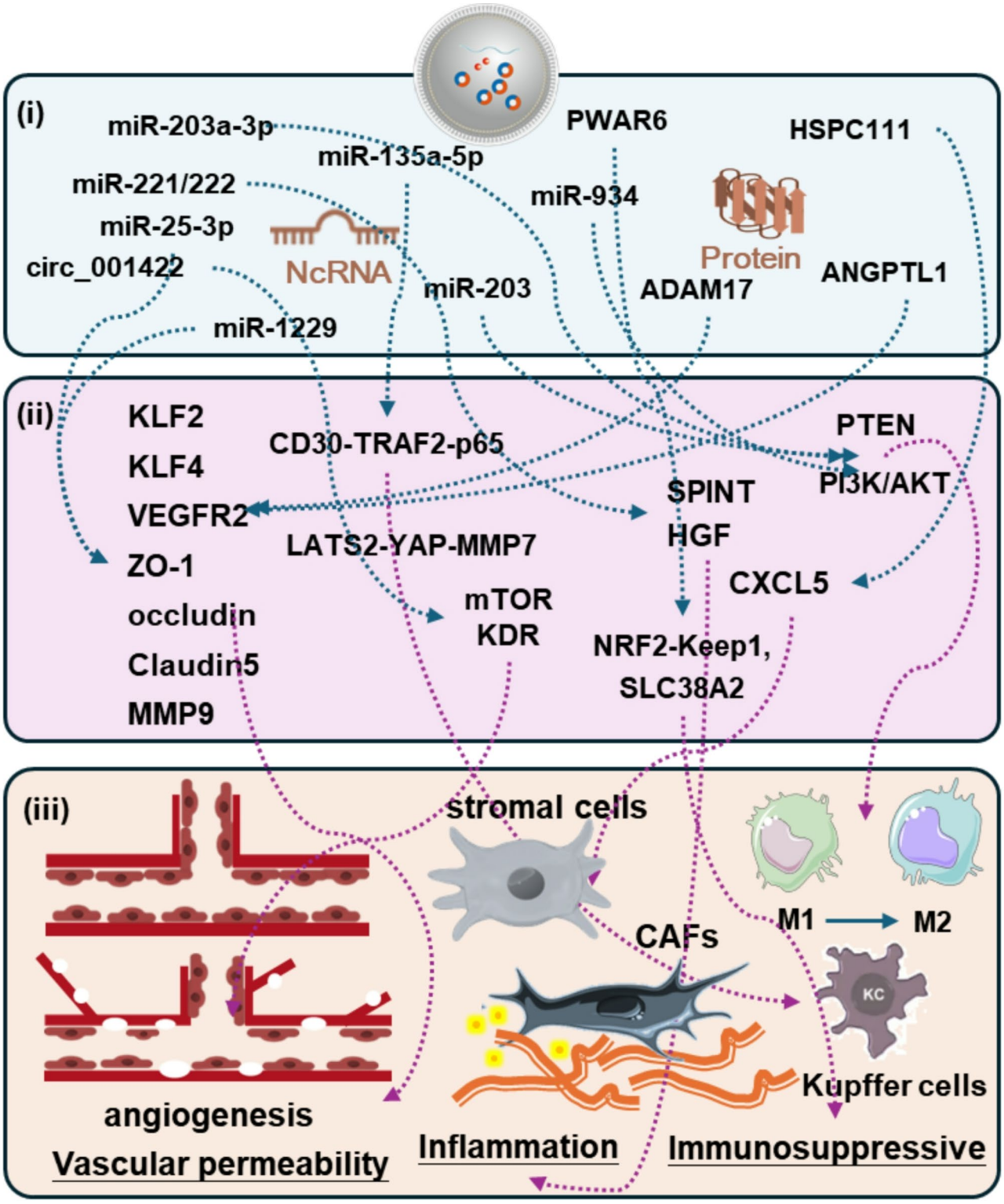


Fig. 1 Effects of exosome on PMN of CRC formation. CRC exosomal ncRNAs and protein can regulate a variety of stromal cells such as CAFs, immune cells such as TAM, et. to promote angiogenesis, EMT and the formation of inflammatory PMNs *via* different signaling pathways

modulating the expression of proteins, including SW480 and SW620, contributing to the formation of PMN [72].

Moreover, the ecological niche of precancerous stem cells (pCSCs)/CSC contains different cytokines, including IL-4, IL-6, IL-8, IL-17a, IL-22, IL-23, IL-33, and interferon (IFN)-γ. These cytokines serve as crucial mediators between pCSC/CSC and their ecological niche. Their involvement is implicated in the onset and progression of adenomatous polyps and sporadic CRC, playing a pivotal role in facilitating the development, progression, and metastasis of CRC [73].

Gut microecology

There are approximately 10^{14} bacteria in the human gut microecology, which is ten times the total number of cells in the human body. Common groups include *Bacteroides*, *Lactobacillus*, *Escherichia coli*, *Enterococcus*, etc [74]. Under normal conditions, *Enterobacteriaceae* maintain homeostasis, thereby contributing to the normal physiological functions of the intestines. This includes the regulation of the body's immunity, metabolizing intestinal angiogenesis, and other physiological processes. The occurrence and development of CRC are intricately linked to an imbalance in *Enterobacteriaceae* [75, 76]. The gut microbiota may enhance CRC and early-onset CRC by promoting an inflammatory environment. Enterobacteria, in particular, contributes to the advancement of CRC through the induction of an aberrant immune response within colorectal tissues. This involves compromising the integrity of the intestinal epithelial barrier and generating tumorigenic toxins that impact intestinal epithelial cells, leading to cellular proliferation. Consequently, this process contributes to the development of a specific immune microenvironment conducive to CRC progression [74]. A functional study in animal models identifies the role of various bacteria, including *Fusobacterium nucleatum* and some strains of *Escherichia coli* and *Bacteroides fragilis*, in CRC development [77]. However, clinical evidence on the specific interactions between the gut microbiota and CRCLM is limited. Recent research indicates a potential link between dysbiosis of the gut microbiota and distant metastasis in CRC. Specifically, studies have shown that CRC patients with liver metastasis exhibit elevated gut microbial α-diversity and increased levels of Escherichia-Shigella compared to CRC patients without liver metastasis [78]. A subsequent study demonstrated that Fusobacterium exhibited consistent and statistically significant enrichment within a cohort of patients diagnosed with CRCLM [79]. Notably, several studies have found that gut microbiota promote CRCLM by facilitating the formation of PMN in liver (Fig. 2).

Regulating the intestinal barrier

A study by Bertocchi discovered that the initiation of the pre-metastatic ecological niche in the liver stems from bacterial dissemination originating from pCRC. In this scenario, the tumor-resident bacterium *Escherichia coli* disrupts the gut vascular barrier (GVB), and the bacterium colonizes the liver before the CRCLM. This colonization gives rise to the establishment of PMN, actively facilitating CRC metastasis. Remarkably, increased numbers of cells expressing elevated levels of PV-1 in primary tumors of CRC patients are linked to hepatic bacterial dissemination and heterochronic distant metastasis. PV-1 emerges as a prognostic indicator for distant recurrence of CRC and is implicated in vascular injury, leading to liver metastasis [80]. Moreover, CRC is recognized to be caused by the transformation of intestinal stem cells (ISCs) into cancer stem cells (CSCs), also referred to as tumor-initiating cells (TICs). ISCs are located in intestinal crypts and are important in maintaining the mucosal barrier. The presence of *Enterobacteriaceae* alongside ISCs involves intricate interactions, and dysbiosis can lead to detrimental effects on the ISC ecosystem, ultimately accelerating the CRC process [81].

Pro-inflammatory and immunosuppression

Tjalsma [82] introduced a bacterial 'driver-passenger' model to explain microbial contributions to CRC progression. According to this model, CRC initiation involves "driver" bacteria, which is replaced by tumor-promoting or tumor-delaying bacteria. *Eubacterium rectale* (E. rectale) acts as a 'driver' bacterium, activating the transcription factor NF-κB, modulating innate and adaptive immune responses in normal colonic epithelial cells, and contributing to cancer development by promoting inflammation [83]. The findings of YIN H suggest that *F.nucleatum* reduces the diversity of gut microbiota in mice, resulting in an imbalance and reorganization of gut microbiota. This alteration influences the secretion of inflammatory cytokines and modulates the hepatic immune response, ultimately promoting CRCLM [78]. In addition, Enterobacteriaceae shape the pro-inflammatory microenvironment by modulating the formation of neutrophil extracellular traps (NETs) to form pro-tumorigenic PMNs for accelerated CRC progression [84]. ZEPEDA-RIVERA et al. identified a novel strain, *F. sphaericum sp. nov.* (Fs), extracted from primary colon cancer tissue. Fs exhibits a metabolic profile and antibiotic resistance consistent with other Clostridium species. This strain is closely associated with human colon cancer epithelial cells and facilitates interleukin-8 (IL-8) secretion with adhesion and immunomodulatory abilities [85].

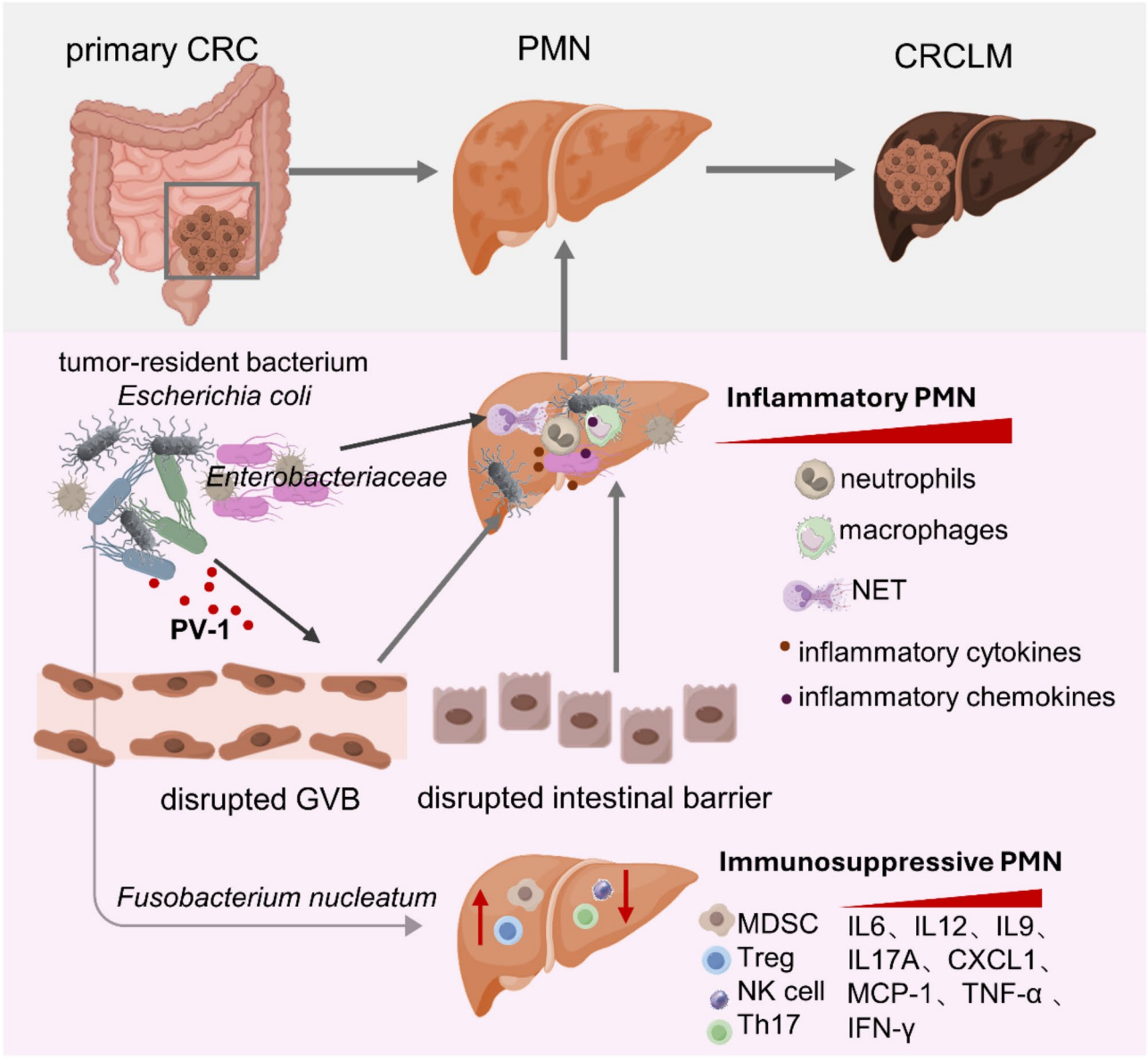


Fig. 2 Effects of gut microecology on PMN of CRC formation. Intestinal bacteria release tumor-promoting inflammatory factors such as IL-8, regulate neutrophil extracellular traps, promote vascular damage, and accelerate immune suppression to create a pro-inflammatory microenvironment

Role of diet

In addition, diet can affect the occurrence and development of CRC by regulating intestinal microbiota. Studies have found that long-term administration of capsaicin, a common food ingredient, can disrupt the intestinal barrier, promote bacterial translocation to the liver, and change the abundance of mucin-associated bacteria (such as Akkermanisa and Muribaculaceae) and bacteria involved in bile acid metabolism, down-regulate the recruitment of natural killer T (NKT) cells in the pre-metastatic niche, hence, promote the CRC liver metastasis [84]. Conversely, supplementation with probiotics or dietary fiber may help prevent or treat liver metastasis in colorectal cancer (CRC). Chuhui Wang et al. found that inulin, cellulose and their mixtures increased the relative abundance of *Bifidobacterium*, *Lactobacillus* and *Lachnospiraceae*, restored the levels of acetate, propionate, isobutyrate and butyrate, regulated the epithelial-mesenchymal transition process, and thus inhibited the liver metastasis of CRC [86].

Limitations

Nevertheless, the study of microbial influences on the pre-metastatic microenvironment faces several limitations, including differences between animal models and human physiology, the complexity of microbial communities, lack of standardized detection methods, challenges

in capturing the spatiotemporal dynamics of the microenvironment, host individual variability, difficulties in establishing causal relationships, and ethical and technical constraints. These factors hinder a comprehensive understanding of the interactions between microbes and the pre-metastatic microenvironment, necessitating advancements in technology and more precise experimental approaches to drive progress in this field.

Microbiome sequencing and metagenomics allow for in-depth analysis of gut microbial composition and functional genes, shedding light on how specific microbial species and their metabolites influence the pre-metastatic niche (PMN). For example, the detection of certain bacteria like Fusobacterium nucleatum and their metabolites (e.g., short-chain fatty acids) can reveal their role in modulating immune cells and promoting an inflammatory microenvironment, which enhances metastasis. Additionally, these techniques help uncover microbial-driven signaling pathways that alter the tumor microenvironment, such as the regulation of neutrophil extracellular traps (NETs) and immune cell infiltration, thus providing new therapeutic targets.

Immune cells

Recent studies have highlighted the pivotal role of immune cells in remodeling the PMN during CRCLM, particularly in establishing an immunosuppressive microenvironment and facilitating early tumor cell dissemination. myeloid-derived suppressor cells (MDSCs), immunosuppressive macrophages and regulatory T cells (Tregs) contribute to PMN formation by secreting IL-10, TGF-β, and other immunosuppressive factors, thereby attenuating antitumor immune responses. Elucidating the dynamic changes and regulatory mechanisms of immune cells within the CRC pre-metastatic niche will provide valuable insights for developing novel immune-based therapeutic strategies to inhibit distant metastasis(Fig. 3).

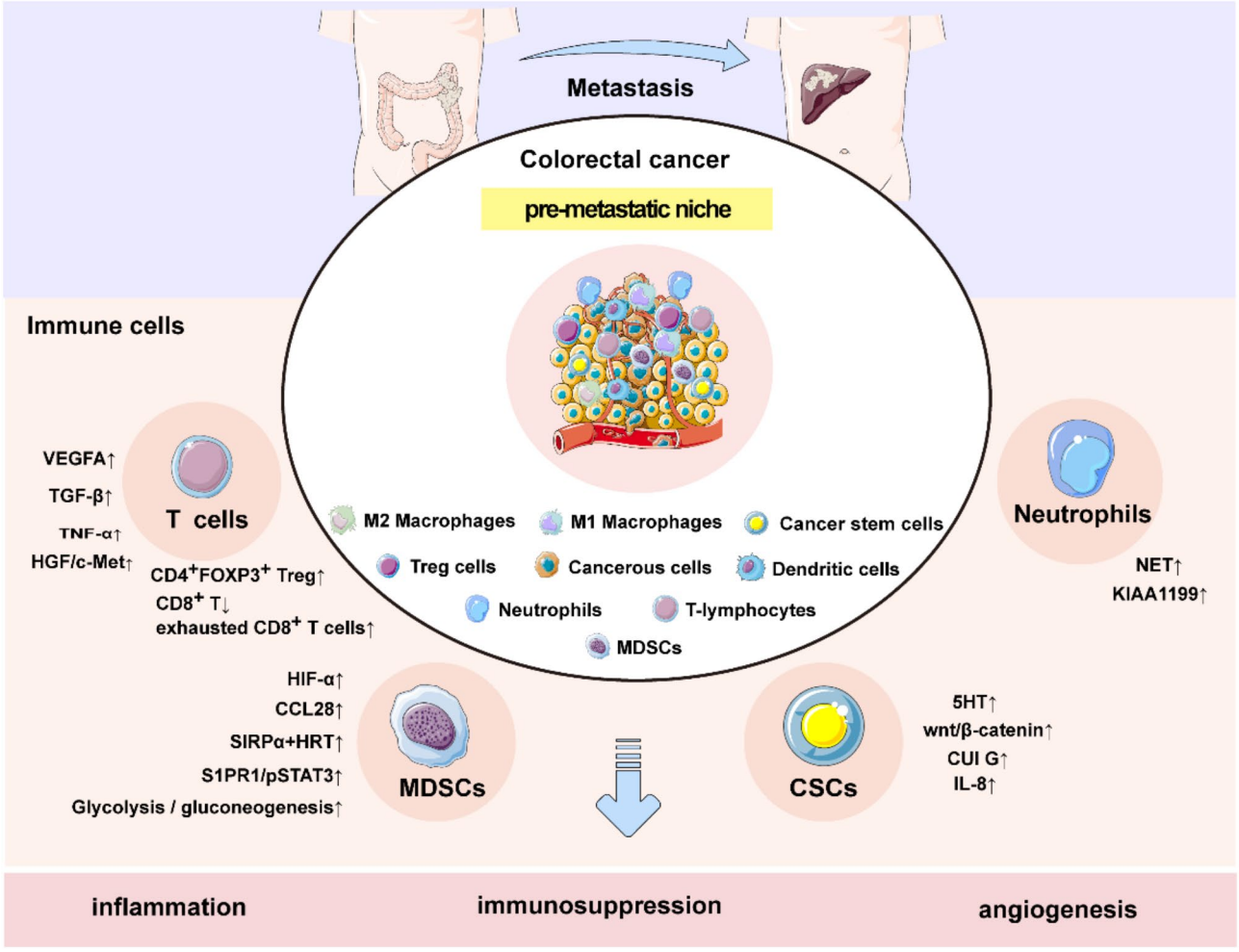


Fig. 3 Effects of immune cells and stem cells on PMN of CRC formation. Immune cells, such as MDSC, T lymphocytes and neutrophils, regulate angiogenesis through multiple signaling pathways and form an immunosuppressive PMN. In addition, other factors such as diet and obesity also promote the formation of PMN by regulating the crosstalk between liver and CRC

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) originate from myeloid progenitor cells in the bone marrow. Typically, these cells would differentiate into dendritic cells (DCs), macrophages, and/or granulocytes. In contrast, under pathological conditions such as tumors and inflammation, these precursor cells will differentiate into MDSCs with immunosuppressive functions under the influence of cytokines including GM-CSF, IL-6, IL-1β, VEGF, IFN-γ, and PGE2. Considering tumor development, MDSCs are attracted and activated by tumor cells, leading them to play a role in promoting tumor growth [87, 88].

ZENG D et al. [89]. elevated levels of MDSCs in the pre-metastatic livers of in situ CRC-homozygous mice and in the peripheral blood of individuals with stage I-III CRC. The mechanisms of MDSC recruitment in the pre-metastatic liver microenvironment of CRC are complex. Tumor cells regulate the generation and accumulation of MDSCs by secreting cytokines or chemokines as well as through interactions with immune cells in the tumor microenvironment. Additionally, factors such as hypoxia, metabolic products (e.g., lactate), and angiogenesis in the tumor microenvironment collectively promote MDSC recruitment. Metabolic reprogramming, such as glycolysis/gluconeogenesis and the upregulation of the HIF-1 signaling pathway in primary tumors, are associated with the infiltration of MDSCs in the liver before metastasis [90]. WANG et al. conducted an analysis of blood and tissue samples from mice using flow cytometry and ELISA assays. They found that pCRC secretes vascular endothelial growth factor A (VEGFA), which in turn stimulates the production of CXCL1 by TAM, thereby recruiting CXCR2-expressing MDSCs into the liver and forming PMNs conducive to liver metastasis. The study highlighted the essential role of the CXCL1-CXCL2 axis in facilitating the recruitment of MDSCs into the pre-metastatic liver and promoting liver metastasis [91]. Abnormally activated hepatocyte intrinsic cell cycle-related kinase (CCRK) activates nuclear factor κB (NF-κB) signaling, which can also increase the transport of chemokines (CXCL1) and promote the infiltration of MDSCs [92]. Additionally, chemokines-like CCL28, CCR/L5, CSF1/CSF1R, CXCL5, et al. were identified as potential mechanisms for recruiting MDSCs [93–95].

The buildup of MDSCs in the liver led to the secretion of immune-suppressing factors and an increase in immune-checkpoint factors, resulting in persistent immunosuppression of PMN. Studies have found that excessive TGF-β secreted by tumor-infiltrating CCR1-G-MDSCs inhibits the immune response of cytotoxic T lymphocytes (CTLs), thereby promoting CRC liver metastasis (CCL9/CCR1 axis-driven chemotactic nanovesicles for attenuating metastasis of SMAD4-deficient colorectal cancer by trapping TGF- β). HMGB1-mediated MDSC infiltration influences hepatocyte GPx4-associated ferroptosis. This reduces CXCL10-dependent infiltration of cytotoxic $CD8^+$ T cells, creating an immunosuppressive microenvironment that impairs anti-metastatic immune responses [96].

The aberrant co-expression of sphingosine-1-phosphate receptor 1 (S1PR1) and signal transducer and activator of transcription-3 (pSTAT3) is linked to CRC metachronous liver metastasis and unfavorable prognosis in CRC. Both in vitro and in vivo, a reciprocal activation loop between S1PR1 and STAT3 enhances CRC cell proliferation, migration, and invasion. The p-STAT3 is an S1PR1-dependent signaling pathway promoting CRC cell growth and liver metastasis. The S1PR1-STAT3-IL-6-MDSCs axis acts in both tumor cells and MDSCs, promoting CRC growth and liver metastasis. S1PR1-STAT3-induced formation of MDSCs in CRC cells with liver PMN promotes organ-specific metastasis [97]. CCL7 secreted by mononuclear MDSCs (Mo-MDSCs) binds to the membrane protein CCR2 of micrometastatic CRC cells, thereby stimulating the JAK/STAT3 pathway to activate dormant cells and promote the formation of liver metastasis or recurrence [98].

T-lymphocytes (T-cells)

T lymphocytes, originating from lymphocyte progenitor cells in the bone marrow, undergo differentiation and maturation within the thymus. Afterward, they are disseminated to various immune organs and tissues via the lymphatic and circulatory systems to carry out immune responses. T lymphocytes play a crucial role in tumor immunity, including cytotoxic T cells (CTLs, recognize and directly kill tumor cells), helper T cells (Th cells, assist in the immune response), regulatory T cells (Tregs, inhibit effective anti-tumor immune responses) and memory T cells (provide long-term immunity by remembering past encounters with tumor antigens).

The liver, being an immune-privileged organ, possesses an immune microenvironment that exerts a substantial influence on the metastatic progression of CRC. With the rapid development of single-cell RNA sequencing (scRNA-seq) methods, an increasing number of immune cell subsets have been identified that promote the progression of CRCLM. NanoString screening was conducted on patients lacking distant metastasis, liver metastasis, and peritoneal carcinomatosis, revealing that elevated expression of $FOXP3^+$ tumor-infiltrating lymphocytes (TILs) exerted a protective effect against metastasis to some degree [99]. Tregs have been identified in elevated numbers in both the peripheral blood and tumors of individuals with CRC. Patient-derived Tregs inhibit the proliferation of autologous T-cells [87]. In the mouse CRCLM model, a decrease in the number

of $CD4^+$ T cells, an increase in the level of $CD4^+FOXP3^+$ Tregs, and upregulation of the HGF/c-Met pathway were confirmed, suggesting that this may be a potential target for intervention in CRCLM [100]. For CRCLM, reports indicate that the chemotherapy drug oxaliplatin may exacerbate liver metastasis of CRC. Analysis of sc-RNA seq data reveals alterations in the liver immune microenvironment towards an immunosuppressive phenotype, characterized by a decrease in T cell populations, particularly $CD8^+$ T cells exhibiting diminished proliferation, activation, and cytotoxic functions, leading to the formation of an immunosuppressive PMN [101].

Multiple studies have demonstrated that exhausted $CD8^+$ T cells (Texs) could serve as a crucial immune cell subset facilitating the progression of liver metastasis. Liu Y [102]. et al. performed scRNA-seq analyses for autologous samples from liver metastasized CRC to disentangle factors shaping TME. They found that Texs and activated Treg were associated with the malignancy. The findings from scRNA-seq analysis of tissues obtained from 16 patients with primary CRC and matched CRCLM patients indicate the following: Exhausted T cells [103] stimulate the epithelial-mesenchymal transition (EMT) program. This occurs via ANGPTL4-SDC1/SDC4 activation of downstream transcription factors, which in turn facilitates the development of liver metastasis.

There is a limited amount of literature available regarding the involvement of T lymphocytes in PMN formation in CRCLM. There are some studies on peritoneal metastasis and bone metastasis. Tregs and T(H)17 cells facilitate the establishment of the pre-metastatic niche (PMN) by inducing VEGF-A, TGF-β, and TNF, which cooperate with T follicular helper (T(FH)) cells and B cells to drive a pro-tumorigenic immune response [104]. Lactate (LA) regulates the expression of CXCL10 and cadherin-11 in CD115(+) progenitors via the PI3K-AKT pathway, promoting osteoclast differentiation and bone metastasis in CRC by recruiting CD4(+) T cells [105].

Neutrophils

Neutrophils play a crucial role in regulating acute injury and repair processes, impacting diverse phenomena such as cancer progression, autoimmunity, and chronic inflammation [106, 107]. Evidence suggests that neutrophils regulate these processes. They also contribute to adaptive immunity by influencing the development of specific adaptive immune responses. Additionally, neutrophils help direct subsequent adaptive immune responses [108].

In patients with CRC, there is an observed increase in neutrophil accumulation at both primary tumor and metastatic sites. Studies have found that high systemic tissue inhibitor of metalloproteinases – 1 (TIMP-1), tissue inhibitor of metalloproteinases (MMP), leads to increased hepatic SDF-1 levels, which in turn promotes neutrophil recruitment to the liver, susceptibility towards CRCLM by triggering the formation of a PMN [109]. Masayoshi, et al. established a metastatic model by orthotopically implanting highly metastatic human colon cancer TK-4 into the cecal wall of nude mice, found that high expression of CXCL1/CXCR2 axis promoted the recruitment of neutrophils and formed an inflammatory PMN [110]. KIAA1199 was found to stimulate the production of CXCL1 and CXCL3 by interacting with TGFBR1/2, activating the TGFβ signaling pathway, thereby driving the accumulation of immunosuppressive neutrophils and promotes immunosuppression and facilitates CRCLM [111].

Neutrophils can also form a meshwork called neutrophil extracellular trap (NET) in primary TME and at metastatic sites. NET is involved in cancer progression, PMN formation, and metastasis, having an important effect on CRC development [84]. In recent years, a new concept has been proposed in which immune cells have the ability to fuse with tumor cells to form tumor-immune hybrid cells (THC). These THC can function as circulating hybrid cells (CHC) and enter the circulation, thereby promoting metastatic formation. Studies have found that the formation of THC is closely associated with the upregulation of NET signaling and the neutrophil degranulation pathway [112]. The mechanisms underlying NET formation in the PMN of CRCLM primarily involve the gut microbiota and fibroblasts. Certain microbiota, such as Fusobacterium nucleatum and bacterial components like LPS, fMLP, and Nigercin, were found can trigger the formation of NETs [113]. FGF19 was found to activate the autocrine effect of IL-1α via the FGFR4-JAK2-STAT3 pathway, mediating the polarization of hepatic stellate cells into inflammatory cancer-associated fibroblasts (iCAF). This further promotes neutrophil infiltration and mediates the formation of NET in the liver PMN through the production of complement C5a and IL-1β, thereby accelerating CRC cell colonization in the liver [114]. Therefore, reducing neutrophil infiltration or blocking NET formation could help interfere with the formation of the PMN and reduce the incidence of CRCLM.

Others

Alcohol consumption may contribute to CRC metastasis by influencing a molecular mechanism that impacts the development of the pre-metastatic ecological niche. NONG et al. [115] employed the Duolink method to assess the interaction between laminin-γ2 (LAMC2) and integrin-β1 (ITGB1). They utilized real-time fluorescence PCR, immunohistochemistry, and Western blotting to measure the expression levels of LAMC2, ITGB1, focal adhesion kinase (FAK), snail, fibronectin, N-cadherin, and special AT-rich sequence binding protein 1 (SATB1).

The findings demonstrated that alcohol enhanced the expression of metastatic markers STAB1 and elevated the levels of pro-inflammatory factors IL-6, IL-1β, and TNF-α. This led to the promotion of EMT-mediated formation of pre-metastatic ecological niches in CRC and subsequently contributed to CRC metastasis through the activation of early interactions between LAMC2 and ITGB1 in rats.

Hepatic growth factor (HGF) triggers the phosphorylation of PU.1, a pioneering transcription factor, subsequently leading to its binding and activation of chromatin regions associated with downstream effector genes. PU.1 enhances histone acetylation at the dipeptidyl peptidase 4 (DPP4) locus. Through precise epigenetic silencing using CRISPR/dCas9 (KRAB) or CRISPR/dCas9 (HDAC), the regulatory elements controlled by individual PU.1 remodeling collectively regulate the expression of DPP4 and the growth of liver metastasis. The HGF-PU.1-DPP4 axis mediates chromatin remodeling of CRCLM. Gene silencing or pharmacological inhibition of each component along this chromatin remodeling axis robustly suppresses liver metastasis [116].

Obesity is an important factor in cancer progression. Tumor-adjacent visceral adipose stromal cells (V-ASCs) secrete IL-6 and HGF, leading to the expansion of metastatic CRC cell compartments ($CD44v6^{+}$). These expanded CRC cells, in turn, secrete neurotrophic factors, including NGF and NT-3, and recruit adipose-derived stem cells (ADSCs) within the tumor mass. Factors derived from visceral adipose tissue promote angiogenesis and metastatic dissemination by activating STAT3. This activation suppresses miR-200a and enhances ZEB2 expression, effectively reprogramming CRC cells into a highly metastatic phenotype. Inhibition of the STAT3 pathway decreases ZEB2 expression, thereby disrupting adipose-releasing protein-maintained metastatic growth [117].

NGUYEN A. et al. identified pyruvate kinase liver and red blood cell (PKLR) as a key driver of metastatic liver colonization through a large-scale in vivo RNAi screen. The expression of PKLR is elevated not only in liver metastatic tumors but also in primary colorectal tumors in individuals with metastatic disease. Glutathione is critically involved in metastasis, and in the context of liver metastasis, PKLR enhances glutathione synthesis by overexpressing GCLC, the rate-limiting enzyme. This elevation of glutathione, a significant endogenous antioxidant, supports cell survival in the tumor cores, particularly under conditions of high cell density and hypoxia, facilitating metastatic hepatic colonization. Notably, PKLR exerts a negative regulatory effect on the glycolytic activity of PKM2, a major isoform of pyruvate kinase responsible for modulating cellular glutathione levels [118]. It can be seen that dietary intake and living habits may also be key factors affecting the formation of PMN in CRC.

Strategies

Despite extensive research on PMN theory over many years, its application in guiding the clinical treatment of colorectal cancer liver metastasis remains underexplored. This article provides a comprehensive review of clinical diagnostic methods, basic research models and potential therapeutic agents targeting PMN (Fig. 4), with the aim of informing future clinical translation efforts.

Diagnosis

Although PMNs have been proven to play an important role that cannot be ignored in tumor metastasis, their characterization and diagnosis remain a challenge. With the advancement of omics technology and organoid technology, the challenging issue of researching PMN in CRCLM has been partially addressed. The integration of radiomics and molecular data, referred to as radiogenomics, provides a unique opportunity to enhance the comprehension of TME heterogeneity, identify specific tumor mutations, and elucidate major tumor activation pathways. This approach can yield promising radiomic profiles from entire organs, serving as valuable surrogate biomarkers to gain insights into "what is going on in the tumor" non-invasively and on a highly individualized level. When addressing CRCLM, the aim is to decipher the early phenotypes of the metastatic ecological niche [119]. Marjaneh Taghavi et al. retrospectively analyzed the initial staged portal phase CT of 91 patients with CRC. They divided them into two groups: patients without liver metastases (at presentation or within 24 months of diagnosis) and patients without liver metastases who had liver metastases at the time of diagnosis but within 24 months of diagnosis. Three predictive models were then developed that included radiomic features, clinical features, and a combination of radiomic and clinical features. Machine-learning-based radiomics analysis of primary staged routine clinical CT imaging can identify valuable biomarkers. These biomarkers can help pinpoint patients at high risk of developing colorectal liver metastasis [120]. John M Creasy et al. studied 120 patients with stage II/III colon cancer, grouped according to 5-year hepatic recurrence, extrahepatic recurrence, or no evidence of disease. They compared radiographic features of the liver parenchyma extracted from CT images between the groups by radiomics technology. Their data showed that CT radiomics is a promising tool to identify those patients at high risk of developing liver metastasis [121]. Francesco Fiz et al. focused on radiomics characteristics of tumor, peritumoral tissue, and non-tumor parenchyma in liver sections from colorectal cancer metastasis. Their radiomics analysis revealed changes in the peritumoral

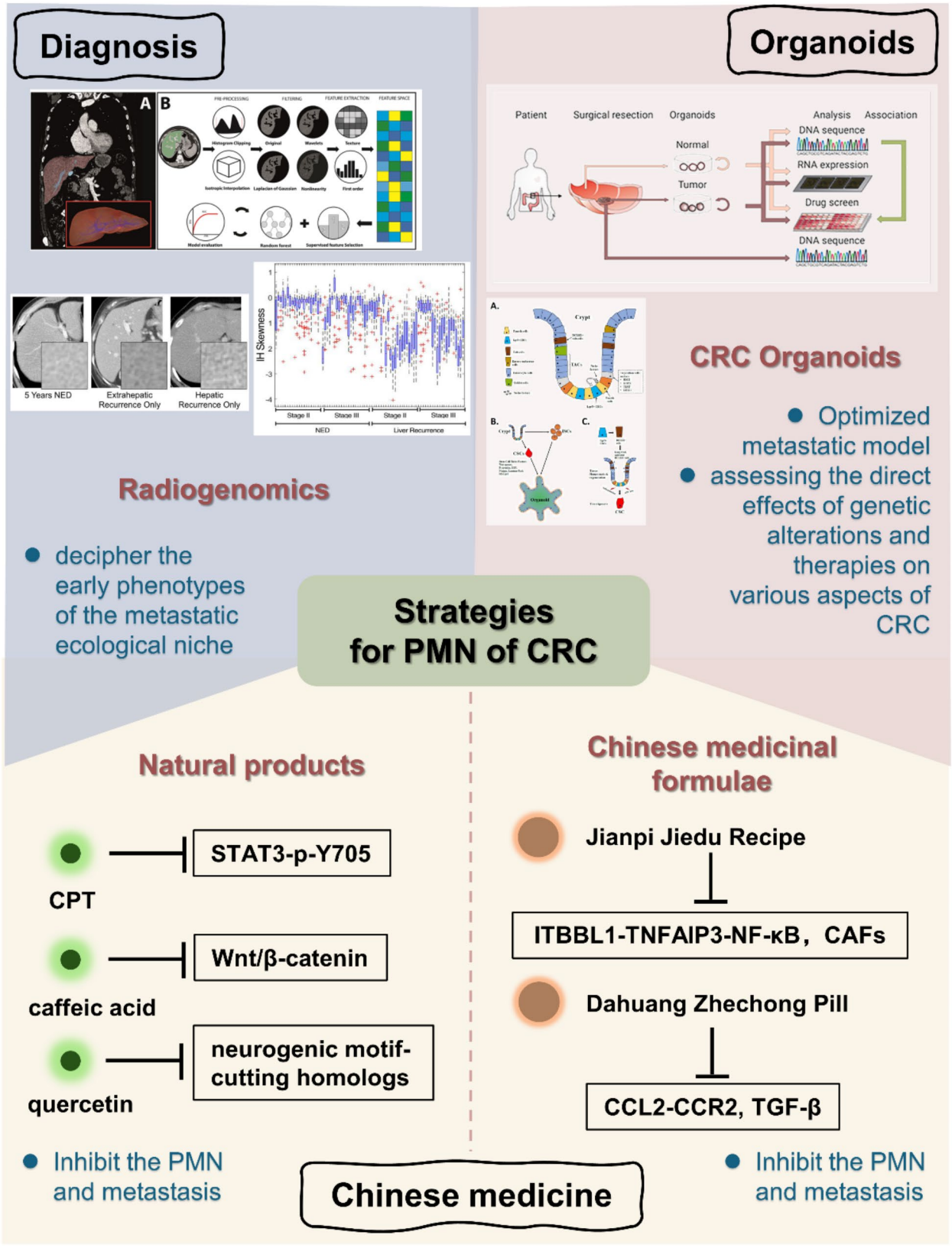


Fig. 4 Prevention and treatment strategies against CRCLM based on PMN. A radiomic approach combined with clinical characteristics is intended to diagnosis metastatic niches early. Organoids derived from mice and patient samples provide improved models and evaluation systems for PMN mechanisms research and drug development in CRCs. Traditional Chinese medicine compound preparations and plant-derived natural ingredients show promise in regulating PMN and potentially preventing CRCLM

tissue that were similar to those observed in the tumor itself. Although, radiographic images showed that this peritumoral tissue appeared identical to the non-tumor liver parenchyma [122]. Furthermore, textural differences identify the peritumoral microenvironment as a separate entity from the normal parenchyma.

Organoids

Organoids are micro-organs with a three-dimensional structure grown in vitro using adult stem cells. Since organoids can simulate the three-dimensional structure and function of real organs in the body, they provide new research methods and treatments for precision medicine. In 2017, organoids were named Technology of the Year by Nature Methods magazine in the life science field. There are several studies utilizing organoids to study liver metastasis of CRC. VAQUERO-SIGUERO N et al. revealed ecotope-dependent clonal selection in mouse-derived organoids (MDOs) using an optical barcoding approach. These results highlight the importance of site-specific ecological niches in driving clonal selection. They emphasize the critical role these niches play in cancer heterogeneity, with significant implications for developing therapeutic strategies [123].

Organoids have also been used to establish tumor metastasis models. Implanting mouse colon cancer organoids into the primary tumor site (i.e., cecum) and metastatic site (i.e., liver) of immunocompromised mice, or using models established through portal vein injection, has proven to be highly effective for studying distant metastatic spread [124]. These models faithfully replicate the fibroblast-rich histology of human CRC liver metastasis. This allows for the investigation of interactions between the liver metastatic microenvironment and cancer-associated fibroblasts (CAFs) [125].

Patient-derived organoids (PDOs) provide an efficient ex vivo platform for assessing the direct effects of genetic alterations and therapies on CRC. They allow for the study of various aspects of CRC, including tumor cell proliferation, differentiation, chemotherapeutic response, and interactions within the tumor microenvironment (TME). This innovative tool holds promise for CRC research, offering the ability to identify the tumors at the core of the disease and replicate their inherent heterogeneity [126]. Shaobo Mo et al. successfully established a living biobank containing 50 CRCLM organoids derived from primary tumors and paired liver metastasis. Using this PDOs system, they predicted the response rate and potential value of clinical prognosis of CRCLM patients treated with FOLFOX or FOLFIRI [127]. Similarly, Fangling Cheng et al. prepared paired 3D organoid cell lines (derived from in situ CRC and derived from CRCLM). The differences in terms of gene expression, sensitivity to chemotherapy could be analyzed by comparing these two types of organoids. Recently, these two organoids are available for public use for researchers [128].

Nevertheless, there are fewer studies related to the application of organoids for pre-metastatic niche. A model proposed with a bone perivascular niche-on-a-chip was constructed to study the interstitial flow mediated cancer cell colonization of breast cancer cell colonization. The complexity of PMN involving multiple stromal cells and components resulting in difficulties for organoid construction. Modeling the PMN is challenging due to the complex and dynamic interactions between tumor and host cells. The PMN involves diverse cell types, including immune cells, stromal cells, and endothelial cells, whose interactions are not fully understood. Therefore, further research and advanced model systems are needed to better simulate the PMN and unravel its intricate mechanisms.

Chinese medicine

There have been a number of studies on modulating PMN for the purpose of treating CRCLM from the active ingredients of herbal medicines and herbal compounds. For instance, the downregulation of pyruvate dehydrogenase kinase 1 (PDK1) significantly reduces CRCLM. This effect is further pronounced when combined with the STAT3-p-Y705 inhibitor cryptotanshinone (CPT, an active ingredient derived from the Chinese herb Salvia miltiorrhiza). Knockdown of PDK1, alone or in combination with CPT, mitigates the impact on anoikis and substantially diminishes the adhesion of CRC cells to fibronectin. Inhibiting PDK1 contributes to decreased CRC cell survival in circulation by upregulating anoikis, while inhibiting STAT3-p-Y705 prevents their settlement in pre-metastatic ecological niches in the liver, ultimately leading to a reduction in liver metastasis [129]. The aforementioned studies mentioned that CSCs are important target cells that form PMNs in CRCLM. Phytochemicals can affect the biology of colorectal CSCs through various pathways. These include modulation of the Wnt/β-catenin pathway, phosphatidylinositol-3-kinase/protein kinase B/mammalian targets of the rapamycin pathway (e.g., caffeic acid [130]), pathways related to neurogenic motif-cutting homologs, differentiation protein pathways (e.g., honokiol and quercetin [131]), Janus kinase signaling and transcriptional pathway activators (e.g., curcumin [132]), and other key signaling pathways. These actions significantly inhibit CSCs and induce apoptosis in CSCs. The targeting of CSCs through these pathways presents the potential for novel therapeutic agents in the treatment of CRC [61].

In addition, some traditional Chinese medicine prescriptions have been reported to inhibit CRCLM by interfering with PMN. A preliminary clinical trial by LI R et al. demonstrated that Jianpi Jiedu Recipe (JPJDR)

emerges as a promising alternative herbal medicine option for preventing and treating CRC metastasis. JPJDR reduces ITGBL1 levels in CRC cell-derived EVs and inhibits CRC cell migration and growth by blocking CAF activation through regulation of ITGBL1-TNFAIP3-NF-κB signaling. Furthermore, JPJDR demonstrates efficacy in reducing CRCLM by regulating the secretion of ITGBL1-rich EVs in CRC. These findings provide experimental support for the clinical utility of JPJDR in controlling CRC metastasis and underscore the viability and importance of targeting EVs in tumor therapy [133]. CHEN conducted a comprehensive analysis of MC38-EGFP-derived exosome adoption using label-free comparative proteomics, quantitative PCR for mRNA expression, immunohistochemistry, immunoassay, and western blotting for protein expression, as well as Masson staining for collagen deposition. Dahuang Zhechong Pill (DZP) significantly diminishes both the quantity and fluorescence intensity of mature TGF-β1 expression, along with reducing fibronectin content and collagen deposition in both splenic and hepatic metastasis models. Additionally, DZP lowers the expression of mature TGF-β1, as well as decreases fibronectin content and collagen deposition. DZP exerts inhibitory effects on CCL2, leading to a substantial reduction in the expression of both CCL2 and its receptor CCR2 in the liver. Moreover, DZP suppresses the lRNA of CRC by inhibiting the CCL2-mediated M2-bias pattern and ameliorating the pro-fibrotic microenvironment to inhibit liver metastasis in CRC [134].

Conclusion

The PMN of CRC is an important cause of CRCLM, and its formation is the result of a combination of several factors including exosomes, cancer stem cells, intestinal flora, and immune evasion. It is still a challenge to translate the current clinical findings on the pre-metastatic microenvironment into practical therapeutic strategies that can be used in the clinic, and no feasible measures have been developed to target the formation of the pre-metastatic microenvironment and thus prevent the formation of liver metastases in colorectal cancer. Promising approaches, such as ongoing studies on exosome inhibitors or immune therapies, may offer novel strategies for preventing liver metastasis by modulating the pre-metastatic microenvironment. In addition, the current clinical methods used for the treatment of colorectal cancer, such as chemotherapy, are still problematic in terms of how to avoid the effects on the pre-metastatic microenvironment and liver metastasis. An in-depth understanding of the mechanism of pre-metastatic microenvironment formation and its role in colorectal cancer liver metastasis is of great significance in finding therapeutic targets to target the pre-metastatic microenvironment and prevent metastasis from occurring. We hope to find ways to target the pre-metastatic microenvironment to treat CRC and reduce liver metastases in the future.

Abbreviations

5-HT	5-hydroxytryptamine
ADAM17	A disintegrin and metalloproteinase 17
ADSCs	Adipose-derived stem cells
BM	Bone marrow
CAFs	Cancer-associated fibroblasts
CCSCs	CRC stem cells
CPT	Cryptotanshinone
CRC	Colorectal cancer
CRCLM	CRC liver metastasis
CSCs	Cancer stem cells
CTCs	Circulating tumor cells
DCs	Dendritic cells
DPP4	Dipeptidyl peptidase 4
dTCs	Disseminated tumor cells
DZP	Dahuang Zhechong Pill
E. rectale	Eubacterium rectale
EMT	Epithelial-mesenchymal transition
EPCs	Endothelial progenitor cells
FAK	Focal adhesion kinase
FFPE	Formalin-fixed and paraffin-embedded
GVB	Gut vascular barrier
HCC	Hepatocellular carcinoma
HGF	Hepatic growth factor
HRT	Hypofractionated radiotherapy
HSCs	Hepatic stellate cells
HSPC111	HBV pre-S2 trans-regulated protein 3
HUHPCs	Human umbilical hematopoietic progenitor cells
IARC	International Agency for Research on Cancer
ICIs	Immune checkpoint inhibitors
ID	Iron deficiency
IDA	Iron deficiency anemia
IFN	Interferon
IL-8	Interleukin-8
ISCs	Intestinal stem cells
ITGB1	Integrin-β1
JPJDR	Jianpi Jiedu Recipe
KCs	Kupffer cells
LA	Lactic acid
LAMC2	Laminin-γ2
LATS2	The large tumor suppressor kinase 2
lncRNAs	Long non-coding RNAs
MDOs	Mouse-derived organoids
MDSCs	Myeloid-derived suppressor cells
miRNAs	MicroRNAs
MMR	Mismatch repair status
MSI	Microsatellite instability
MSI	Microsatellite instability
NcRNAs	Non-coding RNAs
NET	Neutrophil extracellular traps
NETs	Neutrophil extracellular traps
NK	Natural killer
PC	Peritoneal carcinomatosis
PCAM	Pipeline for Characterizing Alternative Mechanisms
pCRC	Primary CRC
pCSC	Precancerous stem cells
PD-L1	Programmed death-ligand-1
PDK1	Pyruvate dehydrogenase kinase 1
PDOs	Patient-derived organoids
PKLR	Pyruvate kinase liver and red blood cell
PMN	Pre-metastatic niche
POSTN	Periostin
ROS	Reactive oxygen species
S1PR1	Sphingosine-1-phosphate receptor 1
SATB1	Special AT-rich sequence binding protein 1
sEV	Small extracellular vesicle
siRNA	Small interfering ribonucleic acid

SIRPα	Signal regulatory protein alpha
SN	Sentinel lymph node
SPEs	Serum-purified exosomes
STAT3	Signal transducer and activator of transcription-3
TAMs	Tumor-associated macrophages
TDEs	Tumor-derived exosomes
TICs	Tumor-initiating cells
TME	Tumor microenvironment
TNF-alpha	Tumor necrosis factor-alpha
V-ASC	Visceral adipose stromal cells
VEGFA	Vascular endothelial growth factor A

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YPL and YC: conceived and designed the review. YPL and YQL: wrote the manuscript, prepared the figures and tables. QYL, HW and XYC: collected the literatures. Contributions from all authors were reviewed and approved before publication.

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Declarations

Conflict of interest
No competing interest.

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