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Exploring molecular and cellular mechanisms of Pre-Metastatic niche in renal cell carcinoma

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Abstract
Renal cell carcinoma (RCC) is among the most frequently occurring types of cancer, and its metastasis is a major contributor to its elevated mortality. Before the primary tumor metastasizes to secondary or distant organs, it remodels the microenvironment of these sites, creating a pre-metastatic niche (PMN) conducive to the colonization and growth of metastatic tumors. RCC releases a variety of biomolecules that induce angiogenesis, alter vascular permeability, modulate immune cells to create an immunosuppressive microenvironment, affect extracellular matrix remodeling and metabolic reprogramming, and determine the organotropism of metastasis through different signaling pathways. This review summarizes the principal processes and mechanisms underlying the formation of the premetastatic niche in RCC. Additionally, we emphasize the significance and potential of targeting PMNs for the prevention and treatment of tumor metastasis in future therapeutic approaches. Finally, we summarized the currently potential targeted strategies for detecting and treating PMN in RCC and provide a roadmap for further in-depth studies on PMN in RCC.

Introduction

As of 2025, RCC is the sixth most common cancer in men, with a relatively high mortality rate [1]. Clear cell renal cell carcinoma (ccRCC) is the major subtype. This subtype represents the predominant proportion, comprising over 70% of documented cases. Approximately 30% of patients experience tumor metastasis, with the most common metastatic sites being the lungs, bones, lymph nodes, liver, adrenal glands, and brain [2, 3]. The organ-specific nature of these metastases leads to variability in treatment responses and outcomes among ccRCC patients with different metastatic sites. Therefore, understanding the complicated mechanisms of the metastatic process in ccRCC is essential for early-stage interventions for metastasis prevention and treatment.

Tumor metastasis is the major cause of death in most cancer patients. Circulating tumor cells (CTCs), which shed from the primary lesion and enter the bloodstream, can infiltrate distant organs and evolve into disseminated tumor cells with higher metastatic potential. Under immune surveillance, most of disseminated tumor cells are exterminated, while the remaining cells enter a dynamic dormant state in distant organs, with the potential to colonize metastatic sites later [4]. According to Stephen Paget's "seed and soil" hypothesis proposed in 1889, disseminated tumor cells, as "seeds", are more likely

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to establish in specific organ niches with favorable "soil" [5].

Extensive research has demonstrated that before tumor cells metastasize to distant organs, the primary tumor can induce changes in the microenvironment of these organs, transforming it into an environment conducive to tumor cell migration, colonization and growth, termed the pre-metastatic niche (PMN) [6]. PMNs are primarily constructed through the interactions between tumor-derived secretory factors (TDSFs), tumor-recruited bone marrow-derived cells (BMDCs), and local host stromal cells and their secreted cytokines. Studies have shown that PMNs facilitate tumor metastasis through various mechanisms, including angiogenesis/vascular permeability, immune suppression, metabolic reprogramming, organotropism and extracellular matrix remodeling [7]. This review explores the different mechanisms by which RCC induces PMN formation and elucidates the characteristics and significance of PMNs in RCC metastasis, offering fresh perspectives for the early detection and prevention of RCC metastasis.

Formation of the PMN

PMNs form before CTCs reach their metastatic sites and are distinct from the tumor microenvironment. PMNs represent a microenvironment that, while lacking tumor cells, is conducive to the survival, colonization, and proliferation of tumor cells [8]. The presence of PMNs indicates that metastasis is an orderly pathological process. Based on the composition and characteristics of PMNs, the progression of tumor metastasis can be categorized into four major stages in a temporal sequence [7, 9], as described below (Fig. 1):

Stage 1: primary tumor initiating PMN formation

In the first stage, the primary tumor initiates the formation of PMNs. As the tumor grows, it releases TDSFs and extracellular vesicles (EVs), such as exosomes and microvesicles. These molecules arrive in distant organs, triggering PMN formation. This stage is marked by the recruitment of BMDCs which induce local inflammation, alter vascular permeability and prepare the extracellular matrix (ECM) for remodeling [10].

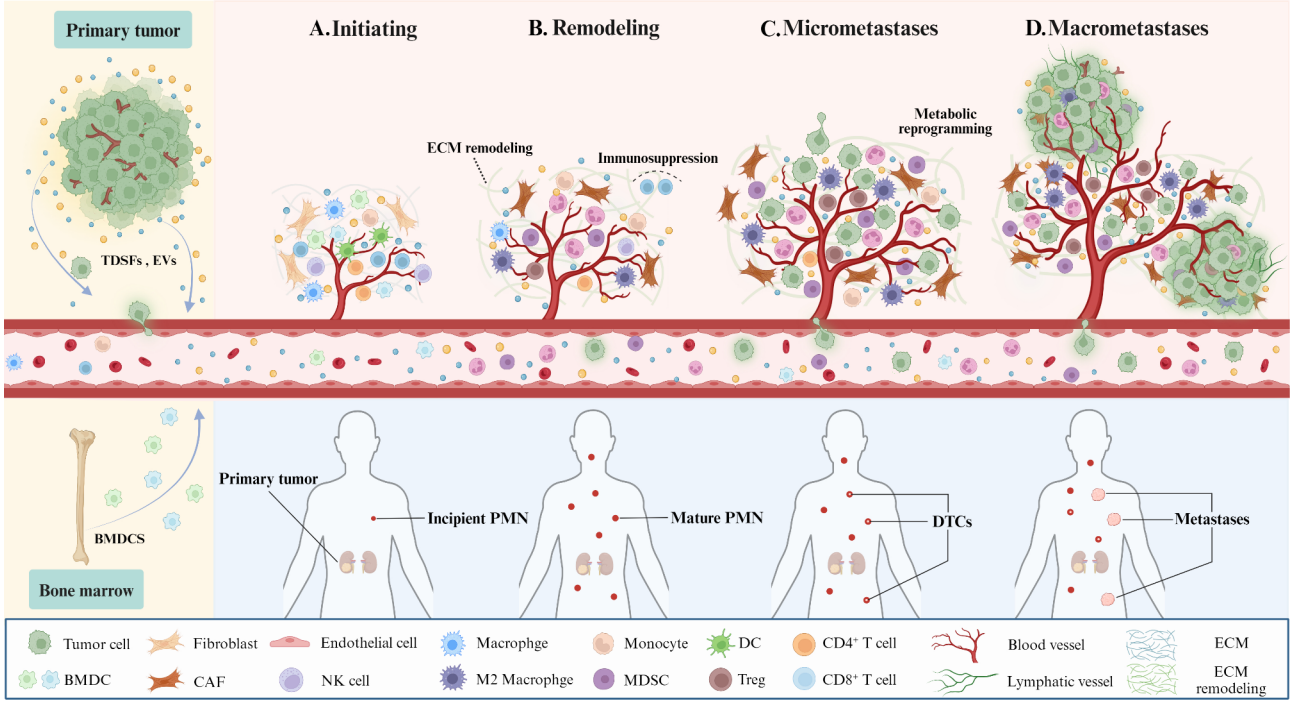

Fig. 1 The formation process of PMN in chronological order. (**A**) During the initiation phase, primary tumor cells release various soluble factors, including TDSFs, EVs and other molecular components. These factors recruit a small number of bone marrow-derived cells to the secondary or distant organs, triggering the formation of an incipient PMN. (**B**) During the remodeling phase, a substantial influx of BMDCs and regulatory/suppressive immune cells is recruited to the early PMN. Under the influence of tumor-derived molecules, a significant proportion of immune cells transition into an immunosuppressive phenotype, resident stromal cells convert to a pro-tumor phenotype, and the ECM undergoes remodeling. This ultimately leads to the establishment of a mature PMN conducive to the colonization and growth of CTCs. **(C)** During the micrometastatic phase, CTCs reach the PMN where some of tumor cells proliferate but are continually eliminated by immune surveillance, while others enter a dormant state until they adapt to the PMN. **(D)** During the macrometastatic phase, PMN supports tumor cells by more angiogenesis and metabolic reprogramming, thereby promoting the growth of metastatic lesions

Stage 2: recruitment of immune-suppressive cells and ECM remodeling

The second stage involves the extensive infiltration of immune-suppressive cells and ECM remodeling. Under the influence of a large number of tumor-secreted factors, normal immune cells are reprogrammed into an immune-suppressive or terminally exhausted state, further exacerbating the immunosuppressive microenvironment within the PMN [11]. Continuously recruited BMDCs secrete various cellular components that interact with stromal cells and resident endothelial cells (ECs). Activated stromal cells undergo significant changes under the influence of TDSFs and BMDCs, including the accumulation of new ECM components and alterations in the physical characteristics of the current ECM [12]. These cells collectively establish a platform enriched with chemokines, growth factors, matrix degrading enzymes, and adhesion molecules [13]with setting up a local immunosuppressive and inflammatory microenvironment that is suitable the arrival and colonization of CTCs.

Stage 3: invasion and colonization by CTCs

In the third stage, CTCs enter the bloodstream and invade various organs. The degree of capillary density and the structural characteristics of capillary walls in each organ influence CTCs extravasation [14]. It has been demonstrated that PMNs attract extravasated tumor cells by increased permeability of blood vessels [15] and VEGFR1-positive hematopoietic progenitor cells aggregated within PMNs enhances the adhesion and dissemination of tumor cells [6]. Tumor cells that successfully arrive PMNs may continue to grow but are continually cleared by immune surveillance, while others enter a dormant state. The reactivation of these dormant cancer cells is a critical factor in metastasis [4]. Cytokines in the PMN can mediate inhibition of proliferation signaling pathways, activate dormancy pathways and facilitate immune evasion, maintaining stable dormancy until the tumor cells adapt to the PMN and reinitiate growth [16, 17]. Finally, the CTCs colonize in the PMN and develop into micrometastases.

Stage 4: progression from micrometastases to macrometastases

The last stage is from micrometastatic tumor cells to detectable macrometastases, a angiogenesis and metabolic reprogramming-driven process. Continued tumor growth necessitates the establishment of a vascular system to supply nutrients. This process is triggered by the recruitment and expansion of ECs, in addition to the activation of supporting cells like pericytes [18]. Bone marrow-derived endothelial progenitor cells are attracted to micrometastatic sites, forming blood vessels that support tumor growth [19]. Studies have shown that enhanced nutrient availability in PMNs directly promotes tumor cell growth. For instance, miR-122 secreted by tumor cells conserves glucose consumption by reducing the metabolism of resident cells in PMNs [20], while lung PMNs rich in palmitate promote metastatic tumor growth through increased p65 acetylation [21]. Metabolic reprogramming in PMNs further increases the metastatic tumor volume, driving the progression from micrometastases to detectable macrometastases.

Mechanisms involved in PMN formation in RCC

Since the mechanisms driving PMN formation vary across different cancers, we here summarize the distinct mechanisms of PMN formation in relation to the unique characteristics of RCC metastasis (Fig. 2).

The mechanisms underlying the formation of the PMN in renal cell carcinoma include angiogenesis and vascular permeability, immunosuppression, ECM remodeling, metabolic reprogramming and organotropism.

Tumor-derived exosomes promoting enhanced angiogenesis

Research has found that exosomes secreted by renal carcinoma cells are abundantly present in blood, urine and other bodily fluids [22]. There are significant differences in the exosomal expression between RCC patients and healthy controls. Therefore, exosomal profiles could serve as potential biomarkers for the detection of RCC [23]. Numerous studies have found that exosomes secreted by RCC have the potential to promote angiogenesis [22]. Therefore, exosomes released from primary tumors prior to metastasis circulate to distant organs, where they may contribute to angiogenesis in the PMN.

Tumors, like normal organs, require the establishment of a vascular supply to meet their demands for oxygen and nutrients, which involves angiogenesis [24]. ccRCC is known for its pronounced vascular phenotype [25]. High-grade ccRCC has been indicated to exhibit greater ECs growth, vascular area and VEGF protein expression levels compared to low-grade renal cell carcinoma [26]. Elevated levels of circulating endothelial progenitor cells have been observed in RCC patients [27]. Studies have found that exosomes from CD105-positive human renal cancer stem cells are stimulate angiogenesis by promoting a pro-angiogenic phenotype in ECs, thus promoting lung PMN formation and increasing the two to three fold metastatic burden [28]. However, this CD105-positive stem cell population might originate from tumor-associated mutated stromal/mesenchymal cells or bone marrow-derived stem cells [29]. In vitro experiments, exosomes containing carbonic anhydrase IX (CA9), released by hypoxic RCC tumor cells, can promote angiogenesis [30]. Similarly, apolipoprotein C1 (ApoC1) present in exosomes from ccRCC cells is delivered to ECs,

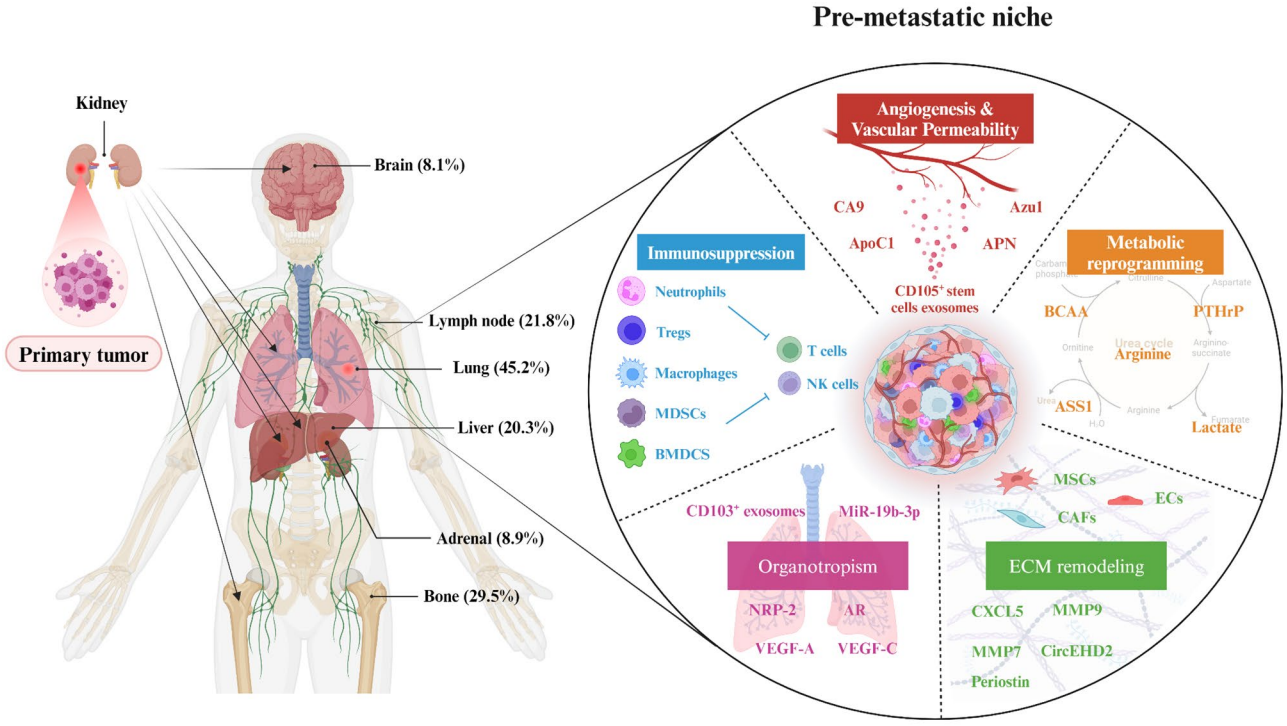

Fig. 2 Mechanisms of the PMN formation in renal cell carcinoma

where it promotes angiogenesis through signal transducer and activator of transcription 3 (STAT3) activation [31]. However, it has also been suggested that ApoC1 is predominantly highly expressed by macrophages in RCC rather than the tumor cells themselves [32]. Furthermore, research has demonstrated that aminopeptidase N (APN) in tumor-derived exosomes promotes enhanced angiogenesis and vascular remodeling in the bone marrow during ccRCC bone metastasis [33].

In addition to angiogenesis, increased vascular permeability within the PMN is a critical step for the infiltration of circulating tumor cells into metastatic sites. Research has found that zurocidin1 (AZU1) overexpressed in ccRCC-derived EVs promote membrane permeabilizing activity for the vascular endothelial cell layer which may facilitate the infiltration of CTCs into distant organs. In addition, EV-AZU1 was specifically detected in the sera of ccRCC patients, but not in healthy controls [34].

Although the majority of the experiments are in vitro, in summary, exosomes secreted by RCC may promote angiogenesis and alter vascular permeability within the PMN of distant organs, thereby facilitating the infiltration and colonization of CTCs.

Immunosuppressive microenvironment

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are key participants in the formation of PMNs and are crucial components in creating an immunosuppressive microenvironment within the PMN [11]. MDSCs are a diverse group from bone marrow, including early-stage myeloid cells, immature macrophages, granulocytes and dendritic cells (DCs). They are mainly classified into two subtypes: monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs). Studies have shown that M-MDSCs mainly impose immunosuppressive effects, while PMN-MDSCs predominantly promote angiogenesis [35].

Researches have showed that elevated levels of circulating MDSCs in RCC patients have been associated with extent of metastasis, cancer stage and prognosis [36–40]. It has been indicated that IL-1β expression in RCC promotes the accumulation of PMN-MDSCs in the periphery [38]. Studies have shown that exosomes derived from RCC, enriched in complement component 3(C3), are taken up by pulmonary macrophages, leading to the secretion of CCL2 and CXCL1. This process facilitates the recruitment of PMN-MDSCs in the lung, thereby establishing an immunosuppressive PMN in the lungs. In vivo experiments, knockdown of C3 expression in renal cell carcinoma-derived exosomes reduces pulmonary metastatic burden in mice by two fold [41]. PMN-MDSCs obtained from the peripheral circulation of RCC patients are known to inhibit the expansion of T cells and reduce IFN-γ production [37, 40]. MDSCs impair T cell immune function primarily by depleting local arginine in the microenvironment [36, 42]. In MDSC, mainly PMN-MDSCs [43]secrete arginase I into the circulation

of RCC patients [40], depleting arginine levels and reducing T cell receptor CD3ζ chain expression, thereby compromising T cell function [44]. In vitro experiments have also found that RCC tumor cells can produce arginase II to deplete L-arginine, leading to decreased CD3ζ chain expression [45].

In conclusion, the recruitment of MDSCs in the circulation or distant organs induced by RCC contributes to the formation of an immunosuppressive pre-metastatic microenvironment, facilitating RCC immune evasion and metastatic progression.

Macrophages

During the formation of the PMN, TDSFs and EVs produced by the primary tumor circulate through the bloodstream and migrate to distant organs, where they recruit bone marrow-derived macrophages to create an inflammatory microenvironment. As the secretion of soluble factors from the primary tumor increases, the recruited bone marrow-derived macrophages and tissue-resident macrophages in distant organs are transformed into tumor-associated macrophages (TAMs) [46]. TAMs predominantly exhibit an M2 polarization phenotype, which promotes tumor progression by stimulating angiogenesis, promoting tumor cell motility and invasion, and attenuating immune response activity [47].

Studies have shown that lung macrophages, upon absorbing exosomes secreted by renal cell carcinoma cells enriched with complement component C3, can remodel the pre-metastatic immune microenvironment in the lungs, enhancing the polarization of TAMs and the recruitment of PMN-MDSCs [41]. Furthermore, numerous in vitro experiments have demonstrated that cytokines and exosomes secreted by renal cell carcinoma cells can promote the polarization of macrophages into TAMs. For instance, The lncRNA AP000439.2, present in exosomes originating from ccRCC, promotes M2 macrophage polarization by activating STAT3 phosphorylation and triggering the NF-κB signaling pathway [48]. Additionally, low expression of SOX17 in ccRCC activates the secretion of CCL5, converting macrophages into TAMs [49]. Moreover, studies have also found that circulating CCL5 can promote the epithelial-mesenchymal transition (EMT) of renal cell carcinoma CTCs, enhancing their malignant phenotype and facilitating lung metastasis [50]. VHL-deficient renal tubular cells representing the early stage of ccRCC initiation secrete IL6 and Oncostatin M (OSM). OSM activates ECs to produce IL-6, which in turn induces macrophage recruitment and polarization [51, 52]. Although in vitro experiments have established a correlation between RCC metastasis and TAMs, it remains to be further investigated whether these secreted factors exert similar effects in PMNs as observed in vitro.

Recent studies have found that microparticles derived from tumors can trigger metabolic and phenotypic alterations in infiltrating macrophages within lung tissue during the initial phase of metastasis. Targeting key altered metabolic pathways in these macrophages could specifically inhibit their pro-tumor functions and reduce tumor metastasis [53]. Although this experiment was not conducted in a renal cell carcinoma model, it provides a new direction for further exploration of macrophages in PMNs within RCC.

Neutrophils

Neutrophils constitute the majority of leukocytes present in human blood and are key players in the inflammatory response [54]. Numerous studies have shown that neutrophils play a crucial role in the formation of PMNs in various cancers [55]. However, research specifically investigating their role in PMN formation in RCC is still limited. Here, we have summarized the potential mechanisms through which neutrophils contribute to PMN formation in RCC.

In peripheral blood from RCC patients, neutrophil levels are significantly elevated, and there is no significant difference in the neutrophil levels between early and late-stage RCC blood samples [56]. This suggests that neutrophils are already extensively recruited into the circulation prior to RCC metastasis, potentially playing a role in the PMN. Research indicates that the secretion of CXC chemokines in metastatic ccRCC triggers systemic inflammatory responses, leading to a significant increase in circulating neutrophils whose kinetic behavior is altered by inflammatory ccRCC cells. It is reported that in circulating inflammatory neutrophils the expression levels of membrane-spanning 4-domains subfamily A (*Ms4a*) family genes were upregulated [57] and that expression of Ms4a6c and Ms4a6d mRNAs was abundant in pro-tumor Siglec-F^{high} neutrophils in lung tumors [58]. This suggests that a group of Siglec-F^{high} neutrophils may also be present in the PMN of metastatic renal carcinoma. These inflammatory neutrophils not only induce angiogenesis in the primary tumor but also facilitates the establishment of PMNs in the lung, thereby promoting cancer cell metastasis. Interestingly, some studies have found that low-metastatic RCC tumor cells express high levels of CXCL5 and IL8, recruiting neutrophils to infiltrate the lungs and establish an immune barrier that inhibits tumor cell growth. Conversely, high-metastatic RCC tumor cells achieve immune escape by silencing the expression of some neutrophil chemotactic factors [59].

Research has identified two distinct polarization states of neutrophils: N1 (highly cytotoxic) and N2 (tumor-promoting). Tumor cells can polarize neutrophils towards a pro-tumor phenotype through the overexpression of immunosuppressive cytokines, such as TGF-β [60]. In

one study, the authors used a specific inhibitor of TGF-β2 to suppress the expression of TGF-β2 in both primary tumors and lung tissues in a renca mouse model. The results showed that this treatment did not affect the growth of the primary tumor but significantly reduced lung metastasis of RCC. Although the authors primarily focused on the immune phenotype changes of infiltrating macrophages in the tumor, it can be inferred from these findings that the expression level of TGF-β2 in the lungs may influence the immune phenotype transformation of neutrophils in the PMN of RCC [61].

Recent studies have highlighted the critical importance of neutrophil extracellular traps (NETs) in immune responses. NETs are formed when neutrophils extrude their decondensed chromatin along with the contents of their granules [62]. Researchers have found that NETs were morphologically detectable in unstimulated peripheral blood leukocytes of RCC patients. Furthermore, the formation of peripheral blood NETs in RCC patients was positively correlated with the survival rate of CTCs [63]. However, there is currently no literature reporting whether the expression levels of NETs in distant organs of RCC are sufficient to form a pro-metastatic niche.

Regulatory T cells

Regulatory T cells (Tregs) are a subset of $CD4^+$T cells characterized by the expression of the regulatory transcription factor forkhead box protein P3 (FoxP3) [64]. Additionally, $CD4^+$T cells that constitutively express the IL-2 receptor α chain (CD25) exert their regulatory functions by suppressing the activity of other T cells [65]. In metastatic renal cell carcinoma (mRCC) patients, a marked increase in Tregs in the blood is observed and their elevated levels are related to poor survival outcomes [66]. $CD4^+CD25^+FoxP3^+$ Tregs isolated from the blood of mRCC patients exhibit immunosuppressive properties, and they inhibit the proliferation of effector T cells in vitro [67]. Studies have shown that culture media derived from renal carcinoma cells, which express high levels of TGF-β, can induce $CD4^+CD25^-$ effector T cells to differentiate into Tregs. In a renca experimental lung metastasis mouse model, by inhibition of TGF-β in lung tissue, the expression of Tregs in the lungs significantly reduced, resulting in to a marked decrease in lung metastasis [68]. A recent study found that exosomes derived from RCC express PD-L2, and these exosomes preferentially accumulate in the kidneys, lungs, and spleen. Overexpression of PD-L2 in exosomes led to an approximately five-fold increase in lung metastasis and a two-fold increase in spleen metastasis in mice. In vivo experiments, it was shown that in the absence of adaptive immunity (in immunodeficient mice), exosomes expressing PD-L2 suppressed tumor growth and metastasis. However, in immunocompetent conditions, TDE-PD-L2 exosomes were captured by immune cells in a PD-1-dependent manner, leading to an increase in Tregs and a suppression of T cell proportions and activity, thereby establishing an immunosuppressive microenvironment at distant metastatic organs [69]. These findings suggest that renal carcinoma cells may promote the formation of an immunosuppressive PMN by secreting high levels of TGF-β and exosomes, which induces the conversion of circulating or distant organ effector T cells into Tregs.

Natural killer cells

Natural killer (NK) cells primarily circulate in the blood, and higher NK cell counts are associated with improved survival rates [70]. Currently, in renal cancer, most studies focus on NK cells infiltrating the solid tumors, because NK cells which are the crucial effector cells in innate immunity, possess inherent cytotoxic activity against both primary and metastatic tumor cells, similar to $CD8^+$ T cells [71]. Currently, in RCC, immunotherapies targeting NK cells are mainly systemic. As a result, research on the local microenvironment of the PMN is almost nonexistent. However, there is some evidence suggesting that NK cells may undergo changes in the PMN of RCC.

NK cells can also be classified into circulating NK cells and tissue-resident NK cells. The roles of peripheral tissue-resident NK cells vary across different cancers [72]. Studies have found that although the proportion of NK cells is reduced in advanced ccRCC, a population of NK cells enriched with tissue-resident markers and reduced cytotoxicity is present [73]. In addition, in vitro studies have found that exosomes from ccRCC cells evade innate immune surveillance by modulating the TGF-β/SMAD pathway, leading to NK cell dysfunction [74]. These studies suggest that there may be a subpopulation of NK cells with impaired immune function in the PMN of renal cancer, which warrants further investigation.

Metabolic reprogramming

The seeding and development of metastatic tumor cells in remote organs require a local environment rich in nutrients and energy, necessitating a metabolic pattern that supports tumor growth. Thus, metabolic reprogramming is a significant feature of PMN formation. It has been found that mutations in seven known RCC genes—*VHL*, *MET*, *FLCN*, *TSC1*, *TSC2*, *FH* and *SDH*—result in dysregulation of specific metabolic pathways. These findings suggest that RCC is a type of metabolic disease [75]. Recent studies indicate that glycogen metabolism is not necessary for in vitro growth of ccRCC cells or in vivo xenograft tumor growth [76]. Other pathways have been incorporated into the metabolic profile of ccRCC such as dysregulation of tryptophan, arginine and glutamine metabolism, as well as elevated lipid and glutathione

biosynthesis [77]. Additionally, high levels of glutathione and dipeptide metabolites are associated with the malignancy of ccRCC [78].

As previously mentioned, MDSCs recruited by RCC tumor cells may deplete arginine in the PMN by releasing arginase, thereby creating a local immunosuppressive environment. Strikingly, arginine levels were significantly reduced in both the tissue and the interstitial fluid in the lungs compared to the kidneys. Arginine deficiency in the pulmonary biological niche induces reactivation of argininosuccinate synthetase-1 (ASS1) in ccRCC metastatic tumor cells through epigenetic mechanisms. This links branched-chain amino acid (BCAA) catabolism with the urea cycle, allowing metastatic tumor cells to generate arginine via BCAAs and endure under distant arginine-limited conditions [79]. Furthermore, TDSFs can alter the metabolic reprogramming of non-tumor cells. For instance, ccRCC tumor cells secrete parathyroid hormone-related peptide (PTHrP), which activates protein kinase A (PKA) to facilitate the browning of perirenal adipose tissue (PAT). The thermogenic effect of PAT leads to excessive lactate release which could be utilized by adjacent cancer cells, enhancing ccRCC invasion and metastasis [80]. However, this metastasis-facilitating niche associated with neighboring adipogenesis is distance-dependent, as primary RCC does not drive the browning of distal adipose tissue.

The mechanisms of metabolic reprogramming in PMNs within RCC are not yet fully understood, most studies have primarily focused on the metabolic reprogramming of metastatic renal tumor cells themselves, which enhances their potential for metastasis. However, analyzing alterations in glucose metabolism, lipid metabolism, amino acid metabolism and other metabolic components in the tumor microenvironment emphasizes the essential role of metabolic reprogramming in PMN formation and development.

Organotropism

Multiple studies have shown that different types of cancers exhibit organ-specific metastasis tendencies. However, the patterns of metastasis to particular organs are influenced by various factors, including anatomical characteristics, hemodynamic properties, the intrinsic traits of tumor cells and the interactions with the PMN [7, 8]. In RCC, the lungs and bones are the most common sites of metastasis.

RCC primarily metastasizes through hematogenous spread. In ccRCC, VEGF-A-induced phenotypes promote the release of tumor cell clusters into circulation, enhancing lung metastasis [81]. In most organs, venous blood circulation flows from the heart to the lungs. The lung capillary network is dense and has small diameters, which facilitates the passive entrapment of larger CTCs. This phenomenon is not unique to RCC but is a common feature of metastasis to the lungs in various types of cancer. Furthermore, tumor cells adhesion to ECs is a key event in hematogenous metastasis. In RCC, neurophilin-2 (NRP-2) expressed on tumor cells interacts with α5 integrins on ECs, facilitating vascular adhesion and extravasation during lung metastasis [82]. These processes are primarily determined by the anatomical structure of the pulmonary vasculature and hemodynamic properties. However, passive entrapment alone does not fully explain the organotropic preference of RCC lung metastases. The PMN plays an additional role in the recruitment and survival of RCC tumor cells.

Research has shown that tumor exosomes with different organ tropisms express distinct integrins, such as integrinα6, β1 and β4 in lung-tropic exosomes [83]. In a study, intravenously injected labeled exosomes derived from renal tumor stem cells in mice were found to predominantly accumulate in the lungs rather than in other organs. This study has shown that $CD103^+$ exosomes secreted by renal tumor stem cell target tumor cells and lung, conferring the higher metastatic capacity of ccRCC to lung [84]. However, as a transmembrane protein, CD103 is primarily expressed on lymphocytes, particularly T cells and dendritic cells and no relevant article has reported CD103 expression in tumor epithelial cells, especially renal tumor cells. Therefore, it is necessary to identify whether renal tumor cells express CD103. Furthermore, the interaction between stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 governs organ-specific metastasis in various tumors including RCC [85]. As RCC is a common genitourinary system disease in men, this gender specificity is significantly related to hormone levels. Studies have found that androgen receptor (AR)-positive ccRCC are more likely to preferentially metastasize to the lungs over lymph nodes. The mechanism may be that AR possibly function by modulation of the VEGF-A or VEGF-C in ccRCC cells to differentially influence the hematogenous and the lymphatic endothelial cells, thus finally affect different metastatic destinations [86]. In conclusion, RCC lung metastasis is likely driven by both passive entrapment due to the lung's architecture and the active promotion of tumor seeding and growth by the PMN.

Similarly, the skeletal system also possesses its own distinct anatomical structure. Capillary structures in the bone marrow, featuring fenestrated ECs termed sinusoids, facilitate the transport of hematopoietic cells, making bone marrow sinusoids more prone to capture CTCs [87]. Compared to the lungs, RCC bone metastases occur at a lower frequency. However, their mechanism is more likely driven by PMN-mediated active recruitment rather than anatomical factors. Osteoblasts, the predominant cell type in bone marrow, can elevate the

expression of adhesion molecules and CCL2 in RCC tumor cells through cytokine secretion where forming a facilitated homing of RCC cells to the bone marrow [88]. Additionally, the expression of CaSR was highest in specimens and cells of patients with bone metastases. In vitro experiments indicate a promoting effect of extracellular calcium on cell migration and proliferation of RCC cells with bone metastasis by highly expressed CaSR and its downstream signaling pathways. High calcium gradients in bone may form a niche promoting RCC bone metastasis [89].

Extracellular matrix remodeling

Tumors and their secreted factors can profoundly influence the host's stromal microenvironment. Components like fibroblasts, mesenchymal stem cells (MSCs), ECs and ECM are pivotal in tumor cell colonization and survival at metastatic sites [7]. The ECM is primarily composed of fibrillar proteins and glycosaminoglycans, forming a network structure within tissues [90]. Research has identified seven genes involved in ECM formation that are upregulated in mRCC: *DCN*, *SLIT2*, *LUM*, *LAMA2*, *ADAMTS12*, *CEACAM6* and *LMO3* [91]. It is a constantly changing structure present across all tissues, with its remodeling tightly regulated through the action of specific enzymes, such as metalloproteinases [92].

The study found that the serum concentration of MMP-7 in local RCC patients was significantly elevated compared to healthy donors, but there was no difference when compared to metastatic RCC patients. These results suggest that the elevated levels of MMP-7 in the circulation associated with RCC may contribute to the remodeling of the pre-metastatic extracellular matrix [93]. Another in vivo experiment showed that, with the progression of RCC lung metastasis, the mRNA expression of MMP-9 in the lungs of mice gradually increased [94]. The primary source of MMP-9 may be the inflammatory cells of bone marrow origin that are recruited, which secrete MMP-9 to aid in the degradation and remodeling of the ECM [95]. In vitro experiments have shown that fibronectin promotes the colonization of mRCC tumor cells [96]. Upregulation of fibronectin in the lungs has been confirmed in other cancer models to facilitate the formation of the PMN and promote lung metastasis [6, 97]. Therefore, if RCC tumor cells secrete certain factors that induce the upregulation of fibronectin in the lungs, it may contribute to the establishment of the PMN in the lung.

Studies have found that the region at the boundary between metastatic tumor tissue and non-tumor tissue in RCC contains abundant extracellular matrix components, particularly periostin. Further validation showed that this periostin, which promotes tumor cells adhesion, is secreted by fibroblasts induced by tumor cells [98]. In another study, it was reported that infiltrating breast tumour cells need to induce stromal periostin expression in the secondary target organ (in this case lung) to initiate colonization [99]. However, this study also found that the lungs of tumor-bearing but non-metastatic mice with breast cancer did not express periostin. This suggests that the expression of periostin in lung tissue is not induced by substances secreted by the primary tumor, but rather by the disseminated tumor cells entering the lungs, which induce ECM remodeling to facilitate colonization. Strictly speaking, this cannot be considered as part of the pre-metastatic niche concept. However, whether RCC induces periostin expression at the metastatic site prior to metastasis remains to be further validated.

The leading role of tumor-associated fibroblasts (CAF) in tumorigenic process has received extensive attention. In vitro experiments have shown that CXCL5 derived from ccRCC promotes the conversion of normal fibroblasts (NF) into CAFs, which can secrete VEGF to induce angiogenesis [100]. Moreover, studies have demonstrated that circEHD2 was upregulated in serum EVs of RCC patients with metastasis. CircEHD2 in EVs can convert lung fibroblasts into CAFs, which secrete pro-inflammatory cytokines like IL-6 to facilitate RCC tumor cell metastasis [101].

Identification of PMN for early diagnosis

The formation of PMNs is a critical step preceding metastasis and holds significant implications for devising approaches for early prevention and detection of metastasis. Currently, imaging techniques for detecting distant metastases in RCC are not yet fully mature. While computed tomography (CT) and positron emission tomography (PET) can precisely identify large metastatic lesions, they are insufficient for detecting early micro-metastases that arise at the initial stages of metastasis [102, 103]. MiRNAs isolated from tumor-derived exosomes exhibit enhanced stability, thereby making them more reliable biomarkers. Furthermore, lncRNAs and circRNAs represent intriguing potential biomarkers [104]. Emerging technologies, such as liquid biopsy [105] and nanoparticle tracking [106], offer promising methods for obtaining early diagnostic information on cancer metastasis by analyzing tumor cells EVs and various biochemical and cellular biomarkers produced during the formation of PMNs.

For example, research has demonstrated that S100A8/A9 serves as a reliable new imaging biomarker that enables visualization of PMNs in a breast cancer mouse model using an S100A8/A9-specific single-photon emission computed tomography (SPECT) probe [107]. Additionally, a novel targeted nanoprobe has been designed by surface-modifying luminescent nanoparticles (LAD NPs) with neutrophil-targeting peptides. Given the correlation

between neutrophils and PMNs, this targeting strategy using luminescent nanoparticles holds promise as an early diagnostic approach for cancer metastasis [108].

However, due to the differing mechanisms of the PMN formation across various cancers, identifying a consistent biomarker indicative of PMN remains challenging. Currently, the most promising strategy is the identification of early metastatic biomarkers specific to particular cancer types.

Therapeutic approaches for PMN

Currently, the initial systemic therapy for mRCC involves the coupling of PD-1 inhibitors and VEGFR-targeted therapies or CTLA-4 inhibitors, which dramatically improve overall survival among individuals with advanced RCC. For patients who have contraindications to immune checkpoint inhibitor (ICI) combination therapy or cannot access ICI treatment, single-agent VEGF tyrosine kinase inhibitors remain the standard treatment [109]. However, treating advanced tumor metastasis remains a significant challenge. Given the important role of PMNs in the early stages of metastasis, targeting the molecules and pathways associated with PMN formation suggests a potential avenue for treating tumor metastasis. We summarize potential therapeutic strategies targeting PMN, focusing primarily on angiogenesis/vascular permeability, ECM remodeling, metabolic reprogramming, tumor exosomes and immune suppression (Fig. 3).

The therapeutic strategies for the PMN can be divided into five directions: targeting PMN angiogenesis and vascular permeability, targeting PMN ECM remodeling, targeting PMN metabolic reprogramming, targeting tumor exosomes and targeting PMN immunosuppression.

Targeting PMN angiogenesis and vascular permeability

VEGFA is expressed at the highest levels in clear cell renal carcinoma among all epithelial cancers [110]. Targeting VEGF and its receptors through anti-angiogenic therapies has been established as one of the most effective treatments for metastatic ccRCC [111, 112]. Studies have found that circSPIRE1 present in RCC exosomes can inhibit angiogenesis and vascular permeability. Nanomedicines composed of circSPIRE1 plasmids show promise in targeting vasculature in PMN to suppress metastatic formation [113]. Moreover, ccRCC with low expression of MCPIP1 can increase vascular permeability by modulating the phosphorylation of VE-cadherin [114]. Therefore, blocking the phosphorylation of VE-cadherin to inhibit vascular permeability in the PMN holds potential therapeutic value. For instance, VE-cadherin is a direct target of the tyrosine kinase inhibitor sunitinib in mRCC [115]. Additionally, research has shown that phenotypic transformation occurs in perivascular cells within metastatic regions, characterized by the lack of vascular smooth muscle cells (vSMCs) and pericytes markers and activation of Krüppel-like (KLF4), which enhances ECM synthesis. These perivascular cells establish a pre-metastatic fibronectin-rich environment. Targeting the expression of KLF4 in perivascular cells has been shown to reduce PMN formation and the colonization of early metastatic tumor cells [116]. To further enhance the specificity of targeting aberrant vasculature in PMN, a novel compound combining vascular-targeting peptides (VTPs) from primary tumor vasculature with the cytokine TNF superfamily 14 (LIGHT) has been developed (LIGHT-VTP). LIGHT-VTP not only normalizes the tumor vasculature and prevents the infiltration of cancer cells but also effectively targets abnormal blood vessels in the PMN, alleviating vascular hyperpermeability and ECM accumulation, thus inhibiting tumor metastasis [117].

Targeting PMN extracellular matrix remodeling

Recent research indicates that fibronectin accumulation aids in the establishment of a tumor-permissive PMN [6]. Based on fibronectin, a targeted ECM deprivation system using self-assembling peptides was developed to construct nanofibers within the ECM of RCC. This system specifically targets fibronectin, ensuring prolonged retention in the ECM while inhibiting fibronectin -mediated pro-tumor signaling pathways, effectively suppressing tumor metastasis and growth [118]. In response to the high expression of MMPs in the PMN, a newly developed MMP inhibitor, ONO-4817, has been demonstrated to effectively suppress lung metastasis of tumor cells in a RCC mouse model [119]. Additionally, an active compound extracted from the seeds of nigella sativa, thymoquinone (TQ), can downregulate the activity and levels of expression of MMP-2 and urokinase-type plasminogen activator (u-PA), thereby reducing adhesion of 786-O renal tumor cells to type I and IV collagen and inhibiting lung metastasis [120]。.

Studies on resident cells within the PMN have revealed a subset of fibroblasts that express cyclooxygenase-2 (COX-2). Knocking out the *Ptgs2* gene, which encodes COX-2, effectively reverses the immunosuppressive characteristics of pulmonary-resident myeloid cells in the PMN [121]. Furthermore, an enzyme-activated assembling peptide, FR17, has been reported to inhibit fibroblast activation, suppress ECM remodeling in the PMN, and reverse vascular instability and angiogenesis. It has also been found to inhibit the mobilization of bone marrow cells to the PMN [122]. Researchers have also designed a liposomal nanovesicle targeted to the lungs, which carries miR-29a-3p to mimic the exosomes secreted by tumors. This liposomal nanovesicle delivery system dramatically downregulates the secretion of type I collagen by pulmonary fibroblasts in vivo, thereby

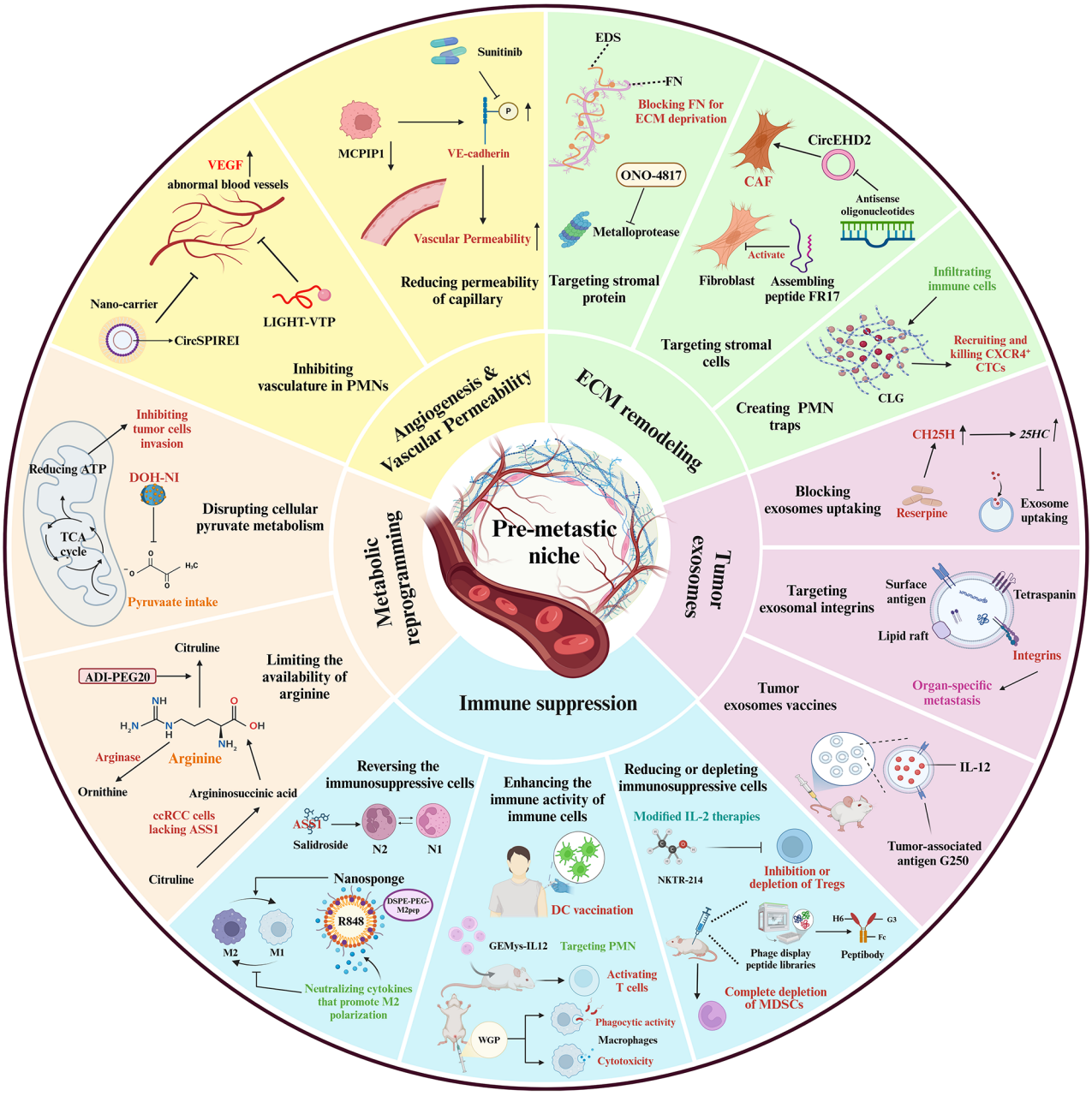

Fig. 3 Therapeutic strategies for the PMN

inhibiting the development of a microenvironment conducive to metastasis [123].Given the pro-tumor functions of CAFs within the PMN, they present promising targets for therapeutic interventions aimed at preventing tumor metastasis. However, the absence of specific markers on their cell surface complicates targeting [124]. Recent findings indicate that targeting circEHD2 in extracellular vesicles with antisense oligonucleotides can significantly inhibit the transformation of fibroblasts into CAFs, thereby suppressing the progression of metastatic renal cell carcinoma [101].

Another research team took a novel approach by developing a hyaluronic acid-based gel system loaded with CXCL12. This gel system (CLG) effectively drives the directional migration of $CXCR4^+$ CTCs toward the CLGs, simulating a PMN. Moreover, CLGs are infiltrated by a large proportion of immune cells, and tumor cells recruited to the CLGs are shown to undergo death within two weeks, reducing the number of CTCs at the initiation stage of metastasis and consequently diminishing $CXCR4^+$ tumor cell lung metastasis [125].

Targeting PMN metabolic reprogramming

Alterations in lipid metabolism are a significant metabolic phenotype of RCC [126]. Recent studies have reported that increased availability of palmitic acid within the PMN can promote the colonization of metastatic tumors in breast and gastric cancers [21, 127]. This finding offers new insights into targeting lipid metabolic reprogramming during PMN formation in metastatic RCC.

Further research has discovered that a polyethylene glycol (PEG) form of arginine deiminase (ADI) can effectively deplete arginine by catalyzing its deamination to citrulline [128]. Cells expressing normal levels of ASS1 like normal renal parenchymal cells can convert citrulline back to arginine. In contrast, cells lacking ASS1, such as ccRCC cells, are unable to synthesize arginine from citrulline. Therefore, ADI exerts anti-tumor activity by limiting the availability of arginine in the PMN for tumor cells, without adversely affecting surrounding normal cells [129]. Although using ADI to deplete arginine appears to be a potential treatment for ccRCC, the efficacy of this therapy may be limited by the capacity of certain tumors to re-express ASS1 [79]. Moreover, recent research has developed a mitochondrial pyruvate carrier (MPC) inhibitor, DOH-NI, which specifically disrupts cellular pyruvate metabolism. By depleting energy supply and paralyzing tumor cell invasion, this inhibitor also impedes the remodeling of ECM in the lungs, thereby prohibiting the formation of the PMN [130].

Due to the current lack of research on metabolic reprogramming in PMNs, there are no specific therapeutic targets or strategies. However, if future studies can combine the targeting of metabolic reprogramming in renal tumor cells with that in PMNs, it could potentially provide a new breakthrough in the treatment of tumor metastasis.

Targeting tumor exosomes

As essential intermediaries in communication between tumors and the microenvironment of target organs, tumor-secreted exosomes play an irreplaceable role in the development of the PMN. Disrupting the communication between tumor exosomes and the PMN may help prevent early tumor metastasis [22, 104]. Studies have shown that exosomal integrins are related to organ-specific metastasis, with integrins α6β4 and α6β1 linked to lung metastasis and integrin αvβ5 connected with liver metastasis. Targeting exosomal integrins can reduce the uptake of exosomes by metastatic organs and consequently diminish metastasis [83]. Additionally, research has found that cholesterol 25-hydroxylase (CH25H) produces 25-hydroxycholesterol, which can inhibit the uptake of tumor exosomes. The antihypertensive drug reserpine has been shown to restore the loss of CH25H, leading to decreased tumor exosome uptake and impaired PMN formation [131]. Furthermore, a novel nanoparticle drug designed to target tumor exosomes has been developed. These nanoparticles co-load a chemotherapeutic agent and a macrophage migration inhibitory factor (MIF) inhibitor, which interact specifically with tumor cells and exosomes, thereby inhibiting MIF within exosomes and suppressing their role in PMN formation [132].

Tumor-derived exosomes express tumor antigens, making them potential candidates for tumor vaccines. One study constructed tumor exosomes anchored with IL-12 in renal cancer cells. These exosomes not only carried the tumor-associated antigen G250 but also produced IL-12, which could promote antigen-specific cytotoxic T lymphocytes in vitro [133, 134]. This approach, leveraging the enhanced immunogenicity and anti-tumor response of exosomes, represents a new strategy for developing exosome-based vaccines against renal cell carcinoma [135]. Another study found that renca mice vaccinated with DCs loaded with tumor exosomes had longer survival times compared to those vaccinated with DCs loaded with tumor cell lysates [136].

Targeting PMN immune suppression

Myeloid-derived suppressor cells

Therapeutic strategies aimed at targeting MDSCs in the PMN can be categorized into two main approaches. The first strategy focuses on reducing the recruitment of MDSCs to the PMN and inhibiting their immunosuppressive activity. In RCC, PMN-enriched MDSC which depletes arginine impair T cell function by generating high levels of arginase I. Research has demonstrated that the use of arginase inhibitors such as N-hydroxy-nor-L-Arg [44] or COX-2 inhibitors can decrease the arginase I expression in MDSCs and subsequently induce lymphocyte-mediated anti-tumor responses [137]. Thus, inhibiting arginase expression in MDSC is a promising adjuvant therapy. Additionally, in a renca mouse model, the selective class I histone deacetylase (HDAC) inhibitor entinostat enhanced the efficacy of PD-1-targeted therapies by suppressing MDSCs function [138]. A novel sponge-like neutrophil membrane-coated nano-system (NM/PPcDG/D) was developed, which demonstrated significant natural affinity for post-surgical inflammatory sites while effectively inhibiting MDSCs recruitment and function, thus suppressing PMN formation and reducing lung metastasis [139].

The second strategy involves the complete depletion of MDSCs. One study employed phage display peptide libraries to create a peptide-Fc fusion protein by fusing sequences coding for the H6 and G3 peptides with sequences encoding the mouse IgG2b Fc region. This peptide-Fc fusion protein, when administered intravenously, led to the complete depletion of MDSCs in peripheral blood, spleen and tumors, without suppressing

pro-inflammatory immune cell types such as DCs [140]. Furthermore, research has indicated that effective concentrations of all-trans retinoic acid (ATRA) can eliminate MDSCs and enhance the specific responses of DCs and T cells. Notably, ATRA treatment is associated with minimal toxicity, indicating its potential for combination therapy with cancer vaccines [141]. Additionally, clinical data suggest that the number and activity of MDSCs are significantly reduced in mRCC patients following treatment with the tyrosine kinase inhibitor sunitinib [37].

Neutrophils

Despite their cytotoxic capabilities, neutrophils are often induced by tumor to promote immune suppression, tumor growth, and metastasis. The neutrophil-to-lymphocyte ratio (NLR) in the circulation of mRCC patients has been established as an individual forecast for recurrence, correlating with poor prognosis [142, 143]. One promising therapeutic strategy focuses on shifting neutrophils from a pro-tumor to an anti-tumor phenotype. Recent studies have emphasized neutrophil activation therapies that utilize a combination of TNF, CD40 agonists and tumor-targeting antibodies to recruit significant numbers of neutrophils. This strategy not only activates the ADCC of neutrophils against tumor cells but also increases complement C5a levels, resulting in the secretion of leukotriene B4 and reactive oxygen species. This induces oxidative damage and T cell-independent destruction of several types of cancer. In vitro studies demonstrate that this combination can activate human neutrophils to lyse human tumor cells, underscoring its potential to eliminate PMN-mediated immune suppression and target disseminated tumor cells [144]. Additionally, research has shown that the natural compound rhodiola rosea effectively prevents nicotine-induced polarization of neutrophils from the N1 to N2 phenotype, inhibiting the formation of the PMN with a tumor-promoting N2 phenotype while enhancing the anti-tumor activity of neutrophils [145]. In ccRCC, tumor cells trigger systemic inflammation through the secretion of chemokines, which further promote metastatic cascades. Inhibiting transcription driven by super-enhancers (SE) using bromodomain and extra-terminal motif inhibitors (BETi) has been found to suppress neutrophil-dependent lung metastasis induced by cancer cell-intrinsic inflammation during ccRCC progression [57].

The migration of inflammatory neutrophils is primarily mediated by adhesion molecules on their membrane [146]. Studies have shown that during early metastasis, inflammatory neutrophils can bind to CTCs directly via the adhesion molecules Mac-1/ICAM-1 and then promote distal metastasis of the tumor [147]. This characteristic of neutrophils, which allows them to target CTCs and migrate toward the PMN, can be leveraged in the design of targeted therapies. However, the short half-life of neutrophils limits their effectiveness for prolonged therapeutic interventions. Consequently, recent research has focused on developing neutrophil membranes as nanoscale drug delivery systems to enhance anti-tumor effects. Activated neutrophil membrane-coated nanoparticles (aNEM NPs) have been developed, which retain functional proteins from activated neutrophils. These aNEM NPs can prevent neutrophil mobilization to both primary tumors and the PMN, as well as prevent neutrophils from adhering to tumor vascular ECs and CTCs. Experimental results from both in vitro and in vivo settings reveal that aNEM NPs can interfere with the formation of CTC-neutrophil clusters [148]. Another innovative approach involved designing a mixed system of neutrophil-iron oxide nanoparticles, which converted live neutrophils (NE) into dead neutrophils ($_{C}$NE) through rapid freezing. This method preserves the structural integrity of neutrophils while preventing the release of NETs. By attaching iron oxide nanoparticles (Mag) to $_{C}$NE, researchers created $_{C}NE^{Mag}$, which targets PMN and depletes tumor-derived secretory factors. Notably, $_{C}NE^{Mag}$ also can reverse macrophages from the M2 phenotype to M1, highlighting its significant therapeutic potential against tumor metastasis [149].

NETs both shield CTCs from damage and support the formation of PMNs, making the targeting of NETs released by neutrophils an effective strategy for inhibiting cancer metastasis [150]. Studies have shown that the transmembrane protein CCDC25 is highly expressed in human ccRCC tissues and cell lines [151]. CCDC25 serves as a receptor for NET-DNA on cancer cells, enabling the sensing of extracellular DNA. Furthermore, NETs-mediated metastasis is abolished in CCDC25 knockout cells [152]. A recent study developed liposomes that stably express CCDC25 via cell membrane nanovesicles, encapsulating DNase1 internally to overcome its short half-life and specifically eliminate NETs [153]. Targeting CCDC25 might offer a promising strategy for the prevention of tumor metastasis. Additionally, peptidylarginine deiminase 4 (PAD4), an enzyme critical for NETs formation, has been targeted with PAD4 inhibitors to block NETs generation, thus inhibiting PMN-induced tumor metastasis [154]. Moreover, the compound AZD7986, which targets tumor-secreted cathepsin C, effectively inhibits the neutrophil infiltrating into the PMN and forming NETs [154].

Macrophages

M2 polarization of macrophages is a primary mechanism underlying macrophage-mediated immunosuppression. Current therapeutic strategies focus on inhibiting M2 polarization of macrophages within the PMN and enhancing their tumoricidal functions. Research has

shown that long non-coding RNAs (lncRNAs), such as lncARSR and AP000439.2, derived from renal cancer cell exosomes, can promote M2 polarization of macrophages via the STAT3 signaling pathway. This indicates that lncRNAs secreted by renal cancer cells and the STAT3 pathway may represent potential therapeutic targets for inhibiting M2 polarization in the PMN [48, 155].

A newly developed macrophage activator, CGP 31,362, delivered via liposomes to the lungs of mice following nephrectomy, has been shown to significantly enhance the tumoricidal functions of lung macrophages, thereby reducing pulmonary tumor metastasis. Early application of such activators in renal cancer may help eliminate PMN formation, potentially preventing tumor recurrence after nephrectomy [156]. Additionally, an innovative antisense oligonucleotide targeting TGF-β2, ISTH0047, has been developed to reinstate the expression of the tumor suppressor factor CD86 in lung macrophages of renca mice [61].

In line with the use of neutrophil membrane nanocarriers, a nanosponging system coated with M2 macrophage membranes has been designed. This system leverages cytokine receptors on the membrane to neutralize cytokines that promote M2 polarization, effectively inhibiting this process. The surface of these nanosponges can also be conjugated with DSPE-PEG-M2pep to specifically target M2 tumor-associated macrophages, while encapsulating the toll-like receptor (TLR) 7/8 agonist R848 within the nanosponges to reverse M1 polarization of macrophages. This strategy has demonstrated effectiveness in inhibiting the progression of RCC in vivo [157]. The nanosponges show promise for clinical applications aimed at reversing macrophage-induced immunosuppression in RCC-associated PMN. Furthermore, studies have indicated that intraperitoneal injection of β-glucan particles (WGP) can retrain the immune phenotype of lung macrophages within the PMN. These trained macrophages exhibit enhanced phagocytic activity and cytotoxicity, as well as increased responsiveness to tumor-derived factors, effectively inhibiting tumor metastasis [158].

Regulatory T cells

IL-2 pioneered immunotherapy in oncology as the first treatment to gain approval [159]. The high-affinity binding of IL-2 is mediated by CD25 which expressed on most Tregs. Therefore, recombinant IL-12 specifically designed to target IL-2 for Tregs not only modulates Tregs function but also enhances immune responses [160, 161]. Preclinical studies have shown that entinostat can downregulate Foxp3 expression and function in Tregs, demonstrating synergistic anti-tumor effects when combined with high-dose IL-2 in RCC models [162]. Although high-dose IL-2 has been approved for use in melanoma and RCC, its efficacy is limited by a short half-life and significant toxicity. Thus, there is a pressing need for new, improved IL-2-based therapies that offer extended half-lives and targeted delivery mechanisms [163].

To date, no modified IL-2 compounds have been approved by regulatory authorities for the therapies for cancer and autoimmune diseases. NKTR-214 is the only such medicine to have completed Phase III trials. However, when combined with sunitinib or cabozantinib for advanced RCC, it failed to achieve its primary efficacy endpoint, as compared to the use of these therapies alone [164]. Other modified IL-2 agents like the AB(389)IL-2, a diphtheria toxin and IL-2 recombinant conjugate, have demonstrated significant reductions in Treg levels in mRCC patients, effectively eliminating Treg-mediated immunosuppression in vivo. Additionally, combining DAB(389)IL-2 with DC vaccines has shown marked improvement in tumor-specific T cell activation among RCC patients [164]. Moreover, long-acting IL-2 immunotherapeutic agents, such as MDNA1, have been designed to address the shortcomings of short half-lives. MDNA1 has exhibited good tolerability in patients with metastatic RCC while eliciting sustained and effective immune responses [165]. Given IL-2's specific targeting of Tregs, there is potential to develop targeted therapies that combine IL-2 with drug delivery systems directed at PMN. This strategy could not only mitigate the systemic side effects of IL-2 but also help reverse immune suppression within the PMN.

Dendritic cells

DCs are pivotal in triggering immune responses through antigen presentation to T cells. This property can be leveraged to induce specific tumor antigen responses in vivo via DC vaccination, which has been validated for safety in clinical settings [166]. Currently, four main strategies are employed to create DC-based cancer vaccines: culturing DCs with autologous tumor tissue obtained from patients, exposing DCs to synthetic peptides or recombinant tumor antigens, transfecting DCs with plasmids encoding tumor-specific antigens, and merging DCs with whole tumor cells through polyethylene glycol-mediated fusion [167]. In mRCC, several clinical trials utilizing DC vaccines have been initiated. The newly developed CMN-001 integrates three signals about antigen presentation, co-stimulatory molecules, and IL-12 secretion into DCs, effectively inducing adaptive T cell responses in vivo [168]. Furthermore, other DC vaccines in combination with sunitinib have shown the capacity to provoke tumor-specific T cell responses in mRCC patients, thereby overcoming the immunosuppressive effects commonly observed in RCC [169–172].

Research has demonstrated that $CD140a^+$ lung fibroblasts can inhibit the antigen-presenting function of

DCs within the PMN through a COX-2-dependent mechanism. This process facilitates the creation of an immunosuppressive microenvironment. Inhibiting the COX-2-PGE2-EP2/EP4 signaling axis improves the efficacy of DC vaccines in targeting metastases [121]. This finding highlights new therapeutic strategies for targeting immunosuppression within PMNs.

Additionally, a unique subset of DCs known as slanDCs has been identified. These slanDCs significantly gather in the draining lymph nodes of metastatic tumors in breast and kidney cancers, while they are absent in primary tumor sites. This observation suggests a strong correlation between slanDCs and the early colonization of metastatic tumors, potentially indicating the formation of PMN. Additionally, slanDCs are capable of secreting high levels of IL-12 and TNF-α, which can facilitate the proliferation and activate T cells and NK cells [173–175]. These DCs may be conducive to reverse PMN immunosuppression.

T cells

Lymphocytes, which include T cells, B cells and NK cells, are the primary immune effector cells responsible for recognizing and clearing tumor cells. The immunosuppression observed in the PMN significantly hampers the anti-tumor effects of these lymphocytes. Restoring and enhancing the anti-tumor capabilities of lymphocytes may potentially eradicate early tumor metastasis. Currently, one of the most effective immunotherapies for metastatic tumor involves immune checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1 antibodies, which help sustain the anti-tumor activity of T cells. Nevertheless, the emergence of treatment resistance presents a major obstacle to their effectiveness [176].A recent review has summarized various adoptive cell therapies aimed at enhancing lymphocyte anti-tumor efficacy in RCC. These treatments encompass T cell receptor (TCR)-engineered T cells, chimeric antigen receptor (CAR) T cells, CAR NK cells and lymphokine-activated killer (LAK) cells [167].

Gene-engineered myeloid cells (GEMys) offer a potential strategy for specifically delivering anti-tumor agents to the PMN. Notably, IL-12 has been demonstrated to enhance the cytotoxic activity of NK cells, increase the generation of cytotoxic T cells and induce the secretion of IFN-γ [177]. By engineering GEMys to express IL-12, these cells can be specifically recruited to the PMN, enabling targeted delivery of IL-12 to the PMN. This strategy aims to reshape the immunosuppressive microenvironment of the PMN and activate antigen presentation and T cell activity, thereby reducing metastasis and improving survival rates in tumor-bearing mice [178]. Furthermore, research has demonstrated that a B7-Fc fusion protein can induce a significant increase in T cells within the tumor-draining lymph nodes of renca mice. When used in conjunction with Tregs depletion, this strategy has been shown to enhance the therapeutic efficacy against tumors [179].

Conclusion and future perspectives

In this review, we present an in-depth exploration of the characteristics of the PMN and the potential mechanisms involved in RCC, focusing primarily on angiogenesis and vascular permeability, immune suppression, metabolic reprogramming, extracellular matrix remodeling and organotropism. Additionally, we discuss potential therapeutic strategies targeting the mechanisms of PMN formation, particularly those related to the remodeling of immune cells and matrix components within the PMN, as well as molecules secreted by tumor cells.

Although studies on PMN in RCC are less extensive than in other cancers, the current advances in technologies and methods have laid a solid foundation for further investigation of PMN in RCC. For example, liquid biopsy technologies (such as exosome analysis and CTC detection) provide novel approaches for the dynamic monitoring of PMN, potentially enabling early diagnosis of RCC metastasis. Moreover, the rapid development of nanomedicine and biocompatible materials has made targeted therapies more effective and specific, offering opportunities for the development of new strategies against PMN. Single-cell multi-omics analysis will be instrumental in revealing the complexity of PMN in RCC and studying the dynamic changes of PMN in RCC metastasis. The use of 3D cell culture systems (such as 3D tumor organoids) and multi-scale models (such as microfluidic chip technology) allows for the study of PMN formation in environments closer to physiological conditions, enabling the simulation of cellular and molecular interactions within PMN. Furthermore, the differences in the PMN microenvironments across various organs and individuals present opportunities for personalized precision medicine in the context of RCC metastasis. With the advancement of big data technologies and artificial intelligence (AI), AI-driven analysis of large-scale metastasis-related data (such as imaging, genomic and clinical data) holds promise for developing predictive models for early metastatic events and personalized treatment strategies. This represents an innovative direction for future metastasis research [180].

Current research on the mechanism of PMN formation in RCC has primarily been conducted using experimental animal models and in vitro cell systems, with no corresponding clinical trials to date. This discrepancy creates significant challenges for clinical translation, particularly in two key aspects. First, there remains a critical knowledge gap in establishing quantitative clinical parameters. Specifically, the threshold concentrations

of tumor-derived exosomes and signaling molecules required for PMN initiation in distant organs need to be determined in human serum. Current experimental models cannot precisely define these critical concentrations for clinical application. Second, the absence of tumor-specific biomarkers presents diagnostic limitations. Currently available markers cannot reliably distinguish whether circulating molecules and exosomes originate from malignant renal cells or other tissue components. This ambiguity hinders clinical detection of tumor-derived factors in pre-metastatic organs of renal cancer patients and prevents accurate assessment of their contribution to subsequent metastatic cascades.

While research on PMN has formed a relatively comprehensive framework, several issues remain to be addressed: (1) What are the dynamic patterns of PMN at different time points (before metastasis, pre-surgery, post-surgery and during relapse)? (2) Is there a relationship between PMN formation and tumor resistance? (3) After the primary tumor is removed, do CTCs persist and promote PMN formation? (4) Do different genetic subtypes of cancer form PMN? (5) Are the monitoring and targeted therapeutic strategies for PMN in mouse models equally effective in humans? (6) How does the microbiome influence PMN formation? These questions require further in-depth investigation for better understanding and validation. In conclusion, we have reviewed the recent advancements in the study of PMN in RCC, with the aim that our insights may offer new strategies and directions for the clinical diagnosis and treatment of RCC metastasis.

Abbreviations

RCC	Renal cell carcinoma
ccRC	Clear cell renal cell carcinoma
mRCC	Metastatic renal cell carcinoma
PMN	Pre-metastatic niche
CTC	Circulating tumor cell
TDSF	Tumor-derived secretory factor
BMDC	Tumor-recruited bone marrow-derived cell
EV	Extracellular vesicle
ECM	Extracellular matrix
EC	Endothelial cell
VHL	Von Hippel-Lindau
VEGF	Vascular endothelial growth factor
MCPIP1	Monocyte chemotactic protein-1-induced protein 1
CA9	Carbonic anhydrase IX
ApoC1	Apolipoprotein C1
STAT3	Signal transducer and activator of transcription 3
MMP	Matrix metalloproteinases
AZU1	Azurocidin1
MDSC	Myeloid-derived suppressor cell
DC	Dendritic cell
M-MDSC	Monocytic myeloid-derived suppressor cell
PMN-MDSCs	Polymorphonuclear myeloid-derived suppressor cell
TLR	Toll-like receptor
PGE2	Prostaglandin E2
TAM	Tumor-associated macrophage
SOX17	SRY-Box transcription factor 17
EMT	Epithelial-mesenchymal transition
OSM	Oncostatin M
PD-1	Programmed cell death protein 1
CTLA-4	Cytotoxic T lymphocyte-associated protein-4
NETs	Neutrophil extracellular traps
C3	Complement component 3
Treg	Regulatory T cell
FoxP3	Forkhead box protein P3
NK	Natural killer
ADCC	Antibody-dependent cellular cytotoxicity
ASS1	Argininosuccinate synthetase-1
BCAA	Branched-chain amino acid
PTHrP	Parathyroid hormone-related peptide
PKA	Protein kinase A
PAT	Perirenal adipose tissue
AR	Androgen receptor
CaSR	Calcium-sensing receptors
NRP-2	Neurophilin-2
MSCs	Mesenchymal stem cells
CAF	Cancer-associated fibroblasts
NF	Normal fibroblasts
TRAIL	Apoptosis inducing ligand
HSV-TK	Herpes simplex virus thymidine kinase
CT	Computed tomography
PET	Positron emission tomography
SPECT	Single-photon emission computed tomography
LAD NPs	Luminescent nanoparticles
ICI	Immune checkpoint inhibitor
vSMCs	Vascular smooth muscle cells
KLF4	Krüppel-like factor 4
VTPs	Vascular-targeting peptides
LIGHT	TNF superfamily 14
HDAC	Class I histone deacetylase
NM/PPcDG/D	Sponge-like neutrophil membrane-coated nano-system
ATRA	All-trans retinoic acid
NLR	Neutrophil-to-lymphocyte ratio
SE	Super-enhancers
BETi	Bromodomain and extra-terminal motif inhibitors
aNEM NPs	Activated neutrophil membrane-coated nanoparticles
NE	Live neutrophils
$_{c}$NE	Dead neutrophils
Mag	Iron oxide nanoparticles
PAD4	Peptidylarginine deiminase 4
WGP	β-glucan particles
TCR	T cell receptor
CAR	Chimeric antigen receptor
LAK	Lymphokine-activated killer
GEMys	Gene-engineered myeloid cells
FN	Fibronectin
EDS	ECM deprivation system
TQ	Thymoquinone
u-PA	Urokinase-type plasminogen activator
CLG	Hyaluronic acid-based gel system loaded with CXCL12
ADI	Arginine deiminase
PEG	Polyethylene glycol
MPC	Mitochondrial pyruvate carrier
CH25H	Cholesterol 25-hydroxylase
MIF	Macrophage migration inhibitory factor

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Declarations

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References

1. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. Cancer J Clin. 2025;75:10–45.
2. Bianchi M, et al. Distribution of metastatic sites in renal cell carcinoma: a population-based analysis. Ann Oncol. 2012;23:973–80.
3. Dudani S, et al. Evaluation of clear cell, papillary, and chromophobe renal cell carcinoma metastasis sites and association with survival. JAMA Netw Open. 2021;4:e2021869.
4. Gerstberger S, Jiang Q, Ganesh K. Metastasis Cell. 2023;186:1564–79.
5. Paget S, THE DISTRIBUTION OF SECONDARY, GROWTHS IN CANCER OF THE BREAST. Lancet. 1889;133:571–3.
6. Kaplan RN, et al. VEGFR1-positive Haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438:820–7.
7. Liu Y, Cao X. Characteristics and significance of the Pre-metastatic niche. Cancer Cell. 2016;30:668–81.
8. Peinado H, et al. Pre-metastatic niches: organ-specific homes for metastases. Nat Rev Cancer. 2017;17:302–17.
9. Prognosis. Targeting of pre-metastatic niche. J Controlled Release. 2020;325:223–34.
10. Peinado H, Lavotshkin S, Lyden D. The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts. Semin Cancer Biol. 2011;21:139–46.
11. Patras L, Shaashua L, Matei I, Lyden D. Immune determinants of the pre-metastatic niche. Cancer Cell. 2023;41:546–72.
12. Jp S. The metastatic niche and stromal progression. Cancer Metastasis Rev 31, (2012).
13. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. Nat Rev Cancer. 2009;9:285–93.
14. Massagué J, Obenauf AC. Metastatic colonization by Circulating tumour cells. Nature. 2016;529:298–306.
15. Zeng Z, et al. Cancer-derived Exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. Nat Commun. 2018;9:5395.
16. Recasens A, Munoz L. Targeting cancer cell dormancy. Trends Pharmacol Sci. 2019;40:128–41.
17. Phan TG, Croucher P. I. The dormant cancer cell life cycle. Nat Rev Cancer. 2020;20:398–411.
18. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009;9:239–52.
19. Gao D, et al. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science. 2008;319:195–8.
20. Fong MY, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. Nat Cell Biol. 2015;17:183–94.
21. Altea-Manzano P, et al. A palmitate-rich metastatic niche enables metastasis growth via p65 acetylation resulting in pro-metastatic NF-κB signaling. Nat Cancer. 2023;4:344–64.
22. Mao W, Wang K, Wu Z, Xu B, Chen M. Current status of research on exosomes in general, and for the diagnosis and treatment of kidney cancer in particular. J Experimental Clin Cancer Res. 2021;40:305.
23. Butz H, et al. Exosomal MicroRNAs are diagnostic biomarkers and can mediate cell–Cell communication in renal cell carcinoma. Eur Urol Focus. 2016;2:210–8.
24. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146:873–87.
25. Pal K, et al. Synchronous Inhibition of mTOR and VEGF/NRP1 axis impedes tumor growth and metastasis in renal cancer. NPJ Precis Oncol. 2019;3:31.
26. Baldewijns MM, et al. High-grade clear cell renal cell carcinoma has a higher angiogenic activity than low-grade renal cell carcinoma based on histomorphological quantification and qRT-PCR mRNA expression profile. Br J Cancer. 2007;96:1888–95.
27. Rs B et al. Increased mobilisation of Circulating endothelial progenitors in von Hippel-Lindau disease and renal cell carcinoma. Br J Cancer 105, (2011).
28. Grange C, et al. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 2011;71:5346–56.
29. Bussolati B, Bruno S, Grange C, Ferrando U, Camussi G. Identification of a tumor-initiating stem cell population in human renal carcinomas. FASEB J. 2008;22:3696–705.
30. Horie K, et al. Exosomes expressing carbonic anhydrase 9 promote angiogenesis. Biochem Biophys Res Commun. 2017;492:356–61.
31. Li Y, et al. ApoC1 promotes the metastasis of clear cell renal cell carcinoma via activation of STAT3. Oncogene. 2020;39:6203–17.
32. Ren L, et al. Systematic pan-cancer analysis identifies APOC1 as an immunological biomarker which regulates macrophage polarization and promotes tumor metastasis. Pharmacol Res. 2022;183:106376.
33. Takeda M et al. Extracellular vesicles secreted from bone metastatic renal cell carcinoma promote angiogenesis and endothelial gap formation in bone marrow in a time-dependent manner in a preclinical mouse model. Front Oncol 13, (2023).
34. Jingushi K, et al. Extracellular vesicles isolated from human renal cell carcinoma tissues disrupt vascular endothelial cell morphology via Azurocidin. Int J Cancer. 2018;142:607–17.
35. Myeloid-derived. Suppressor cell heterogeneity and subset definition. Curr Opin Immunol. 2010;22:238–44.
36. Ochoa AC, Zea AH, Hernandez C, Rodriguez PC. Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma. Clin Cancer Res. 2007;13:s721–6.
37. Ko JS, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res. 2009;15:2148–57.
38. Najjar YG, et al. Myeloid-Derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with intratumoral expression of IL1β, IL8, CXCL5, and Mip-1α. Clin Cancer Res. 2017;23:2346–55.
39. Rinchai D, et al. Integrated transcriptional-phenotypic analysis captures systemic Immunomodulation following antiangiogenic therapy in renal cell carcinoma patients. Clin Translational Med. 2021;11:e434.
40. Rodriguez PC, et al. Arginase I–Producing Myeloid-Derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res. 2009;69:1553–60.
41. Zhang Y et al. Complement C3 of tumor-derived extracellular vesicles promotes metastasis of RCC via recruitment of immunosuppressive myeloid cells. *Proceedings of the National Academy of Sciences* 122, e2420005122 (2025).
42. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol. 2005;5:641–54.
43. Zea AH, et al. Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. Cancer Res. 2005;65:3044–8.
44. Rodriguez PC, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-Cell receptor expression and Antigen-Specific T-Cell responses. Cancer Res. 2004;64:5839–49.
45. Tate DJ, et al. Effect of arginase II on L-arginine depletion and cell growth in murine cell lines of renal cell carcinoma. J Hematol Oncol. 2008;1:14.
46. Wang Y, et al. Pre-metastatic niche: formation, characteristics and therapeutic implication. Sig Transduct Target Ther. 2024;9:1–31.
47. Cassetta L, Pollard JW. Targeting macrophages: therapeutic approaches in cancer. Nat Rev Drug Discov. 2018;17:887–904.
48. Shen T, et al. Exosomal AP000439.2 from clear cell renal cell carcinoma induces M2 macrophage polarization to promote tumor progression through activation of STAT3. Cell Communication Signal. 2022;20:152.
49. Wang C, et al. Targeting a positive regulatory loop in the tumor-macrophage interaction impairs the progression of clear cell renal cell carcinoma. Cell Death Differ. 2021;28:932–51.
50. Guan Y, et al. CCL5 promotes the epithelial-mesenchymal transition of Circulating tumor cells in renal cancer. J Translational Med. 2024;22:817.
51. Nguyen-Tran H-H, Nguyen T-N, Chen C-Y, Hsu T. Endothelial reprogramming stimulated by Oncostatin M promotes inflammation and tumorigenesis in VHL-Deficient kidney tissue. Cancer Res. 2021;81:5060–73.
52. Nguyen T-N, Nguyen-Tran H-H, Chen C-Y, Hsu T. IL6 and CCL18 mediate Cross-talk between VHL-Deficient kidney cells and macrophages during development of renal cell carcinoma. Cancer Res. 2022;82:2716–33.

53. Kersten K, et al. Uptake of tumor-derived microparticles induces metabolic reprogramming of macrophages in the early metastatic lung. Cell Rep. 2023;42:112582.
54. Summers C, et al. Neutrophil kinetics in health and disease. Trends Immunol. 2010;31:318–24.
55. Chen E, Yu J. The role and metabolic adaptations of neutrophils in premetastatic niches. Biomark Res. 2023;11:50.
56. Minárik I, et al. Regulatory T cells, dendritic cells and neutrophils in patients with renal cell carcinoma. Immunol Lett. 2013;152:144–50.
57. Nishida J, et al. Epigenetic remodelling shapes inflammatory renal cancer and neutrophil-dependent metastasis. Nat Cell Biol. 2020;22:465–75.
58. Engblom C, et al. Osteoblasts remotely supply lung tumors with cancer-promoting SiglecFhigh neutrophils. Science. 2017;358:eaal5081.
59. López-Lago MA, et al. Neutrophil chemokines secreted by tumor cells Mount a lung antimetastatic response during renal cell carcinoma progression. Oncogene. 2013;32:1752–60.
60. Fridlender ZG, et al. Polarization of Tumor-Associated neutrophil phenotype by TGF-β: N1 versus N2 TAN. Cancer Cell. 2009;16:183–94.
61. Huber-Ruano I, et al. An antisense oligonucleotide targeting TGF-β2 inhibits lung metastasis and induces CD86 expression in tumor-associated macrophages. Ann Oncol. 2017;28:2278–85.
62. Herre M, Cedervall J, Mackman N, Olsson A-K. Neutrophil extracellular traps in the pathology of cancer and other inflammatory diseases. Physiol Rev. 2023;103:277–312.
63. Wen L, et al. Cooperation between the inflammation and coagulation systems promotes the survival of Circulating tumor cells in renal cell carcinoma patients. Front Oncol. 2019;9:504.
64. Saleh R, Elkord E. FoxP3 T regulatory cells in cancer: prognostic biomarkers and therapeutic targets+. Cancer Lett. 2020;490:174–85.
65. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med. 2001;193:F41–46.
66. Rw G et al. Frequency of regulatory T cells in renal cell carcinoma patients and investigation of correlation with survival. Cancer Immunol Immunotherapy: CII 56, (2007).
67. Finke JH, et al. Sunitinib reverses Type-1 immune suppression and decreases T-Regulatory cells in renal cell carcinoma patients. Clin Cancer Res. 2008;14:6674–82.
68. Liu VC, et al. Tumor evasion of the immune system by converting CD4 + CD25– T cells into CD4 + CD25 + T regulatory cells: role of Tumor-Derived TGF-β. J Immunol. 2007;178:2883–92.
69. Liu T, et al. PD-L2 of tumor-derived exosomes mediates the immune escape of cancer cells via the impaired T cell function. Cell Death Dis. 2024;15:1–9.
70. Remark R, et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. Clin Cancer Res. 2013;19:4079–91.
71. Chiossone L, Dumas P-Y, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol. 2018;18:671–88.
72. Björkström NK, Ljunggren H-G, Michaëlsson J. Emerging insights into natural killer cells in human peripheral tissues. Nat Rev Immunol. 2016;16:310–20.
73. Xu W, et al. Progressive natural killer cell dysfunction in advanced-stage clear-cell renal cell carcinoma and association with clinical outcomes. ESMO Open. 2025;10:104105.
74. Y X. et al. Negative regulation of tumor-infiltrating NK cell in clear cell renal cell carcinoma patients through the Exosomal pathway. Oncotarget 8, (2017).
75. Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol. 2010;7:277–85.
76. Xie H, et al. Glycogen metabolism is dispensable for tumour progression in clear cell renal cell carcinoma. Nat Metab. 2021;3:327–36.
77. Wettersten HI, Aboud OA, Lara PN, Weiss RH. Metabolic reprogramming in clear cell renal cell carcinoma. Nat Rev Nephrol. 2017;13:410–9.
78. Hakimi AA, et al. An integrated metabolic atlas of clear cell renal cell carcinoma. Cancer Cell. 2016;29:104–16.
79. Sciacovelli M, et al. Dynamic partitioning of branched-chain amino acids-derived nitrogen supports renal cancer progression. Nat Commun. 2022;13:7830.
80. Wei G, et al. The thermogenic activity of adjacent adipocytes fuels the progression of CcRCC and compromises anti-tumor therapeutic efficacy. Cell Metab. 2021;33:2021–e20398.
81. Kats-Ugurlu G, et al. Circulating tumour tissue fragments in patients with pulmonary metastasis of clear cell renal cell carcinoma. J Pathol. 2009;219:287–93.
82. Cao Y, et al. Neuropilin-2 promotes extravasation and metastasis by interacting with endothelial A5 integrin. Cancer Res. 2013;73:4579–90.
83. Hoshino A, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527:329–35.
84. Wang L, et al. CD103-positive CSC exosome promotes EMT of clear cell renal cell carcinoma: role of remote MiR-19b-3p. Mol Cancer. 2019;18:86.
85. Pan J, et al. Stromal derived Factor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. Mol Cancer. 2006;5:56.
86. Huang Q, et al. Androgen receptor increases hematogenous metastasis yet decreases lymphatic metastasis of renal cell carcinoma. Nat Commun. 2017;8:918.
87. Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9:274–84.
88. Schüler Y, et al. Osteoblast-secreted factors enhance the expression of dysadherin and CCL2-dependent migration of renal carcinoma cells. Int J Cancer. 2012;130:288–99.
89. Joeckel E, et al. High calcium concentration in bones promotes bone metastasis in renal cell carcinomas expressing calcium-sensing receptor. Mol Cancer. 2014;13:42.
90. Huang J, et al. Extracellular matrix and its therapeutic potential for cancer treatment. Sig Transduct Target Ther. 2021;6:1–24.
91. Th H et al. Differential gene expression profiling of matched primary renal cell carcinoma and metastases reveals upregulation of extracellular matrix genes. Annals Oncology: Official J Eur Soc Med Oncol 28, (2017).
92. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15:786–801.
93. Jung K, Ramankulov A, Schrader M, Miller K, Lein M. Circulating matrix Metalloproteinase-7: an early or metastatic marker for renal cell carcinoma?? Clin Chem. 2008;54:1927–9.
94. Wu Y, Li Y-Y, Matsushima K, Baba T, Mukaida N. CCL3-CCR5 axis regulates intratumoral accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis Process1. J Immunol. 2008;181:6384–93.
95. Ahn G-O, Brown JM. Matrix Metalloproteinase-9 is required for tumor vasculogenesis but not for angiogenesis: role of bone Marrow-Derived myelomonocytic cells. Cancer Cell. 2008;13:193–205.
96. Knowles LM, Gurski LA, Maranchie JK, Pilch J. Fibronectin matrix formation is a prerequisite for colonization of kidney tumor cells in fibrin. J Cancer. 2015;6:98–104.
97. Wu S, et al. Matrix stiffness-upregulated LOXL2 promotes fibronectin production, MMP9 and CXCL12 expression and BMDCs recruitment to assist pre-metastatic niche formation. J Experimental Clin Cancer Res. 2018;37:99.
98. Bakhtyar N, et al. Clear cell renal cell carcinoma induces fibroblast-mediated production of stromal Periostin. Eur J Cancer. 2013;49:3537–46.
99. Malanchi I, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature. 2012;481:85–9.
100. Hc YL. Q. & Y H. Renal clear cell carcinoma-derived CXCL5 drives tumor-associated fibroblast formation and facilitates cancer progression. Pathol Res Pract 244, (2023).
101. T H. et al. Extracellular vesicle-circEHD2 promotes the progression of renal cell carcinoma by activating cancer-associated fibroblasts. Mol Cancer 22, (2023).
102. Ns M et al. F-18 Fluorodeoxyglucose positron emission tomography in the evaluation of distant metastases from renal cell carcinoma. J Clin Oncology: Official J Am Soc Clin Oncol 21, (2003).
103. de A D et al. L. Current Challenges in Diagnosis and Assessment of the Response of Locally Advanced and Metastatic Renal Cell Carcinoma. *Radiographics: a review publication of the Radiological Society of North America, Inc* 39, (2019).
104. Guo Y, et al. Effects of exosomes on pre-metastatic niche formation in tumors. Mol Cancer. 2019;18:39.
105. Cimadamore A, et al. Emerging molecular technologies in renal cell carcinoma: liquid biopsy. Cancers. 2019;11:196.
106. Hu L, Wickline SA, Hood JL. Magnetic resonance imaging of melanoma exosomes in lymph nodes. Magn Reson Med. 2015;74:266–71.
107. Sakaguchi M. S100-SPECT uncovers cellular and molecular events of pre-metastatic niche formation and following organ-specific cancer metastasis. Theranostics. 2017;7:2649–51.
108. Zheng H, et al. Early diagnosis of breast cancer lung metastasis by nanoprobe-based luminescence imaging of the pre-metastatic niche. J Nanobiotechnol. 2022;20:134.
109. Young M, et al. Renal cell carcinoma. Lancet. 2024;404:476–91.

110. Goteri G, et al. Expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1alpha (HIF-1alpha), and microvessel density in endometrial tissue in women with adenomyosis. Int J Gynecol Pathol. 2009;28:157–63.
111. Renal cell carcinoma. Lancet. 2009;373:1119–32.
112. Choueiri TK, Kaelin WG. Targeting the HIF2–VEGF axis in renal cell carcinoma. Nat Med. 2020;26:1519–30.
113. Shu G, et al. Exosomal circSPIRE1 mediates glycosylation of E-cadherin to suppress metastasis of renal cell carcinoma. Oncogene. 2023;42:1802–20.
114. Marona P, et al. MCPIP1 downregulation in clear cell renal cell carcinoma promotes vascularization and metastatic progression. Cancer Res. 2017;77:4905–20.
115. Polena H, et al. The tyrosine-kinase inhibitor Sunitinib targets vascular endothelial (VE)-cadherin: a marker of response to antitumoural treatment in metastatic renal cell carcinoma. Br J Cancer. 2018;118:1179–88.
116. Murgai M, et al. KLF4-dependent perivascular cell plasticity mediates pre-metastatic niche formation and metastasis. Nat Med. 2017;23:1176–90.
117. He B, et al. Remodeling of metastatic vasculature reduces lung colonization and sensitizes overt metastases to immunotherapy. Cell Rep. 2020;30:714–e7245.
118. Wang L, et al. Transformable ECM deprivation system effectively suppresses renal cell carcinoma by reversing Anoikis resistance and increasing chemotherapy sensitivity. Adv Mater. 2022;34:2203518.
119. Muraishi Y, Mitani N, Fuse H, Saiki I. Effect of a matrix metalloproteinase inhibitor (ONO-4817) on lung metastasis of murine renal cell carcinoma. Anticancer Res. 2001;21:3845–52.
120. Liou Y-F, et al. Thymoquinone inhibits metastasis of renal cell carcinoma cell 786-O-SI3 associating with downregulation of MMP-2 and u-PA and suppression of PI3K/Src signaling. Int J Med Sci. 2019;16:686–95.
121. Gong Z, et al. Lung fibroblasts facilitate pre-metastatic niche formation by remodeling the local immune microenvironment. Immunity. 2022;55:1483–e15009.
122. Zhou Y, et al. Peptide nano-blanket impedes fibroblasts activation and subsequent formation of pre-metastatic niche. Nat Commun. 2022;13:2906.
123. Yan Y, et al. Inhibiting collagen I production and tumor cell colonization in the lung *via* miR-29a-3p loading of exosome-/liposome-based nanovesicles. Acta Pharm Sinica B. 2022;12:939–51.
124. Dong G, Chen P, Xu Y, Liu T, Yin R. Cancer-associated fibroblasts: key criminals of tumor pre-metastatic niche. Cancer Lett. 2023;566:216234.
125. Ieranò C, et al. CXCL12 loaded-dermal filler captures CXCR4 expressing melanoma Circulating tumor cells. Cell Death Dis. 2019;10:1–13.
126. Heravi G, Yazdanpanah O, Podgorski I, Matherly LH, Liu W. Lipid metabolism reprogramming in renal cell carcinoma. Cancer Metastasis Rev. 2022;41:17–31.
127. Fang Y, et al. LAMC1-mediated preadipocytes differentiation promoted peritoneum pre-metastatic niche formation and gastric cancer metastasis. Int J Biol Sci. 2022;18:3082–101.
128. Yoon J-K, Frankel AE, Feun LG, Ekmekcioglu S, Kim KB. Arginine deprivation therapy for malignant melanoma. CPAA. 2012;5:11–9.
129. Yoon C-Y, et al. Renal cell carcinoma does not express argininosuccinate synthetase and is highly sensitive to arginine deprivation via arginine deiminase. Int J Cancer. 2007;120:897–905.
130. Lv M, et al. ROS-responsive core–shell nano-inhibitor impedes pyruvate metabolism for reinforced photodynamic therapy and interrupted pre-metastatic niche formation. Acta Biomater. 2024;182:288–300.
131. Ortiz A, et al. An Interferon-Driven Oxysterol-Based defense against Tumor-Derived extracellular vesicles. Cancer Cell. 2019;35:33–e456.
132. Deng M, et al. Curbing exosome communications via introducing artificial membrane receptors for metastatic pancreatic cancer therapy. Adv Mater. 2023;35:2303736.
133. Y Z, Xh W, Cl GC, L., Jm Z. [Preparation of renal cancer vaccine of IL-12-anchored exosomes and its antitumor effect in vitro]. Zhonghua Zhong Liu Za Zhi [Chinese Journal of Oncology] 32, (2010).
134. Zhang Y, et al. Interleukin-12-anchored exosomes increase cytotoxicity of T lymphocytes by reversing the JAK/STAT pathway impaired by tumor-derived exosomes. Int J Mol Med. 2010;25:695–700.
135. Zhang Y, et al. Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response in vitro: A novel vaccine for renal cell carcinoma. Int J Oncol. 2010;36:133–40.
136. Gu X, Erb U, Büchler MW, Zöller M. Improved vaccine efficacy of tumor exosome compared to tumor lysate loaded dendritic cells in mice. Int J Cancer. 2015;136:E74–84.
137. Rodriguez PC, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med. 2005;202:931–9.
138. Orillion A, et al. Entinostat neutralizes Myeloid-Derived suppressor cells and enhances the antitumor effect of PD-1 Inhibition in murine models of lung and renal cell carcinoma. Clin Cancer Res. 2017;23:5187–201.
139. Xia C, et al. Sponge-like nano-system suppresses tumor recurrence and metastasis by restraining myeloid-derived suppressor cells-mediated immunosuppression and formation of pre-metastatic niche. Acta Biomater. 2023;158:708–24.
140. Qin H, et al. Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice. Nat Med. 2014;20:676–81.
141. Mirza N, et al. All-trans-Retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. Cancer Res. 2006;66:9299–307.
142. Immunomonitoring. Prognostic relevance of neutrophils in clinical trials. Sem Cancer Biol. 2013;23:200–7.
143. Change in. Neutrophil-to-lymphocyte ratio in response to targeted therapy for metastatic renal cell carcinoma as a prognosticator and biomarker of efficacy. Eur Urol. 2016;70:358–64.
144. Linde IL, et al. Neutrophil-activating therapy for the treatment of cancer. Cancer Cell. 2023;41:356–e37210.
145. Tyagi A, et al. Nicotine promotes breast cancer metastasis by stimulating N2 neutrophils and generating pre-metastatic niche in lung. Nat Commun. 2021;12:1–18.
146. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol. 2007;7:678–89.
147. Spicer JD, et al. Neutrophils promote liver metastasis via Mac-1–Mediated interactions with Circulating tumor cells. Cancer Res. 2012;72:3919–27.
148. Zeng W, et al. Neutrophil nanodecoys inhibit tumor metastasis by blocking the interaction between tumor cells and neutrophils. ACS Nano. 2024;18:7363–78.
149. Han X, et al. Bacterial Magnetosome-Hitchhiked Quick-Frozen neutrophils for targeted destruction of Pre-Metastatic niche and prevention of tumor metastasis. Adv Healthc Mater. 2023;12:2301343.
150. Adrover JM, McDowell SAC, He X-Y, Quail DF, Egeblad M. NETworking with cancer: the bidirectional interplay between cancer and neutrophil extracellular traps. Cancer Cell. 2023;41:505–26.
151. Qian Z, et al. Coiled-coil domain containing 25 (CCDC25) regulates cell proliferation, migration, and invasion in clear cell renal cell carcinoma by targeting the ILK-NF-κB signaling pathway. FASEB J. 2024;38:e23414.
152. Yang L, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature. 2020;583:133–8.
153. Wang Z, et al. Cell membrane derived liposomes loaded with DNase I target neutrophil extracellular traps which inhibits colorectal cancer liver metastases. J Controlled Release. 2023;357:620–9.
154. Xiao Y, et al. Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. Cancer Cell. 2021;39:423–e4377.
155. Zhang W, et al. Renal cell carcinoma-derived exosomes deliver LncARSR to induce macrophage polarization and promote tumor progression via STAT3 pathway. Int J Biol Sci. 2022;18:3209–22.
156. Dinney CPN, et al. Therapy of spontaneous lung metastasis of murine renal adenocarcinoma by systemic administration of liposomes containing the macrophage activator CGP 313621. Cancer Res. 1991;51:3741–7.
157. Jiang Y, et al. Macrophage-Derived nanosponges adsorb cytokines and modulate macrophage polarization for renal cell carcinoma immunotherapy. Adv Healthc Mater. 2024;13:2400303.
158. Ding C, et al. Inducing trained immunity in pro-metastatic macrophages to control tumor metastasis. Nat Immunol. 2023;24:239–54.
159. Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol. 2014;192:5451–8.
160. Dikiy S, et al. A distal *Foxp3* enhancer enables interleukin-2 dependent thymic Treg cell lineage commitment for robust immune tolerance. Immunity. 2021;54:931–e94611.
161. Hernandez R, Põder J, LaPorte KM, Malek TR. Engineering IL-2 for immunotherapy of autoimmunity and cancer. Nat Rev Immunol. 2022;22:614–28.
162. Pili R, et al. Immunomodulation by entinostat in renal cell carcinoma patients receiving High-Dose Interleukin 2: A multicenter, Single-Arm, phase I/II trial (NCI-CTEP#7870). Clin Cancer Res. 2017;23:7199–208.
163. Overwijk WW, Tagliaferri MA, Zalevsky J. Engineering IL-2 to give new life to T cell immunotherapy. Annu Rev Med. 2021;72:281–311.

164. Raeber ME, Sahin D, Karakus U, Boyman O. A systematic review of interleukin-2-based immunotherapies in clinical trials for cancer and autoimmune diseases. eBioMedicine. 2023;90:104539.
165. Huijts CM, et al. Phase 1 study of everolimus and low-dose oral cyclophosphamide in patients with metastatic renal cell carcinoma. Cancer Immunol Immunother. 2019;68:319–29.
166. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. Cell Res. 2017;27:74–95.
167. Wang Y, et al. Evolution of cell therapy for renal cell carcinoma. Mol Cancer. 2024;23:8.
168. DeBenedette M, Gamble A, Norris M, Horvatinovich J, Nicolette CA. A review of the clinical experience with CMN-001, a tumor RNA loaded dendritic cell immunotherapy for the treatment of metastatic renal cell carcinoma. Hum Vaccines Immunotherapeutics. 2023;19:2220629.
169. Matsushita H, et al. A pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with Sunitinib for metastatic renal cell carcinoma. J Immunother Cancer. 2014;2:30.
170. Figlin RA, et al. Results of the ADAPT phase 3 study of Rocapuldencel-T in combination with Sunitinib as First-Line therapy in patients with metastatic renal cell carcinoma. Clin Cancer Res. 2020;26:2327–36.
171. Barata PC, Rini BI. Treatment of renal cell carcinoma: current status and future directions. Cancer J Clin. 2017;67:507–24.
172. Amin A, et al. Survival with AGS-003, an autologous dendritic cell–based immunotherapy, in combination with Sunitinib in unfavorable risk patients with advanced renal cell carcinoma (RCC): phase 2 study results. J Immunother Cancer. 2015;3:14.
173. Toma M, et al. Accumulation of tolerogenic human 6-sulfo LacNAc dendritic cells in renal cell carcinoma is associated with poor prognosis. Oncoimmunology. 2015;4:e1008342.
174. Vermi W, et al. SlanDCs selectively accumulate in carcinoma-draining lymph nodes and marginate metastatic cells. Nat Commun. 2014;5:3029.
175. Schäkel K, et al. Human 6-Sulfo LacNAc-Expressing dendritic cells are principal producers of early Interleukin-12 and are controlled by erythrocytes. Immunity. 2006;24:767–77.
176. Edwards SC, Hoevenaar WHM, Coffelt SB. Emerging immunotherapies for metastasis. Br J Cancer. 2021;124:37–48.
177. Brunda MJ, et al. Antitumor and antimetastatic activity of Interleukin 12 against murine tumors. J Exp Med. 1993;178:1223–30.
178. Kaczanowska S et al. Genetically engineered myeloid cells rebalance the core immune suppression program in metastasis. Cell 184, 2033–2052.e21 (2021).
179. Liu A, Hu P, Khawli LA, Epstein AL. Combination B7-Fc fusion protein treatment and Treg cell depletion therapy. Clin Cancer Res. 2005;11:8492–502.
180. Bojmar L, et al. Multi-parametric atlas of the pre-metastatic liver for prediction of metastatic outcome in early-stage pancreatic cancer. Nat Med. 2024;30:2170–80.

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