Journal of Translational Medicine

REVIEW

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Invadopodia in cancer metastasis: dynamics, regulation, and targeted therapies

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Abstract

Pseudopodia and invadopodia are dynamic, actin-rich membrane structures extending from the cell surface. While pseudopodia are found in various cell types, invadopodia are exclusive to tumor cells and play a key role in cancer progression. These specialized structures enable tumor cells to degrade the extracellular matrix, breach tissue barriers, and invade surrounding tissues and blood vessels, thus facilitating metastasis. Extensive research has elucidated the distinct structure of invadopodia, the signaling pathways driving their formation, and their interaction with the tumor microenvironment. Integrin- and Src kinase-mediated signaling pathways regulate invadopodia dynamics. This review explores the mechanisms underlying invadopodia stabilization and highlights recent insights into their regulation by the tumor microenvironment. Particular emphasis is placed on the role of cell surface signaling in modulating invadopodia activity and the intracellular targeting of matrix metalloproteinases (MMPs) in enhancing invasive potential. A deeper understanding of invadopodia-driven cancer cell migration and metastasis provides valuable implications for therapeutic development. These findings support the potential for receptor-mediated and molecularly targeted therapies to inhibit tumor metastasis, improve clinical outcomes, and enhance the efficacy of existing cancer treatments.

Keywords Invadopodia, Metastasis, Matrix metalloproteinase, Membrane receptor, Therapeutic targets

Introduction

The invasion-metastasis cascade refers to the process by which cancer cells migrate from the primary tumor to distant sites of metastasis. This sequence includes local invasion, intravasation into the circulatory system, survival in circulation, extravasation into peripheral tissues, and colonization of the metastatic site [1–3] (Fig. 1). Basement membrane disruption precedes metastatic events, while its complete loss marks the onset of primary tumor dissemination. Once this occurs, the tumor mass is classified as malignant [4]. Malignant tumors increase their invasive capacity by forming invadopodia, which degrade barrier tissues and facilitate invasion into the surrounding environment. Invadopodia are actin-rich membrane protrusions localized on the surface of tumor cells. During tumorigenesis and progression, cancer cells use invadopodia to degrade the extracellular matrix (ECM) in a spatially regulated manner, particularly at the invasive front [5]. The most significant effector molecules enabling ECM degradation by invadopodia are the matrix metalloproteinases (MMPs), with membrane type-1 MMP (MT1-MMP) playing a predominant role in basement membrane disintegration. MT1-MMP is directly anchored to the membrane surface of invadopodia in cancer cells, cleaving and degrading ECM components. Together, these

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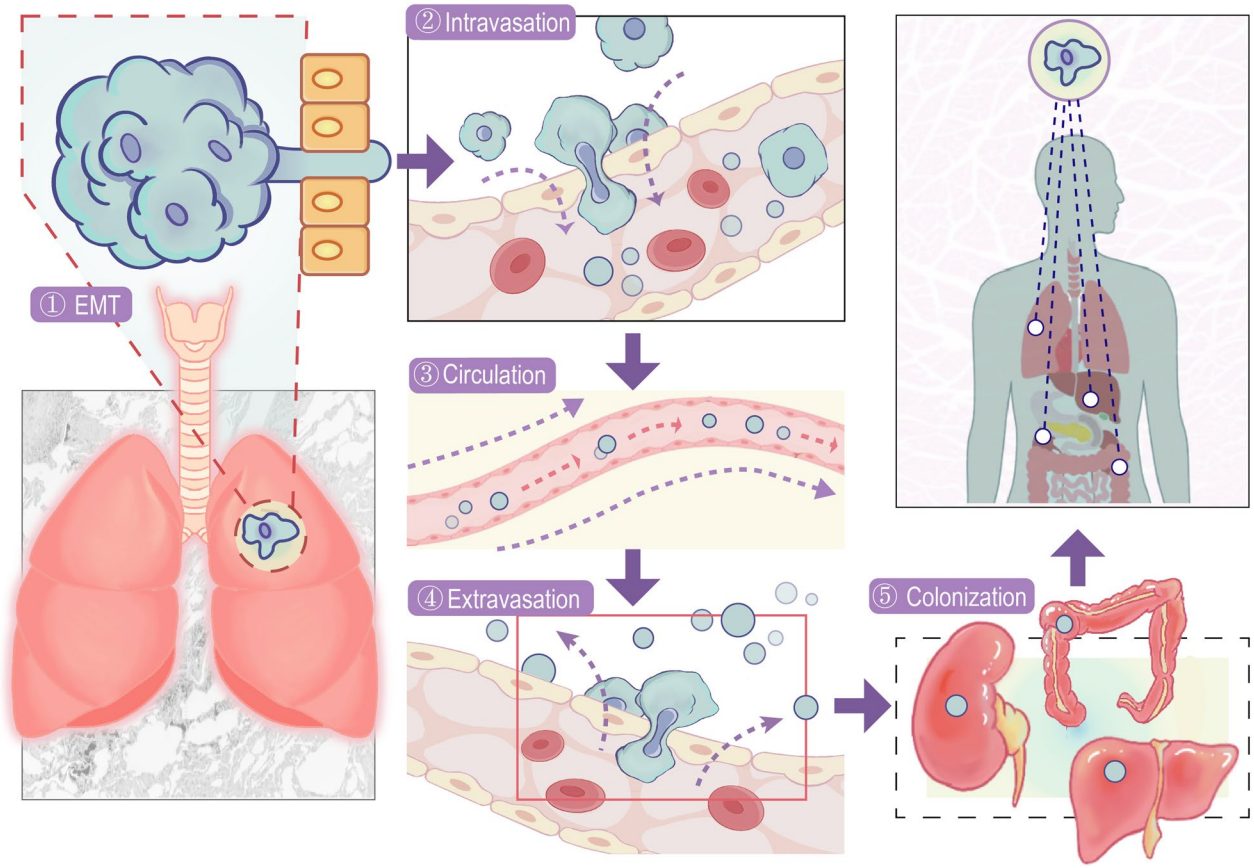

Fig. 1 The cascade of tumor metastasis. (1) Epithelial-mesenchymal transition (EMT): Tumor cells undergo phenotypic changes, allowing detachment from the primary tumor and acquiring migratory and invasive properties. (2) Intravasation: Tumor cells infiltrate surrounding tissues and enter the bloodstream or lymphatic vessels, facilitated by extracellular matrix (ECM) degradation and interactions with endothelial cells. (3) Circulation: Tumor cells, referred to as circulating tumor cells (CTCs), traverse the vascular system to reach distant sites. (4) Extravasation: CTCs exit the bloodstream and invade distant tissues by adhering to and penetrating the endothelial barrier. (5) Colonization: Tumor cells adapt to the new microenvironment, interact with stromal components, and establish secondary metastatic lesions

mechanisms facilitate both local invasion and distant metastasis of cancer cells [6]. Investigating the molecular mechanisms underlying invadopodia formation and the intracellular trafficking of MMPs may provide effective strategies for inhibiting metastasis. Potential therapeutic approaches include inhibiting MMP activity, interfering with cell surface signal transduction pathways, and targeting actin cytoskeletal reorganization [7]. These approaches aim to reduce the invasiveness of cancer cells, limiting their spread and metastatic potential. By precisely modulating these pathways, it may be possible to reduce cancer cell invasiveness while minimizing effects on normal cells [8, 9]. This article explores the regulatory mechanisms governing invadopodia formation and function to advance strategies for limiting cancer metastasis.

Characteristics of invadopodia in cancer

Tarone and Marchisio, in 1985, identified ventral membrane protrusions enriched in actin and phosphotyrosine as key structures mediating cell attachment to the ECM. These structures became known as podosomes [5, 10]. In the same year, Chen, Parsons, and their colleagues demonstrated that Src kinase localized to sites of cell-ECM interaction and proposed that ECM degradation occurred specifically at these sites [11]. Building on this, Chen, in 1989, established that Src-enriched ECM degradation sites corresponded to actin-rich protrusions, initially referred to as podosomes but subsequently renamed invadopodia [12]. By 1994, invadopodia-associated proteolytic activity in human cancer cells was characterized for the first time, linking these structures directly to tumor invasion and ECM remodeling [13]. This section examines the key features of podosomes and invadopodia, focusing on their

Table 1 The characteristics of podosomes and invadopodia

Parameters/properties	Podosomes	Invadopodia
Cell type	Monocyte/macrophage lineage cells (macrophages, dendritic cells, bone marrow-derived osteoclasts), endothelial cells, smooth muscle cells, fibroblasts, neural crest cells	Cancer cells (such as breast cancer, cervical cancer, pancreatic cancer, lung cancer, squamous cell carcinoma, gastric cancer, colorectal cancer, liver cancer, bone cancer, and melanoma)
Size	Diameter: 0.5–1 μm, Height: 0.5–0.8 μm	Diameters: 0.5–3 μm, Height: 2–5 μm
Structure	F-actin-rich puncta consisting of core (F-actin, ARP2/3 complex, WASP, WIP, CDC42, cortactin, cofilin), Ring (β2 and β3 integrins, vinculin, paxillin, talin) and cap (crosslinked and bundled filaments)	F-actin-rich puncta consisting of core (F-actin, ARP2/3 complex, N-WASP, WIP, CDC42, cortactin, cofilin, Diaphanous-related-formins, dynamin 2, fascin, cysteine-rich protein 2, MT1-MMP, TKS5, MenalNV), Ring (β1 and β3 integrins, vinculin, paxillin, zyxin, ILK)
Main functions	Adhesion, Migration, Mechanosensation, Phagocytosis (macrophages)	ECM degradation, Mechanosensing
Relationship with diseases	Normal physiological migration (such as wound healing and immune surveillance). Abnormal activation may lead to chronic inflammation or fibrosis	Directly drive tumor metastasis and drug resistance, significantly associated with poor patient prognosis
Commonality	Dependent on actin dynamics assembly and Rho family GTPases (such as Cdc42, Rac1). Involved in cell-environment interactions	Share regulatory mechanisms with actin reorganization and Rho GTPases, both requiring localized membrane protrusion and the generation of mechanical forces

similarities and differences in morphology, composition, structure, turnover, and function. Table 1 summarizes the distinct characteristics of these structures.

Podosomes and invadopodia are specialized cytoskeletal structures on the cell surface that regulate cell migration, adhesion, and invasion. Podosomes are primarily found in normal, metabolically active cells, including dendritic cells, macrophages, monocytes, and osteoclasts. They also form in endothelial cells, smooth muscle cells, and transformed fibroblasts [14]. These structures are small, measuring 0.5–1 μm in diameter and 0.5–0.8 μm in height [15], and are short-lived, typically persisting for only a few minutes. Structurally, podosomes contain a branched actin core enriched with proteins essential for actin polymerization, surrounded by a peripheral zone containing β2 and β3 integrin receptors along with associated proteins such as vinculin, talin, and paxillin [16]. Functionally, podosomes primarily contribute to processes including cell migration and leukocyte extravasation.

Invadopodia are primarily found in cancer cells, including those derived from head and neck cancer, melanoma, bladder cancer, breast cancer, and prostate cancer [17]. Compared to podosomes, invadopodia are larger, with diameters reaching 3 μm and heights up to 5 μm [18]. Although fewer in number, invadopodia persist significantly longer, often exceeding an hour [19].

Structurally, invadopodia are outward projections of the cell membrane enriched with filamentous actin (F-actin), actin-associated proteins such as cortactin and N-WASP, adhesion molecules, signaling proteins, membrane remodeling factors, and matrix-degrading enzymes [5]. The primary function of invadopodia is to facilitate tumor cell invasion by degrading the ECM, allowing entry into blood vessels, and promoting metastasis. These structures typically form on the side of the cell adjacent to the basement membrane, where they localize and secrete MMPs, including MT1-MMP, MMP2, and MMP9, at the tips of the protrusions to promote ECM degradation [20].

A key distinction between podosomes and invadopodia is their ability to degrade ECM components. Podosomes, which exhibit a high turnover rate and are present in large numbers per cell, typically mediate widespread but shallow ECM degradation. Invadopodia are fewer in number, longer-lived, and facilitate localized, deeper ECM degradation [5]. Cortactin, an actin-binding protein, is closely associated with invadopodia formation, primarily through its interactions with other proteins. Overexpression of cortactin is frequently observed in various invasive cancers, making it a widely used marker for invadopodia [21]. Moreover, vinculin has been suggested as a marker for podosomes [22], while Nck adaptor protein 1 (NCK1) has been identified as specific to invadopodia [23].

The temporal stages of the formation of the invadopodia

High-resolution live-cell microscopy studies have categorized invadopodia formation into three distinct phases: precursor core assembly, stabilization, and maturation [24]. The initiation phase primarily depends on the actin-cortactin complex, which rapidly recruits neural Wiskott-Aldrich syndrome protein (N-WASP), actin-related protein 2/3 (Arp2/3) complex, and cofilin within seconds [16]. At this stage, the invadopodia precursor core remains highly unstable. Approximately 20 s later, the adapter protein tyrosine kinase substrate with five SH3 domains (TKS5) is recruited, anchoring the precursor core to phosphatidylinositol 3,4-bisphosphate (PI(3,4)P2) via its Phox homology (PX) domain [25]. This step is critical for stabilization. PI(3,4)P2 formation at the plasma membrane occurs progressively and requires activation of phosphatidylinositol 3-kinase (PI3 K), which catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) into phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3). The 5′-phosphatase SH2-containing inositol 5′-phosphatase 2 (SHIP2) then converts PI(3,4,5)P3 into PI(3,4)P2 [26]. Approximately 2–3 min after precursor assembly begins, TKS5 binds PI(3,4)P2, securing the structure to the membrane [27]. Invadopodia stabilization occurs through ECM adhesion mediated by β1 integrin. During this process, the actin regulatory proteins Mena and Arg kinase are recruited via integrin α5 and integrin β1, respectively [28]. Activation of Arg kinase by β1 integrin signals the transition of invadopodia into the maturation stage. At this stage, Arg kinase phosphorylates cortactin at the Y421 site, recruits NCK1, and activates the N-WASp-Arp2/3 complex through NCK1 and CDC42 [28, 29]. This complex drives further polymerization and nucleation of F-actin, facilitated by cofilin proteins [24, 30]. This process continues for several minutes, leading to the protrusion and full maturation of the invadopodia. Following maturation, the polymerization of actin in the core region supports the gradual extension of the pseudopod to form a protrusion. Simultaneously, MMPs are recruited to the invadopodia, enabling ECM degradation [31].

The stability of invadopodia is critical

The process of stabilizing invadopodia can be compared to docking a cargo ship. It involves securing the cargo ship, represented by the actin-cortactin complex, firmly to the shore, symbolized by the plasma membrane PI(3,4)P2, using a prepared anchor, TKS5. The key components involved in stabilizing invadopodia are depicted in Fig. 2.

Phosphoinositides support invadopodia formation

Phosphoinositides are lipid components of cell membranes, mainly localized to the inner leaflet of the plasma membrane and intracellular organelles. They play essential roles in actin cytoskeleton remodeling [32] and function as key regulators of intracellular signaling and membrane trafficking [33]. Phosphoinositides are dynamically regulated by kinases and phosphatases, generating seven distinct species that localize to specific subcellular regions. Among these, phosphatidylinositol derivatives, particularly PI(3,4)P2, PI(4,5)P2, and PI(3,4,5)P3, are crucial for invadopodia formation [26].

During the early stages of invadopodia formation, localized conversion of PI(3,4,5)P3 to PI(3,4)P2 generates binding sites for TKS5 scaffolding proteins [27]. This interaction facilitates actin filament nucleation, which is essential for maintaining membrane protrusions and directing the delivery of metalloprotease MT1-MMP to invadopodia [34]. Moreover, the localized production of PI(4,5)P2, mediated by phosphatidylinositol-4-phosphate 5-kinase type I alpha (PIP5 K1α) upon activation by ADP-ribosylation factor 6 (ARF6) recruits key invadopodia components, including N-WASP, cofilin, and dynamin-2. ARF6, localized at the plasma membrane, further regulates endosomal positioning and tubulation through activation of the βPIX-Rac3-GIT1 signaling axis, ensuring the efficient delivery of MT1-MMP to invadopodia [35]. A more detailed discussion of MT1-MMP trafficking is provided in the following sections. PI(4,5)P2 serves as a substrate for the generation of PIP3 via PI 3-kinase, which then activates AKT and promotes invadopodia formation [36]. Invadopodia are often positioned near multivesicular late endosomes, and the secretion of exosomes further supports their formation, facilitating invasive cellular behaviors [37]. At invadopodia, PI(4,5)P2 functions as a platform for exosome secretion and the assembly of key components essential for invadopodia formation [38].

SHIP2 assists in the formation of berthing sites

SH2-containing inositol 5-phosphatase 2 (SHIP2) plays a significant role in stabilizing invadopodia by regulating phosphoinositide conversion. SHIP2 removes the 5′ phosphate group from PI(3,4,5)P3, converting it to PI(3,4)P2 [39], supporting invadopodia formation and stability. Moreover, SHIP2 interacts with various molecules to regulate invadopodia stability, among which Mena is particularly significant [40]. Mena, an Ena/VASP

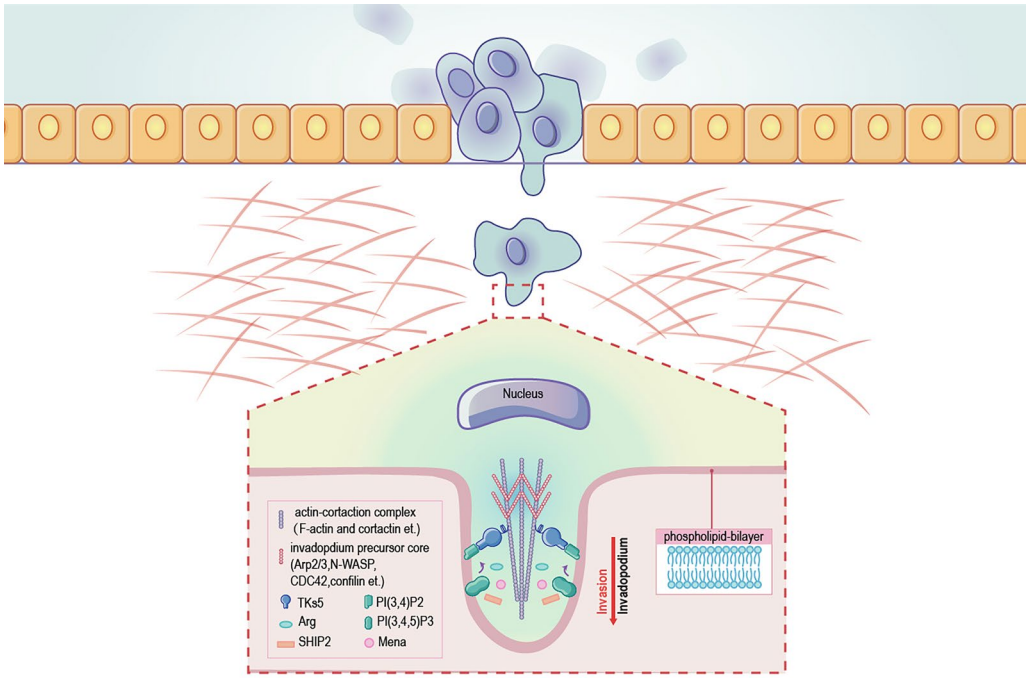

Fig. 2 Schematic representation of the structural stability of invadopodia. (Top) Cancer cells extend invadopodia to penetrate the basement membrane and degrade the extracellular matrix (ECM), facilitating local matrix remodeling and promoting invasion into surrounding tissues. (Bottom) Invadopodia formation is triggered by actin polymerization, driven by key regulators such as the Arp2/3 complex, N-WASP, and CDC42. Structural stability is primarily maintained by cortactin and TKS5, which reinforce the actin network and promote invadopodia maturation. Lipid signaling, particularly the conversion of phosphatidylinositol (PI(3,4)P2) to PI(3,4,5)P3 via SHIP2, further stabilizes invadopodia by regulating proteins such as Arg and Mena. These stabilized structures facilitate the targeted delivery of proteases and ECM degradation, supporting the continuous invasion of cancer cells into the surrounding matrix

protein family member, is essential for invadopodia formation and maturation. It promotes actin polymerization to stabilize invadopodia and co-localizes with cortactin and F-actin at invadopodial sites [41].

It has been shown that Mena is upregulated in several cancers, including breast, pancreatic, colon, gastric, and cervical cancers, and melanoma. In breast cancer, a specific splicing isoform of Mena, known as MenaINV, is strongly associated with cancer cell invasiveness [42]. MenaINV enhances invadopodia activity by inhibiting protein tyrosine phosphatase 1B (PTP1B), a phosphatase that regulates key receptors such as c-Met and EGFR and promotes cell invasion and migration [42, 43]. Moreover, MenaINV expression is regulated by the NOTCH signaling pathway [44], further contributing to tumor cell invasion. By increasing tyrosine phosphorylation of cortactin, particularly at Y421, MenaINV promotes invadopodia assembly, which represents a key regulatory step in tumor cell invasion [42].

SHIP2 plays a dual role in cancer, acting as a tumor suppressor or an oncogene, depending on the cancer type and cellular context. As a tumor suppressor, SHIP2 expression is downregulated in gastric cancer and invasive squamous cell carcinoma, leading to PI3 K/AKT pathway activation and subsequent tumor growth and proliferation [45, 46]. In glioblastoma cells, SHIP2 regulates focal adhesion dynamics through PI(4,5)P2, inhibiting cell migration [47, 48]. SHIP2 can also act as an oncogene, being activated by AKT in several cancers, including ER-negative breast cancer, hepatocellular carcinoma (HCC), colorectal cancer, and laryngeal squamous cell carcinoma, where its expression is upregulated [49, 50]. This duality in SHIP2 function may be attributed to its enzymatic activity, which differentially affects PI3 K/Akt signaling across cancer types. The "phosphatidylinositol phosphate (PIP) hypothesis" provides a possible explanation, proposing that PI(3,4,5)P3 and PI(3,4) P2 distinctly regulate Akt phosphorylation at Thr308 and Ser473, leading to diverse cancer cell responses and biological behaviors [51]. In cancers with elevated SHIP2 expression, its role in promoting invadopodia formation enhances ECM degradation, increases cell migration, and drives tumor metastasis. This highlights the context-dependent function of SHIP2 in cancer progression, particularly in facilitating tumor cell invasion and metastasis through invadopodia [26].

TKS5 as the anchor hook for invadopodia

The stabilization of invadopodia precursors is essential for their formation and function, with TKS proteins playing a central role in this process. These proteins, characterized by their SH3 and PX domains, mediate key protein–protein and protein-lipid interactions, facilitating the recruitment of molecules necessary for assembling and anchoring invadopodia precursors to the plasma membrane [52]. 靶向TKS5蛋白可能成为有效抑制.

TKS proteins are key regulators of cancer cell invasion, particularly during the transition of invadopodia precursors into fully functional structures [53]. Their activity is regulated through tyrosine phosphorylation by Src tyrosine kinase, a modification required for their function [54]. As a scaffolding protein, TKS5 stabilizes the invadopodia core complex, anchors it to the plasma membrane, and promotes its maturation [27]. Among the TKS family members, TKS5 plays a particularly significant role in invadopodia dynamics by recruiting adaptor proteins NCK1 and NCK2, which activate the N-WASP–Arp2/3 complex, thereby enhancing invadopodia stability and expansion [55]. Moreover, TKS5 contributes to matrix degradation by facilitating the localization and activation of proteases. Through its interaction with Rab40b, TKS5 anchors vesicles containing MMPs to invadopodia, increasing protease activity and promoting cellular invasion [56, 57]. In addition to structural and proteolytic functions, TKS proteins regulate reactive oxygen species (ROS) at the invadopodial membrane. They localize NADPH oxidase (NOX) to invadopodia, where ROS are compartmentalized to prevent damage from free radicals while acting as second messengers to regulate signaling pathways important for invadopodia activity and cancer cell invasion [58, 59].

Another member of the same protein family, TKS4, is also localized to invadopodia in Src-transformed cells, which plays an important role in their assembly. Studies on TKS4-deficient cells indicate that despite elevated TKS5 levels, these cells fail to degrade ECM components, suggesting that TKS4 is required for recruiting MT1-MMP to invadopodia [25]. Recent findings suggest another role of TKS4 in epithelial-mesenchymal transition (EMT)-like processes. In HCT116, colon cancer cells lacking TKS4, mesenchymal morphology, increased motility, and reduced cell–cell adhesion have been observed. Furthermore, these cells exhibit a loss of E-cadherin, disruption of apical-basal polarity, and upregulation of fibronectin and the transcription factor Snail2. However, the precise mechanism by which TKS4 deficiency induces EMT in these cells remains unclear [60].

Rho GTPases are core molecules

Rho GTPases are key regulators of cell migration, invasion, and invadopodia formation. Specifically, CDC42 is involved in the assembly of invadopodia precursors, while RhoA supports their maturation. Similarly, RhoC contributes to invadopodia development, RhoG plays a role in their degradation, and Rac1 promotes their formation [61]. Together, these GTPases coordinate invadopodia turnover.

A key Rho GTPase, CDC42, plays a central role in both invadopodia formation and ECM degradation, remaining active throughout the invadopodia lifecycle [62]. During assembly, CDC42 activity peaks, driving actin core formation, which serves as the structural foundation of invadopodia. However, reduced CDC42 activity is observed during disassembly, contributing to invadopodia breakdown. This dynamic regulation is mediated by the effector protein CIP4, which links active Rho GTPases to the signaling terminator ARHGAP17 in a phosphorylation-dependent manner. ARHGAP17 localizes to the invadopodium ring in the early stages, restricting CDC42 activity to the actin core. As invadopodia transitions to ECM degradation, ARHGAP17 shifts to the core, inactivating CDC42 and initiating disassembly [63]. Cooperation between CDC42 and RhoA enhances ECM degradation. This interaction is facilitated by the effector IQGAP1, which directs the delivery of MMPs to the invadopodial membrane. Through its association with the exocyst complex, IQGAP1 ensures efficient ECM degradation, promoting tumor cell invasion [64].

RhoC is crucial for invadopodia development. The spatiotemporal activation of RhoC is confined to the region surrounding the invadopodium actin core, where it regulates cofilin phosphorylation through the ROCK/LIMK pathway [65]. This localized activation directs cofilin's severing activity to the invadopodium core, promoting the formation of free barbed ends necessary for actin polymerization and cofilin turnover. These processes generate the protrusive force required for invadopodia extension [66]. Meanwhile, RhoG mainly plays a degradative role in the dynamic regulation of tumor cell invasion. While Rac1 supports invadopodia assembly, RhoG promotes disassembly. This process involves the activation of RhoG by the guanine nucleotide exchange factor SGEF (ARHGEF26). Active RhoG enhances paxillin phosphorylation, stimulating invadopodia disassembly. Silencing RhoG or SGEF increases invadopodia stability [67]. During tumor invasion, RhoG promotes the degradation of invasive pseudopodia, not to suppress invasion but to facilitate invadopodia turnover, thereby accelerating tissue invasion.

Regulation of invadopodia by membrane-mediated signaling and dynamics

The plasma membrane of tumor cells functions as a critical interface between the cell and its surrounding microenvironment, playing a central role in regulating invasion and metastasis. Membrane receptors on the cell surface mediate signal transduction, coordinating the complex processes involved in tumor cell migration and invadopodia formation.

The cell surface signal transduction mechanism regulates invadopodia

The cancer cell surface contains receptors that activate signal transduction mechanisms upon ligand binding, facilitating communication between tumor cells and their microenvironment [68]. Tumor cells regulate invadopodia formation by sensing and processing these signals. Various mechanisms governing invadopodia formation and function are illustrated in Fig. 3. Receptor-ligand binding transmits signals through autocrine or paracrine mechanisms, triggering intracellular cascades that converge on key pathways involved in invadopodia formation, including Src, PI3 K, and Rho family GTPases. Tumor cells must integrate receptor-mediated signaling for environmental sensing and migration while coordinating actin remodeling in a temporally and spatially controlled manner to facilitate invadopodia formation. This section examines the major receptor families and signaling pathways implicated in this process across different tumor types.

Integrins

Integrins serve as the primary cellular receptors for ECM components, including collagen, laminin, fibronectin, and vitronectin [69, 70]. These receptors play a central role in mediating cell-ECM interactions, which are essential for various cellular processes. The integrin family consists of alpha and beta subunits that form 24 distinct heterodimers [71], each characterized by specific ligand-binding properties and biological functions.

Integrins perform several fundamental roles in cellular biology. They function as principal adhesion receptors, anchoring cells to ECM components. Upon engagement with the ECM, integrins recruit specific signaling, scaffolding, and cytoskeletal proteins to adhesion sites. This recruitment activates intracellular signaling pathways, facilitating cancer cell invasion and metastasis [72]. Further, integrins act as mechanosensors, detecting and responding to changes in the tumor microenvironment (TME), thus regulating signal transduction and cellular adaptation. Among integrins,

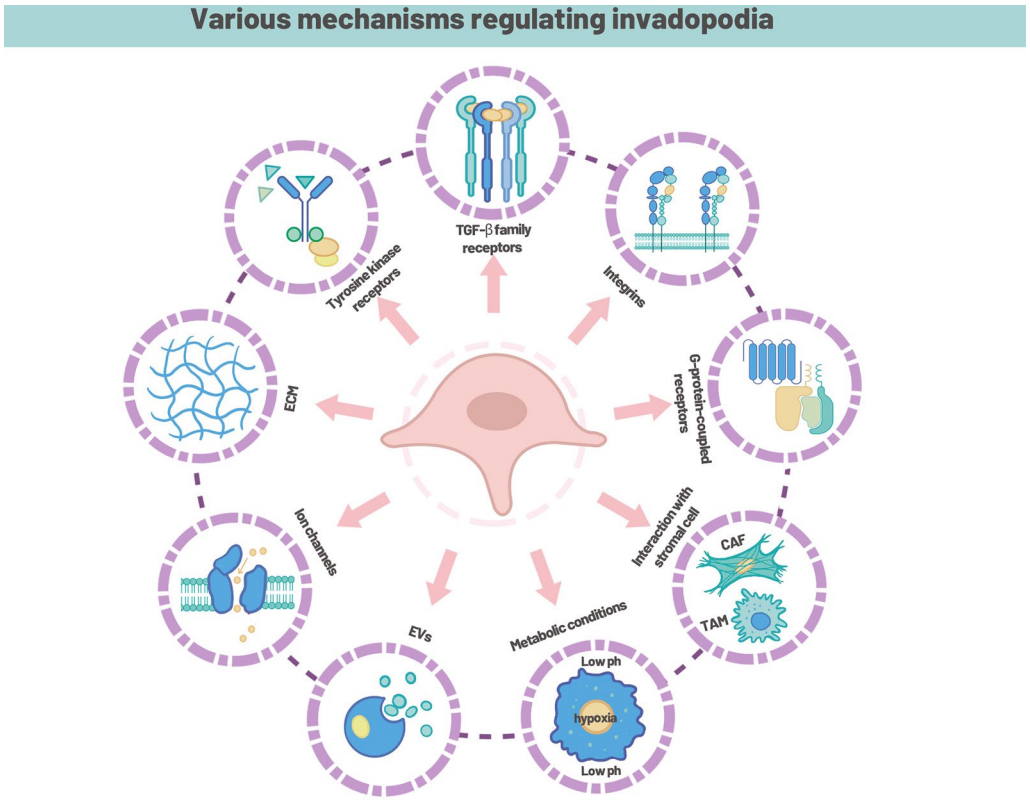

Fig. 3 Mechanisms regulating invadopodia formation and function. This diagram illustrates the complex mechanisms regulating invadopodia, specialized cellular structures implicated in extracellular matrix (ECM) degradation. Key factors include (1) Tyrosine kinase receptors, such as EGFR, which activate downstream signaling pathways to promote invadopodia formation; (2) TGF-β family receptors, which regulate cellular invasion and motility; (3) Integrins, which mediate adhesion to ECM components and regulate cytoskeletal dynamics; (4) G-protein-coupled receptors (GPCRs), which contribute to cytoskeletal remodeling; (5) Stromal interactions, where cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) provide supportive signals; (6) Metabolic conditions, including hypoxia and low pH, which increase invadopodia activity; (7) Extracellular vesicles (EVs), which facilitate intercellular communication; (8) Ion channels, which maintain intracellular ionic homeostasis; and (9) ECM components, which serve as both structural substrates and regulators of invadopodia dynamics. These interconnected mechanisms collectively drive invadopodia-mediated ECM degradation and tumor invasion

β1-integrin is the most extensively studied in the context of invadopodia.

The functions of β1-integrin subtypes vary across cancer types. In glioblastoma, α2β1, α3β1, α5β1, and α6β1 integrins promote tumor cell invasiveness [73]. In melanoma, α3β1, α5β1, and α6β1 integrins regulate invadopodia function through interactions with fibroblast activation protein (FAP), fibronectin, and laminin [17]. In breast cancer, β1-integrin interacts with either α2 or α5 integrins, depending on the microenvironmental context [74]. Moreover, the hypoxia-induced carbonic anhydrase IX (CAIX)-β1 integrin axis has been implicated in invadopodia formation and ECM degradation, reinforcing the role of β1-integrin in tumor invasion and metastasis [75]. Integrins are involved throughout the different stages of invadopodia formation. During the initiation phase, β1-integrin interacts with the tyrosine kinase Arg, leading to cortactin phosphorylation, which promotes invadopodia formation and maturation [28, 76]. In the stabilization phase, α5β1-integrin enhances cell adhesion to the ECM and supports actin polymerization, maintaining invadopodia structural stability [77]. In the functional execution phase, β1-integrin activation enhances ECM degradation [78, 79]. Specifically, β1-integrin regulates Na^+/H^+ exchanger 1 (NHE1), which acidifies the extracellular environment surrounding invadopodia, therefore facilitating ECM protein degradation [80]. Moreover, β1-integrin mediates the targeted transport of MMPs, including MMP2, MMP9, and MT1-MMP, to invadopodia, enhancing their degradative activity [81]. The resulting extracellular acidification strongly correlates with tumor progression, highlighting the central role of β1-integrin in tumor invasion [82].

Integrins also contribute to key signaling pathways regulating cell invasion. It has been shown that when *RAD23B* is knocked down, CRC cells' migration,

invasion, and metastasis are impaired. This occurs due to suppressing the integrin signaling pathway, inhibiting Rac1 and CORO1 C localization at the cell edge, and suppressing invasive protrusions and invadopodia [83]. Integrins are highly sensitive to ECM stiffness. A rigid ECM promotes integrin activation and the recruitment of focal adhesion proteins, which aid in forming adhesion plaques [84, 85]. These focal adhesions contain integrin-associated proteins, such as vinculin, paxillin, and Rho GTPases, which play critical roles in cytoskeletal organization and the assembly of invadosomes and lamella [86, 87]. Integrins also collaborate with growth factors and G protein-coupled receptors to promote cell invasion and migration. Recent studies highlight the mechanosensitive role of CAV1/caveolae in collagen fiber recruitment, highlighting their contribution to invadopodia formation. The topology and composition of these specialized plasma membrane domains are precisely regulated to support their function. CAV1/caveolae mediate the inward deformation of the plasma membrane and integrin binding, both of which are essential for invadopodia assembly. The proteolytic cleavage of collagen fibers further regulates invadopodia formation through the invadopodia-MT1-MMP axis, which controls the endocytosis of caveolae-dependent β1-integrin [88]. As tumor cells migrate through dense ECM and encounter newly formed collagen fibers, invadopodia and caveolae undergo dynamic recycling. MT1-MMP is recycled back to the plasma membrane, reinforcing invadopodia formation and improving invasive activity [6, 89, 90].

These findings suggest that invasive cancer cells exploit key membrane subdomains, such as caveolae, invadopodia, and clathrin-coated pits, to support matrix remodeling and tumor invasion. The coordinated activity of these subdomains highlights the caveolae-invadopodia axis as a critical regulator of cellular invasion. Importantly, this axis represents a promising therapeutic target for interventions to control tumor cell invasion.

G protein-coupled receptors

G protein-coupled receptors (GPCRs) are heptahelical membrane proteins that regulate cell morphology, adhesion, and migration by activating heterotrimeric G proteins [91]. Lysophosphatidic acid (LPA) receptors, a subset of GPCRs, are critical for invadopodia formation. Autotaxin, an enzyme involved in LPA production, promotes invadopodia formation and enhances fibrosarcoma cell invasion and metastasis by activating the LPA4 receptor and the Rap1/Rac1 signaling pathway, regulating the downstream effector WASP [92]. In ovarian cancer, LPA signaling facilitates invadopodia assembly by promoting Gαi2 translocation, where it interacts with β-pix and Src [93]. This pathway modulates CDC42 and RhoA activity in melanoma, further supporting invadopodia formation [94].

Endothelin-1 (ET-1) signals through its receptors, ETA and ETB, to regulate invadopodia assembly and facilitate matrix degradation during cancer cell invasion and metastasis [95]. This process requires precise coordination of Rho GTPases [96]. ET-1 induces invadopodia assembly in melanoma cells by activating CDC42 through Gi signaling while inhibiting RhoA activity [94]. In ovarian cancer, ET-1-mediated invadopodia formation involves a molecular complex coordinated by β-arrestin 1 (β-arr1) [97], whereas in breast cancer, the activation of the GPCR Kisspeptin receptor (KISS1R) promotes invadopodia assembly through β-arrestin 2 (β-arr2) and the ERK1/2 signaling pathway, with signaling crosstalk between KISS1R and EGFR [98].

Chemokine receptors, including CXCR4 and CCR3, also regulate invadopodia formation [99]. CXCR4 activation by SDF1α in breast cancer stimulates Abl kinase, facilitating MT1-MMP transport to invadopodia and enhancing ECM degradation [100]. The CXCL12/CXCR4 axis also regulates cortactin phosphorylation via Arg in glioma cells, controlling invadopodia formation and invasion [101]. In lung cancer, CCL7 increases MMP-9 transport to invadopodia via CCR3, promoting collagen degradation and ECM invasion, contributing to metastasis [102]. These GPCR-mediated pathways play a key role in tumor metastasis.

Tyrosine kinase receptors

Receptor tyrosine kinases (RTKs) constitute a large family of proteins that regulate cancer cell growth, proliferation, survival, invasion, and metastasis [103]. Activation of specific RTKs triggers downstream signaling cascades that integrate with signals from the TME, collectively promoting invadopodia formation. Among these receptors, the EGFR is a key regulator of invadopodia formation in various cancer cell lines, exerting its effects primarily through ligand-dependent signaling. EGF-induced activation of EGFR increases actin polymerization via the N-WASP-Arp2/3-cofilin axis, facilitating invadopodia assembly [104, 105]. EGFR signaling promotes invadopodia maturation through Src activation and cortical actin phosphorylation [106, 107]. Amplified EGFR signaling may involve crosstalk with other cell surface receptors, including CD44, CD147, CDC42, MET, and the KISS1R, forming a network that collectively regulates invadopodia formation [108].

Platelet-derived growth factor (PDGF) and its receptors are also critical regulators of invadopodia formation, particularly in breast and pancreatic cancers [109, 110]. In breast cancer, the EMT-related transcription factor Twist1 promotes invadopodia formation by upregulating

PDGFRα expression, which activates Src signaling, facilitating ECM degradation and enhancing tumor cell migration and metastasis in vivo [111]. In pancreatic cancer, PDGF signaling, combined with genetic mutations such as β-catenin activation, *KRAS* mutations, and p53 loss, significantly enhances the invasive capacity of tumor cells. These aberrations trigger autocrine PDGF signaling, significantly improving invadopodia formation and ECM degradation, thereby increasing tumor cell invasiveness [110].

TGF-β family receptors

TGF-β family members regulate various cellular functions by binding to type I and type II receptor transmembrane kinases, initiating downstream signaling cascades through both Smad2/3-dependent and non-Smad2/3-dependent pathways [112]. TGF-β plays an important role in tumor progression by activating transcription factors involved in EMT, including Snail, Slug, ZEB1, ZEB2, and Twist, therefore enhancing cancer cell invasiveness and migration [113, 114]. In bladder cancer, TGF-β has been implicated in regulating the interplay between EMT and invadopodia formation. This occurs through the induction of Transgelin expression, a myosin-binding protein that modulates the actin cytoskeleton. Transgelin regulates actin dynamics and promotes tumor cell invasion and metastasis [115].

In breast cancer, TGF-β regulates the activity of the FAK-Src signaling pathway by interacting with focal adhesion components, such as Hic-5. This interaction promotes matrix degradation by activating Rac1 and RhoC-ROCK signaling pathways [116]. Moreover, both the lipoma-preferred partner (LPP) and SHC adaptor protein (SHCA) have been identified as critical mediators in TGF-β-induced invadopodia formation. These proteins play a vital role in tumor cell migration and metastasis [117, 118]. In triple-negative breast cancer (TNBC), the natural compound Isotoosendanin, which targets TGF-β signaling, significantly inhibits EMT and invadopodia formation, effectively reducing TNBC cell invasiveness and metastasis [119]. These findings highlight the central role of TGF-β in breast cancer invasion and metastasis, highlighting its potential as a therapeutic target.

Ion channels

Calcium and sodium ion channels play critical roles in regulating intracellular Ca^{2+} and Na^{+} concentrations, which are essential for invadopodia formation, ECM degradation, and tumor cell migration [120, 121]. These ion channels and their associated signaling pathways regulate Src kinase activity, invadopodia assembly, and the secretion of ECM-degrading enzymes [122].

The TRPM7 channel, located in invadopodia, regulates actin dynamics by establishing a Ca^{2+} gradient across the cell membrane. TRPM7 functions as an ion channel and a kinase, possessing a serine/threonine kinase domain. This kinase domain regulates actin polymerization and dynamics through the phosphorylation of G-protein signaling 1 and regulatory factors involved in regulating MHC-II subtypes A–C [123, 124]. Furthermore, calmodulin (CaM) is pivotal in regulating Src tyrosine kinase activity during tumor cell invasion. In response to EGF, CaM translocates from the nucleus to the cytoplasm, binding to Src and NHE1. This interaction promotes invadopodia formation and enhances chemotaxis [125, 126].

The voltage-dependent calcium channel CaV2.2 plays a crucial role in stabilizing cortactin through deubiquitinase USP43 [121], supporting invadopodia formation and increasing tumor invasiveness. Furthermore, stromal interaction molecule 1 (STIM1) and Orai1 facilitate invadopodia activity in melanoma cells by stimulating ECM degradation and calcium influx through store-operated calcium entry (SOCE). Activation of SOCE by STIM1 serves as a key regulator of calcium sensitivity and is essential for invadopodia formation [127].

In prostate cancer cells, Na^{+} influx through the Na^{+}/Li^{+}-coupled channel NALCN regulates intracellular calcium oscillations, which are co-regulated by SOCE, NCX, NCLX, SERCA, and ROS. This interplay leads to Src kinase activation, supports invadopodia formation, and facilitates the secretion of Ca^{2+}-dependent ECM-degrading enzymes. NALCN-mediated Na^{+} influx interacts with intracellular Ca^{2+} oscillations through the same co-regulatory network, further sustaining Src kinase activation and invadopodia formation while promoting the release of Ca^{2+}-dependent proteolytic enzymes [120].

Research on cell surface signal transduction mechanisms provides a solid foundation for developing clinical strategies that target invadopodia. A deeper understanding of these signaling pathways allows the identification of novel therapeutic targets and the development of specific drugs. These targeted therapies can be combined with conventional treatments such as radiotherapy or chemotherapy, offering the potential to improve tumor metastasis control and advance overall cancer treatment outcomes and patient prognosis.

Regulation of invadopodia by dynamic changes in the cell membrane

Invadopodia are protrusive extensions of the tumor cell membrane, and their formation is closely associated with membrane integrity and fluidity. Cholesterol is essential for maintaining the structural stability and fluidity of animal cell membranes, both of which are essential for invadopodia function [128]. Disruptions in cholesterol metabolism can alter membrane properties, affecting the formation of lipid rafts and invadopodia. These changes significantly affect the invasive and metastatic potential of tumor cells [129].

Estrogen-related receptor α (ERRα) has been shown to regulate endothelial cell migration and invasion by reprogramming intracellular cholesterol metabolism. This occurs through the activation of hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), which increases membrane fluidity, promoting invadopodia formation and contributing to EMT [130]. Hydroxyacyl-CoA dehydrogenase subunit alpha (HADHA) catalyzes the final steps of long-chain fatty acid β-oxidation in the mitochondria [131]. Recent studies have demonstrated that HADHA is regulated by miR-612, where miR-612 suppression leads to HADHA upregulation and subsequent activation of fatty acid β-oxidation. Through the SREBP2/HMGCR cascade, this process produces sufficient acetyl-CoA and ATP to support cholesterol biosynthesis, facilitating invadopodia formation. Moreover, miR-612 and HADHA interact dynamically with cortactin and caveolin-1 to remodel the F-actin cytoskeleton in HCC, thus increasing invadopodia formation [132].

A deeper understanding of how tumor cells detect and respond to changes in the microenvironment through the plasma membrane and membrane receptors is essential. These processes regulate invadopodia formation and invasive tumor cell migration. Various factors in the TME, including mechanical forces, cell–cell interactions, and metabolic changes, affect membrane composition and receptor activity, collectively shaping tumor cell invasiveness. Investigating these regulatory mechanisms provides important insights into tumor biology and enables the identification of invadopodia-specific regulatory pathways across different cancer types. Moreover, these findings offer potential therapeutic targets, which will be further discussed in subsequent articles was written here.

MMPs facilitate invadopodia-mediated matrix degradation

Zinc-dependent endopeptidases, such as MMPs, are involved in the formation of pre-metastatic niches. Tumor metastasis not only depends on the characteristics of cancer cells that spread from the primary tumor but also requires the establishment of a favorable microenvironment in distant organs for tumor cell growth, known as the metastatic niches [133]. Meanwhile, The core function of MMPs is the degradation and remodeling of the ECM, which paves the way for invasion and metastasis through peripheral tissues. They primarily mediate the function of invadopodia. The maturation of invadopodia requires the targeted delivery and exocytosis of MMP2, MMP9, and MT1-MMP [134]. The appearance of these MMPs is often regarded as a marker of functionally mature invadopodia. It has been shown that MT1-MMP specifically targets invadopodia and plays a central role in matrix degradation [6]. Extracellular vesicles (EVs) are widely recognized for their role in intercellular communication and the regulation of physiological processes through molecular transport. Based on previous research on vesicles and exosomes [135–138], this paper will explore the loading, transport, and release of MMPs from this perspective. The transport pathway of MMPs is depicted in Fig. 4.

Targeted transport to invadopodia

Invadopodia serve as specialized sites for the accumulation and release of proteases, including MMPs [139]. Studies have demonstrated that MT1-MMP plays a key role in invadopodia formation and undergoes intracellular transport through vesicular trafficking mechanisms. One well-characterized pathway involved in this process is the SNARE complex, a family of membrane-associated proteins that facilitate membrane fusion by bridging adjacent membranes [140]. The formation of invadopodia and subsequent tumor cell invasion depends on SNARE-mediated protein transport. This process not only allows ECM degradation but also contributes to increased cell migration [141–145]. The SNARE complex consists of v-SNARE proteins, such as VAMP, which are located on vesicular membranes, and t-SNARE proteins, including syntaxin and SNAP-25, which are present on target membranes [146]. These SNARE proteins are essential for facilitating membrane fusion during the release of EVs and are also involved in regulating the intracellular trafficking of MT1-MMP. VAMP3, for instance, has been identified as a key regulator of vesicular cargo transport, specifically mediating the transfer of MT1-MMP from the cell surface to newly forming vesicles [147]. VAMP7 is essential for directing MT1-MMP to invadopodia, highlighting its role in the spatial organization of these MMPs during invasive processes [141]. Recent studies have identified TOM1L1 as a key regulator of MT1-MMP translocation to invadopodia in ERBB2-driven breast cancer cells [148]. TOM1L1 functions as an important downstream target of the MAPK signaling pathway and has been shown to regulate its serine phosphorylation in

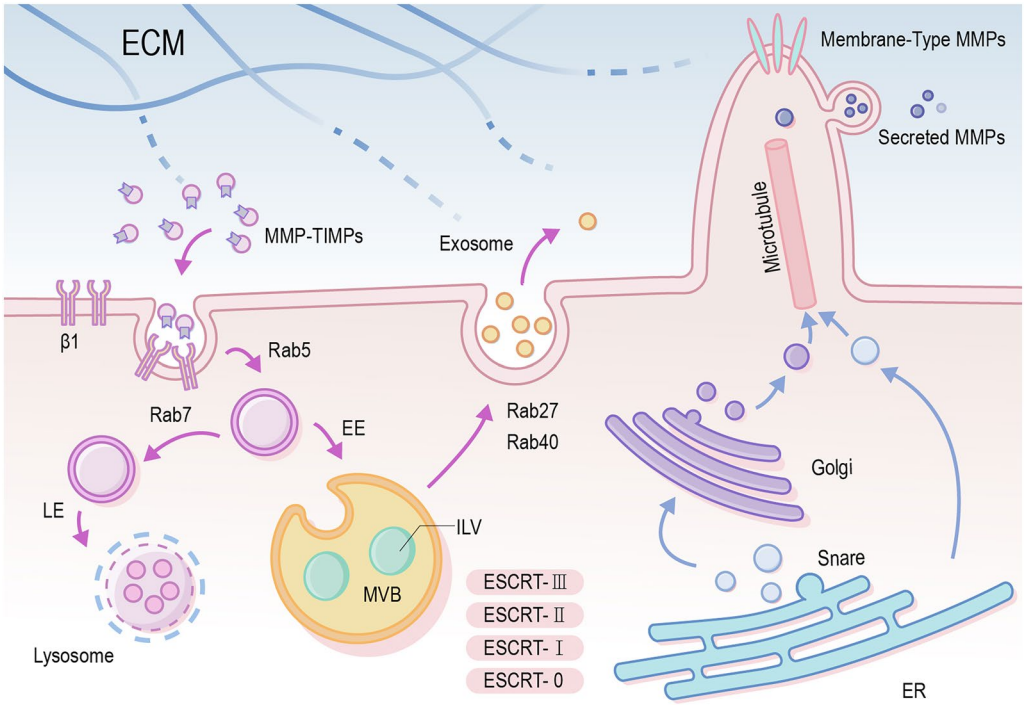

Fig. 4 The Transport Pathway of matrix metalloproteinases (MMPs). The right-side blue-arrow pathway depicts the synthesis, maturation, and secretion of MMPs. MMPs are synthesized in the endoplasmic reticulum (ER) and transported to the Golgi apparatus for maturation. Mature MMPs are then trafficked along microtubules to the plasma membrane via SNARE protein-mediated vesicular transport, where they facilitate extracellular matrix (ECM) degradation. A subset of MMPs remains anchored as membrane-type MMPs at the invadopodial membrane, directly exerting proteolytic activity. Another subset is secreted into the tumor microenvironment, contributing to ECM remodeling and tumor invasion The left-side purple-arrow pathway represents the endocytic recycling of MMPs. MMP activity at the plasma membrane is regulated by β1 integrin-mediated endocytosis. Internalized MMPs initially enter early endosomes (EE) and are then sorted into late endosomes (LE) under the regulation of Rab5 and Rab7. Within late endosomes, MMPs are either transported to lysosomes for degradation or directed into the multivesicular body (MVB) pathway. Through the endosomal sorting complex required for transport (ESCRT) machinery, MMPs are incorporated into intraluminal vesicles (ILVs). Finally, ILVs containing MMPs are released into the tumor microenvironment as exosomes, facilitating distant ECM degradation and enhancing tumor cell invasiveness

melanoma, controlling the precise localization of MT1-MMP at invadopodia [149].

In oncology, the interaction between invadopodia and MMPs is highly synergistic and important to tumor invasiveness and metastatic potential. This collaboration allows tumor cells to degrade the basement membrane, invade blood vessels and lymphatics, and eventually form distant metastases. A deeper understanding of the molecular mechanisms underlying this process is essential for identifying therapeutic targets and developing novel strategies for cancer treatment.

Facilitating invasion via the EVs pathway

Studies have demonstrated that EVs serve as key carriers of MMPs and play crucial roles in various cancer models. EVs derived from 8701-BC breast cancer cells and HT-1080 fibrosarcoma cells have been shown to contain enzymatically active MMP-9. Similarly, exosomes isolated from the ascites of ovarian cancer patients contain MMP-2 and MMP-9, both of which contribute to ECM degradation [150, 151]. Moreover, mature MMPs, such as MT1-MMP secreted by G361 melanoma cells and MMP-13 secreted by nasopharyngeal carcinoma cells, are highly enriched in EVs [152]. The quantity of EVs released, along with the levels of proteolytic enzymes they transport, correlates with the invasive potential of various cancer cell lines in vitro [153]. These findings highlight the key role of EVs in tumor progression. EV secretion is closely associated with invadopodia formation. Invadopodia serve as docking sites for multivesicular bodies (MVBs), and these structures are mechanistically associated with EV secretion. Recent studies have shown that MVBs, marked by CD63 and Rab27a, localize to invadopodia regions, reinforcing the spatial and functional relationship between these cellular components. Inhibition of key molecules involved in invadopodia formation, including TKS5, N-WASP, and cortactin, significantly reduces exosome secretion [16, 37, 154, 155]. Constitutive activation

of PI3 K induces invadopodia formation and improves EV secretion, suggesting a feedback loop that reinforces both processes [156]. The transport and activity of proteolytic enzymes via EVs have been well-documented, highlighting their clinical relevance in tumor progression. Comprehensive reviews by Thuault et al. [31], Shimoda M, and Khokha R [157, 158] have thoroughly detailed the underlying mechanisms and molecular interactions that govern these processes, providing insight into the complex interplay between invadopodia, EV secretion, and tumor progression, providing insight into the complex interplay between invadopodia, EV secretion, and tumor progression.

Incorporating transmembrane cargo into EVs primarily relies on endosomal sorting mechanisms [159]. The endosomal sorting complex required for transport (ESCRT) plays a fundamental role in the formation of intraluminal vesicles (ILVs) and MVBs. Initially, ESCRT-0 and ESCRT-I complexes recruit cargo to the limiting membrane of the endosome. Then, ESCRT-II and ESCRT-III facilitate membrane budding and scission, leading to ILV formation [160, 161]. The classical ESCRT pathway is regulated through interaction between syntenin and the ESCRT-associated protein ALG-2 interacting protein X (ALIX, also known as programmed cell death 6-interacting protein). This interaction bridges cargo to the ESCRT-III subunit VPS32 (CHMP4), promoting membrane remodeling and ensuring efficient cargo sorting [162].

Recycling of MMPs supports invasion

MT1-MMP is partially recycled and relocalized to invadopodia, where it supports their invasive functions, while the remaining MT1-MMP is packaged into EVs to be secreted into the extracellular space. This secretion contributes to ECM remodeling and signal transduction [152]. Approximately 80% of internalized MT1-MMP is recycled, and its dynamic balance on the plasma membrane is maintained primarily through the coordinated regulation of endocytosis and exocytosis.

Specific signals within the intracellular C-terminus of MT1-MMP are essential for its targeted delivery during molecular sorting [163]. The PDZ-binding motif facilitates recycling by interacting with the sorting protein SNX27, which recruits the retrograde complex to Rab7a-positive endosomes, allowing the recycling of MT1-MMP to invadopodia [164]. Although MT2-MMP contains a class III PDZ-binding motif (EWV), it does not interact with SNX27, highlighting the specificity of this recycling mechanism [164, 165]. Studies have reported that cancer cells primarily rely on the endosome/lysosome recycling pathway to transport internalized MT1-MMP back to the plasma membrane [37, 90, 148]. β1-integrin plays an important role in this process by facilitating MT1-MMP internalization through phosphorylation of Thr residues, leading to its co-internalization with MT1-MMP into Rab5-positive early endosomes. MT1-MMP is then transported to Rab7-positive late endosomes, where it becomes available for invadopodia formation [166]. Chloride intracellular channel 3 (CLIC3) interacts with Rab25 to regulate the recycling of α5β1-integrin from late endosomes to the plasma membrane, thus promoting the invasiveness of pancreatic and ovarian cancer cells [167].

Blocking SOCE does not affect the endocytosis of MT1-MMP but significantly disrupts its post-endocytic recycling to the plasma membrane. This disruption leads to the accumulation of MT1-MMP in Rab5-positive early endosomes [168]. The proteolytic activity of MT1-MMP is often associated with its interaction with tissue inhibitors of metalloproteinases 2 (TIMP2) [169]. Disruption of the pro-metastatic gene *NEDD9* alters the distribution of MT1-MMP across different endosomal compartments, impairing its recycling pathway. As a result, this disruption interferes with the targeted delivery of the TIMP2/MT1-MMP complex to late endosomes and increases the activity of ARF6, a small GTPase that regulates MT1-MMP recycling [170]. Furthermore, apurinic/apyrimidinic endonuclease 1 (APE1) interacts with ARF6 in a redox-dependent manner, regulating MT1-MMP trafficking. APE1 prevents excessive internalization of MT1-MMP, ensuring its proper relocalization to the plasma membrane [171]. These regulatory mechanisms are vital for tumor cell invasion, as they control the cycling, endocytosis, and recycling of MT1-MMP, all of which are essential for tumor cell migration and metastasis.

The research on developing anticancer therapies targeting invadopodia

Growing evidence suggests that invadopodia plays a pivotal role in cancer invasion and metastasis. This highlights the potential of targeting their regulatory factors as a promising strategy for treating malignant cancers. The formation of invadopodia is regulated by multiple factors, as discussed earlier.

Table 2 provides a systematic overview of the major regulatory factors involved in the formation and function of invadopodia, including actin-remodeling proteins, adaptor molecules, receptor tyrosine kinases, intracellular kinases, small GTPases, stromal interactions, tumor microenvironmental cues, and functional proteases. These components coordinate cytoskeletal remodeling, signal transduction, and matrix degradation, collectively driving invadopodia biogenesis and the acquisition of invasive capacity. Notably, several regulators—such as cortactin, Tks5, EGFR, SRC, and MMPs—are frequently dysregulated in metastatic tumors and correlate with enhanced invasiveness and poor prognosis, highlighting

Table 2 Various regulatory factors of invadopodia

Type	Main components	Research
Actin regulatory proteins	Cortactin	miR-182 inhibits invadopodia formation by targeting cortactin [172] LanCL2 activates the STAT3/Cortactin signaling pathway to promote invadopodia formation [173]
	N-WASP	miR-182 expression inhibits invadopodia formation by suppressing the Cdc42/N-WASP pathway [172] LMP1 promotes invadopodia formation by activating the Cdc42/N-WASP signaling axis [174]
	Arp2/3	Pimozide inhibits invadopodia formation by targeting subunits of the Arp2/3 complex [175]
	Formin	Downstream targets of the Wnt5a/Dvl2 pathway, Formin, and Fascin, synergistically promote actin assembly in invadopodia [176]
	Actin	Targeting actin inhibits cancer cell motility [177]
	Fascins	Fascin influences invadopodia formation through metabolic pathways [178]
	Cofilin	The interaction between cofilin and Rhoc leads to cofilin phosphorylation, affecting invadopodia formation [66]
Adaptor proteins	Tks5	The MAP1B-cortactin-Tks5 axis regulates invadopodia formation [179]
	Paxillin	The kindlin-3-leupaxin-paxillin signaling pathway regulates invadopodia stability [180]
Receptor protein	TGF-β	TGF-β induces EMT and invadopodia formation [115] Targeting TGF-β inhibits invadopodia formation [119]
	EGFR	The EGFR-Src-Arg-cortactin pathway mediates invadopodia maturation [106]
	PDGF	Increased levels of PDGF/phospho-Src promote invadopodia formation and enhance MMP activity [110]
	MET	Met phosphorylation of Fis1 Tyr38 promotes mitochondrial fission and affects invadopodia formation [181]
Kinases	ABL	The downstream Abl signaling of CXCR4 plays a role in invadopodia formation and function [100]
	SRC	ERβ promotes invadopodia formation through the ICAM1/p-Src/p-Cortactin signaling pathway [182]
	PTK2B	Pyk2 regulates invadopodia formation in breast cancer cells [183]
	FAK	EB1 restricts invadopodia formation and matrix protein degradation in breast cancer cells through FAK [184]
	ERK	ERK promotes invadopodia formation by activating cortactin [185]
	PAK	The PAK1/Cortactin pathway promotes invadopodia turnover and invasion [186]
GTPases	CDC42	The CDC42/N-WASP/Arp2/3 signaling pathway regulates invadopodia formation [187]
	Rho/Rac	Rho-Rac signaling regulates invadopodia sensing and formation [188]
	Dynamin	Dynamin-2 enhances the rigidity of actin bundles in invadopodia [189]
	ARF6	The novel ARF6-PI3 K-AKT pathway promotes invadopodia formation [190]
Interaction with stromal cell	CAF	CAF synergistically promotes invadopodia-mediated migration and invasion in oral squamous cell carcinoma [191]
	TAM	TAM interaction with cancer cells regulates the Notch1/Mena INV signaling pathway to promote invadopodia formation [44]
Tumor microenvironment	PH	CAIX regulates pH and affects the function of invadopodia [192]
	Hypoxic	The hypoxic tumor microenvironment promotes invadopodia formation through the collaboration of the LPA1 receptor and EGFR [105]
	Matrix environmental	The extracellular matrix regulates invadopodia formation [193]
Functional protease	MMPs	Research on the close association between invadopodia and MMPs [6, 7, 194, 195]

their translational potential as diagnostic or therapeutic targets [9]. Dissecting this regulatory landscape may reveal vulnerable nodes for therapeutic intervention. Precision targeting of invadopodia-related pathways may offer a strategy to suppress metastatic dissemination while sparing normal cellular functions, thus presenting a compelling opportunity for translational anti-metastatic therapy.

To identify proteins that could serve as therapeutic targets for regulating invadopodia function, the study by Meirson et al. [9] provides a comprehensive list of protein families that may be inhibited by therapeutic drugs, including GPCRs, ion channels, receptors, non-receptor tyrosine kinases, phosphatases, transporters, cytokines, growth factors, and proteases. These findings offer valuable insights for further research on the regulatory mechanisms of invadopodia and their

potential drug targets. Building on this, we further summarize the key therapeutic strategies targeting the genes involved in the positive regulation of invadopodia, along with the relevant signaling pathways implicated in these processes. As summarized in Table 3, multiple genes positively regulate invadopodia formation, and several therapeutic agents have been developed to target these regulatory factors. These include upstream signaling molecules, such as EGFR and Src family kinases, cytoskeletal scaffolds like Tks5 and Cortactin, and proteolytic enzymes, including MMP2 and MMP9. Targeting these key regulators through inhibition may provide novel therapeutic strategies to disrupt invadopodia-driven metastasis.

The inhibition of invadopodia formation has emerged as a promising therapeutic avenue to suppress tumour invasion and metastasis. Pharmacological agents targeting key signalling pathways and proteolytic enzymes involved in invadopodia dynamics have shown encouraging results in preclinical and early clinical settings [9]. Notably, the EGFR tyrosine kinase inhibitor erlotinib and the Src family kinase inhibitor dasatinib have been demonstrated to effectively suppress invadopodia formation and tumour cell invasiveness across multiple cancer models [106, 210]. Dasatinib markedly inhibits invadopodia assembly, reduces metastatic potential, and sensitizes tumour cells to chemotherapy. Phase II clinical trials in solid tumours, including HER2-positive breast cancer, have reported delayed disease progression in a subset of patients, although overall efficacy remains variable [211]. Erlotinib has shown robust anti-invasive effects in both in vitro and in vivo models of head and neck squamous cell carcinoma and triple-negative breast cancer, primarily through attenuation of matrix degradation capacity and cellular motility [212, 213]. At the level of proteolytic regulation, Marimastat,

Table 3 Therapeutic strategies targeting invadopodia regulatory

Molecular target	Representative Drug(s)	Related pathway	Putative impact on invadopodia
ABL2	imatinib, nilotinib, dasatinib	TGFβ1/Smad signaling pathway	Inhibition of invadopodia maturation [196]
AKT2	triciribine	PI3 K/AKT2 signaling pathway	Inhibition of invadopodia formation [132]
BRAF	sorafenib, vemurafenib, dabrafenib	Ca2 +/CAM-PYK2 signaling pathway	Inhibition of invadopodia formation [197]
CXCL12	NOX-A12	Jak/Vav/Rho GTPase signaling pathway	Inhibition of MMP activity in invadopodia [198]
CXCR4	POL6326, BL-8040, burixafor	SDF1α/CXCR4 signaling pathway	Inhibition of MMP-mediated stimulation of invadopodia [100]
EGFR	cetuximab, erlotinib, panitumumab, tesevatinib, nimotuzumab	EGFR-Src-Arg-cortactin signaling pathway	Inhibition of invadopodia maturation [106]
ERBB2	trastuzumab, varlitinib, tesevatinib, afatinib, pertuzumab,	Tyrosine kinases signaling pathway	Inhibition of MMP trafficking to invadopodia [148]
ITGB3	abciximab, cilengitide	Fak and Src signaling pathway	Inhibition of invadopodia formation, maturation, and MMP activation [199]
KRAS	AZD4785	Ral effector signaling pathway	Inhibition of invadopodia formation [200]
MAPK1	MAP kinase1 inhibitor, binimetinib, ulixertinib	K-Ras/MAPK/ERK2/MMP signaling pathway	Inhibition of MMP transcription, protein abundance, and enzymatic activity [201]
MAPK8	aplidine	MAPK signaling pathway	Inhibition of invadopodia formation [202]
MET	crizotinib, tivantinib, cabozantinib, amuvatinib	c-Met/LanCL2/STAT3/Cortactin signaling pathway	Inhibition of invadopodia formation [203]
MTOR	ridaforolimus, dactolisib, vistusertib, apitolisib	PI3 K/Akt/mTOR signaling pathway	Inhibition of invadopodia formation and matrix degradation [204]
MMP14	rebimastat, marimastat, prinomastat	ARL4 C-IQGAP1-MMP14 signaling pathway	Inhibition of invadopodia maturation and matrix degradation [205]
NFKB1	triflusal, thalidomide	NF-kB signaling pathway	Inhibition of invadopodia formation [206]
NOTCH1	OMP-52M51	Notch1/Mena INV signaling pathway	Inhibition of invadopodia formation [44]
PDGFR	dasatinib, sunitinib, pazopanib, axitinib, tivozanib	PDGFRα-La/SSB-LAMB1 signaling pathway	Inhibition of invadopodia formation [207]
PDK1	dichloroacetic acid	PDK1-AKT signaling pathway	Inhibition of invadopodia formation [156]
PIK3 CA	dactolisib, pictilisib, buparlisib	PI3 K-AKT signaling pathway	Inhibition of invadopodia formation [208]
SRC	dasatinib, saracatinib, nintedanib	EGFR-Src-Arg-cortactin signaling pathway	Inhibition of invadopodia formation and maturation [106]
STAT3	OPB-31121	STAT3/Cortactin signaling pathway	Inhibition of invadopodia formation [203]
TGFB1	dalantercept	ERK signaling pathway	Inhibition of invadopodia formation [209]

Table 4 Clinical trials evaluating the anti-metastatic inhibition of invadopodia-targeted drugs

Drug	Gene	Cancer type	Interference	Clinical trial (NCT number)
Aflibercept	*EGFR ERBB2 ERBB3 ERBB4*	Colorectal Cancer	Aflibercept + Oxaliplatin + 5-Fu + Folinic acid Aflibercept + Folfiri	NCT00851084 NCT00561470
		Prostatic Cancer	Aflibercept + Docetaxel + Prednisone or Prednisolone	NCT00519285
Bevacizumab	*VEGFA*	Breast Cancer	Abraxane + Bevacizumab Carboplatin + Bevacizumab + Herceptin	NCT00281528 NCT01004172
		Colorectal Cancer	Pembrolizumab + Bevacizumab + Binimetinib 5-Fluorouracil + Bevacizumab + Leucovorin + Oxaliplatin	NCT03475004 NCT00508872
		Esophagogastric Adenocarcinomas	Capecitabine + Oxaliplatin + Bevacizumab	NCT00447330
Cabozantinib	*MET*	Prostate cancer	Cabozantinib	NCT01834651
		Non small cell lung cancer	Cabozantinib	NCT02132598
Cetuximab	*EGFR*	Colorectal Cancer	Cetuximab Cetuximab + Oxaliplatin Cetuximab + Oxaliplatin + Capecitabine	NCT00083720 NCT00125034 NCT00444678
Dasatinib	*ABL2 PDGFRBSRC*	Breast cancer	Dasatinib Dasatinib + Zoledronic acid	NCT00371254, NCT00410813 NCT00566618
		Head and neck cancer	Dasatinib	NCT00507767
Erlotinib	*EGFR ERBB2*	Non small cell lung cancer	Erlotinib + Romidepsin	NCT01302808
		Esophageal Cancer	Erlotinib + 5-Fluorouracil + Leucovorin + Oxaliplatin	NCT00539617
Everolimus	*MTOR*	Breast Cancer	Letrozole + Lapatinib + Everolimus Everolimus + Vinorelbine + Trastuzumab	NCT01499160 NCT01305941
Sirolimus	*MTOR*	Prostate Cancer	Carboplatin + Docetaxel + Sirolimus	NCT02565901
Sorafenib	*BRAF PDGFRB*	Advanced or Metastatic Urothelial Cancer	Sorafenib Tosylate	NCT00112671
		Colorectal Cancer	Sorafenib + Mfolfox6	NCT00865709
Sunitinib	*PDGFRB*	Melanoma	Sunitinib Malate	NCT00462982
		Adenocarcinoma of the Gastroesophageal Junction	Sunitinib Malate + Capecitabine	NCT00891878

a broad-spectrum inhibitor of matrix metalloproteinases (MMPs), effectively inhibits the enzymatic activity of several MMPs, particularly those critically involved in invadopodia-driven extracellular matrix degradation. Despite initial promise in early-phase trials, marimastat's development was hampered by dose-limiting musculoskeletal toxicity. More than 50 MMP inhibitors have failed in clinical trials, largely due to suboptimal trial design, inappropriate clinical endpoints, and adverse toxicity profiles [214, 215]. These findings underscore the necessity for next-generation MMP inhibitors with improved selectivity and tumour-targeting capabilities. Collectively, these pharmacological interventions validate the therapeutic potential of targeting invadopodia-associated signalling pathways and enzymatic functions. With advances in molecular precision and drug delivery technologies, such strategies may complement existing anti-metastatic regimens and contribute meaningfully to the clinical management of solid tumours.

In principle, invadopodia inhibitors may effectively suppress cancer metastasis, potentially providing patients with an extended survival period and optimal surgical timing. However, according to the"grow or go"theory, cancer cell invasion and proliferation are negatively correlated, meaning these processes cannot occur simultaneously [216]. Thus, targeting invadopodia alone may not be sufficient for patients with metastatic disease to stop the growth of established metastatic lesions. This suggests that effectively treating metastatic cancer may require strategies that inhibit both tumor invasion and proliferation [217]. A combination of invadopodia inhibitors with cytotoxic therapies may provide an effective treatment approach by simultaneously reducing metastasis and controlling tumor proliferation. Inhibiting invadopodia formation may also increase the cytotoxic effects of chemotherapeutic drugs, therefore improving

therapeutic outcomes. Studies have shown that cancer cell extravasation relies on invadopodia [3, 194, 218], providing a strong rationale for targeting invadopodia at any stage of disease progression to prevent metastasis. To determine whether drugs that regulate invadopodia-related proteins are being evaluated in clinical trials for distant metastasis, a search was conducted on ClinicalTrials to identify ongoing studies investigating the anti-metastatic effects of such drugs (Table 4).

Most of the drugs that are currently under investigation target kinases such as EGFR, VEGFR, and PDGFR. These kinases play important roles in regulating the elongation and stability of the actin core, the delivery and secretion of MMPs, and the turnover of protrusions [219]. Clinical trials provide valuable data from the human physiological environment, helping us understand the effects of drugs on specific tumor types. This understanding can guide the development of more targeted strategies to inhibit invadopodia and improve efficacy while reducing side effects.

Conclusion and outlook

Invadopodia, specialized protrusions in tumor cells, have gained significant attention due to their role in tumor invasion and metastasis. Early research on tumor biology using 2D and 3D models has provided insights into the multi-stage processes governing invadopodia formation. The TME, where invadopodia forms, is highly dynamic, allowing cells to detect and respond to minor fluctuations that promote tumor invasion and dissemination. Thus, it is important to thoroughly investigate the signal-sensing capabilities of tumor membrane surface receptors and the ensuing signaling pathway activation, which precisely controls the development and functioning of invadopodia.

Future studies should focus on the mechanical changes of invadopodia during matrix degradation, particularly by examining their dynamic characteristics from a mechanical perspective and integrating this analysis with the invadopodia turnover process. Such investigations would allow a more precise characterization of the structural integrity and functional properties of invadopodia. Moreover, integrating previous research on invadopodia structure and regulatory mechanisms may provide a theoretical foundation for targeted therapeutic strategies aimed at modulating invadopodia formation and activity. These studies may identify novel therapeutic targets, offering potential strategies to mitigate metastatic cancer by inhibiting invadopodia, therefore advancing the development of effective anti-metastatic therapies.

Acknowledgements
Not applicable.

Author contributions
ZH and MZ conducted the research study and drafted the manuscript. YD, JL and GZ provided assistance during the revision and drafting process. XP and HL contributed to the conceptual framework, supervised the study, and revised the manuscript. All authors carefully reviewed the final manuscript and approved it for publication.

Funding
This research was funded by the National Natural Science Foundation of China (No.82303373), Liaoning Provincial Local Science and Technology Development Fund (2024020198-JH 6/1008), Shenyang Science and Technology Program Project (114/2400023041), and Shenyang Young and Middle-aged Science and Technology Talents Support Program (RC230583).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 22 February 2025 Accepted: 21 April 2025
Published online: 16 May 2025

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