Probing the Protrusions: Lamellipodia and Filopodia in Cancer Invasion and Beyond

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Abstract

The dynamic protrusions of lamellipodia and filopodia have emerged as crucial players in tumor progression and metastasis. These membrane structures, governed by intricate actin cytoskeletal rearrangements, facilitate cancer cell migration, invasion, and interaction with the tumor microenvironment. This review provides a comprehensive examination of the structural and functional attributes of lamellipodia and filopodia, shedding light on their pivotal roles in mediating cancer invasion. Navigating through the intricate landscape of cancer biology, the review illuminates the intricate signaling pathways and regulatory mechanisms orchestrating the formation and activity of these protrusions. The discussion extends to the clinical implications of lamellipodia and filopodia, exploring their potential as diagnostic and prognostic markers, and delving into therapeutic strategies that target these structures to impede cancer progression. As we delve into the future, the review outlines emerging technologies and unexplored facets that beckon further research, emphasizing the need for collaborative efforts to unravel the complexities of lamellipodia and filopodia in cancer, ultimately paving the way for innovative therapeutic interventions.

Keywords: Lamellipodia; filopodia; cancer invasion; metastasis; mechanobiology

1. Introduction

Cancer cell migration is a critical factor in cancer progression, facilitating the invasion and dissemination of cancer cells from primary tumors to establish metastases in distant organs. This process is complex and involves the integration of signaling pathways that regulate cell adhesion, cytoskeleton reorganization, and interactions with the tumor microenvironment [1–3]. Among others, collective movement, mesenchymal migration, and amoeboid migration are crucial aspects of cancer migration, each characterized by distinct mechanisms and implications in the progression of cancer. Collective cell movement involves the coordinated migration of groups of cancer cells. it is characterized by cells moving as sheets, strands, clusters, or ducts, and is regulated by cadherin-based junctions maintaining supracellular properties like collective polarization and force generation [4]. It has been seen in various cancers and relies on cell-cell adhesion mechanisms to maintain the integrity and directionality of the group. Studies have shown that beta1 integrins play a critical role in the invasive migration of multicellular clusters, such as in primary melanoma explants. Disruption of beta1-integrin function can lead to the detachment of individual cells and switch to amoeboid migration, highlighting the plasticity in tumor cell migration strategies [5]. Whilst, mesenchymal migration enables cancer cells to move individually. It is characterized by the formation of focal adhesions and the elongation of the cell body. The transition from mesenchymal to amoeboid migration can be induced by changes in the microenvironment, such as confinement and low adhesion, allowing mesenchymal cells to switch to a fast amoeboid phenotype [6]. Amoeboid migration is characterized by high plasticity, allowing cancer cells to move independently of adhesions, often through squeezing and deforming their cell body. This mode can be induced under certain conditions, such as hypoxia, which triggers a collective-to-amoeboid transition promoting the dissemination of amoeboid-moving single cells from collective invasion strands. This

process involves hypoxia-inducible factors (HIF-1) and demonstrates the adaptive capability of cancer cells to environmental challenges [7].

Cellular dynamics in cancer progression have become a focal point of research, unveiling the intricate roles played by subcellular structures like lamellipodia and filopodia. Lamellipodia, broad, sheet-like protrusions, and filopodia, slender, finger-like extensions, are dynamic membrane structures crucial for cellular movement and interaction within the complex tumor microenvironment. They are dynamic extensions emanating from the leading edge of migrating cells, orchestrating directed cell movement through the intricate interplay of the actin cytoskeleton. Lamellipodia, characterized by a broad, flattened morphology, drive cell migration by promoting adhesion to the extracellular matrix (ECM) and facilitating the establishment of focal contacts. On the other hand, filopodia, with their slender, finger-like appearance, are involved in cellular probing, sensing the microenvironment, and guiding directional movement [8,9]. Comprising a dense network of actin filaments, lamellipodia and filopodia exhibit distinct molecular and structural features. Lamellipodia are enriched with branched actin networks, largely regulated by the Actin-Related Protein 2/3 (Arp2/3) complex and the Wiskott-Aldrich Syndrome Protein (WASP) [8,10,11]. In contrast, filopodia exhibit bundled actin filaments regulated by proteins like fascin and the Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) family members [9,12–14]. These structural variances contribute to their specialized functions in cellular motility and invasion.

The relevance of lamellipodia and filopodia in cancer progression lies in their pivotal roles in the tumor invasion and metastatic cascade [9]. Cancer cells exploit these structures to navigate through the intricate matrix of the tumor microenvironment, invade surrounding tissues, and disseminate to distant sites [15]. Lamellipodia-driven migration facilitates the invasion of cancer cells by promoting ECM degradation and enabling efficient interaction with neighboring cells [16,17]. Moreover, filopodia play a crucial role in guiding cancer cells through the complex extracellular milieu, aiding in processes such as intravasation and extravasation during metastasis [18]. Beyond their physical contributions to invasion, these membrane protrusions actively participate in signal transduction pathways. They respond to extracellular cues, translating environmental signals into intracellular responses that modulate cellular behaviors. The dysregulation of these signaling processes contributes to aberrant cancer cell motility and invasiveness, making lamellipodia and filopodia promising targets for therapeutic intervention [10,19,20].

This review aims to provide a comprehensive synthesis of current knowledge regarding the structural and functional intricacies of lamellipodia and filopodia in the context of cancer progression. By critically evaluating existing literature and integrating findings from diverse studies, we seek to offer a holistic understanding of how these protrusions contribute to cancer cell invasion and metastasis. Furthermore, this review serves as a platform for discussing the regulatory mechanisms governing lamellipodia and filopodia dynamics in cancer cells. Insights into the signaling pathways and key molecular players involved will be dissected, providing a roadmap for potential therapeutic interventions. Beyond elucidating their roles in cancer progression, this review will explore the clinical implications of lamellipodia and filopodia, considering their potential as diagnostic and prognostic markers.

2. Lamellipodia and Filopodia: Structure and Function

2.1. Definition and characteristics of lamellipodia

Lamellipodia are characterized by a complex and dynamic actin cytoskeleton organization. The actin filaments within lamellipodia form a dense meshwork, creating a broad, sheet-like protrusion at the leading edge of migrating cells. This organization is largely regulated by the Arp2/3 complex and WASP, orchestrating the polymerization of actin filaments in a branched pattern [8]. The dynamic interplay between actin assembly and disassembly within lamellipodia allows for rapid membrane protrusion and retraction, facilitating efficient cell movement. Lamellipodia are essential for promoting cell motility by facilitating adhesion to the ECM and establishing focal contacts. The leading edge of lamellipodia contains membrane ruffles, allowing for dynamic interactions with the surrounding environment [19]. The continuous extension and retraction of lamellipodia create a wave-like pattern, propelling the cell forward. This dynamic behavior is crucial for

processes such as chemotaxis, where cells navigate gradients of signaling molecules, and haptotaxis, where cells move along concentration gradients of matrix-bound factors [10,21,22].

2.2. Definition and characteristics of filopodia

Filopodia, in contrast to lamellipodia, exhibit a slender, finger-like morphology characterized by the formation of tightly bundled actin filaments. These actin bundles are organized in a parallel fashion, providing structural stability to the protrusion. Key regulatory proteins involved in filopodia formation include fascin and members of the Ena/VASP family [9,23,24]. The bundling of actin filaments imparts rigidity to filopodia, allowing them to serve as exploratory antennae for the cell. Filopodia play a critical role in cellular sensing and directional migration. These structures are enriched with receptors and sensors that enable cancer cells to perceive the surrounding microenvironment. Through interactions with the ECM and neighboring cells, filopodia contribute to the transmission of external signals into the cell, influencing migratory responses [25,26]. Moreover, filopodia are involved in guiding cells during directional migration, facilitating the navigation of cancer cells through complex tissue architectures and aiding in processes such as intravasation and extravasation during metastasis [18].

Together, lamellipodia and filopodia contribute to the directional motility of cancer cells by extending the cell's leading edge and interacting with the extracellular matrix, thus aiding in metastasis and invasion. Lamellipodia act as a motor pulling the cell forward, while filopodia serve sensory or exploratory functions [9].

3. Role of Lamellipodia and Filopodia in Cancer Progression

3.1. Lamellipodia- and filopodia-driven cancer cell migration and invasion

Lamellipodia are instrumental in mediating cancer cell invasion by orchestrating the degradation of the ECM. Lamellipodia play a role in forming invadopodia – specialized membrane structures that focus proteolytic activity, facilitating localized ECM degradation [9]. This process is facilitated by the secretion of matrix metalloproteinases (MMPs) and other proteolytic enzymes, enabling cancer cells to breach physical barriers and invade surrounding tissues [27]. The dynamic protrusions of lamellipodia create focal points for the assembly of integrins, transmembrane receptors that connect the cell to the ECM, fostering adhesion and promoting localized matrix degradation [28,29]. In aggressive cancers, elevated expression of MMPs in lamellipodia correlates with increased invasive potential. The spatiotemporal regulation of ECM degradation by lamellipodia is critical for tumor progression, allowing cancer cells to navigate through the intricate matrix and invade neighboring tissues [30]. Additionally, lamellipodia extend beyond their role in ECM degradation to facilitate dynamic interactions with neighboring cells. Adhesion structures formed by lamellipodia, such as focal adhesions, not only promote stable attachments to the ECM but also enable communication between cancer cells and surrounding stromal or immune cells [31]. This intercellular crosstalk influences collective migration, allowing groups of cancer cells to coordinate their invasive behavior [9]. Through these interactions, lamellipodia contribute to the creation of a permissive microenvironment that supports invasion and further potentiates metastasis.

Filopodia, with their slender and elongated morphology, serve as sensory extensions that guide cancer cells through the complex terrain of the tumor microenvironment. Rich in receptors and adhesion molecules, filopodia sense and respond to chemotactic gradients, allowing cancer cells to navigate toward specific regions within the tumor. Through dynamic probing and sensing, filopodia facilitate the recognition of guidance cues, such as growth factors and chemokines, directing cancer cells towards blood vessels or areas of increased stromal support. This guidance is critical for the spatial organization of cancer cells within the tumor, influencing invasion patterns and ultimately shaping the metastatic potential of the cancer [25,26]. Filopodia play a crucial role in the metastatic cascade, particularly during extravasation, where cancer cells exit the bloodstream and invade distant tissues [18]. As cancer cells intravasate into the bloodstream, filopodia aid in their interaction with endothelial cells, facilitating the adhesion to and transmigration through the vascular endothelium [32]. Once in the extravascular space, filopodia continue to contribute to metastasis by guiding cancer cells through the foreign microenvironment. The ability of filopodia to establish contacts

with neighboring cells, including stromal cells and other cancer cells, enhances their invasive potential. Filopodia-driven invasion is thus a key determinant in the establishment of secondary tumor foci, shaping the metastatic spread of cancer cells [9].

3.2. The roles of lamellipodia and filopodia in immune cells' activity during malignancy

Lamellipodia and filopodia on macrophages and T cells play crucial roles in malignancy, largely due to their involvement in cell movement, environmental sensing, and immune responses. Macrophage filopodia assist in the phagocytic uptake of particles, aiding in pathogen clearance. They capture pathogens by various mechanisms, including surfing along the filopodial shaft towards the cell body and sweeping actions. These mechanisms are crucial for the phagocytic uptake of particles and, by extension, plays a significant role in tumor immunity where phagocytosis of cancer cells is involved [33]. In T cells, lamellipodia and filopodia play a crucial role in crossing endothelial barriers, a process vital during immune surveillance and inflammation. Rho GTPases, which regulate cytoskeletal dynamics, are essential for T-cell polarization and migration [34]. A study by Doh, Song, and Kwon (2013) found that T cells utilize lamellipodia to sense topography of endothelial cell layers and filopodia to sense nuclei of endothelial cells, crucial for optimal intraluminal path finding during transendothelial migration [35]. Ward (2009) described shear-facilitated chemokine-induced adhesive filopodia on crawling T lymphocytes that scan the endothelial surface for potential sites of transendothelial migration, indicating a novel mode of lymphocyte locomotion over endothelial cells before extravasation [36]. Shulman et al. (2009) found that endothelial-presented chemokines triggered high-affinity lymphocyte function-associated antigen 1 (LFA-1) and adhesive filopodia underneath crawling lymphocytes, which were critical for lymphocyte crawling and probing for transendothelial migration sites [37].

4. Regulation of Lamellipodia and Filopodia in Cancer Cells

The intricate dynamics of lamellipodia and filopodia in cancer cells are tightly regulated by a complex network of signaling pathways and regulatory proteins (**Figure 1**) [38–40]. At the forefront of regulating cytoskeletal dynamics are the Rho family of small GTPases, particularly RhoA, Rac1, and Cdc42. These GTPases act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. The activation of Rho GTPases orchestrates the intricate processes of lamellipodia and filopodia formation [38,39,41]. Activation of RhoA promotes actomyosin contractility, influencing the rear retraction of the cell during migration. This counteracts the protrusive forces generated by lamellipodia, ensuring coordinated cell movement. Rac1 activation stimulates lamellipodia formation by promoting actin polymerization at the leading edge [9,29,39,42]. WASP and Arp2/3 complex are critical regulators of lamellipodia formation, acting downstream of Rac1 activation. Activated by Rac1, WASP binds to the Arp2/3 complex, promoting the nucleation of new actin filaments. This nucleation initiates the formation of branched actin networks characteristic of lamellipodia. Through the branching of actin filaments, Arp2/3 complex facilitates the creation of a dendritic network that drives the protrusion of the plasma membrane during lamellipodia formation. Overall, the WASP-Arp2/3 pathway exemplifies the intricate molecular machinery that governs the dynamics of lamellipodia, allowing cancer cells to extend and retract their leading edges during migration [43–45]. Next, activation of Cdc42 induces filopodia formation by promoting the bundling of actin filaments. It engages with proteins like Ena/VASP, facilitating the elongation of filopodia [9,39]. Ena/VASP family of proteins enhance actin filament elongation and bundling, promoting the formation of parallel actin bundles characteristic of filopodia. They interact with actin barbed ends, inhibiting capping and facilitating filament growth [14,46]. Ena/VASP proteins contribute to filopodia elongation, stability, and navigation through the tumor microenvironment, ultimately influencing cancer cell invasion and metastasis [47,48]. Overall, the coordinated activity of regulatory proteins, such as WASP and Ena/VASP, ensures the precise orchestration of lamellipodia and filopodia dynamics, allowing cancer cells to respond to external stimuli and navigate through complex tissue environments.

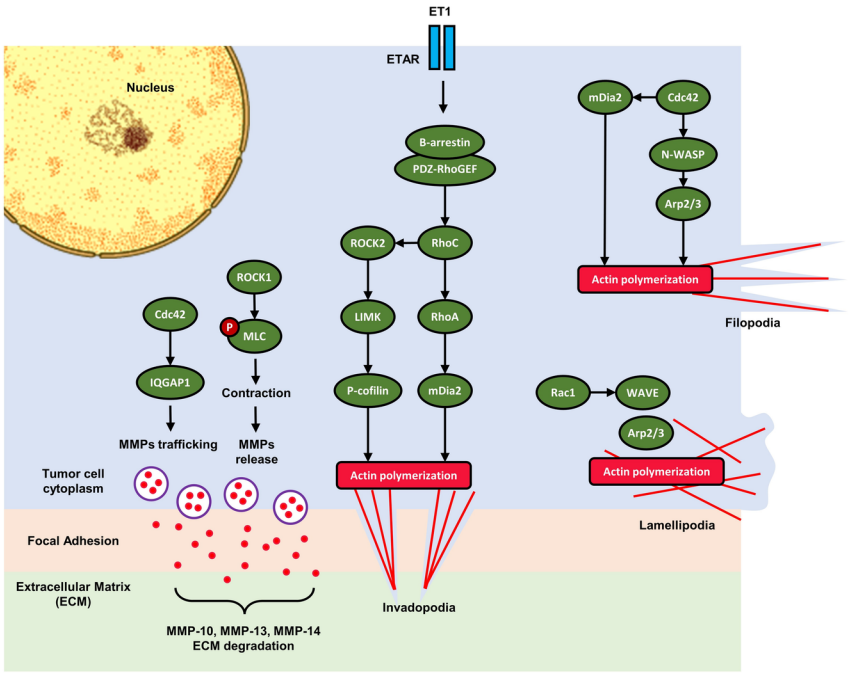

Figure 1: Molecular Pathways in Tumor Cell Invasion and Migration. This diagram illustrates the complex intracellular signaling pathways that govern the invasion and migration of tumor cells. Key components include the endothelin-1 (ET-1) signaling through endothelin A receptor (ETAR) and endothelin B receptor (ETBR), which leads to actin polymerization via multiple pathways. Proteins like RhoC, RhoA, Rac1, and Cdc42 are small GTPases that play pivotal roles in cytoskeletal dynamics. ROCK1/2 are Rho-associated protein kinases involved in actin-myosin contraction. LIMK and p-cofilin are implicated in actin filament stabilization and turnover. mDia2, IQGAP, N-WASP, WAVE, and the Arp2/3 complex are crucial for actin nucleation and polymerization, forming structures such as filopodia and lamellipodia. These processes are aided by the release of matrix metalloproteinases (MMPs) like MMP1, MMP13, MMP14, and MMP10, which remodel the extracellular matrix to facilitate tumor cell movement. (ET-1: Endothelin-1; ETAR: Endothelin A receptor; RhoC, RhoA, Rac1, Cdc42: Small GTPases involved in cytoskeletal organization; ROCK1/2: Rho-associated protein kinases; LIMK: LIM domain kinase; p-cofilin: phosphorylated cofilin; mDia2: Mammalian diaphanous-related formin 2; IQGAP: IQ motif containing GTPase activating protein; N-WASP: Neural Wiskott-Aldrich syndrome protein; WAVE: WASP family verprolin-homologous protein; Arp2/3: Actin-related protein 2/3 complex; MMPs: Matrix metalloproteinases)

The delicate balance and spatiotemporal regulation of Rho GTPase activity determine the type and extent of protrusions formed, playing a central role in the migratory and invasive behavior of cancer cells. These GTPases activate downstream effectors such as Rho-associated protein kinases (ROCK1/2) that facilitate the phosphorylation of myosin light chains (MLC), leading to increased actomyosin contractility. Simultaneously, LIM kinases (LIMK) phosphorylate cofilin (p-cofilin), which prevents actin filament depolymerization, thus stabilizing the cytoskeleton. Meanwhile, proteins like mDia2 and N-WASP, in conjunction with the Arp2/3 complex, promote actin nucleation, a process fundamental to the polymerization of actin filaments, giving rise to the dynamic restructuring of the cytoskeleton. Concurrently, the cell coordinates the trafficking and releasing of matrix metalloproteinases (MMPs) via molecules such as IQGAP. MMPs are proteolytic enzymes that degrade components of the extracellular matrix, clearing a path through the tissue and enabling the invasive behavior of the tumor cell [49].

The proteins WASP and WASP-family Verprolin-homologous protein (WAVE), integral to Rho GTPase

signaling, are key regulators of the actin cytoskeleton, playing crucial roles in the formation of lamellipodia and filopodia. They are activated by Rho family GTPases, such as Rho, Rac1, and Cdc42, leading to the formation of cellular protrusions essential for cell motility. In cancer, this mechanism becomes pivotal, as the dynamic rearrangement of the actin cytoskeleton facilitates invasive and metastatic behaviors of tumor cells. These proteins serve as critical activators of the actin cytoskeleton, which plays a central role in the morphological and motile capabilities of cancer cells. The activation of specific Rho GTPases - Rho, Rac1, and Cdc42 - by WASP and WAVE leads to the formation of actin stress fibers, membrane ruffles, lamellipodia, and filopodia. These actin structures are not merely components of cellular architecture but are actively involved in the directional motility of cancer cells, a key process in the invasion of surrounding tissues and the progression to metastasis. The actin cytoskeleton's dynamic reorganization, facilitated by these proteins, enables cancer cells to form cellular protrusions essential for their movement and interaction with the extracellular matrix. This interaction is particularly important for the cells' ability to degrade barriers, a prerequisite for invasive behavior. Moreover, these structures enable the cells to navigate complex extracellular environments, aiding in their metastatic spread. Furthermore, by influencing the actin dynamics and cell motility, these proteins emerge as potential targets for therapeutic interventions aimed at mitigating cancer metastasis [50,51].

Colorectal cancer, the third leading cause of cancer-related deaths globally, has been linked to WAVE2 expression. Studies indicate WAVE2's association with liver metastasis, disease progression, and the activation of TGF-β1 and YAP1 signaling pathways in colorectal cancer. WAVE2's role is critical in colorectal liver metastasis, particularly through its regulation by TGF-β1 in the cancer immune microenvironment. In cervical cancer, which predominantly affects women aged 35-44, overexpression of SH3BP1 has been found to increase Rac1 and WAVE2 activity, enhancing invasion, migration, and chemoresistance. WAVE2 has also been implicated in the invasiveness and motility of pancreatic cancer cells. It is shown that WAVE2 interacts with alpha-actinin 4 (ACTN4), affecting cell movement and invasiveness. Prostate cancer research indicates the involvement of WAVE2 in cell invasion and metastasis, particularly through its interaction with PIP3 and Rac1-induced actin reorganization. WAVE2 is also significant in breast cancer, the most common cancer among women worldwide. It contributes to the formation of lamellipodial protrusions in cancer cells and is associated with aggressive cancer types like triple-negative breast cancer (TNBC). The binding of WAVE2 to the Arp2/3 complex plays a crucial role in breast cancer cell migration and invasion, with implications for potential therapeutic targets [51].

Integrins, transmembrane receptors that link the ECM to the actin cytoskeleton, also play a pivotal role in regulating lamellipodia and filopodia dynamics [29,52]. Integrin engagement with the ECM initiates signaling cascades that influence focal adhesion formation and cytoskeletal rearrangements. Integrins activate focal adhesion kinase (FAK) and Src kinases, initiating downstream signaling events that regulate the activity of Rho GTPases. Focal adhesions serve as anchoring points for actin filaments and contribute to the stability of lamellipodia and filopodia [53]. The turnover of these focal adhesions is essential for dynamic cell movement. The interplay between integrins, focal adhesion signaling, and Rho GTPases coordinates the adhesive and protrusive forces required for effective cell migration and invasion in the tumor microenvironment [16].

5. Clinical Implications

As these cellular protrusions play pivotal roles in cancer progression, their dysregulation holds potential as diagnostic and prognostic markers and provides a foundation for developing targeted therapeutic strategies.

5.1. Diagnostic and prognostic markers

Examining the expression levels of key proteins associated with lamellipodia and filopodia dynamics holds promise as diagnostic and prognostic markers in various cancers. Aberrant expression or overactivation of proteins involved in the formation and regulation of these protrusions may serve as indicators of invasive potential and disease progression. Elevated expression of Rho GTPases, such as Rac1 and Cdc42, or overexpression of regulatory proteins like WASP and Ena/VASP, may correlate with more aggressive cancer phenotypes. Increased levels of these proteins might be indicative of enhanced migratory and invasive ca-

pabilities in cancer cells [47,54,55]. Additionally, techniques such as immunohistochemistry and molecular profiling can be employed to assess the expression levels of these proteins in tumor tissues. High-throughput analyses can provide a comprehensive profile of the molecular landscape, aiding in the identification of potential biomarkers for specific cancer types.

Advanced imaging techniques offer a non-invasive means to visualize and quantify lamellipodia and filopodia in cancer cells, providing valuable information for diagnosis and prognosis. Real-time observation of cancer cells using live-cell imaging allows for the dynamic visualization of lamellipodia and filopodia. This technique enables researchers and clinicians to monitor the migratory behavior of cancer cells and assess the impact of therapeutic interventions [56,57]. Super-resolution microscopy techniques, such as stimulated emission depletion (STED) microscopy and stochastic optical reconstruction microscopy (STORM), surpass the diffraction limit, providing detailed insights into the subcellular structures of lamellipodia and filopodia [58]. This can aid in precise characterization and quantification of these protrusions, offering valuable information for diagnostic purposes. Utilizing a combination of expression profiling and advanced imaging techniques, clinicians can gain a more comprehensive understanding of the invasive potential of cancer cells, enabling tailored therapeutic strategies.

5.2. Therapeutic targeting strategies

Targeting key proteins involved in the regulation of lamellipodia and filopodia dynamics presents a promising avenue for therapeutic intervention. Small molecules and inhibitors designed to disrupt the formation or activity of these cellular protrusions have the potential to impede cancer cell invasion. Small molecules targeting Rho GTPases, such as Rac1 and Cdc42, can interfere with the signaling pathways responsible for lamellipodia and filopodia formation (**Table 1**). These inhibitors aim to disrupt the cytoskeletal dynamics, inhibiting the protrusive forces driving cancer cell migration [59–61]. Meanwhile, targeting the WASP-Arp2/3 complex pathway with specific inhibitors can disrupt the nucleation and branching of actin filaments, inhibiting lamellipodia formation. Such inhibitors may attenuate the invasive potential of cancer cells [45]. Therapeutic strategies can also be designed to target signaling pathways that regulate lamellipodia and filopodia dynamics. By modulating these pathways, it is possible to influence the migratory behavior of cancer cells and impede their invasive capabilities. Small molecules targeting integrins or FAK can disrupt the signaling cascades that link extracellular signals to the actin cytoskeleton. This interference may hinder the formation and stability of lamellipodia and filopodia [62–64]. Developing inhibitors specific to Ena/VASP proteins could hinder the bundling and elongation of actin filaments in filopodia. This approach may prove effective in curtailing the formation and function of filopodia in cancer cells [65]. Tailoring therapeutic strategies to interfere with the molecular machinery governing lamellipodia and filopodia dynamics represents a novel approach in cancer treatment, particularly for malignancies with a strong invasive component.

Table 1. Characteristics, Regulation and Implications of Lamellipodia and Filopodia in Cancer Cell Progression

Feature	Lamellipodia	Filopodia
Definition	Broad, sheet-like protrusions at the leading edge of migrating cells, essential for cell movement.	Thin, spike-like protrusions from the leading edge of migrating cells, playing roles in sensing the cellular environment and directionality.
Key Cytoskeletal Components	Actin filaments arranged in a branched network, primarily regulated by the Arp2/3 complex.	Tightly bundled actin filaments, elongated by formins and enabled by fascin.
Primary Regulators	Rac1 GTPase stimulates the Arp2/3 complex to initiate actin polymerization.	Cdc42 GTPase activates formins to promote actin polymerization and bundling.

Feature	Lamellipodia	Filopodia
Signaling Molecules	- Rac1 - Arp2/3 complex - WAVE complex	- Cdc42 - Ena/VASP proteins - Formins
Pathways Involved	**Rho GTPase signaling**: - Rac1 activation leads to WAVE complex recruitment, activating the Arp2/3 complex for actin nucleation. **PI3K/Akt signaling**: - Promotes Rac1 and Arp2/3 complex activities, enhancing lamellipodia formation and cell migration. - Influences the activity of proteins that control actin polymerization and depolymerization, regulating the dynamic rearrangement of the actin cytoskeleton for lamellipodia extension.	**Rho GTPase and PI3K/Akt signaling**: - Cdc42 activation triggers formin-mediated actin elongation. Formins are actin-binding proteins that nucleate the elongation of unbranched actin filaments. The PI3K/Akt pathway can influence the activity of formins directly or indirectly through Cdc42. **FAK-Src signaling**: - Facilitates integrin-mediated signaling, enhancing filopodia formation for cell adhesion and migration.
Role in Cancer	Lamellipodia are crucial for cancer cell migration, invasion, and metastasis by facilitating cell movement through the ECM.	Filopodia contribute to cancer cell invasion and metastasis by probing the environment, forming contacts with the ECM, and directing migration.
Targeted Therapies	Inhibitors targeting Rac1 or the Arp2/3 complex to disrupt lamellipodia formation and hinder cancer cell migration.	Small molecules or peptides inhibiting Cdc42 activity or formin function to prevent filopodia formation and impair metastatic potential.

Cellular protrusions of immune cells can also be employed as a therapeutic modality to treat cancer. Weiskopf and Weissman (2015) explored the role of macrophages in antibody therapies for cancer, highlighting their ability to perform antibody-dependent phagocytosis [66]. This process significantly involves the use of filopodia for initial contact and engagement with target cells, emphasizing the critical role of macrophage filopodia in cancer therapies. Pathria, Louis, and Varner (2019) discussed the critical pathways regulating the recruitment, polarization, and metabolism of tumor-associated macrophages (TAMs) during tumor progression. The ability of TAMs to phagocytose tumor cells, a process that involves the use of filopodia, was highlighted as a potential therapeutic strategy [67]. Jaiswal, Chao, Majeti, and Weissman (2010) discussed how macrophages act as mediators of tumor immunosurveillance. They emphasized the role of macrophages in the recognition and phagocytic clearance of cancer cells, a process that involves the dynamic use of filopodia [68].

6. Future Perspectives

6.1. Emerging technologies for studying lamellipodia and filopodia

First, the advent of single-cell omics technologies holds tremendous promise for dissecting heterogeneity within cancer cell populations [69]. By analyzing individual cells, researchers can uncover variations in lamellipodia and filopodia dynamics that may be masked in bulk analyses. Single-cell RNA sequencing and proteomics can provide a nuanced understanding of how individual cancer cells modulate protrusion dynamics in response to microenvironmental cues. Second, continuous advancements in live-cell imaging technologies offer unprecedented opportunities to capture the dynamic behavior of lamellipodia and filopodia in real-time. High-speed, high-resolution microscopy combined with super-resolution techniques enables

detailed visualization of these cellular protrusions. Additionally, the integration of multi-dimensional imaging modalities, such as fluorescence and label-free imaging, enhances the spatiotemporal resolution, allowing for a comprehensive exploration of protrusion dynamics in diverse physiological contexts [56]. Third, computational tools for quantitative image analysis are evolving rapidly, providing researchers with the ability to extract precise measurements and quantitative data from imaging experiments. Automated algorithms can track the dynamics of lamellipodia and filopodia, enabling the quantification of parameters such as protrusion length, speed, and branching patterns. These tools facilitate large-scale data analysis and contribute to a more systematic understanding of the factors influencing protrusion dynamics [70–72].

6.2. Unexplored aspects and potential areas for research

The interplay between cancer cells and the immune system remains an underexplored aspect of protrusion dynamics. Investigating how lamellipodia and filopodia influence immune cell interactions within the tumor microenvironment could uncover novel mechanisms of immune evasion and immune-mediated control of cancer cell invasion. The role of extracellular vesicles (EVs) in mediating communication between cancer cells and the tumor microenvironment is also an emerging area of interest. Understanding how lamellipodia and filopodia contribute to the release and uptake of EVs may unveil new dimensions of intercellular communication that impact cancer progression and metastasis. Additionally, the influence of mechanical forces on lamellipodia and filopodia dynamics is an area ripe for exploration. Investigating how physical cues, such as substrate stiffness and fluid shear stress, modulate protrusion formation and function could provide insights into the biomechanics of cancer cell invasion. While much of the existing research relies on two-dimensional cell cultures, the transition to three-dimensional models more closely mimics the *in vivo*microenvironment. Examining lamellipodia and filopodia dynamics within 3D cultures or organoids can offer a more realistic representation of cancer cell invasion, providing valuable information for translational research [73–75].

6.3. Integration of computational models in understanding protrusion dynamics

Agent-based models (ABMs) simulate the behavior of individual agents (cells) within a defined environment [76]. Integrating ABMs allows researchers to simulate and analyze the emergent properties of lamellipodia and filopodia in response to various stimuli. These models provide a platform for exploring how individual cells contribute to collective invasive behavior and how perturbations at the cellular level propagate through the system. Developing mechanistic computational models that incorporate biochemical and biomechanical processes involved in protrusion dynamics enables a more detailed understanding of the underlying regulatory networks. Computational simulations can predict the effects of genetic or pharmacological interventions on lamellipodia and filopodia dynamics, guiding experimental design and hypothesis generation. Leveraging machine learning and data-driven approaches can uncover hidden patterns within large datasets generated from imaging experiments. Integrating computational algorithms with experimental data facilitates the identification of novel correlations and predictive models, enhancing our understanding of the factors influencing protrusion dynamics [77].

7. Conclusion

In this comprehensive exploration of lamellipodia and filopodia in cancer, key findings underscore the critical roles these cellular protrusions play in invasion, metastasis, and intercellular communication within the tumor microenvironment. The intricate regulation of lamellipodia and filopodia by signaling pathways and regulatory proteins influences cancer cell behavior, emphasizing their significance as therapeutic targets. The insights gained from studying lamellipodia and filopodia have profound implications for cancer research and treatment. Diagnostic and prognostic markers associated with these protrusions offer potential avenues for personalized medicine. Therapeutic targeting strategies aimed at disrupting the dynamics of lamellipodia and filopodia may curb the invasive potential of cancer cells, presenting novel approaches in the fight against metastatic disease. As we conclude this exploration, a call for continued investigation and collaboration resonates strongly. The uncharted territories of immune interactions, extracellular vesicle communication, mechanical influences, and three-dimensional modeling present exciting opportunities for future research. The integration of computational models and advanced technologies is essential for unraveling the complex-

ities of lamellipodia and filopodia dynamics, and collaboration across disciplines will drive transformative advancements in our understanding of cancer biology.

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