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Wnt/PCP signaling contribution to carcinoma collective cell migration and metastasis

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Abstract

Our understanding of the cellular mechanisms governing carcinoma invasiveness and metastasis has evolved dramatically over the last several years. The previous emphasis on the epithelial-mesenchymal transition as a driver of the migratory properties of single cells has expanded with the observation that carcinoma cells often invade and migrate collectively as adherent groups. Moreover, recent analyses suggest that circulating tumor cells within the vasculature often exist as multicellular clusters, and that clusters more efficiently seed metastatic lesions than single circulating tumor cells. While these observations point to a key role for collective cell migration in carcinoma metastasis, the molecular mechanisms driving collective tumor cell migration remain to be discerned. Wnt/PCP (planar cell polarity) signaling, one of the non-canonical Wnt signaling pathways, mediates collective migratory events such as convergent extension during developmental processes. Wnt/PCP signaling components are frequently dysregulated in solid tumors, and aberrant pathway activation contributes to tumor cell migratory properties. Here we summarize key studies that address the mechanisms by which Wnt/PCP signaling mediates collective cell migration in developmental and tumor contexts. We emphasize Wnt/PCP component localization within migrating cells, and discuss how component asymmetry may govern the spatiotemporal control of downstream cytoskeletal effectors to promote collective cell motility.

Introduction

Metastasis is a complex, multi-step process whereby cancer cells invade into surrounding tissues, access and traverse the vasculature, disseminate throughout the body, and survive and proliferate at secondary sites to colonize distant metastatic lesions (1). While the molecular underpinnings of primary tumor initiation and growth have been extensively explored, mechanisms governing metastatic behavior remain poorly understood. Failure to clinically address metastasis is a barrier to successful therapeutic intervention, and is

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responsible for the majority of cancer-related deaths (2). The initial steps of the metastatic cascade require activation of pathways that promote cell migration, which are often distinct from the molecular programs regulating transformation and proliferation. Cancer cells aberrantly activate a multitude of developmental migratory pathways, giving rise to invasiveness, metastasis, and poor patient survival (3).

Cell migration is a finely regulated, fundamental biological process critical to tissue rearrangement events from developmental morphogenesis to wound healing. Migration can occur in response to a variety of stimulants, such as chemokines and growth factors (a process known as chemotaxis) (4–7), currents and electric fields (galvanotaxis) (8–10), and the physical properties of the surrounding environment (haptotaxis or durotaxis) (11, 12). These stimuli engage diverse intracellular signaling pathways that instruct motility-associated cytoskeletal dynamics. Migratory modes may be classified into two major subtypes: single cell migration and collective cell migration, where multiple adherent cells move as a coordinated single unit in a sheet or cluster. Single cell migration has been the subject of extensive study *in vitro* and contributes to diverse cell motility events *in vivo*, including tissue morphogenesis during development (13), immune surveillance (14), and cancer metastasis (15). Collective cell migration plays critical roles in many *in vivo* biological processes including blood vessel formation (16), convergent extension (17), and branching morphogenesis (18). Accumulating evidence suggests that carcinoma invasiveness and metastasis may rely at least in part on collective cell migration (19), contrasting with classic metastasis models that focus on epithelial-mesenchymal transition (EMT) in single tumor cells as the primary initiator of dissemination. Indeed, recent observations suggest that metastatic lesions may be largely seeded by polyclonal groups, while single cell seeding may represent only a fraction of metastatic colonization events (20–23). A better understanding of cell signaling pathways that govern collective cell migration may thus identify novel therapeutic targets in patients with aggressive and late-stage disease.

Migration is often directed by gradients of environmental stimuli. Directional migration requires the establishment of cell polarity driven by the asymmetric localization of cellular components into specific domains, and breakdown of cellular polarity programs is associated with many developmental defects and disease states. Wnt/planar cell polarity (Wnt/PCP) signaling, a branch of non-canonical Wnt signaling, is critical to the establishment and maintenance of polarity in epithelial tissues. Classically, Wnt/PCP signaling maintains cell polarization in the planar axis across the surface of an epithelial sheet, orthogonal to the apical-basal axis. In this context, Wnt/PCP signaling relies upon the asymmetric distribution of core protein complexes within individual cells, and this asymmetry is propagated across the tissue through intercellular protein-protein interactions (24). Wnt/PCP signaling is essential for both collective and single cell migration during embryonic development (25, 26). In both static epithelial tissues and migrating cells, non-canonical Wnt ligands provide global instructional cues necessary for proper Wnt/PCP signaling (27–29). Importantly, core Wnt/PCP components are dysregulated in a variety of solid tumors and have been directly implicated in promoting tumor cell migration and metastasis (30).

The emerging role of collective cell migration in metastatic dissemination

Historically, the EMT program has been favored as the major driver of carcinoma dissemination. In this model, individual primary tumor cells undergo EMT and acquire mesenchymal characteristics that enable them to leave the primary tumor site and travel to distant tissues, suggesting that metastatic lesions arise from the clonal outgrowth of single tumor cells (31) (Figure 1A). However, several recent studies have provided evidence that metastases can be efficiently seeded by polyclonal cell clusters (20–22) and that carcinoma cells invade almost exclusively in a collective manner (32) (Figure 1B). Importantly, Bronsert et al. demonstrated that cells at the invasive front rarely exhibit mesenchymal morphology or expression of EMT markers (32). These studies together suggest that primary carcinoma invasion generally involves the collective migration of groups of cohesive cells into adjacent tissue rather than the dispersal of individual carcinoma cells, leaving the necessity of EMT to metastatic dissemination unclear (33).

Collective migration and invasion have been most extensively studied in breast cancer, where analysis of both mouse and human samples suggest that clusters of primary tumor cells may significantly contribute to critical steps of metastatic dissemination. To better understand the role of collective invasion in metastasis, Cheung et al. (34) developed an *ex vivo* 3D culture model and observed that tumor organoids derived from several genetically-engineered mouse models of mammary carcinoma invade via the extension of multi-cellular strands of tumor cells into a collagen matrix. Importantly, tumor cells leading this collective invasion express the basal epithelial marker keratin 14 (K14) and lack expression of markers for EMT. Consistently, aggressive K14-positive cells are enriched at the invasive front at the tumor-stroma border of primary MMTV-PyMT tumors and in the lung metastases (34). In a separate study, these investigators observed that the majority of MMTV-PyMT metastatic lung lesions arise from genetically heterogeneous clusters of disseminated cells rather than from single disseminated cells (23). Further, reduced K14 expression results in fewer metastatic outgrowths, suggesting that the formation of collectively invading K14-positive protrusions promotes metastasis (23).

These observations are consistent with findings that circulating tumor cells (CTCs) disseminate from primary mouse and human tumors into the bloodstream as both clusters and individual cells. Importantly, CTC clusters in transit likely result from collective migration from the primary tumor, rather than aggregation of single tumor cells within the vasculature following intravasation (20, 23, 35). Although both single cell and collective cell migration modes likely occur during metastasis, the relative contribution of each mode to successful metastatic colonization likely differs. Indeed, a recent study of CTCs in breast cancer (20) revealed that CTC clusters exist at a lower frequency than individual CTCs, but have 23- to 50- fold higher metastatic potential. Consistent with these findings, high levels of CTC clusters in the bloodstream correlate with adverse outcomes in breast cancer patients (20). Further, analysis of CTCs in small-cell lung cancer patients demonstrated that CTC clusters are better protected from anoikis and are more resistant to cytotoxic drugs during dissemination via the bloodstream compared to single CTCs (21).

The heterogeneity of primary tumors and their microenvironments has made it difficult to determine if precise signals dictate the mode of migration; some studies suggest that a combination of microenvironment, genetics, and signaling cues influence the migration mode selected by a primary tumor cell (12, 36). Curiously, the structure of the tumor microenvironment and ECM components seems to promote collective migration in spontaneous murine colorectal tumors (37). In invasive regions of tumors with thick and straight collagen bundles, collectively migrating sheets and strings of connected tumor cells are present. However, in invasive regions of tumors with poorly organized, short and curly collagen fibers, isolated single cancer cells are present (37). This is particularly interesting in light of findings that bundling of collagen fibers provides migration tracks for breast carcinoma cells, and bundled collagen correlates with poor patient survival (38). Further study is required to address how combinations of cell-intrinsic factors such as gene mutations and cell-extrinsic factors such as microenvironment composition dictate the mode of cell migration.

From these and other studies, a picture is emerging whereby collective cell migration is a major contributor to the metastatic dissemination of carcinoma. However, our understanding of the molecular underpinnings of collective cell migration significantly lags that of single cell migration. A better understanding of the signaling pathways governing collective migration may allow for the identification of novel therapeutic targets and opportunities for intervention into metastatic disease.

Collective cell migration

Migration is a multi-step process that begins with cellular polarization in response to a migration-promoting stimulus, followed by extension of membrane protrusions in the direction of the stimulus, adhesion of the protrusions to the extracellular matrix (ECM), generation of tractional forces which allow movement of the cell body, and disassembly of adhesion points and retraction at the cell rear (13, 39). Although collective and single cell migration share these broad mechanistic features, collective cell migration additionally requires the maintenance of cell-cell adhesion within the migrating collective, propagation of polarity cues across the collective, and the establishment of distinct cell leader cells follower cell populations.

Leader cells are localized at the leading front of a migrating collective, a position that allows leaders to both sense and respond to external cues such as cell-ECM interactions and soluble chemoattractants such as chemokines or growth factors. Indeed, cells become leaders in response to these external cues, polarize similarly to individually migrating cells, and promote migration of the collective cell group (40–42). Maintenance of leader and follower cell identity is essential for migration of the collective; ablation or separation of the leader cell from a collectively migrating epithelial sheet disrupts both the directionality and persistence of migration as well as the collective nature of the migrating cells, demonstrating that the establishment of leader cells is required for the collective migratory mode (43–45). To investigate differences in gene expression between leader and follower cells, a recent study (46) analyzed the transcriptomes of individual cells in a neural crest cell model of collective migration. Single cell RNA sequencing (scRNA-seq) analysis of leader-like and

follower-like cells revealed differentially expressed genes dependent on cellular location within the migrating collective. For example, cells at the invasive front exhibit elevated expression of genes associated with motility pathways including Rho GTPases, actin cytoskeleton signaling, and Wnt/PCP signaling (46). These observations suggest that differences in gene expression are important for the establishment of distinct cell populations required for collective migration. Additionally, cell morphology varies significantly between leader and follower cells. While leader cells have a more polarized and spindle-like shape, follower cells tend to remain tightly-packed and maintain their epithelial sheet-like organization (47). Further, cell-cell interactions between leaders and followers are critical to the initiation of polarity within leader cells, demonstrating that leader cell polarization is reliant upon both external stimuli and intercellular communication within the migrating collective (48).

Despite differences in the necessity of cell-cell interactions to the persistence of cellular migration, both individual and collective cell migration share mechanisms for the establishment of front-rear polarity. Front-rear polarity is tightly regulated by asymmetric activity of intracellular signaling molecules in the Rho family GTPases that govern cytoskeletal rearrangements (49) and the orientation of membrane trafficking (50, 51). Rho GTPases are active and stimulate downstream targets when bound to GTP and inactive when bound to GDP. They are activated by guanine nucleotide exchange factors (GEFs), which induce exchange of GDP for GTP, and inactivated by GTPase-activating proteins (GAPs), which catalyze GTP hydrolysis to GDP (52). The establishment of front-rear polarity by Rho GTPase activity generally occurs through mutual antagonism between Rac1 and RhoA. Typically, Rac1 at the leading edge of the cell promotes the formation of pro-migratory membrane protrusions such as lamellipodia (53–56) and engagement of integrins with the ECM to promote adhesion and stabilize protrusions (57, 58). Rac1 promotes membrane protrusion by activating the WAVE family of actin nucleation promoting factors, such as the Arp2/3 complex (59). At the trailing edge of the cell, RhoA regulates adhesion disassembly and retraction through activation of its effector Rho kinase (ROCK), which promotes actomyosin contractility and protrusion collapse (59–61). The mechanisms underlying the mutual antagonism of Rac1 and RhoA involve both spatial and temporal regulation of these GTPases by GAPs and GEFs. Here, Rac1 inhibits RhoA at the leading edge by activating a Rho specific GAP, while RhoA inhibition of Rac1 at the trailing edge occurs via ROCK phosphorylation of a Rac-specific GAP, leading to Rac1 inactivation (59). However, these interactions are complex, as individual GTPases are regulated by multiple GEFs, and one GEF can act upon multiple GTPases. Moreover, many of these interactions result in feedback and feed-forward loops. These features permit context-specific activation of signaling events at specific subcellular localizations with precise kinetics (62).

Wnt/PCP signaling

Wnt/PCP signaling is the most extensively studied non-canonical β-catenin-independent Wnt signaling pathway (63). Wnt/PCP was first identified through genetic studies in *Drosophila*, where defects in hair and sensory bristles on the wings were observed upon disruption of pathway components. In vertebrates, Wnt/PCP signaling is critical to tissue organization during embryogenesis and adult tissue homeostasis. For example, proper

localization of Wnt/PCP complexes is crucial for proper orientation of limb bud chondrocytes (64), hair follicles (65), and cochlear stereocilia (66) in the mouse, as well as the directional beating of multiciliated cells (25). Wnt/PCP signaling is maintained by the asymmetric distribution of highly conserved core Wnt/PCP components. The seven-pass transmembrane receptor Frizzled (*Drosophila* Fz/mammalian Fzd), the atypical cadherin Flamingo (Fmi/Celsr), and the intracellular scaffolds Dishevelled (Dsh/Dvl) and Diego localize to the distal membrane, while the proximal membrane is marked by Fmi, the transmembrane tetraspanin scaffold Van Gogh (Vang/Vangl) and the intracellular scaffold Prickle (Pk). Planar polarity across the tissue is achieved by the establishment of the asymmetric distribution of core Wnt/PCP protein complexes within individual cells through intracellular antagonism between opposing complexes (67–70). Intercellular protein-protein interactions between Wnt/PCP protein complexes on adjacent cells then transmit that asymmetry to neighboring cells (71–73) (Figure 2A). This propagation of asymmetry across many cell distances allows for integration of global cues into locally polarized cellular behavior. Although the molecular mechanisms enforcing intracellular mutual antagonism are not completely understood, accumulating evidence suggests that post-translational modifications and directed protein trafficking contribute to the establishment and enforcement of asymmetry. Studies in both *Drosophila* and mammalian models have demonstrated that newly synthesized Wnt/PCP components are selectively trafficked to proximal or distal regions, and proximal-distal trafficking may support proper localization of mis-localized complexes (74–77). Further, post-translational modifications such as ubiquitination of Wnt/PCP components support asymmetric localization by providing local regulation at distal and proximal membranes (78–83), suggesting that local negative regulation of complex components is essential for generating global polarity within an epithelial tissue.

In addition to maintaining and enforcing polarity within static epithelial tissues, Wnt/PCP signaling regulates cell migration events essential for proper embryonic development. In the context of a motile cell, antagonism between opposing core Wnt/PCP complexes both within a single cell and on adjacent cells, analogous to the asymmetry in static tissues, appears to drive context- and tissue-specific cytoskeletal rearrangements through the activation of downstream effectors. Activation of Wnt/PCP signaling occurs by non-canonical Wnt ligand (e.g. Wnt5a or Wnt11) binding to Fzd receptor at the plasma membrane, which recruits and activates Dvl. Dvl serves as both a scaffold and activator of downstream effector components such as Rho family GTPases and c-Jun N-terminal kinase (JNK), which in turn modulate actin cytoskeleton organization to promote cellular motility (84)(Figure 2B).

Defects in vertebrate Wnt/PCP signaling during development predominantly manifest as impaired convergent extension (CE), a form of collective cell migration. Closure of the neural tube, a key step in nervous system development, is driven by CE movements of the neuroepithelium (17). Failure of this process can result in neural tube defects (NTDs) such as spina bifida and exencephaly (partially opened neural tube at the spinal cord and head regions, respectively) or craniorachischisis (fully opened neural tube)(85–89). Importantly, mutations in core Wnt/PCP components genes such as *Vangl1*, *Vangl2, Dvl1 and Dvl2, Fmi*, and *Fzd3* and *Fzd6* have all been linked to NTDs (89–92). Looptail (*Lp*) mice, which possess a dominant negative mutation in the *Vangl2* gene, have been studied in the greatest

detail (93). Homozygous *Lp/Lp* mice die *in utero* and exhibit craniorachischisis, among other phenotypes. Based on cell labeling experiments and morphological measurements, *Lp/Lp* mice display extensive CE defects of the neuroepithelium, which result in a wider, shorter, and opened neural tube compared with wild-type mice (93). Similar defects have been observed in *Xenopus* embryos expressing dominant negative *Dvl* constructs (94).

Consistent with the critical importance of Wnt/PCP signaling in coordinating cell migration events during development, aberrant Wnt/PCP pathway activity may be a significant contributor to tumor malignancy. Dysregulation of core Wnt/PCP components has been reported to promote cell migration, invasion, or metastasis in breast (95–101), brain (84), ovarian (102), prostate (103), gastric (104), and colorectal cancers (105, 106). The contribution of Wnt/PCP signaling to the progression of such diverse tumor types suggests that pathway dysregulation may be a common feature of solid tumor malignancy (30). Importantly, mechanistic studies of Wnt/PCP in development and cancer contexts suggests that Wnt/PCP component localization may be critical to the defining features of collective cell migration, including cell polarization at the leading edge of a migrating collective and maintenance of cell-cell contact within the migrating collective.

Wnt/PCP signaling contribution to carcinoma collective cell migration

In vitro *motility studies*

An abundance of evidence suggests that Wnt/PCP signaling is a major driver of migration, invasion, and metastasis in a wide variety of solid tumor types (30), and likely mediates both single and collective migratory modes. Historically, *in vitro* assays such as the scratch migration and Boyden chamber methods have been employed to explore mediators of cell motility in numerous cell types, but these results are likely skewed toward the single cell migratory mode. Table 1 summarizes the major studies implicating Wnt/PCP involvement in cell motility, classified by tumor type and motility mode based on the assays employed. While only a handful of studies have specifically examined Wnt/PCP involvement in collective carcinoma cell migration, it is important to emphasize that studies utilizing classical assays implicating single cell migration do not preclude Wnt/PCP contribution to the collective cell migration mode.

Wnt/PCP signaling contribution to collective cell migration has been best described in gastric cancer (104), ovarian cancer (102), and melanoma (107). In gastric cancer (104), the expression of Wnt/PCP pathway-activating ligand Wnt5a correlates with aggressive disease and promotes collective cell migration of gastric cancer cells *in vitro.* Immunohistochemical analysis of 237 human gastric cancer cases revealed that Wnt5a is expressed in 30% of cases, and Wnt5a expression is associated with depth of invasion and degree of lymph node metastasis. Stimulation of gastric cancer cells with Wnt5a enhances cell migration *in vitro*, while collective migration of gastric cancer cells in a scratch assay is impaired by Wnt5a knockdown. Membrane ruffling and high levels of actin filaments is observed at the leading edge of collectively migrating control cells, but not Wnt5a knockdown cells. Additionally, live cell fluorescent imaging revealed that Wnt5a modulation also impacts focal adhesion dynamics. In control cells, paxillin-containing focal adhesions disassemble as new adhesions are formed at the leading edge, whereas turnover of focal adhesions is impaired in Wnt5a

knockdown cells. Consistent with these findings, Wnt5a stimulation enhances the activities of focal adhesion kinase (FAK) and Rac1, suggesting that Wnt5a drives Wnt/PCP-mediated modulation of cytoskeletal components at the leading edge critical to migration of the collective. In melanoma cells (107), Wnt5a promotes membrane ruffling and enhanced actin dynamics at the leading edge. Further, Wnt5a enhances collective migration in a Fzd5 dependent manner, supporting a role for Wnt5a and Wnt/PCP-mediated cytoskeletal remodeling in collective cell migration. Additionally, the Wnt/PCP receptor Fzd7 has been implicated in ovarian cell collective migration (102). Fzd7 is highly expressed in the Stem-A and Mes molecular subtypes of ovarian cancer, both of which are associated with poor patient prognosis. Fzd7 knockdown impairs the rate of collective migration in a scratch migration assay compared to control cells via modulation of Rac1 and RhoA activities and actin cytoskeletal rearrangements (102).

In summary, studies examining Wnt/PCP signaling contribution to both single and collective cancer cell migration modes suggest that regulation of Rac1 and RhoA cytoskeletal effectors by Wnt/PCP components and complexes is fundamental to both migration modes across diverse cancer types. Consistent with the spatial and temporal regulation of Rac1 and RhoA required for cellular motility, Wnt/PCP complex asymmetrical distribution is likely critical to proper polarization of cellular migration machinery in both motility modes.

Wnt/PCP component localization

Significant effort has been put into understanding how the Wnt/PCP component asymmetry required for epithelial tissue polarization influences cell motility. Specifically, several studies have focused on determining the localization of Wnt/PCP components and complexes within motile cells, and how this localization impacts the activity of downstream effectors such as RhoA and Rac1. Findings in both developmental and cancer contexts suggest that Wnt/PCP signaling at the leading edge of a motile cell or motile cell collective polarizes actin cytoskeletal components to promote the formation or suppression of migratory protrusions through these GTPases. Given the similarities between single motile cells and leader cells of a migrating collective, Wnt/PCP contributions to leading edge biology in both contexts will be discussed here.

Wnt/PCP component localization has been most extensively studied in breast cancer, where non-canonical Wnt ligands, such as Wnt11 and Wnt5a, drive invasive cellular behaviors (95–97). Luga et al. found that stimulation of invasive MDA-MB-231 breast cancer cells with Wnt11 results in the asymmetric distribution of core Wnt/PCP complexes along migratory protrusions at the leading edge of a single migrating breast cancer cells (97) in a manner analogous to the asymmetric distribution critical for epithelial cell planar polarity and tissue maintenance. Specifically, these investigators found that Fzd/Dvl complexes are localized to the tip of protrusions, while Vangl/Pk complexes are localized to the non-protrusive membrane at the base of migratory protrusions (97). Zhang et al. (100) demonstrated that Arhgap21/23, members of the Rho GAP family, co-localize with Pk1 at non-protrusive membranes lateral to migratory protrusions in migrating breast cancer cells. In this context, Pk1 and Arhgap21/23 function to inhibit protrusive activity through regulation of RhoA. However, Rac1 or RhoA activity modulation by Fzd/Dvl complexes at

protrusive membrane tips was not demonstrated. Nevertheless, these observations support a mechanism in which classic Wnt/PCP asymmetry is repurposed to orient cytoskeletal machinery to support promigratory activity at the leading edge of protrusions and inhibit promigratory protrusions on adjacent membranes. In this model of single cell migration, Wnt/PCP signaling drives directed cell migration in response to stimulation with activating Wnt ligands. Importantly, others have reported different Wnt/PCP component localization within single migrating MDA-MB-231 cells. Daulat et al., reported that Pk1 localizes to the leading edge of lamellipodia, but did not report the localization of other Wnt/PCP components. This discrepancy may be explained by differences in experimental design, including analysis of endogenous versus ectopically expressed, epitope-tagged proteins and the addition or omission of exogenous Wnt ligand. Regardless, additional studies will be required to fully characterize Wnt/PCP component localization and its contribution to single cell migration.

In the collective cell migratory mode, asymmetric distribution of Wnt/PCP complexes at the leading edge of leader cells has not been reported. Instead, Wnt/PCP complexes in leader cells are differentially distributed between the free leading edge and the trailing edge that maintains contact with follower cells. Thus, maintenance of cell-cell adhesion may result in a distinct distribution of Wnt/PCP components that allows for polarization cues to be propagated to neighboring cells and among the entire migrating collective. For example, in a migrating collective of breast cancer cells, Vangl is localized to the leading edge of leader cells in the migratory collective (98). Taken together with the independent observation that Pk1 also localizes to leading edge lamellipodia (99), it appears that the Vangl/Pk complex may reside at the protrusive edge of a leader cell. In a migrating collective of *Xenopus* neural crest cells, Dsh localizes to the trailing edge of the leader cell. Notably, Wnt11 ligand and Fzd7 receptor also co-localized at the trailing end of leader cells (108). In aggregate, these observations suggest that Wnt/PCP complexes asymmetrically distribute within the leader cell of a migrating collective, with Vangl/Pk at the leading edge and Fzd/Dvl at the trailing edge, resulting in asymmetrical activation of RhoA in the trailing region (108). Thus, asymmetrical distribution of Fzd/Dvl and Vangl/Pk opposing complexes within a leader cell may allow for proper orientation of RhoA and Rac1 activity to promote protrusive activity at the leading edge of the leader cell and limit non-productive protrusive activity elsewhere. The net effect is the directed migration of the entire collective (Figure 2C).

These apparently divergent sets of observations suggest that Wnt/PCP complex engagement of downstream effectors may be context- or ligand-dependent. For example, in a single migrating breast cancer cell, Pk1 and Arhgap21/23 function to inhibit protrusive activity through regulation of RhoA at the non-protrusive membrane at the leading edge (100). However, in a migrating collective, RhoA is instead regulated by a Fzd/Dvl complex at the trailing edge of the leader cell where it limits protrusive activity to ensure pro-migratory protrusions form only at the leading edge (108). These observations suggest that members of Wnt/PCP opposing complexes may differentially impact Rho GTPase activity through interactions with Rac1- or RhoA-specific GAPs and GEFs to locally impact cytoskeletal dynamics based on complex localization within a single cell or within the larger cell collective. Additionally, Wnt/PCP complex distribution may be dictated by Wnt ligand instruction. In *Xenopus* ectoderm, for example, Wnt5a and Wnt11 have the capacity to

orient the accumulation of Pk and Vang away from the source of Wnt ligand expression (28). Finally, Wnt/PCP complex localization and activity may be cell-cell contact-dependent, a notion supported by the importance of the leader-follower cell interaction to proper polarization and migration of the entire collective.

The biology of follower cells is perhaps the most poorly understood aspect of collective cell migration. For example, the mechanisms by which follower cell character and polarity are maintained and propagated across the migrating collective have not been fully elucidated, but evidence suggests that direct cell-cell interactions (109) and contact inhibition of locomotion (CIL) are involved (48). Mechanistically, CIL is a multi-step process in follower cells that involves sensing contact with neighboring cells, transmission of this signal from the plasma membrane to the cytosol, and intracellular signaling that instructs or restrains formation of migratory protrusions, dependent upon the extent of cell-cell contacts (110). In the relatively well-characterized context of neural crest cell migration, CIL prevents formation of protrusions between neighbors and polarizes the group so that the protrusive activity is limited to cells at the leading edge in an N-Cadherin- (7) and Wnt/PCP-dependent manner (111). Previous reports in *Xenopus* suggest that neural crest cells utilize N-Cadherin- and Wnt/PCP-mediated CIL to both initiate and maintain collective polarity (59). For example, *Xenopus* explants expressing a dominant-negative form of Dsh (DshDep+), which specifically inhibits the Wnt/PCP pathway but does not impact Dsh involvement in canonical Wnt signaling (85), were compared to wildtype neural crest explants. In wildtype explants, leader cells polarize and extend protrusions at the leading edge, whereas follower cells do not. However, in DshDep+ explants, cells do not polarize and instead extend large protrusions in all directions, resulting in cells crawling on top of each other (108). These findings demonstrate that Wnt/PCP signaling is critical to maintaining polarity of the entire collective, and may act by promoting CIL in follower cells to limit protrusive activity within the follower cell population.

A hallmark of Wnt/PCP signaling in maintaining epithelial integrity within an epithelial sheet is the propagation of polarization cues across many cell distances through intercellular signaling via opposing core complexes on adjacent cells. Follower cells must maintain epithelial sheet integrity through cell-cell contact with each other, and enforce collective-wide polarity through cell-cell contact with leader cells. Although a requirement for Wnt/PCP complex intercellular coupling has not been demonstrated in a migrating collective of cancer cells, collective cell migration in *Drosophila* has provided the most striking evidence that Wnt/PCP signaling propagation throughout a collective is critical for migration of the entire collective. The *Drosophila* egg chamber is a well-characterized model of collective cell migration in which border cells delaminate from the epithelium and migrate towards the anterior border of the oocyte, carrying non-motile polar follicle cells along with them in a migrating collective (112). Consistent with findings in vertebrate models and cancer cells, defects in Wnt/PCP signaling impair collective cell migration in this model by disrupting RhoA-governed protrusive activity. Most interesting, however, was the observation that Wnt/PCP signaling in the non-migratory epithelial polar follicle cells is critical to the coordinated movement of the entire collective. Here, Fz and Vang mediate intercellular communication between the border cells and polar follicle cells via opposing complexes on adjacent cells (112). These studies suggest that similar intercellular

communication among follower cells, and between leader and follower cells, helps reinforce polarization of the entire collective in a manner similar to Wnt/PCP pathway function in static epithelial tissues.

Further examples from vertebrate development suggest that asymmetric distribution of core Wnt/PCP complexes may support CIL and maintenance of epithelial sheet character within the follower cell population. Pk asymmetrically localizes to the anterior membrane of neuroepithelial cells in zebrafish (113), and appears to regulate the localization of Vangl2 to the same portion of the membrane in *Xenopus* (114). Additionally, Dvl and Fmi have been shown to asymmetrically localize to the anterior-posterior faces of neural midline cells in chick embryos (115). Additional studies will be required to rigorously determine whether Wnt/PCP component asymmetry is required for CIL within the follower cell population of a migrating collective, and to assess the degree to which Wnt/PCP complexes contribute to the maintenance of the epithelial sheet state of migrating collectives.

Conclusions and future directions

Findings from both developmental and cancer contexts demonstrate that Wnt/PCP signaling is critical to collective cell migration. Key aspects of collective cell migration that distinguish it from single cell migration, including the establishment and maintenance of leader and follower cells and intercellular communication within the migrating collective, appear to rely heavily upon Wnt/PCP signaling function. Indeed, collective migration is at least partially dependent upon asymmetric localization of Fzd/Dvl and Vangl/Pk complexes within leader cells, perhaps to properly orient downstream Wnt/PCP cytoskeletal effectors Rac and Rho. Although the Wnt/PCP activating ligand Wnt11 co-localizes with trailing edge Fzd/Dvl complexes in the leader cell, it remains unclear if Wnt11 is specifically driving localization or activity of Wnt/PCP core components for downstream effectors. Future studies will be required to fully elucidate the requirements for asymmetry within the leader cell. Additionally, findings that Wnt/PCP components promote polarization of both migratory leader cells and follower cells suggest that Wnt/PCP signaling may be critical to collective cell migration by propagating local polarity cues across the entire migrating collective. This raises the possibility that Wnt/PCP signaling may be important for maintaining epithelial sheet organization and integrity during collective migration. Nonetheless, Wnt/PCP signaling appears to play critical roles in both single cell and collective cell migratory modes, and the asymmetrical distribution of components appears critical to key aspects of collective cell migration.

While collective cell migration has been historically underappreciated and understudied in cancer biology, an abundance of recent studies clearly demonstrate that this migratory mode contributes significantly to carcinoma metastatic dissemination. This conclusion in turn highlights the need for a better understanding of the drivers and mechanisms underlying collective cell migration across multiple tumor types. Wnt/PCP signaling components appear to asymmetrically distribute within leader cells and govern the localization and activity of actin cytoskeletal effectors Rac1 and RhoA. Importantly, Wnt/PCP signaling may also propagate cytoskeletal polarization throughout the migrating collective, promoting pro-migratory protrusion formation at the leading edge while suppressing protrusions within the

trailing collective. While these findings provide initial mechanistic insight, many questions remain concerning the nature of leader and follower cells, factors that instruct collective versus single migratory modes, and roles for Wnt/PCP at other stages of the metastatic cascade. Additionally, though genetic knockout animals support a role for Wnt/PCP signaling in collective cell migration *in vivo* during development, no studies using genetic or xenograft models of cancer or primary tumor tissues have been reported. I*n vivo* and *ex vivo* studies focused on core component modulation within the context of a metastatic tumor will likely markedly improve our understanding of Wnt/PCP involvement.

Current findings suggest that Wnt/PCP signaling is a prime candidate for therapeutic intervention into metastatic disease. For example, the contribution of Wnt/PCP to the establishment and maintenance of epithelial tissue polarity, and its involvement in the propagation of polarity both across tissues and within migrating collectives, raises the possibility that Wnt/PCP signaling may be critical to CTC cluster formation and maintenance within the circulation. Translationally, therapeutic intervention that disrupts Wnt/PCP signaling and component asymmetry may reduce CTC cluster formation or render CTC clusters in circulation unstable. This could elicit significant clinical benefit since CTC clusters have higher metastatic potential and are more resistant to anoikis and cytotoxic drugs than single CTCs. Future studies are needed to assess Wnt/PCP pathway activity in single CTCs and CTC clusters, and to determine how modulation of Wnt/PCP signaling impacts their viability and metastatic potential.

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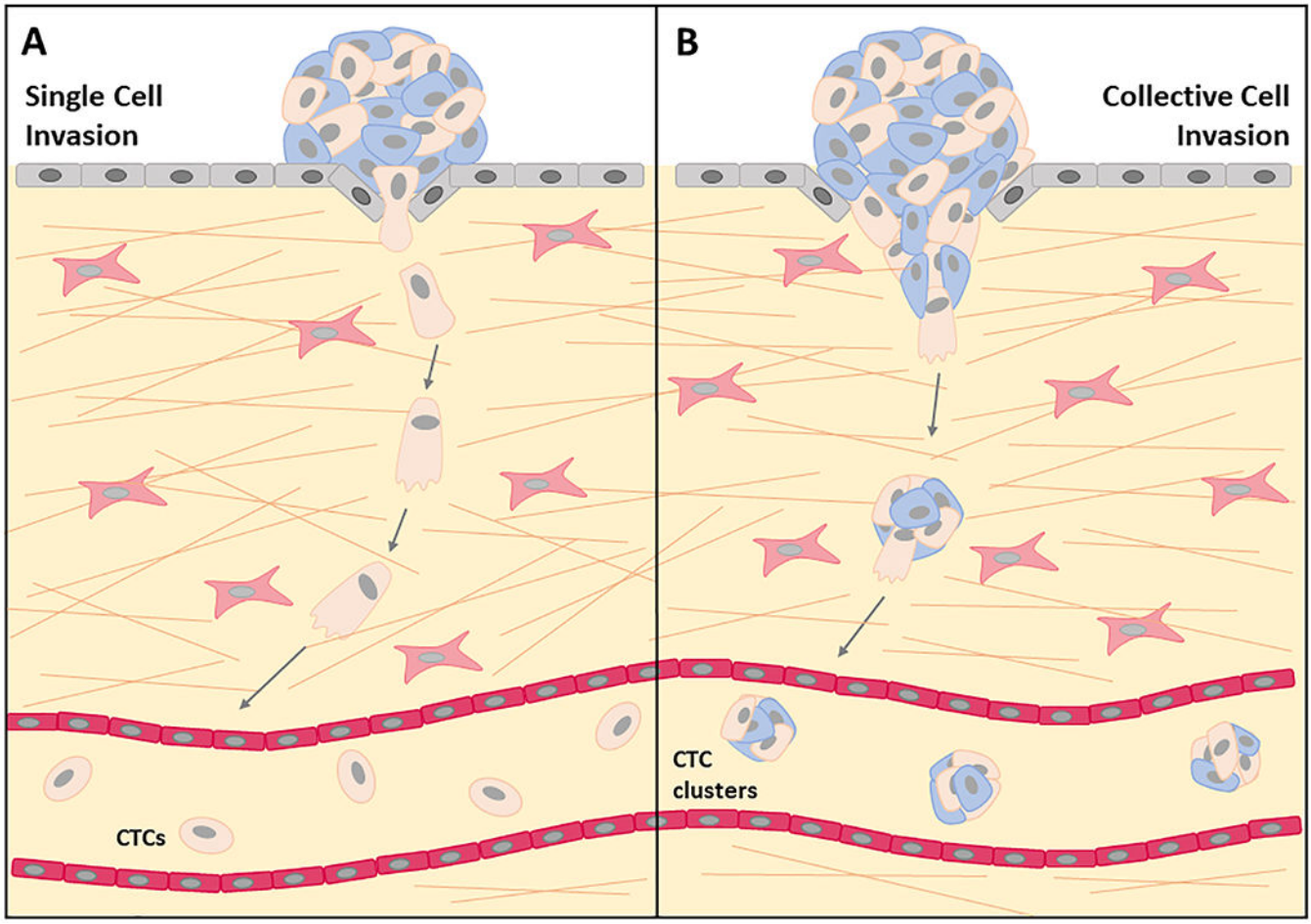

Figure 1. Carcinoma cell metastatic dissemination is mediated by multi-modal cell migration.
(A) In the single cell invasion model, individual primary tumor cells undergo EMT to acquire mesenchymal characteristics that enable them invade into the tumor stroma, circulate in the vasculature as individual cells, and seed clonal metastatic lesions (31). **(B)** In the collective cell invasion model, groups of cohesive cells invade into the tumor stroma, circulate in the vasculature as clusters of cells, and seed polyclonal metastatic lesions (20–22, 32).

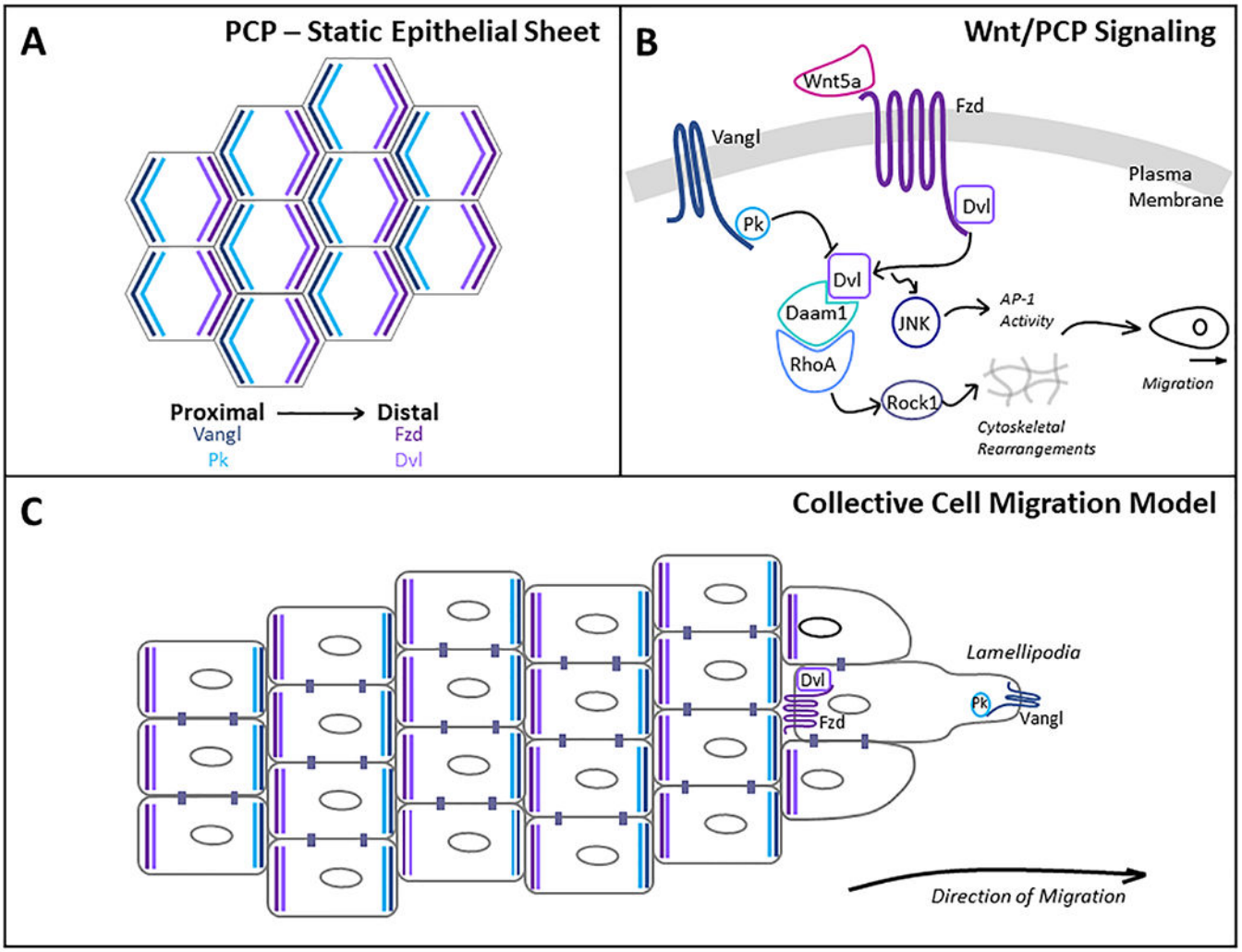

Figure 2. Asymmetric distribution of Wnt/PCP pathway core components is critical to epithelial tissue planar polarity and collective cell migration.

(A) Planar polarity (orthogonal to the apical-basal axis) is established with the asymmetric distribution of core Wnt/PCP protein complexes within a single cell via intracellular antagonism between opposing complexes. Intercellular protein-protein interactions between Wnt/PCP protein complexes on adjacent cells then transmit asymmetry across the collective. **(B)** Activation of Wnt/PCP signaling occurs by non-canonical Wnt ligand (e.g. Wnt5a or Wnt11) binding to Fzd receptor at the plasma membrane, which recruits and activates Dvl. Dvl serves as both a scaffold and activator of downstream effector components such as Rho family GTPases and JNK, which in turn modulate actin cytoskeleton organization to promote cellular motility. Adapted with permission from Elsevier (30). **(C)** Findings from both developmental and cancer contexts demonstrate that collective migration is at least partially dependent upon asymmetric localization of Wnt/PCP core component complexes within leader and follower cells. In our working model, Fzd/Dvl and Vangl/Pk complexes asymmetrically distribute within leader cell and follower cells to orient downstream

Wnt/PCP cytoskeletal effectors RhoA and Rac1 activity to promote protrusive activity at the leading edge of the leader cell and limit non-productive protrusive activity elsewhere.

Table 1.

Wnt/PCP signaling contribution to cancer cell migration

Wnt/PCP Component	Component Function	Tumor Type	Downstream Effectors Involved	Impact on Cytoskeleton	Migration Mode	Reference
Wnt5a	Activating ligand	Breast	JNK, c-Jun		single cell	95
		Breast	RhoA		single cell	96
		Gastric	Rac	altered actin dynamics at leading edge, altered focal adhesions	collective	104
		Melanoma		altered membrane ruffling, altered actin dynamics at the leading edge	collective	107
Wnt11	Activating ligand	Breast		altered ability to form pro-migratory protrusions	single cell	97
		Prostate			single cell	103
		Colorectal	JNK, c-Jun		single cell	105
Fzd5	Transmembrane receptor	Melanoma			collective	107
Fzd6	Transmembrane receptor	Breast		altered ability to form pro-migratory protrusions	single cell	97
Fzd7	Transmembrane receptor	Breast		altered ability to form pro-migratory protrusions	single cell	97
		Ovarian	RhoA, Rac1	altered actin dynamics	collective	102
Dvl1	Cytoplasmic scaffold	Breast		altered ability to form pro-migratory protrusions	single cell	97
Dvl2	Cytoplasmic scaffold	Breast			single cell	97
Dvl3	Cytoplasmic scaffold	Breast			single cell	97
Vangl1	Transmembrane scaffold	Breast			single cell	98
		Breast		altered ability to form pro-migratory protrusions	single cell	97
		Glioma			single cell	84
Vangl2	Transmembrane scaffold	Breast			single cell	101
Pk1	Cytoplasmic scaffold	Breast		altered ability to form pro-migratory protrusions	single cell	97
		Breast		altered lamellipodia formation, altered cell shape and polarization, altered focal adhesion stability	single cell	99
		Breast	RhoA	altered actin dynamics, altered focal adhesion dynamics	single cell	100