Wnt/Planar cell polarity signaling: A new paradigm for cancer therapy

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Abstract
The evolutionarily conserved and developmentally important Wnt signaling pathway has traditionally been regarded as a critical player in tumorigenesis through the canonical Wnt/β-catenin cascade. Nevertheless, accumulating evidence based on recent research has revealed the previously unacknowledged role of noncanonical Wnt/planar cell polarity (PCP) signaling in cancer progression, invasion and metastasis, and angiogenesis. This review describes the PCP signaling pathway and its ever-expanding components and modulators, highlights the most recent studies that provide insight into the link between PCP signaling and cancer, and, finally, proposes a model by which PCP signaling may promote cancer development. This review underscores the emerging theme that deregulated PCP signaling contributes to tumorigenesis, providing new potential targets for cancer therapy. [Mol Cancer Ther 2009;8(8):2103–9]

Introduction
The Wnt signaling pathway is highly conserved in eukaryotes and is one of the most important signaling pathways. It is used extensively during animal development to regulate diverse processes including cell proliferation, differentiation, polarity, and migration, which are fundamental to embryogenesis. Additionally, deregulation of Wnt signaling has been implicated in a broad range of pathological processes including degenerative diseases and cancer (1). Despite the complexity of this pathway, a combination of developmental, genetic, and biochemical analyses have greatly enriched our understanding of the Wnt pathway, and the list of Wnt signaling branches and components has exploded (2). It is evident that multiple extracellular, cytoplasmic, and nuclear regulators intricately modulate Wnt signaling. Wnt signals are transduced by at least two distinct pathways: the well-established canonical Wnt/β-catenin pathway and the β-catenin independent noncanonical Wnt pathway. Noncanonical Wnt signaling is very diverse and is still evolving into more and more branches (3). Among them, Wnt/Ca2+ signaling and Wnt/planar cell polarity (PCP) signaling are relatively better characterized. In Wnt/Ca2+ signaling, Wnt-Frizzled (Fzd) binding activates phospholipase C via G proteins, leading to the increase of intracellular Ca2+ levels and activation of downstream effectors including protein kinase C (PKC). The Wnt/Ca2+ pathway plays an important role in development and is implicated in cancer (4). For example, Wnt5a mediates melanoma metastasis via the induction of epithelial to mesenchymal transition (EMT) through the PKC/Ca2+ cascade (5).

Wnt/PCP signaling is the first described and most extensively studied among the several noncanonical Wnt pathways (6, 7). Drosophila serves as an excellent model system for PCP signaling because planar cell polarity occurs visibly in several external structures, including the precisely aligned hairs on wing cells, the perfectly arranged ommatidia in the facet eye, and the bristles on the thorax. In fact the term “planar cell polarity” is derived from the study of tissue polarity necessary to generate polarization within the plane of the epithelium, along an axis perpendicular to the apical-basal axis of the cell (8). Subsequently, planar cell polarity was also shown in several cellular processes in vertebrates such as convergent extension (CE) movements of mesenchymal cells during gastrulation, ordered arrangement of hairs of mammalian skin and cilia of respiratory tract, orientation of stereocilia in the organ of Corti, and orientation of axon extension (9).

PCP Signaling Pathway
Genetic screens in Drosophila and subsequent work in vertebrates have helped establish that the PCP pathway is evolutionarily conserved and constitutes three sequential steps (10). Initially, upstream PCP components provide a long range signal for the global direction of polarity across the entire tissue. These components include Four-jointed, Dachsous, Fat and Atrophin (11). This directional signal is then interpreted by a set of core PCP components to establish planar polarity within individual cells along the axis of polarity. Fzd, Dishevelled (Dvl), Celsr, Vangl, and Prickle
PCP Signaling and Cancer

With a growing number of molecules in PCP signaling identified, our understanding of PCP signaling has exploded in the past several years. The role of PCP signaling is critical in multiple embryonic processes including embryo morphogenesis and the formation of most embryonic tissues and organs (15–17). It is noteworthy that embryonic development shares many similarities with cancer development. In the 19th century, Lobstein and Cohnheim speculated that tumorigenesis recapitulates aspects of development (18). In 1982 Nusse and Varmus made the groundbreaking discovery that Wnt1 is proto-oncogene activated in mouse mammary tumors prior to the discovery of its role in embryonic development over the following two decades (19). In fact, conserved signaling pathways involved in embryogenesis including Wnt, Hedgehog, and Notch are disregulated in tumorigenesis, supporting the theory that cancer is a manifestation of development gone awry (20). Although canonical Wnt/β-catenin signaling is a critical player in tumorigenesis and β-catenin has gained recognition as an important target for cancer therapy (21), accumulating evidence draws our attention to the previously unappreciated role of PCP signaling in cancer development (22, 23). Summarized below are recent studies suggesting that PCP signaling is closely linked to cancer and plays diverse roles in tumorigenesis.

Role of PCP Signaling in Tumor Metastasis

Metastasis accounts for about 90% of all deaths from cancer. As tumors progress, cancer cells develop the ability to invade through surrounding tissues and basement membranes to form secondary tumors at sites distinct from the primary tumor. Metastatic tumor formation consists of a series of discrete processes. An improved understanding of molecular basis of metastasis is of vital importance to develop effective targeted therapy (24). Although the molecular mechanisms involved in metastasis are still incompletely understood, the factors associated with cell adhesion and cell migration are commonly accepted to be critical in tumor invasion and metastasis (25, 26). Given the central role of PCP signaling in the modulation of cell adhesion, motility, and movement in diverse developmental morphogenetic contexts, it comes as no surprise that deregulated PCP signaling is implicated in tumor metastasis.

Characterization of a variety of PCP components and modulators has shown their involvement in metastasis. Wnt5a is a typical noncanonical Wnt that transduces β-catenin independent signals in most cases; although, it can
activate β-catenin signaling depending on receptor context (2). Wnt5a has been shown to promote metastasis of melanoma, gastric cancer, and breast cancer by activating Rac and JNK (27-29). Among the 10 members of the Fzd gene family, Fzd7 is unique in that it can activate different branches of Wnt signaling. In *Xenopus*, Fzd7 can activate noncanonical Wnt signaling in morphogenesis, and Fzd7-mediated PCP signaling regulates cell movements via activation of JNK and Rho (30-32). Fzd7 also mediates PCP signaling to regulate bone morphogenesis in chick (33). In humans, Fzd7 promotes migration of hepatocellular carcinoma and invasion of colon cancer cell lines, which seems to be mediated by noncanonical Wnt signaling (34-36). Recently, Fzd10 was shown to play a critical role in the metastasis of synovial sarcoma. Mechanistically, Fzd10 activates the Dvl-Rac1-JNK axis of PCP signaling, with no effect on nuclear β-catenin accumulation and Tcf/Lef reporter activity (37). Downstream of Fzd, three Dvl members (Dvl1, Dvl2, and Dvl3) have been identified in human. A recent study investigating the clinical significance of Dvl expression in non-small cell lung cancer (NSCLC) specimens showed that the expression of Dvl1 and Dvl3 are significantly higher in nodal metastases than primary tumors. Intriguingly, although Dvl1 expression is correlated with β-catenin expression in the metastases, there is no correlation between Dvl3 and β-catenin expression in primary tumors or metastases. Corroborating these histological data, exogenous expression of Dvl1 and Dvl3 each promotes the

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<th>Table 1. Vertebrate PCP components and modulators, their role in PCP signaling, and their involvement in cancer</th>
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<td><strong>Upstream PCP components</strong></td>
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invasive ability of lung cancer cells but has differential effects on β-catenin protein levels (38). This suggests that metastasis of NSCLS may be mediated not only by Dvl1 via the canonical Wnt pathway, but also by Dvl3 in a β-catenin independent manner. Further analysis is needed to clarify whether Dvl3 mediates NSCLS metastasis through PCP pathway or other noncanonical Wnt pathways like the Wnt/β-catenin pathway.

Van Gogh-like 1 (Vangl1) is the human homolog of Drosophila PCP gene van gogh/strabismus. Suppression of Vangl1 expression inhibits growth of hepatocellular carcinoma (39). Interestingly, later this 4-transmembrane protein was identified as a binding partner of metastasis suppressor KIAA/CD82 and renamed as KIAA COOH-terminal interacting tetraspin (KITENIN) (ref. 40). Vang1 promotes mouse colon cancer and squamous cancer metastasis, and its expression is higher in tumor mucosa and metastatic tissues from gastric cancer patients and metastatic lymph nodes from head and neck squamous cell carcinoma (40, 41). Moreover, loss of Vang1 by siRNA suppresses colon cancer metastasis in mice, confirming the metastasis-promoting function of Vang1 (42). Vangl1 promotes colorectal cancer cell migration and invasion by forming a functional complex with Dvl and PKCδ to modulate cell motility (43). This study suggests that upregulation of Vangl1 takes over PCP signaling to promote tumor invasion and metastasis.

 Eph receptors are the largest family of receptor tyrosine kinases. Their ligands, ephrins are membrane-anchored proteins that are classified as glycosylphosphatidylinositol-anchored ephrinA and transmembrane ephrinB. Eph/ephrin signaling plays critical roles not only in embryonic development, but also in the homeostasis of adult organs by regulating a variety of cellular processes such as cell adhesion versus repulsion, cell motility and migration, and cell-cell communication (44). This may be partly due to the unique feature of the Eph/ephrin system to initiate bidirectional signaling that impacts not only Eph receptor-expressing cells, but also ephrin ligand-expressing cells. In particular, reverse signaling transduced via the intracellular domain of ephrinB can regulate the interaction of the C-terminal PDZ-binding motif of ephrinB with other proteins affecting cytoskeletal organization and cell adhesion. Members of the ephrinB family are often overexpressed in cancer cells and are associated with angiogenesis and metastasis (45). Although several studies provide insight into how ephrinB1-mediated reverse signaling contributes to invasion of gastric and pancreas cancer cells (46, 47), recent evidence reveals that intracellular domain of ephrinB1 interacts with Dvl and modulates PCP signaling to control cell adhesion and movement in development (48-50). This opens up the possibility to explore the potential crosstalk between ephrinB and PCP signaling in the context of tumor cell invasion and metastasis.

 Collagen triple helix repeat containing protein 1 (Cthrc1) is a secreted glycoprotein containing a short collagen-like motif with 12 Gly-X-Y repeats. Cthrc1 was first identified as a gene transiently expressed in the arterial wall upon injury, possibly supporting vascular remodeling by limiting collagen matrix deposition and promoting cell migration (51). Cthrc1 is aberrantly upregulated in the majority of human solid tumors and seems to be associated with cancer invasion and metastasis (52, 53). Intriguingly, Cthrc1 was recently characterized as a novel Wnt coreceptor that specifically activates PCP signaling. Cthrc1 knockout synergizes with Vang2 mutation leading to PCP defects in mice. At cellular level, Cthrc1 synergizes with Wnt5a or Dvl2 to activate PCP downstream effectors RhoA and Rac. Biochemical study shows that Cthrc1 promotes the clustering of Wnt/Fzd/Ror2 complex, providing convincing evidence that Cthrc1 is specifically engaged in PCP signaling (54). Because the molecular mechanism by which Cthrc1 promotes cell migration remains elusive, it is tempting to investigate whether abnormal modulation of PCP signaling by Cthrc1 contributes to invasiveness of Cthrc1-overexpressing tumor cells.

Role of PCP Signaling in Angiogenesis

Angiogenesis, the formation of new blood vessels, is essential not only for the growth of primary tumors but also for tumor metastasis. Angiogenesis facilitates metastasis by increasing the chance that tumor cells enter the blood circulation and by providing nutrients and oxygen for growth at the metastatic sites. Angiogenesis is a complex process involving endothelial cell proliferation along with a myriad steps in which endothelial cells divide, invade the basement membrane, migrate, and eventually undergo differentiation and capillary tube formation (55). Wnts have emerged as a novel class of angiogenic factors that promote angiogenesis, and recent evidence indicates that both Wnt/β-catenin and noncanonical Wnt signaling are involved in angiogenesis (56, 57). Using a chemical genetic approach, the potent anti-angiogenic natural product fumagillin was identified as a specific inhibitor of noncanonical Wnt signaling, suggesting a link between noncanonical Wnt signaling and angiogenesis (58). Further study showed that inhibition of PCP signaling by the fumagillin analog TNP-470 disrupts endothelial cell growth, polarity, and migration. Furthermore, Dvl2 mutants capable of mediating canonical Wnt/β-catenin but that are deficient in PCP signaling also disrupt endothelial cell growth, polarity, and migration. Interestingly, ΔDIX-Dvl2, a Dvl1 mutant that selectively abrogates Wnt/β-catenin signaling, and the PCP downstream effectors Daam1, diversin, or invisin can rescue the defects caused by TNP-470. Moreover, Wnt5 mutant zebrafish with impaired PCP signaling display angiogenesis defects such as defective intersegmental vessels, reduced dorsal aortas, and reduced posterior cardinal veins (59). Taken together, these findings provide strong support for the emerging role of PCP signaling in angiogenesis.

Complex Role of PCP Signaling in Cancer Development

Although the large body of evidence discussed above delineates the significant involvement of PCP signaling in tumor invasion, metastasis, and angiogenesis, the role of PCP signaling in cancer development is still controversial.
One would expect that PCP signaling can suppress tumor-igenesis given the well-recognized upregulation of Wnt/β-catenin signaling in tumorigenesis and the documented antagonism between canonical and noncanonical Wnt signaling (60). Indeed, several components of the PCP signaling pathway can suppress tumor progression, including the protocadherin family members Fat, Dachsous, and Paraxial protocadherin (PAPC). Although the basic outline emerges that Fat and Dachsous act as tumor suppressors by engaging downstream signaling networks to regulate transcription and control cell growth, it is still an open question to determine the extent to which control of cell polarity by Fat PCP signaling is involved in the tumor suppression function of Fat (61). PAPC can promote PCP signaling by sequestration of Sprouty with its cytoplasmic tail (62). Given that Sprouty negatively regulates Ras signaling and is frequently downregulated in cancer (63), PAPC might enhance tumor development by antagonizing Sprouty and promoting PCP signaling. Contrary to this prediction, protocadherin-8 (PCDH8), the human ortholog of PAPC, has tumor suppression function and is inactivated through either mutation or epigenetic silencing in about one third of all breast carcinomas (64). It is conceptually simple to assign the tumor suppressor ability of PCDH8 to the adhesion activity mediated by the extracellular domain of PCDH8 given that most of PCDH8 mutations found in breast tumors reside at the extracellular cadherin repeat. However, one PCDH8 mutation is localized in the intracellular domain, implying that intracellular signaling may be also important for tumor suppression function of PCDH8 (64). Further investigation is needed to address the status of PCP signaling in PCDH8 inactivated breast cancer. Moreover, the tumor suppressor or oncogenic activity of PCDH8 should also be surveyed in other tumors given that another protocadherin protocadherin-PC is implicated in the progression of prostate cancer (65).

Noncanonical Wnt5a acts as an oncogene or tumor suppressor gene in a context-dependent manner. Antisense Wnt5a mimics Wnt1-mediated mammary epithelial cell transformation (66). Wnt5a is downregulated in hematopoietic malignancies, thyroid carcinoma, breast cancer, colorectal cancer, and hepatocellular carcinoma, and in some cases there is an inverse correlation between Wnt5a level and β-catenin level (67–72). Taken together, these studies support the tumor suppressor function of Wnt5a by antagonizing Wnt/β-catenin signaling. Nevertheless, in other contexts, especially in cancers of advanced stages, Wnt5a is upregulated and has oncogenic properties promoting tumor invasion, metastasis, and angiogenesis as discussed in previous sections. In this aspect, PCP signaling is analogous to transforming growth factor β (TGFβ) signaling, which plays multiple roles in cancer development with early tumor suppressive effects through growth inhibition but later tumorigenic effects, including increased tumor cell motility and invasion and induction of angiogenesis (73). Therefore, it is appealing to propose a model in which PCP signaling has biphasic role in carcinogenesis by acting early as tumor suppressor but later stimulating cancer progression via the regulation of tumor invasion, metastasis, and angiogenesis (Fig. 2).

Modulation of PCP Signaling for Cancer Therapy

This review makes the first attempt to comprehensively cover the impact of PCP signaling on different features of cancer development and outlines an emerging picture that PCP signaling plays important roles in tumorigenesis. Therefore, it is critical for us to take advantage of this new understanding to develop novel diagnostic and therapeutic approaches for cancer. In fact, some components and modulators of PCP signaling such as Vangl1, EphrinB, and Cthrc1 have the potential to serve as diagnostic biomarkers for advanced metastatic tumors, given that their expression levels are higher in malignant tumors and are correlated with reduced patient survival rates (41, 45, 52, 53). In addition, inhibition of the function of PCP signaling molecules Vangl1 by siRNA may suppress colon cancer progression and metastasis (42), supporting the targeting of PCP signaling components as promising cancer therapies. Compared with canonical Wnt/β-catenin signaling, which plays a crucial physiologic role not only in embryonic development but also in the maintenance of adult tissue homeostasis, current research suggests that PCP signaling functions primarily in embryo morphogenesis. Thus, modulation of PCP signaling in cancer patients is less likely to interfere with homeostasis and represents a better avenue for cancer therapy.

Nevertheless, several issues must be sorted out in order to conceptualize effective therapeutic strategies for targeting PCP signaling. First, unlike the field of canonical
Wnt/β-catenin signaling in which a straightforward readout system has been used to identify small molecule compounds targeting this pathway (74), the field of PCP signaling lacks a well-established pathway readout system, hindering the utilization of small molecule screening to discover compounds that specifically modulate PCP signaling for cancer therapy. Fortunately, TNP-470 was serendipitously identified as the first small molecule capable of specifically inhibiting PCP signaling (58, 59). This example provides guidance for future small molecule screening for PCP signaling modulators using zebrafish high resolution pheno-typing resources until a better PCP pathway readout system is developed. Second, some components of PCP signaling have multifaceted functions. For example, unrelated to its function in PCP signaling, Fat has tumor suppressor function through regulation of transcription and cell growth. Undoubtedly, it will benefit cancer patients if we can target downstream effects of Fat PCP signaling while preserving its tumor suppression function. Third, tumors have recognized abilities to acquire resistance to therapeutics, so combined therapy is absolutely necessary. It is well acknowledged that PCP signaling can crosstalk with other pathways including canonical Wnt/β-catenin signaling, bone morphogenetic proteins (BMP) signaling, and fibroblast growth factor (FGF) signaling to regulate embryo morphogenesis (15). Therefore, it is worth investigating their crosstalk in the context of cancer development to develop potent cancer therapy strategies.

In conclusion, we are now in a good position to approach cancer from the perspective of developmental biology, which will continue to provide valuable insights into the mechanisms and roles of PCP signaling. Realizing that the role of PCP signaling in cancer is a budding area, there is no doubt that in-depth dissection of the role of PCP signaling in cancer development will uncover novel and powerful therapeutic approaches for cancer treatment in the coming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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