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Vangl1 and Vangl2: planar cell polarity components with a developing role in cancer

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Abstract

Cancers commonly reactivate embryonic developmental pathways to promote the aggressive behavior of their cells, resulting in metastasis and poor patient outcome. While developmental pathways such as canonical Wnt signaling and epithelial-to-mesenchymal transition have received much attention, our understanding of the role of the planar cell polarity (PCP) pathway in tumor progression remains rudimentary. Protein components of PCP, including a subset that overlaps with the canonical Wnt pathway, partition in polarized epithelial cells along the planar axis and are required for the establishment and maintenance of lateral epithelial polarity. Significant insight into PCP regulation of developmental and cellular processes has come from analysis of the functions of the core PCP scaffolding proteins Vangl1 and Vangl2. In particular, studies on zebrafish and with *Looptail* (*Lp*) mice, which harbor point mutations in *Vangl2* that alter its trafficking and localization, point to roles for the PCP pathway in maintaining cell polarization along both the apical–basal and planar axes as well as in collective cell motility and invasiveness. Recent findings have suggested that the Vangls can promote similar processes in tumor cells. Initial data-mining efforts suggest that *VANGL1* and *VANGL2* are dysregulated in human cancers, and estrogen receptor (ER)-positive breast cancer patients whose tumors exhibit elevated *VANGL1* expression suffer from shortened overall survival. Overall, evidence is beginning to accumulate that the heightened cellular motility and invasiveness associated with PCP reactivation may contribute to the malignancy of some cancer subtypes.

Keywords

polarity; planar cell polarity; Vangl1; Vangl2; developmental pathways; cell migration; invasiveness; breast cancer

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Introduction

Planar cell polarity (PCP), or the organization of epithelial cells along the planar axis orthogonal to the apical–basal axis, is critical for the fidelity of embryonic development and the proper structuring of multicellular tissues. For example, in the mouse embryo, PCP is responsible for events as diverse as ensuring proper neural tube closure (Kibar *et al.* 2001, Murdoch *et al.* 2001a) and orienting mouse hairs toward the posterior of the animal (Devenport & Fuchs 2008). Loss of proper PCP leads to a variety of developmental disorders, and recent studies have begun to highlight a link between PCP signaling and cancer. As the reactivation of developmental pathways is a central theme in tumor initiation and progression, the essential roles of PCP in development and tissue organization make this pathway a prime candidate for further exploration as a significant contributor to tumor progression. Analysis of the role of PCP components in cell migration and metastasis as mimicry of embryonic convergent extension (Luga & Wrana 2013) underscores the relevance of PCP signaling to late-stage cancer.

Noncanonical Wnt signaling encompasses a variety of pathways, including PCP, Wnt/calcium signaling, and Wnt–Ror signaling (Gao & Chen 2010). While the noncanonical Wnt pathways can share components such as Dvl with the canonical arm, the pathways diverge in their downstream events and cellular outcomes. The canonical Wnt pathway, which involves the stabilization of cytosolic β -catenin, is largely involved in the regulation of genes controlling cellular proliferation and differentiation. On the other hand, noncanonical signaling, which does not require cytosolic β -catenin, generally results in cytoskeletal rearrangements through the small GTPases Rac and Rho (Anastas & Moon 2013). The shared components between the two pathways probably allow crosstalk between canonical and noncanonical signaling (Mikels & Nusse 2006, Grumolato *et al.* 2010, Gao *et al.* 2011), while components unique to PCP such as the cell surface Vangl proteins can provide the scaffolding necessary for the assembly of PCP signaling complexes. While mutations in the VANGLs and other PCP proteins have been implicated in a variety of human diseases, including neural tube defects (Kibar *et al.* 2007, Lei *et al.* 2010), cystic renal disease (Goggolidou 2013), congenital heart disease (Wu *et al.* 2011), and lung diseases (Yates & Dean 2011), the role of PCP pathway components in cancer is an understudied topic and thus deserving more attention. This review focuses on the function of the Vangls, core PCP proteins not shared by canonical Wnt signaling, and highlights the growing evidence for roles of VANGL proteins in cancer progression.

Vangl structure

The mammalian homologues of the *Drosophila melanogaster* protein Van Gogh (Vang/Strabismus), Vangl1 and Vangl2, each contain four transmembrane domains and intracellular N- and C-termini (Murdoch *et al.* 2001a). Figure 1 illustrates Vangl2 protein topology and highlights the key regulatory elements of the mouse and human forms, which share 99% amino acid identity. Human VANGL1 and VANGL2 are 72% identical, and although the Vangls lack any known enzymatic activity, they contain important protein–protein interaction domains: a C-terminal coiled-coil domain and a PDZ-binding motif (Murdoch *et al.* 2001a). Vangl proteins can also homo- and hetero-oligomerize, processes

that do not require the N- or C-terminal cytoplasmic regions (Belotti *et al.* 2012). Vangl2 has been shown to contain a conserved plasma membrane-targeting motif in the C-terminal cytoplasmic domain; mutations in this site disrupt Vangl2 trafficking from the Golgi to the plasma membrane (Guo *et al.* 2013).

It appears that much of Vangl function relies on its cellular localization. Critical *Vangl2* mutations identified in the *Looptail (Lp)* mouse are located in the C-terminal cytoplasmic region and result in severe neural tube defects (Kibar *et al.* 2001, Murdoch *et al.* 2001a). Mutant Vangl2 protein is retained in the endoplasmic reticulum, has reduced stability, and is degraded by the proteasome (Iliescu *et al.* 2011). Further effects of these mutants in development are detailed below. Notably, it has been reported that Wnt5A-induced phosphorylation of two N-terminal clusters in Vangl2 regulates its asymmetric localization in developing mouse limb bud chondrocytes (Gao *et al.* 2011).

Biochemically, the functions of Vangl1 and Vangl2 appear nearly identical, and the observed differences in their effects upon *in vivo* manipulation probably result from the differences in temporal or spatial expression patterns. Although alterations to *Vangl2* result in more severe developmental defects, suggesting a more central role for this form in early tissue organization, both proteins have been implicated in tumor progression.

Vangls in development

PCP has been studied extensively in *Drosophila* and has been reviewed in detail previously (Peng & Axelrod 2012). In flies, PCP is best characterized mechanistically in the developing wing epithelium, where asymmetric apical localization of protein complexes drives consistent distal positioning of wing hairs (Fig. 2A). The proteins Prickle, Vang, and Flamingo (*Drosophila* Fmi/mammalian CELSR) form a complex on the proximal side of each cell, while Frizzled (*Drosophila* Fz/mammalian Fzd), Dishevelled (Dsh/Dvl), Diego, and Fmi form a complex on the distal side. Positive feedback maintains this segregation through two general mechanisms. First, each complex restricts the subcellular localization of its opposing complex; for example, Vang and Prickle prevent proximal Dsh localization (Tree *et al.* 2002). In addition, opposing complexes on neighboring cells interact. For example, proximal Vang and distal Fz on adjacent cells interact to reinforce and propagate the polarity signal through the tissue (Wu & Mlodzik 2008). Abolishing the activities of the 'core module' proteins disrupts PCP and leads to aberrant phenotypes that are propagated beyond the manipulated cells, demonstrating that intercellular communication drives PCP. Asymmetric localization of the components of this 'core module' has also been observed in the developing *Drosophila* eye and thorax.

In vertebrates, most developmental studies have focused on Vangl2. Alterations to Vangl2 lead to severe developmental defects in multiple organs, providing fundamental insight into PCP function. Most notably, depletion of *Vangl2* in zebrafish (*Danio rerio*) causes a severe reduction in the body length due to defective convergent extension (Jessen *et al.* 2002, Ciruna *et al.* 2006), where a tissue elongates along one axis while narrowing along a perpendicular axis. This process can occur through two mechanisms: via collective cell migration where cells polarize and migrate along the elongating anterior–posterior axis (Fig.

2B, left panel) or via cell intercalation where cells polarize and migrate along the narrowing medial–lateral axis (Fig. 2B, right panel; reviewed by Tada & Heisenberg (2012)). *Vangl2* is necessary for both modes of convergent extension in zebrafish (Sepich *et al.* 2000, Jessen *et al.* 2002).

In mice, the *Lp* mutation was first described by Strong & Hollander (1949) and subsequently mapped to *Vangl2* in 2001 (Kibar *et al.* 2001, Murdoch *et al.* 2001a). The *Lp/+* heterozygote shows a ‘looped’ tail, wobbly head movements, and delays in neural tube closure, while *Lp/Lp* homozygous mice die *in utero* and suffer from severe neural tube closure defects such as craniorachischisis and spina bifida (van Abeelen & Raven 1968, Wang *et al.* 2006), again resulting from impaired convergent extension (Ybot-Gonzalez *et al.* 2007). In both mice and zebrafish, loss of *Vangl2* function also results in the dysregulation of a variety of cellular processes such as deficient cell differentiation (Park & Moon 2002, Lake & Sokol 2009), migration (Park & Moon 2002, Glasco *et al.* 2012), hair alignment (Lopez-Schier & Hudspeth 2006, Devenport & Fuchs 2008), and cilia localization, commonly studied in the developing embryonic mouse cochlea (Montcouquiol *et al.* 2003, Borovina *et al.* 2010). Further, *Lp/Lp* mice exhibit erroneous organogenesis, including abnormal heart looping (Henderson *et al.* 2001) and branching defects in the lungs (Yates *et al.* 2010a) and kidneys (Yates *et al.* 2010b). A common theme among *Lp/Lp* phenotypes is deficient cellular migratory and invasive behavior, mirroring the hypothesized role of the Vangls in cancer. Interestingly, neural tube defects in the *Vangl1* knockout mouse are not as penetrant as in *Lp/Lp* mice, but loss of both *Vangl1* and *Vangl2* leads to a much higher frequency of craniorachischisis (Torban *et al.* 2008, Song *et al.* 2010), suggesting that *Vangl2* plays the predominant role in development. *VANGL1* and *VANGL2* loss-of-function mutations have been identified in patients who suffer from neural tube defects, suggesting that Vangl proteins regulate similar processes in mice and humans (Kibar *et al.* 2007, Lei *et al.* 2010). Thus, although the most dramatic effects of *Vangl2* inactivation are consequences of impaired convergent extension, many other critical cellular processes are probably disrupted.

While the effects of mutations in *Vangl2* and other PCP components have been described in many contexts, the molecular causes underlying the developmental defects observed in *Vangl2^{Lp/Lp}* animals remain to be fully clarified. Interestingly, although the *Lp/Lp* mutant is often used as a model for loss of function, its phenotype is more severe than that observed for the *Vangl2* knockout. One possible explanation for this discrepancy may be attributed to the trafficking and localization of the mutant protein. The mutant *Vangl2^{Lp}* protein is synthesized and trapped in the endoplasmic reticulum (Iliescu *et al.* 2011), where it can interact with other PCP components (including *Vangl1*) to prevent their trafficking to the plasma membrane. A true *Vangl2* knockout animal has less severe developmental defects probably because the function of *Vangl1* and other PCP effectors is not affected as strongly as in the *Lp/Lp* animal (Yin *et al.* 2012).

Studies on trafficking components also underscore the notion that trafficking and localization of PCP components are essential for proper development. Loss-of-function mutations in *Sec24b*, a component of COP II anterograde vesicles, lead to open neural tubes and loss of cochlear polarity reminiscent of mutations in key PCP components. *Sec24b* is

essential for trafficking Vangl2 from the endoplasmic reticulum to the Golgi, and mutant *Vangl2^{Lp}* proteins are not properly sorted into anterograde vesicles (Merte *et al.* 2010). Further studies using *Vangl2^{-/-}* animals will be necessary to determine the true extent of Vangl2 function in development.

Although Vangl2 is generally thought to regulate PCP rather than apical–basal polarity, there is evidence of regulatory overlap between the two polarity modes. In the developing mouse uterus, female *Lp/Lp* mutants show some errors in apical–basal polarity, including loss of columnar epithelial cell morphology, apical filamentous actin, and lateral E-cadherin (Fig. 2C; Vandenberg & Sassoon 2009). *Lp/Lp* embryos or embryos overexpressing *Vangl2* have disrupted adherens junctions in their developing neural tubes, along with a loss of cortical actin and cadherins (Lindqvist *et al.* 2010). In mouse embryonic day 6.5, epiblasts knockout of the core PCP component Prickle1, which in *Drosophila* interacts with Vang and prohibits mislocalization of Dsh and Fz (Peng & Axelrod 2012), leads to misoriented cell division and loss of properly localized laminin, actin filaments, E-cadherin, and PKC ζ . *Prickle1^{+/-}/Vangl2^{Lp/+}* mice appear to pheno-copy these effects, suggesting that a *Vangl2*–*Prickle1* genetic interaction is involved in epiblast polarity (Tao *et al.* 2009). These observations suggest that in addition to simply localizing to the apical–basolateral boundary, Vangl2 may assist in maintaining apical–basal polarity.

Scribble, part of a complex that defines the basolateral domain and prevents expansion of the apical domain (reviewed by Humbert *et al.* (2006)), interacts with the Vangls (Kallay *et al.* 2006, Anastas *et al.* 2012) and also functions as a PCP effector in *Drosophila* (Courbard *et al.* 2009). In mice, Scribble mutations give rise to open neural tubes (Murdoch *et al.* 2001b) and misaligned cochlear cilia (Montcouquiol *et al.* 2003) similar to the phenotypes observed in *Vangl2^{Lp/Lp}* animals. The loss of apical–basal polarity in the *Lp/Lp* uterus is accompanied by mislocalization of Scribble (Vandenberg & Sassoon 2009), and it is possible that Vangl2 affects apical–basal polarity in part through Scribble. Vangl2 may also be critical to establishing apical–basal polarity in early embryo development. In *Xenopus* oocytes and early embryos, Vangl2 interacts with aPKC, part of the machinery defining the apical domain. Loss of Vangl2 or aPKC disrupts apical–basal polarity by preventing proper expression and localization of specific mRNAs important in early development (Cha *et al.* 2011). Although generally considered as two distinct pathways, there may be unexplored crosstalk between PCP and apical–basal polarity involving the Vangl proteins.

While stem cell maintenance is more closely associated with canonical Wnt signaling (Reya & Clevers 2005, Nusse 2008), Vangl2 and noncanonical Wnt signaling are required for stem cell maintenance in at least one context. During muscle regeneration, the satellite stem cell population expands to generate sufficient differentiated progeny to repair damage. This expansion is stimulated by Wnt7a, which induces polarized localization of Vangl2, and mice lacking Wnt7a are deficient in satellite cells during regeneration. Vangl2 is necessary for this stem cell expansion, as *Vangl2* knockdown prevents the Wnt7a-stimulated expansion (Le Grand *et al.* 2009). Further, Vangl2 expression in muscle fibers is induced by nitric oxide to promote stem cell expansion (Buono *et al.* 2012), demonstrating that other mechanisms of regulating Vangl2 and PCP signaling remain to be explored.

VANGLs in cancer

Decades of studies have demonstrated that PCP signaling is essential for development, but its involvement in adult tissue maintenance is poorly understood. As PCP signaling can regulate processes such as differentiation, cell motility, and tissue organization in development, it has been hypothesized that the key components of PCP such as VANGL1 and VANGL2 might also be dysregulated in cancers.

The Cancer Genome Atlas (TCGA) project has analyzed hundreds of tumor samples from numerous tumor types. Table 1 summarizes VANGL1 and VANGL2 expression levels and copy number trends in breast, ovarian, uterine, and prostate cancer from TCGA datasets. While *VANGL1* expression levels are upregulated in 5% of invasive breast carcinomas compared with healthy tissue, *VANGL1* expression shows no clear trend in ovarian, uterine, or prostate cancers. By contrast, *VANGL2* is consistently upregulated and amplified in breast, ovarian, and uterine carcinomas (Cerami *et al.* 2012, Cancer Genome Atlas Research Network *et al.* 2013, Gao *et al.* 2013). Notably, *VANGL2* transcript overexpression in 24% of invasive breast carcinomas correlates with the amplification of the gene in 13% of patient tumors, raising the possibility that elevated VANGL2 levels contribute to disease progression.

Anastas *et al.* (2012) had previously demonstrated that elevated *VANGL1* expression predicts an increased risk of tumor recurrence in breast cancer patients. In Fig. 3, we used the Kaplan–Meier plotter (KM plotter) dataset, which combines gene expression and clinical data from Gene Expression Omnibus (GEO), TCGA, and The European Genome-phenome Archive (EGA), to assess the correlation of *VANGL1* expression and overall breast cancer patient survival. (Similar data with *VANGL2* were not available at the time of our analysis for this review.) Interestingly, *VANGL1* expression does not correlate with the overall survival in the total breast cancer patient population, but higher *VANGL1* expression is associated with reduced overall survival in the estrogen receptor (ER)-positive subset of patients (Fig. 3; Gyorffy *et al.* 2010, 2012). These observations imply that the importance of PCP signaling in cancer progression may be context dependent. We hypothesize that aberrant engagement of the PCP signaling pathway may have more dramatic effects on tumor progression in cancer subtypes that are classically considered less aggressive, such as ER-positive breast cancers.

Unfortunately, the KM plotter dataset does not segregate the luminal A and luminal B breast cancer subtypes, thus it is unclear whether elevated *VANGL1* expression correlates with tumors already predisposed to aggressive behavior. Similarly, the relationship between ER and PCP signaling remains unclear. The noncanonical ligand *WNT11*, which signals in PCP pathways (Gao 2012), is regulated by estrogen during development (Mohamed *et al.* 2004, Lin *et al.* 2007). Furthermore, recent microarray data suggest that *VANGL1* is down-regulated by estrogen in the pituitary gland (Kim *et al.* 2011), while in MCF7 breast cancer cells *VANGL1* expression may be increased by estrogen (Carroll *et al.* 2006, Al Saleh *et al.* 2011). These studies suggest that connections between PCP pathways and ER signaling exist but remain unexplored and that *VANGL1* regulation by estrogen may be cell type or tissue dependent. Although it is unknown whether *VANGL1* or *VANGL2* is a direct transcriptional

target of ER, a better understanding of the crosstalk between ER and PCP signaling would provide valuable insights into breast cancer biology. In addition, as Vangl probes become more reliable and tumor analyses more prevalent, it will be of interest to determine whether *VANGL* expression correlates with overall, relapse-free, and metastasis-free survival in other cancers, and if *VANGL2* is more highly prognostic than *VANGL1* given its more prominent role in development.

Although gene expression and copy number data implicate *VANGL1* and *VANGL2* in cancer progression, their mechanisms of action in cancer remain largely unknown. Knockdown of *VANGL1* reduces the migration of the aggressive MDA-MB-231 human breast cancer cell line, and in wound healing assays *VANGL1* localizes to the leading edge of lamellipodia where it forms a complex containing the PCP effector SCRIBBLE. This complex is not detected in tightly packed non-transformed mammary cells, suggesting that altered *VANGL* signaling contributes to breast cancer cell motility (Anastas *et al.* 2012). In a separate study, treatment with exosome-containing conditioned media from L-cell fibroblasts stimulated breast cancer cell motility dependent on *VANGL1* and other PCP components. *VANGL1* localized to the base and arms of cell protrusions in individual migrating cells (see Fig. 4), where it co-localized with *PRICKLR1*, while *FZD6* and *DVL1* localized to the tips of these protrusions (Luga *et al.* 2012) resulting in a segregation of PCP components similar to that observed both in *Drosophila* tissues (Peng & Axelrod 2012) and in developing axons (Shafer *et al.* 2011). Interestingly, *PRICKLE1* knockdown suppressed metastasis but not primary tumor growth rate in MDA-MB-231 cells co-transplanted with L-cells, demonstrating that PCP proteins probably play roles in tumor progression beyond initiation and growth. Although the MDA-MB-231 cells activate PCP signaling through autocrine WNT11 secretion, L-cell-derived exosomes were critical for WNT11 availability, demonstrating that cooperativity with the tumor microenvironment can augment PCP signaling (Luga *et al.* 2012).

In other cancer types, *VANGLs* have been generally shown to promote proliferation and invasion. *VANGL1* knockdown in multiple cancer cell lines (such as colon, gastric, head and neck, hepatocellular, and oral cavity squamous cell carcinoma) produced a variety of phenotypes, including reduced cell proliferation, invasion, *AP-1* transcriptional activity, and xenograft growth (Yagyu *et al.* 2002, Lee *et al.* 2009, Ryu *et al.* 2010, Hwang *et al.* 2011, Yoon *et al.* 2013). *VANGL1* is also known to interact with the metastasis suppressor Kai1 (CD82) in colon cancer, but the functional significance of this interaction is not clear (Lee *et al.* 2004). *VANGL2* and other PCP components are overexpressed in chronic lymphoid leukemia (CLL), and CLL cells undergo PCP-driven migration (Kaucka *et al.* 2013).

On the other hand, *VANGLs* may inhibit processes associated with tumor progression in some contexts. During zebrafish gastrulation, *Vangl2* regulates the matrix metalloproteinase 14 (MMP14) by decreasing its availability at the plasma membrane through FAK-dependent endocytosis (Williams *et al.* 2012a). A similar role for *VANGL2* in extracellular matrix remodeling has been suggested in cancer cells, as *VANGL2* inversely correlates with cell motility and cell surface MMP2/MMP14 levels in a fibrosarcoma cell line (Cantrell & Jessen 2010, Williams *et al.* 2012b). Furthermore, PCP signaling may suppress canonical Wnt signaling (Mikels & Nusse 2006, Gao *et al.* 2011), which functions in cancer tumor-

initiating cell maintenance (Anastas & Moon 2013). For example, *Vangl2* expression has been observed to attenuate canonical Wnt signaling upstream of β -catenin by recruiting Dvl1 from cytoplasmic puncta to the plasma membrane (Park & Moon 2002). Additional studies have demonstrated that *VANGL2* promoter methylation is associated with increased tumor grade and *BRAF* mutation in colon cancer, and that *VANGL2* overexpression in cell lines decreases canonical Wnt signaling (Piazzi *et al.* 2013). These observations suggest that *VANGL2* can act as a tumor suppressor in canonical Wnt-dependent tumors, further supporting the hypothesis that the role of PCP components in cancer is context dependent. Figure 5 summarizes many of the observed effects of *VANGL* proteins on the properties of cell lines derived from various tumor types.

The roles of immediate downstream *VANGL* effector proteins necessary to induce tumor responses are also under investigation. In breast cancer cells stimulated with L-cell conditioned media, knockdown of *PRICKLE1* reduces metastasis (Luga *et al.* 2012). Further, *PRICKLE1* expression is elevated with many PCP components in CLL (Kaucka *et al.* 2013), suggesting that it promotes PCP signaling in tumors. On the other hand, the role of *SCRIBBLE* in tumor progression appears to be more complex. Although Anastas *et al.* (2012) showed that high *SCRIBBLE* expression is correlated with an increased risk of relapse, other studies have shown that *SCRIBBLE* expression is decreased in breast tumors (Navarro *et al.* 2005, Zhan *et al.* 2008). *Scribble* mutations in *Drosophila* contribute to aberrant neoplastic growth (Brumby & Richardson 2003) and *SCRIBBLE* is a proposed tumor suppressor in humans (Navarro *et al.* 2005). However, as *SCRIBBLE* regulates polarity through both *VANGL*-dependent and -independent mechanisms, its contributions to PCP-driven tumor progression remain to be addressed.

Conclusions

The striking developmental defects observed in *Vangl2^{Lp/Lp}* mice arise from alterations to core cellular processes such as migration and invasion, processes that are also critical for tumor progression. Mounting patient and mechanistic data suggest that dysregulation of PCP components, including the *VANGL* proteins, probably promotes tumor aggressiveness. Reactivation of this conserved pathway generally increases invasion and metastasis, the cause of the vast majority of cancer deaths (Valastyan & Weinberg 2011). A molecular model encompassing many of the key observations to date is illustrated in Fig. 6 and emphasizes the potential importance of PCP component segregation in the leading edge structures of migrating tumor cells. Importantly, the effects of aberrant *VANGL* regulation on tumors may be context dependent and play stronger roles in typically less aggressive diseases, where PCP signaling might synergize with oncogenic pathways to promote metastasis. For example, the observation that high *VANGL1* expression correlates with reduced survival in ER-positive breast cancer patients suggests that the engagement of PCP signaling could facilitate the shift of a traditionally less aggressive tumor to a more aggressive state. In this regard, *VANGL* expression or other hallmarks of PCP engagement could serve as prognostic markers for ER-positive patients at risk for disease progression.

Although high *VANGL1* expression is associated with poorer prognosis in some patients, neither *VANGL1* nor *VANGL2* has been identified as an oncogene, suggesting that they may

function later in tumor development to amplify the hallmarks of metastatic disease rather than acting directly in tumorigenesis. It is therefore possible that elevated PCP signaling is acquired after initial tumor formation, and future studies are necessary to explore the mechanisms by which this pathway is reactivated. As has already been suggested, the tumor microenvironment could play a key role in PCP reactivation (Luga *et al.* 2012), a concept that warrants further exploration.

In aggressive epithelial tumors, cells lose their typical apical–basal polarity and eventually undergo epithelial-to-mesenchymal transition (EMT). EMT increases the migratory and invasive capabilities of tumor cells and is strongly associated with the expansion of tumor-initiating cells, which may be responsible for increased relapse and metastasis (Valastyan & Weinberg 2011). Altered VANGL2 signaling leads to defects in apical–basal polarity in several developmental systems (Tao *et al.* 2009, Vandenberg & Sassoon 2009, Cha *et al.* 2011) and contributes to satellite stem cell maintenance in muscle regeneration (Le Grand *et al.* 2009), suggesting that unexplored crosstalk occurs between the orthogonal polarity modes. Although several studies have demonstrated that VANGL1 contributes to invasiveness of several cancers (e.g. Ryu *et al.* 2010, Yoon *et al.* 2013), the potential for VANGLs to promote EMT and regulate tumor-initiating cells has not been reported. The extent to which PCP components contribute to EMT should be explored, as VANGLs play important roles in analogous developmental pathways. Future studies will further illuminate the diverse processes regulated by the Vangls and will probably demonstrate substantial overlap between its functions in development and cancer.

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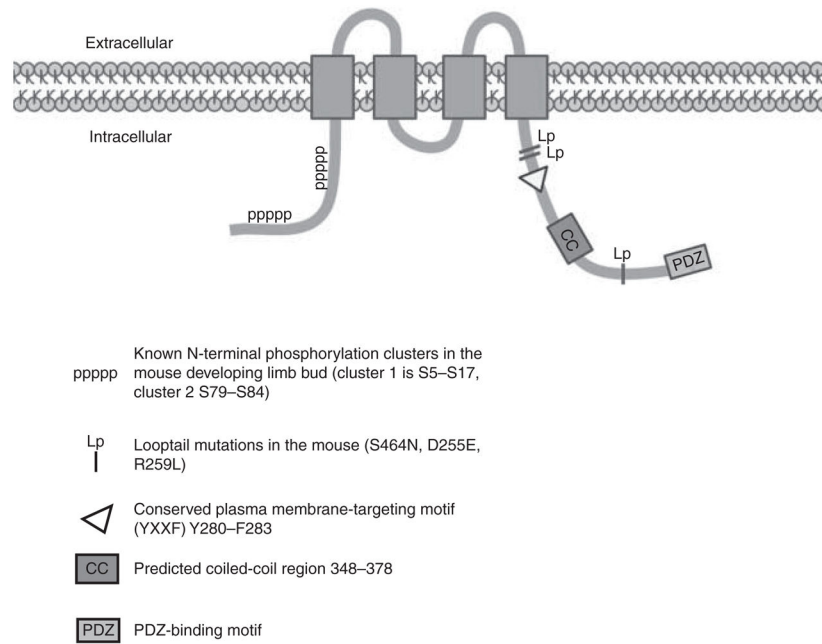


Figure 1. Vangl2 structure. The Vangls are cell surface proteins featuring four transmembrane domains as well as intracellular amino- and carboxy-terminal domains. The N-terminal domain of Vangl2 contains two serine-rich regions whose phosphorylation in response to Wnt5a has been reported to regulate its asymmetric distribution. The C-terminal domain is the region mutated in the *Lp* mouse and it contains two protein–protein interaction motifs and a plasma membrane-targeting motif.

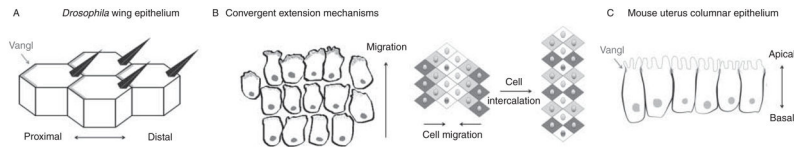


Figure 2.

Cellular and developmental processes regulated by Vangl proteins. (A) In the *Drosophila* wing, PCP protein complexes asymmetrically localize on opposite ends of the cell with Vang, Prickle, and Fmi at the proximal (shaded) end. This asymmetric organization maintains cell planar polarity, allowing for distal hair (black spikes) orientation. (B) Convergent extension can occur via two mechanisms. In collective cell migration (left panel), the graded asymmetric distribution of PCP components (shaded in gray) follows the direction of elongation and contributes to cell motility. In cell intercalation (right panel), polarized cells simultaneously migrate inward and intercalate to elongate tissue structure. (C) VANGL2 is localized apically (shaded) to the plasma membrane of the columnar epithelium of the mouse uterus, and is particularly enriched near cell–cell contacts. Loss of VANGL2 results in a loss of columnar epithelial organization and apical–basal polarity, disrupting tissue development and function.

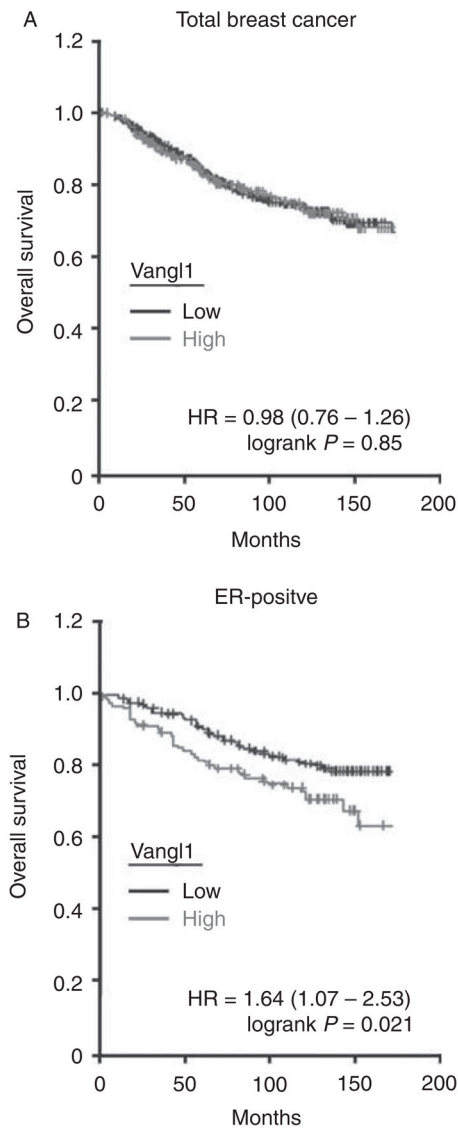


Figure 3. *VANGL1* overexpression correlates with worsened prognosis in ER-positive breast cancer patients. Kaplan–Meier plots depicting overall survival in months of (A) all breast cancer patients ($n=747$), and (B) only ER-positive breast cancer patients ($n=377$) are illustrated. Patient cohorts were divided based on *VANGL1* expression levels with the upper tertile depicted in gray. (www.kmplot.com, Affymetrix HG-U133A, HG-U133 Plus 2.0, and HG-U133A 2.0 microarrays, Access date: 20 February 2014).

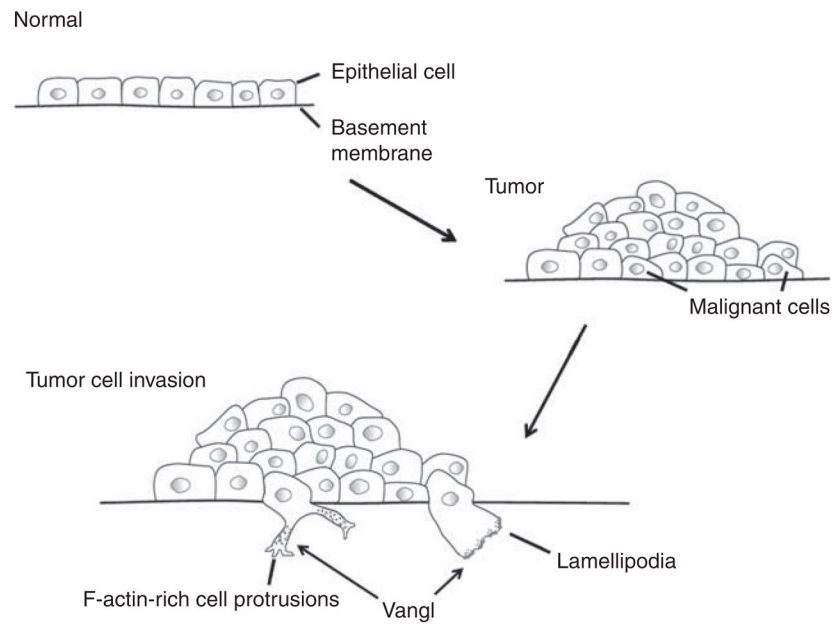


Figure 4. Model of VANGL1 distribution in invasive breast cancer. In a breast cancer cell line (MDA-MB-231), VANGL1 was found at the leading edge of lamellipodia and along the arm of F-actin-rich protrusions of motile cells and was required for efficient cell motility. These observations suggest that breast cancer cells concentrate VANGL in leading protrusions to facilitate invasiveness.

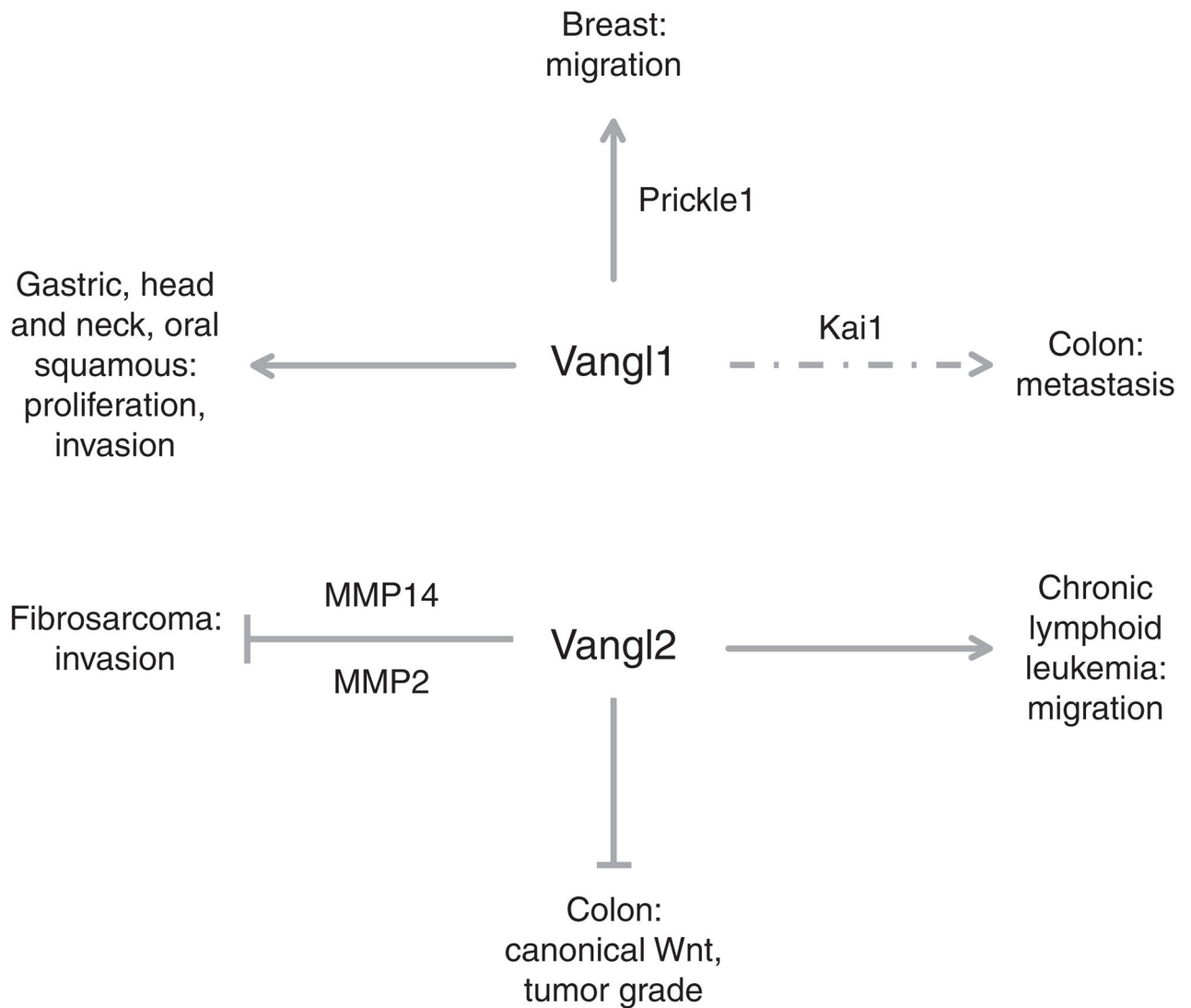


Figure 5. Effects of *VANGL* expression on cancer cell line phenotypes. Pointed arrows indicate an augmenting function for Vangls in mediating the indicated phenotypes for various cancers, while bar-ended arrows indicate suppressing effects. The broken arrow indicates a tentative functional connection. Gene names next to arrows indicate proteins that act in a concerted fashion with Vangls to produce the phenotype.

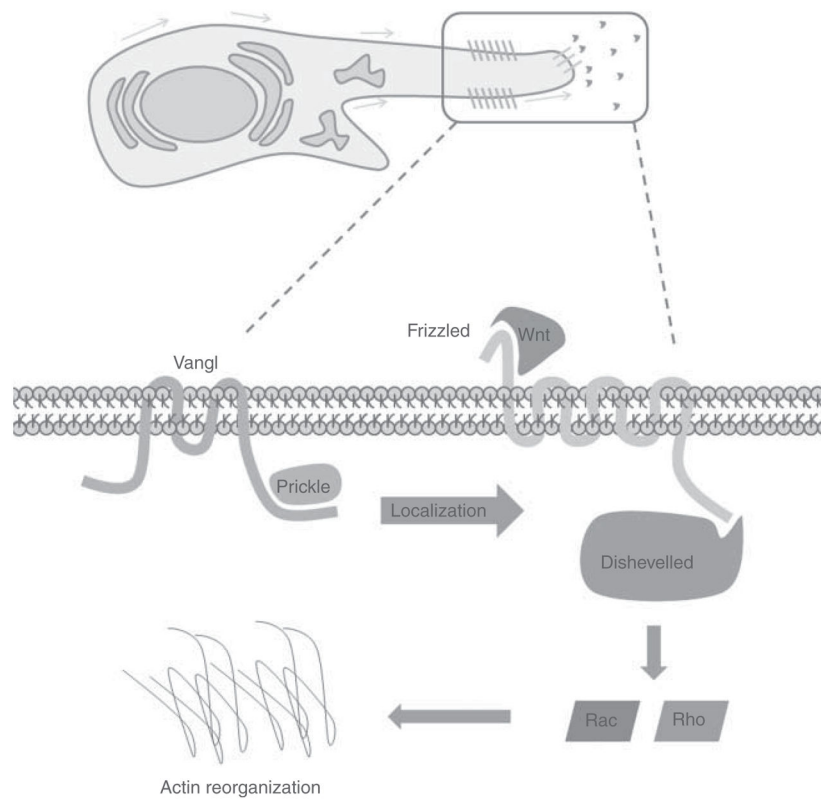


Figure 6. Molecular model of Vangl signaling in cancer cell motility. Vangl1 and Prickle form a complex at the base and arms of cell protrusions, which reinforces the polarity of these structures in migrating cancer cells, segregating Fzd and Dvl to the tips. Fzd receptors bind Wnt ligand to transduce signals via Dishevelled, leading to Rac and Rho activation, actin cytoskeletal reorganization, and ultimately cellular motility.

Table 1

Vangl dysregulation in endocrine-related cancers

Cancer	Expression	Copy number
VANGL1		
Breast invasive carcinoma	5% (53/988) up	<1% (6/988) amplified <1% (4/988) homozygous deletion
Ovarian serous cystadenocarcinoma	5% (14/261) up 2% (5/261) down	2% (13/570) amplified <1% (4/570) homozygous deletion
Uterine corpus endometrial carcinoma	4% (13/333) up <1% (2/333) down	None (0/363) amplified <1% (1/363) homozygous deletion
Prostate adenocarcinoma	4% (8/195) up 2% (4/195) down	None (0/197) amplified <1% (1/197) homozygous deletion Access date: 20 February 2014
VANGL2		
Breast invasive carcinoma	24% (236/988) up	13% (127/988) amplified
Ovarian serous cystadenocarcinoma	14% (36/261) up	5% (29/568) amplified
Uterine corpus endometrial carcinoma	10% (34/333) up	5% (19/363) amplified
Prostate adenocarcinoma	3% (6/195) up	<1% (1/197) amplified 2% (3/197) homozygous deletion Access date: 20 February 2014

The relative expression levels and copy number alterations for *VANGL1* (upper panel) and *VANGL2* (lower panel) in various tumor types are summarized as the percent of samples with altered message or copy number (number of samples with alteration/total number), followed by the type of alteration.