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.

Key Words
- polarity
- planar cell polarity
- Vangl1
- Vangl2
- developmental pathways
- cell migration
- invasiveness
- breast cancer

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 have 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.

![Vangl2 structure](image-url)
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, deletion 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 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.
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 Vangl 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 Vangl2Lp/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 Vangl2Lp protein is synthesized and trapped in the endoplasmic reticulum (Ilescu 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 Vangl2Lp 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 adherent 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 PKCζ. Prickle1+/−/Vangl2Lp/+ mice appear to phenotype 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 (Coubard 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 Vanlg2Lp/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 Vanlg2 affects apical–basal polarity in part through Scribble. Vanlg2 may also be critical to establishing apical–basal polarity in early embryo development. In Xenopus oocytes and early embryos, Vanlg2 interacts with aPKC, part of the machinery defining the apical domain. Loss of Vanlg2 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 Vanl proteins.

While stem cell maintenance is more closely associated with canonical Wnt signaling (Reya & Clevers 2005, Nusse 2008), Vanl2 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 Vanlg2, and mice lacking Wnt7a are deficient in satellite cells during regeneration. Vanlg2 is necessary for this stem cell expansion, as Vanlg2 knockdown prevents the Wnt7a-stimulated expansion (Le Grand et al. 2009). Further, Vanlg2 expression in muscle fibers is induced by nitric oxide to promote stem cell expansion (Buono et al. 2012), demonstrating that other mechanisms of regulating Vanlg2 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.

### Table 1  Vanlg dysregulation in endocrine-related cancers

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

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.
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 downregulated 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.

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).
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

![Figure 4](http://erc.endocrinology-journals.org)

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.
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 Vangl2Lp/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.

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

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References


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