The planar cell polarity protein VANGL2 coordinates remodeling of the extracellular matrix

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Keywords: fibronectin, extracellular matrix, gastrulation, invadopodia, migration, MMP14/MT1-MMP, planar cell polarity, VANGL2, zebrafish

Abbreviations: ECM, extracellular matrix; FAK, focal adhesion kinase; MMP14, membrane type-1 matrix metalloproteinase; PCP, planar cell polarity; VANGL2, Vang-like 2

Understanding how planar cell polarity (PCP) is established, maintained, and coordinated in migrating cell populations is an important area of research with implications for both embryonic morphogenesis and tumor cell invasion. We recently reported that the PCP protein Vang-like 2 (VANGL2) regulates the endocytosis and cell surface level of membrane type-1 matrix metalloproteinase (MMP14 or MT1-MMP). Here, we further discuss these findings in terms of extracellular matrix (ECM) remodeling, cell migration and zebrafish gastrulation. We also demonstrate that VANGL2 function impacts the focal degradation of ECM by human cancer cells including the formation or stability of invadopodia. Together, our findings implicate MMP14 as a downstream effector of VANGL2 signaling and suggest a model whereby the regulation of pericellular proteolysis is a fundamental aspect of PCP in migrating cells.

During zebrafish gastrulation, PCP is defined as the elongation and mediolateral alignment of cells as they engage in polarized behaviors including collective or group migration.1-4 Over a decade ago it was recognized that homologs of proteins regulating PCP in cuticular structures of Drosophila melanogaster also control PCP in gastrula cells.2,5,6 Zebrafish gastrulation mutant embryos such as trilobite/vangl2 exhibit a PCP phenotype characterized by shortened and broadened embryonic body axes.2,7,8 It is generally thought that vertebrate PCP signaling regulates the formation, polarization, and/or stabilization of actin-rich membrane protrusions.9 This concept is largely based on data from the fly wing epithelium demonstrating that PCP proteins restrict the formation and localization actin-rich structures.10 Indeed, Rho family small GTPases are known regulators of the actin cytoskeleton and influence gastrulation cell movements in the Xenopus laevis embryo.11-13 Disruption of membrane protrusive activity in the zebrafish gastrula is thought to underlie the PCP defect in trilobite/vangl2 mutant embryos.2 However, in migrating cell populations the establishment of PCP must be coordinated with other proteins/pathways regulating motility including those affecting ECM remodeling and cell-matrix adhesion.14 Therefore, identification of additional proteins regulating gastrulation cell movements and determination of how they interact with PCP signaling is crucial.

Previously our lab demonstrated that Mmp14 is required for PCP and exhibits a strong genetic interaction with kny`)k9oyc1w4,15 a Wnt co-receptor necessary for proper gastrulation cell movements.4 Subsequently we showed that a fibronectin- and laminin-containing ECM network develops coincidently with the timing of PCP establishment.16 By late gastrulation stages fibronectin and laminin form two layers; one between the ectoderm and mesoderm germ layers and a second localizing beneath (and surrounding) deep mesodermal and endodermal cells.16 Notably, utilizing a cancer cell culture model we further demonstrated that human VANGL2 regulates cell surface MMP14 expression, MMP2 activation, and invasion through an ECM barrier.17 Taken together, our previous data suggested a mechanistic connection between the establishment of PCP in migrating cells and matrix metalloproteinase-dependent ECM remodeling.

In our recent work, we hypothesized that the transmembrane PCP protein VANGL2 directly regulates cell surface levels of MMP14 by controlling its trafficking to or from the plasma membrane.18 To test this possibility we transfected human fibrosarcoma HT-1080 cells with either VANGL2 or control siRNAs and performed various endocytosis and recycling assays. These cells are frequently used to address mechanisms of MMP14 trafficking and localization to specific vesicular compartments.19,20 Utilizing a biochemical assay based on biotin labeling of cell surface proteins followed by shifting the temperature to 37°C, it became clear that VANGL2 knockdown cells have a defect in MMP14 endocytosis. Significantly, loss of VANGL2 function did not globally disrupt trafficking as indicated by endocytosis of transferrin.

In the embryo VANGL2 homologs are thought to function at the plasma membrane but their expression has been reported in

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Submitted: 03/15/12; Revised: 04/05/12; Accepted: 04/06/12
http://dx.doi.org/10.4161/cib.20291

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both membrane and vesicular compartments, a finding that we also observed in HT-1080 cells. Thus it was unclear whether VANGL2 signaling acted at the cell surface to influence MMP14 internalization. Previous data showed that endocytosis of MMP14 could be regulated downstream of focal adhesion kinase (FAK) phosphorylation at Y-397. This suggests that MMP14 trafficking might be coordinated with integrin function and the formation of cell-matrix adhesions. Indeed, we demonstrated that VANGL2-dependent effects on cell surface MMP14 required Y-397 phosphorylation of FAK. These results suggest that in HT-1080 cells VANGL2 regulates MMP14 endocytosis at the level of the plasma membrane.

Based on our cell culture data we further hypothesized that zebrafish trilobitevangl2 mutant embryos have increased matrix metalloproteinase activity. By extracting total embryo protein under conditions that maintain enzymatic activity, we were able to perform protease assays using fluorescent gelatin and collagen IV substrates. We found that trilobitevangl2 mutant embryos have significantly more protease activity than wild-type controls and that this activity could be suppressed using broad-spectrum or Mmp14-specific inhibitors. Moreover, by knocking down Mmp14 in Vangl2 loss of function embryos we were able to suppress the gastrulation cell movement defect indicating that Mmp14 acts downstream of Vangl2. We next determined whether loss of Vangl2 function during gastrulation affected formation of an ECM network. By immunolabeling for fibronectin and performing confocal microscopic imaging we showed that trilobitevangl2 mutant embryos have decreased ECM. These biochemical and molecular genetic data indicate that Vanl2-dependent regulation of Mmp14 activity is required for fibronectin remodelining in the zebrafish gastrula embryo. It is also likely that Mmp14 acts on additional ECM and non-ECM substrates to influence PCP during gastrulation.

In the fly wing epithelium, Van Gogh restricts or localizes the activity of other PCP proteins to specific polarized cellular domains. We therefore wondered whether human VANGL2 regulates cell surface proteolytic activity and focal matrix degradation at polarized plasma membrane structures including protrusions and invadopodia. First, we incubated HT-1080 cells on fluorescent gelatin for 20 h and quantified the total degradation area in relation to cell number. Here, the focal ECM degradation areas detected resembled footprints or tracks created by protease activity that is associated with membrane protrusions (Fig. 1A). Our data show that VANGL2 siRNA transfected cells have significantly more degradation areas per cell than controls (Fig. 1A and B) though the average size is not increased in VANGL2 knockdown cells (Fig. 1C). In contrast to the degradation areas produced by membrane protrusions, invadopodia are dot-like F-actin-rich structures that are formed at certain cell-matrix contact sites and exhibit increased Mmp14 activity and ECM degradation. To visualize invadopodia, we incubated HT-1080 cells on fluorescently labeled gelatin for 5 h prior to fixation and imaging. We identified actin-positive punctae that both co-labeled with cortactin and overlapped with foci of matrix degradation (Fig. 1D). These structures are thus considered invadopodia and were quantified in VANGL2 and control non-targeting siRNA transfected cells. Our results indicate that VANGL2 knockdown cells have more invadopodia than controls (Fig. 1E and F). Notably, the size of invadopodium and their associated matrix degradation spots appeared larger in VANGL2 knockdown cells than controls (Fig. 1E). However, because HT-1080 cells are highly motile on 2D ECM substrates, we were unable to quantify the focal degradation spots produced specifically by the invadopodia of individual cells. Together, our results support the notion that increased cell surface proteolytic activity in VANGL2 knockdown cells increases total focal matrix degradation and affects the formation or stability of invadopodia. Our data are consistent with observations that loss of Mmp14 function disrupts both invadopodia formation and proteolytic activity.

In summary, we have demonstrated that the PCP protein VANGL2 regulates MMP14 endocytosis and cell surface activity and that this membrane-tethered protease functions downstream of zebrafish Vangl2 to influence both ECM remodeling and gastrulation cell movements. Together with other work, these data suggest that the regulation of vesicular trafficking events may be a broadly applicable mechanism underlying the establishment of PCP in diverse cell types. We have now shown that human VANGL2 also impacts the focal degradation of ECM and the formation or stability of invadopodia. It will now be important to determine how zebrafish Vangl2 function influences polarized cell behaviors underlying collective or group migration. During gastrulation, migrating cells interact with ECM proteins but do not plow through or invade an ECM barrier. In this context, we suggest that tight regulation of cell surface MMP14 activity at cell-matrix focal adhesions is required to restrict membrane protrusive activity to specific cellular domains.

Materials and Methods

Control and VANGL2 knockdown cells were generated as described. The QCM gelatin invadopodia assays were performed and quantified according to manufacturer instructions (Millipore, ECM670). In each experiment 20,000 HT-1080 cells were plated per well of an 8-well chamber slide. The total number of degradation spots formed after 20 h incubation (including both invadopodia and other protrusive membrane structures) was quantified from three independent experiments (12 images per experiment = 36 fields of view analyzed, > 500 cells per condition). The number of invadopodia formed after 5 h was also quantified from three independent experiments (20 cells per experiment = 60 total cells per condition). Only cells with at least one invadopodium were analyzed. Cortactin antibody labeling (Millipore clone 4F11; 1:500 dilution) was detected using a mouse Cy5 secondary antibody (Jackson ImmunoResearch; 1:400 dilution). Statistical significance was calculated utilizing the unpaired Student’s t-test.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grants to J.R.J. from the American Cancer Society (RSG 0928101) and National Science Foundation (IOS 0950849).
Figure 1. VANGL2 regulates the focal degradation of ECM by HT-1080 cancer cells. (A) Total degradation areas observed after 20 h incubation on FITC-labeled gelatin (actin-labeled images also shown). Quantification of (B) average number of degradation areas formed (normalized to total cell number) and (C) average size of each degradation area with standard deviations and medians (black boxes). (D) Invadopodia formed after 5 h incubation on FITC-labeled gelatin as visualized by phalloidin (to label actin), cortactin, and matrix degradation (arrows). Area within the white box is magnified in adjacent panel. (E) Invadopodia formation in control non-targeting (NT) and VANGL2 siRNA transfected cells. Arrows denote matrix degradation spots co-localizing with actin foci (only one spot is highlighted per cell). (F) Quantification of the average number of invadopodia per cell with standard deviations and medians (black boxes). (B and F) Asterisks indicate p-value less than 0.01. Scale bars = 10 μm.
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