In vitro culture models elucidate a role for planar cell polarity signaling in endothelial cell migration

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Statement of Purpose: During development, embryos elongate through a process known as convergence and extension in which individual cells in the embryo tissues elongate and produce oriented protrusions that enable coordinated motion of the entire cellular sheet (not just individual cells), in a specific direction. Planar cell polarity (PCP) signaling occurs during this process to allow individual cells to align and coordinate with neighboring cells in sheets of tissue[1]. PCP signaling is also critical during other coordinated cell migration processes that occur during development. For example, the PCP mutant *Vangl1* and *Vangl2* knockout mice mutants show defects in convergence and extension and neural tube closure and in humans, defective PCP signaling result in conditions such as spina bifida. The mechanism through which PCP signaling coordinates cell migration during tissue development is not well understood. An in vitro model to understand how PCP coordinates cell migration will provide a tool to better understand how defects in PCP signaling result in disease. The objective of this research was to utilize in vitro cell migration models to understand how the PCP proteins *Vangl1* and *Vangl2* influence cell migration.

Methods: We conducted three different cell migration assays using endothelial cells with normal or modified levels of *Vangl1* or *Vangl2*. To elevate Vangl protein levels we infected human umbilical vein endothelial cells (HUVEC) with GFP-tagged *Vangl1* or *Vangl2* or GFP only lenti-virus. To knockdown protein expression we used siRNA. Using these different cell populations we conducted the following cell migration assays in serum free medium containing fibroblast growth factor (FGF): (1) *Wound closure assay*: We seeded HUVEC into a 6-well plate, incubated for 24h and then generated a wound in the cell sheet using a plastic tip. (2) *Cell tracking assay*: We seeded 1000 or 12500 HUVEC into a 96-well plate, incubated for 24h, stained the cell nuclei using Hoechst and then characterized the average speed and persistence of cells migrating in the confluent cell sheet by obtaining images every 15 minutes using an automated high-throughput microscope. (3) *Cell tracking on nanogrooves:* We followed the same method as (2) but seeded the cell sheet on a nano-grooved elastomeric surface coated with fibronectin, created by molding an elastomer on the surface of a diffraction grating. For each cell population we characterized the distribution of *Vangl*1 and 2 in cells within the confluent sheet and at the wound edge using immunofluorescence.

Results: In the wound closure assay, over-expression of the *Vangl* proteins increased HUVEC migration by 35%. Similarly, knockdown using siRNA targeting *Vangl1* or 2 produced a 10-20% increase in wound closure rate. In cell-tracking experiments on flat surfaces, when compared to uninfected WT cells, sparse *Vangl1*GFP cells migrated faster with increased persistence (like the wound closure assay) however confluent *Vangl1*GFP cells migrated slower with decreased persistence. In contrast, both sparse and confluent *Vangl2*GFP cells behaved no differently to WT cells in the cell-tracking assay. To understand the difference between the effect of elevating *Vangl1* and 2 on cells in confluent sheets versus at a wound edge we imaged the protein location. No difference was observed in the location of *Vangl1* (Fig. 1) or *Vangl2* (data not show) in cells within confluent sheets or at the wound edge.

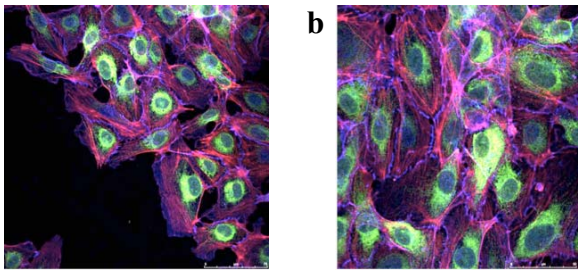

Fig1: Vangl1-GFP in cells at (a) wound and (b) in sheet Vangl1 (Green), actin (red), VE-Cadherin (Blue)

In the wound closure assay loss of cell-cell contact occurs and the cells experience a directional bias to move into the wounded region. In the cell-tracking assay at sparse cell densities the cells have no cell-cell contact and no directional bias. When the cell-tracking assay is conducted at confluent densities the cells experience no loss of cell-cell contact and no directional bias. Depending on the assay Vangl1 and 2 have different effects on cell migration. For Vangl1 loss of cell-cell contact seems most important while for Vangl2 a directional bias seems most important. To isolate the influence of a directional bias we developed a system to perform the cell-tracking assay on a nano-grooved surface. In this case there is no loss of cell-cell contact but the nano-grooves do provide a directional preference to the cells in the sheet: the average cell migration rate of WT cells on the grooved surfaces increased relative to WT cells on flat surfaces (Fig.2).

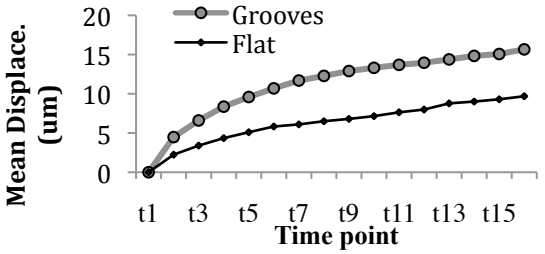

Fig2: WT cell migration on grooved versus flat surfaces

Conclusions: Our results suggest that the effect on cell migration of elevating Vangl depends the level of cell-cell contact and the presence of a directional bias. Our future work will test the prediction that on a nano-grooved surface Vangl1 elevated cells will migrate slower than WT and that Vangl2 cells will migrate faster than WT.

References:

[1] Roszko I et al. Semin Cell Dev Biol. 2009 *in press*