

A Novel Role for PECAM in the Regulation of Leukocyte Transendothelial Migration

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Mechanical traction between platelet endothelial cell adhesion (PECAM) on a transmigrating leukocyte and PECAM at the endothelial cell border initiates leukocyte diapedesis in areas of inflammation. The PECAM-initiated signaling pathway mediates transendothelial migration (TEM) by promoting the movement of a storage membrane compartment, the lateral border recycling compartment (LBRC), to the site of TEM. Previously, we demonstrated that kinesin light chain 1, variant 1 (KLC1c) regulates TEM by controlling the targeted delivery of the LBRC to the TEM site. The goal of this study was to identify and characterize KLC1c-interacting proteins in endothelial cells and to elucidate the molecular pathways through which KLC1c moves the LBRC and facilitates TEM.

We performed an unbiased proteomics screen using FLAGAPEX2-tagged KLC1c expressed in endothelial cells to label neighboring proteins. Biotinylated proteins were affinity-purified and analyzed via mass spectrometry. In parallel, we conducted mass spectrometry on proteins co-immunoprecipitated with KLC1c. We utilized high-performance computing and AlphaFold-Multimer to model potential protein interactions between KLC1c and candidate binding partners.

Across both datasets, we identified a convergent set of top candidate interactors, many of which are associated with cytoskeletal dynamics, membrane trafficking, and scaffolding—hallmarks of KLC1 function. PECAM was the only transmembrane protein identified. AlphaFold Multimer modeling revealed PECAM as a direct binding partner for KLC1c. Notably, the PECAM ITIM1 motif (previously shown to be essential for TEM) docked onto the 6TPR domain on KLC1c, a region typically associated with cargo and adaptor binding. Co-immunoprecipitation experiments in endothelial cells expressing Flag-tagged KLC1c confirmed this interaction.

Our data suggest that PECAM is not only involved in the initiation of TEM but also in the final stages of recruitment of the LBRC. To test this, we are synthesizing novel peptides to disrupt PECAM:KLC1c interactions. We are using RFdiffusion to generate 3D peptide backbones, followed by ProteinMPNN to optimize the final amino acid sequences. If successful, these could serve as potent and selective anti-inflammatory therapies.

