MECHANICS UNDERLIES IMPAIRED ANGIOGENESIS AND ENDOTHELIAL MOSAICISM IN CEREBRAL CAVERNOUS MALFORMATIONS

Apeksha Shapeti (1), Jorge Barrasa Fano (1), Janne de Jong (1), Abdel Rahman Abdel Fattah (1), E.Planus (2), Eva Faurobert (2), Hans Van Oosterwyck (1,3)

1. Biomechanics Section, KU Leuven, Belgium; 2. Institute for Advanced Biosciences, France; 3. Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium

Introduction

The underlying mechanics of pathogenic angiogenesis in the context of vascular disease progression remains poorly understood due to the challenges in force quantification in 3D multicellular systems. Cerebral Cavernous Malformation (CCM) is one such disease characterized by leaky, tumor-like vessels. ROCK1-dependent intracellular tension is one factor responsible for CCM lesions[1]. Further, CCM mutant endothelial cells (ECs) can form mosaic sprouts during invasion by attracting wild-type (WT) ECs[2]. This work combines 3D angiogenic assays, traction force microscopy (TFM), live confocal imaging, perturbation of mechanosignaling pathways, and scRNA sequencing to elucidate the role of impaired mechanics in this endothelial mosaicism for lesion formation.

Methods

We coupled a 3D in-vitro angiogenic invasion assay using extracellular matrix(ECM)-mimicking PEG with 3D TFM. PEG composition was modulated to mimic various stiffnesses, ligand binding, degradability and angiogenic cues. Hydrogels were mixed with either fluorescent beads or fluorescent gelatin for confocal image-based quantification of 3D ECM deformations or ECM degradation respectively. 3D tractions were inferred from ECM deformations around invading non-mosaic or mosaic (with WT-ECs) sprouts for control, CCM2-silenced, CCM2+ROCK1-silenced, and CCM2+ROCK2-silenced conditions. Live overnight imaging was used to visualize dynamics of non-mutant EC recruitment. Finally, immunostaining and scRNA sequencing were used to investigate the modified mechanotransductive machinery at the protein and gene expression levels.

Results

Mutant CCM2 ECs exert higher tractions, show increased ECM degradation, and invade further during both mosaic and non-mosaic 3D angiogenic sprouting. Non-mutant WT-ECs show increased invasion in CCM2-depleted mosaics where they are restricted to follower position by the hyper-angiogenic mutant ECs. These effects are rescued through further silencing of ROCK1 but not ROCK2, or upon treatment with blebbistatin (myosin inhibitor). Fascinatingly, WT-ECs in mosaic mutant sprouts display mutant-like morphologies with increased actin stress fibers, β1 integrin dependent focal adhesions, and nuclear invasion, but surprisingly not higher cell-ECM forces. Dynamic live imaging revealed the capacity of WT cells to follow mechanically active mutant cells through cell contact, as well as to migrate in tunnels formed by mutant cells through ECM degradation. Force analysis of migrating mosaic cell pairs shows that the leading mutant EC uses pulling forces (on the ECM and on the WT EC) to lead the forward movement of the cell pair. Finally, scRNA sequencing of CCM ECs isolated from the 3D mosaic assay showed upregulation of signalling pathways previously identified in human lesions and mouse models confirming the biological relevance of our in-vitro findings.

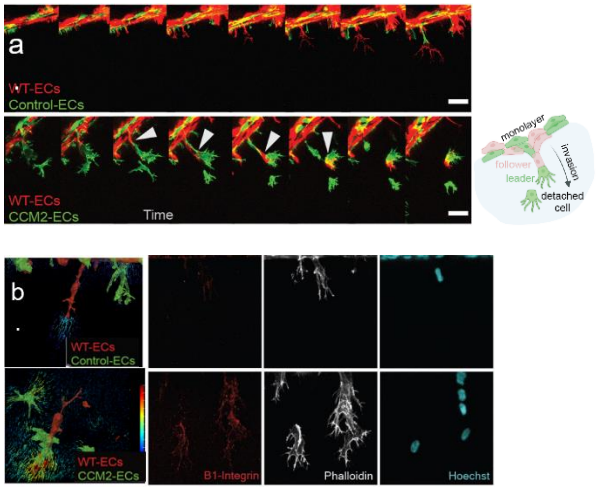

Figure 1: a. Dynamic recruitment of WT ECs(red) by mutant ECs(green) b. Representative renders of 3D displacements of ECM by mosaic sprouts, and immunostaining

Discussion

CCM2 loss leads to a ROCK1-dependent increase in force exertion and ECM degradation by mutant ECs which fuels the invasion of both mutant and WT-ECs. A mechanical continuum forms between the CCM2 mutant leader and the WT followers in which the mutant pulls strongly on the ECM while WT followers do not. Our novel 3D TFM workflows combined with multicellular in-vitro systems provide new tools for identifying disease mechanisms.

References

1. Lisowska et. al. J Cell Sci, 131.15, 2018
2. Vannier et al. Angiogenesis, 24, 843–860, 2021