Dysregulation of the Ephrin B2/EphB4 Ratio in Human Cerebral Cavernous Malformations is Associated with Endothelial Cell Dysfunction in vitro

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No Disclosures
Cerebral cavernous malformations (CCMs) are comprised of disorganized clusters of immature vasculature. Recent studies have demonstrated that cavernous endothelium undergoes an endothelial-to-mesenchymal transition by which endothelial-specific behavior is lost. EphrinB2 has been implicated in the endothelial-to-mesenchymal transition literature but has not previously been studied in CCM. We hypothesize that the Ephrin family of axon guidance factors (AGFs) contribute to the formation of cerebral cavernous malformation and may lead to impaired endothelial cell adhesions and thereby increase risk of hemorrhage. Here, we investigate (1) EphrinB2 ligand and EphB4 receptor expression in CCM (2) the impact of altered EphrinB2:EphB4 ratio on brain endothelial cell function.
Cell lines and patient primary cells
Primary patient-derived CCM cell lines were established and were compared to normal human endothelial cell controls (HBMVEC = Human Brain Microvascular Endothelial Cells; HUVEC = Human Umbilical Vein Endothelial Cells). Endothelial cells (CCM Patients A, B, C CD31+) were selected for using CD31 Endothelial Cell Dynabeads (Invitrogen #11155D). Mesenchymal cells (CCM patients A, B, C CD31-) were analyzed separately.

Western Blot analysis
Endothelial (CD31+) and mesenchymal (αSMA) markers were assessed and relative expression of EphrinB2 and EphB4 were studied by Western Blot.

Angiogenesis assay
Angiogenic potential was compared between primary CCM ECs and healthy vascular controls cells using tube formation as a surrogate. CCM CD31+ ECs, HBMVECs and HUVECs were grown to ~80% confluence and split onto a 48-well dish coated with Matrigel Matrix Basement Membrane (Corning, Tewksbury, MA). Cells were re-suspended in 0.5% serum EGM2. After incubation at 37°C for 24 hours, formation of tube-like structures was evaluated. Images were captured on live cells at 100x magnification and the number of completed junctions was quantified using ImageJ software (National Institutes of Health, Bethesda, MD).
Primary patient-derived cavernoma cell lines were established from 3 patients:

A. 16yoM with sporadic CCM

B. 6yoF with genetics pending

C. 21moF with CCM3
RESULTS

Characterization of CCM cell lines

CCM endothelial cells are effectively isolated and express endothelial marker and have low mesenchymal marker expression, as opposed to CCM mesenchymal cells.
RESULTS

Tube Formation of endothelial cells

At baseline, CCM CD31+ ECs demonstrate impaired angiogenesis compared to healthy vascular endothelial controls.

Control endothelial cells demonstrate polygons and capillary-like formation; CCM ECs demonstrate abnormal morphology with redundant branching and impaired capillary-like formation.
CCM ECs have lower expression of both EphrinB2 and EphB4 as compared to control normal EC but keep the same EphrinB2/EphB4 ratio.

Ephrins expression in CCM endothelial cells

CD31 + cells

CD31 - cells

A

B

C

HBMVEC

HUVEC

EphB4

GAPDH

EphrinB2

EphB4

EphrinB2

GAPDH

Ratio

EphrinB2/GAPDH

Ratio

EphB4/GAPDH

Ratio

EphrinB2/EphB4

C

CD31 + cells

nEphrinB2/nEphB4

CD31 - cells

nEphrinB2/nEphB4
DISCUSSION

- Ephrin family AGFs have previously been shown to play a role in aberrant cerebrovascular endothelial behavior in arteriovenous malformations. We hypothesized that Ephrin expression may play a role in cerebral cavernomas (CCM)

- CCM lesions undergo endothelial-to-mesenchymal transition and both endothelial and mesenchymal cells may contribute to the pathology

- Primary cerebral cavernous malformation cell lines demonstrate abnormal angiogenesis in vitro compared to normal healthy controls

- CCM cell lines have altered expression of EphrinB2 and EphB4 as compared to normal vascular endothelial cell controls

- This preliminary data suggests that dysregulation of the EphrinB2:EphB4 signaling cascade may play a role in CCM pathology, with potential utility as a diagnostic and therapeutic target. Further investigation is warranted.