SMCs/pericytes Migration Defect Induced by CCM3 Knockout is a Major Contributor to CCM

Yun He, Hianfeng Zhang, Alexander Vortmeyer and Wang Min

Department of Pathology Interdepartmental Program in Vascular Biology and Transplantation, Yale School of Medicine, New Haven, CT

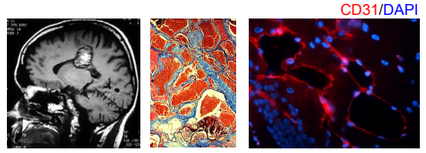
ABSTRACT

Cerebral cavernous malformations (CCMs) account for 10-15% CNS vascular malformations, but the cellular and molecular events causing CCMs remain poorly characterized. CCM1, CCM2 and CCM3 genes have been identified by lineage analysis from human CCM. Recently we have reported that CCM3-mediated VEGFR2 signaling was critical for vascular development by ubiquitous or specific deletion in the vascular endothelium. Here we report mice bearing CCM3 specific deletion in SMC/pericyte all can generate human-like CCM lesions, even in as young as E11.5 embryos. Pathological CCM lesions in CCM3 SMCs/pericytes-specific knockout (smKO) old mice were same as human lesions comprising closely clustered, enlarged capillary channels (caverns) with a single layer of endothelium without mature vessel wall elements or normal intervening brain parenchyma. But the CCM lesions in embryonic smKO all loosely wrapped by pericytes and astrocytes, and the pericyte coverage rate of smKO was almost half less than that of WT in E15 embryos. We found smKO pericytes migration was slower than WT both in vitro and in vivo, and expression of migration related adhesion, polarization and contractile cytoskeleton protein reduced in CCM3 smKO smooth muscle cells. Our conclusion is that migration defect induced by poorly adhesion and dispersed golgi in smKO is a major contributor to cerebral cavernous malformations.

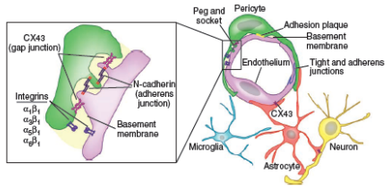
BACKGROUND

1. Cerebral Cavernous Malformations (CCM)

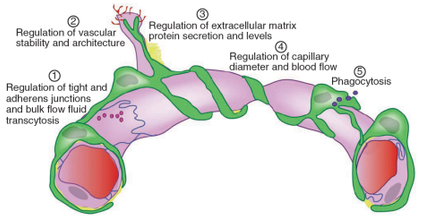
   clustered and enlarged capillary channels with a single layer of endothelial cells



2. CCM occurred at the blood-brain barrier site, which is composed of neurovascular cells, such as endothelial cells, pericytes, astrocytes, vascular smooth muscle cells, neurons and brain macrophages.



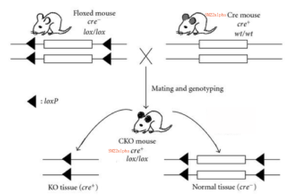
3. Pericytes play a unique role in barriergenesis and in maintaining homeostasis.



4. CCM3 (PDCD10) focal adhesion targeting (FAT) homology domain interacts with paxillin LD motifs.

METHODS

1. Schematic diagram of generating a CCM3 sm22alpha conditional knockout mouse from breeding a tissue-specific sm22alpha Cre mouse (top right) with a mouse carrying CCM3 homozygous floxed gene (top left).



2. Trichrome and H&E stain in human brain tissue and mouse smKO brain tissue
3. Vasualize SMC/pericytes in vivo by SM22 α (+)/Tomato mice
4. FITC-Dextran perfusion of cerebrovasculature
5. Isolation of primary aorta SMCs
6. Isolation of primary pericytes from mouse brain
7. Spreading and migration function assay
8. Tissue and cell immunofluorescence stain
9. Quantification and Statistical Analysis

RESULTS

Table 1. Quantification and statistics of CCM lesion incidence of CCM3 SMC-specific knockout

strain	E11.5-E20			d1-adult		
	Total embryo No.	No. of embryo with CCM lesion	percentage of embryo with CCM lesion	Total mouse No.	No. of mouse with CCM lesion	percentage of mouse with CCM lesion
WT	102	0	0	34	0	0
smko	96	96	100% *	30	30	100% ★

X^2 test: *and★ $p<0.01$

Figure 1. CCM3 gene was successfully deleted in smooth muscle cell/pericyte.

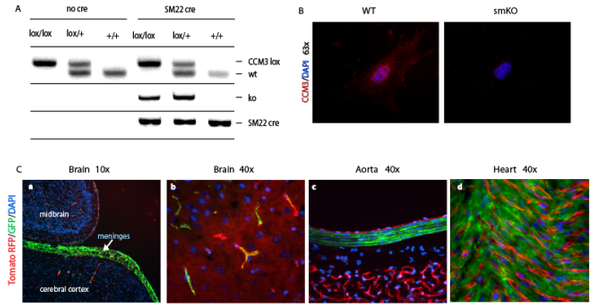


Figure 2. CCM lesions were found in all of CCM3 smKO adult mice.

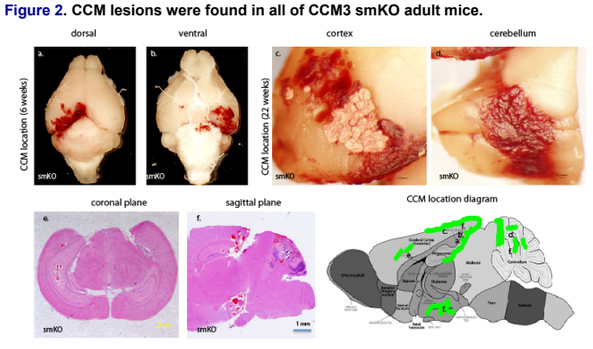


Figure 3. CCM lesions in smKO adult mice showed similar pathological phenotype as human CCM.

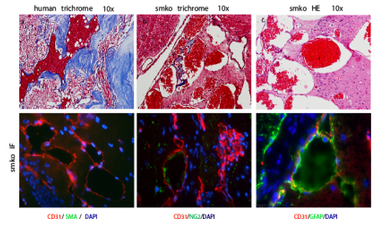


Figure 4. CCM lesions were also found in all of embryonic and neonatal mice brain.

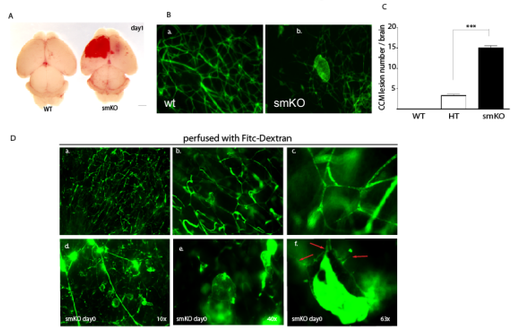


Figure 5. CCM lesion at embryonic stage showed endothelial cells were surrounded by pericytes, which was different from adult smKO mice.

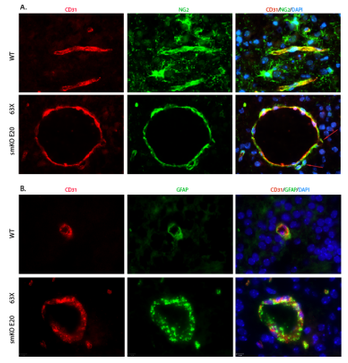


Figure 6. In vivo, pericytes of CCM3 smKO migrated slower than WT embryo.

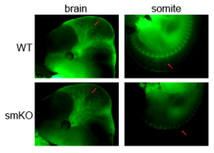


Figure 7. Pericyte coverage was half less in CCM3 smKO brain capillary than in WT brain.

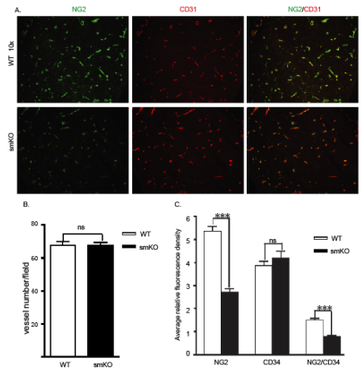


Figure 8. In vitro, CCM3 smKO SMC/pericyte spread (A) and migrated (B) slower than WT.

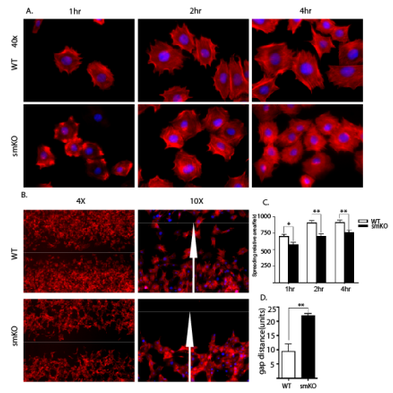


Figure 9. Focal adhesion molecules paxillin decreased in CCM3 smKO pericytes.

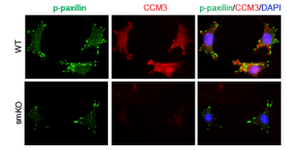


Figure 10. Contractile cytoskeleton protein reduced in CCM3 smKO smooth muscle cells.

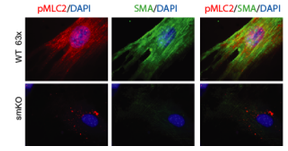
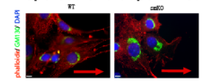


Figure 11. Dispersed and depolarized golgi were found in smKO pericytes.



"→" showed migration direction

CONCLUSIONS

1. CCM3 SMCs/pericytes conditional knockout mice model can mimic human cerebral cavernous malformation disease.
2. At smKO mouse embryonic stage E11.5, CCM lesion could be detected, suggested CCM lesion maybe exist in human early stage embryo, and along with the age become detectable.
3. ECs in early stage CCM lesion were loosely covered by pericytes and astrocytes, but in late stage they were lost.
4. In vivo and in vitro smKO SMCs/pericytes migrated slower than WT, and resulted in pericyte coverage less than WT, indicated CCM3 is critical for pericyte migration.
5. Reduced focal adhesion, decreased contractile cytoskeleton protein and dispersed and depolarized golgi maybe contribute to slow migration and cerebral cavernous malformation.

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