Supplemental Materal

Smooth Muscle PPARγ Plays a Critical Role in Formation and Rupture of Cerebral Aneurysms in Mice In Vivo

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Supplemental Methods

Genotyping: Genotyping of the transgenes was performed by PCR of tail DNA using the following primers: *S-P467L* transgene: 5'-TATCTTCTAACTGGGTGGTGGTG-3' and 5'-GAGGAGAGTTACTTGGTCGTTCA-3'; *E-V290M* transgene: 5'CAGCTCACAAAGGAACAATAACAG-3' and 5-CTCCATAGTGAAATCCAGAAG-3'. Both models have been extensively backcrossed onto the C57BL/6J genetic background.

Pharmacological Treatments: Pioglitazone was suspended in water (20 mg/kg/day) whereas GW9662 (1 mg/kg/day) and MLN4924 (100 mg/kg/day) were dissolved in water. In C57BL/6 mice, drugs were administered intraperitoneally starting 24 hours post aneurysm induction surgery. A search of the literature suggested these are the most commonly used doses to achieve maximal desired effect.[1-3] Control mice (C57BL/6) were administered vehicle. Mice that died or were sacrificed due to deterioration based on neurological exam within 48 hours of aneurysm induction surgery were excluded from the study (see Supplemental Table S1). Neurological examinations were then carried out daily using a previously described method.[4-7] Mice were euthanized when they developed neurological signs (lethargy/decreased activity, circling in one direction, weakness or paralysis of one or more limbs, and/or hunched back), or experienced weight loss >20% of baseline.[4-7] All asymptomatic mice were euthanized 3 weeks after aneurysm induction surgery.

Ang-II was obtained from Bachem (Torrance, CA), pioglitazone was obtained from Takeda (Deerfield, IL), GW9662 was obtained from Santa Cruz Biotechnology (Dallas, TX), and MLN4924 was obtained from Cayman (Ann Arbor, MI). All other reagents were obtained from Sigma (St Louis, MO).

Blood pressure analysis. Systolic blood pressure was recorded in conscious restrained mice using the tail-cuff method (Visitech Systems BP-2000).[4,8] After training, blood pressures were measured before aneurysm induction surgery and weekly until day 21 of the study. Baseline systolic blood pressure (SBP) for all mice in different experiments was between 100-120 mmHg.

Gene expression analysis. After examination for aneurysms, cerebral arteries of the circle of Willis, including the basilar artery and middle cerebral arteries were harvested, rapidly frozen in liquid nitrogen, and stored at -80^{o} Celsius. RNA was harvested in TRIzol and reversed transcribed as described previously.[9] PCR was performed using primer assays from Life Technologies (Table S2). Due to very small size of cerebral aneurysms in mice, we were unable to perform Western blotting to quantify relative level of specific proteins.

Supplemental References

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Table S1: Mice excluded from cerebral aneurysm analysis.

	Control Group		**Experimental Group**	
	Total Excluded	Aortic Aneurysms	Total Excluded	Aortic Aneurysms
Pioglitazone vs. control	5 *(of 18)*	0	2 *(of 17)*	1
GW9662 or MLN4924 vs. control	6 *(of 18)*	1	GW 1 *(of 9)*	0
			MLN 0 *(of 9)*	0
E-V290M vs. non-transgenic	2 *(of 15)*	1	3 *(of 16)*	0
S-P467L vs. non-transgenic	1 *(of 11)*	1	1 *(of 17)*	0

Table S2: TaqMan Primers and Probes

Gene	Catalog #	Company
CD68	Mm03047343_m1	Applied Biosystems (ABI)
Cxcl1	Mm.PT.58.42076891	Integrated DNA Technologies (IDT)
MCP-1	Mm.PT.58.56a.42151692	Integrated DNA Technologies (IDT)
TNFα	Mm.99999068_m1	Applied Biosystems (ABI)
MMP3	Mm.00440295_m1	Applied Biosystems (ABI)
MMP13	Mm.01168713_m1	Applied Biosystems (ABI)
MMP9	Mm.PT.58.10100097	Integrated DNA Technologies (IDT)
Cul3	Mm. PT.58.1917739	Integrated DNA Technologies (IDT)
Keap1	Mm. PT.58.5093463	Integrated DNA Technologies (IDT)
MMP3	Mm.00477786_m1	Applied Biosystems (ABI)

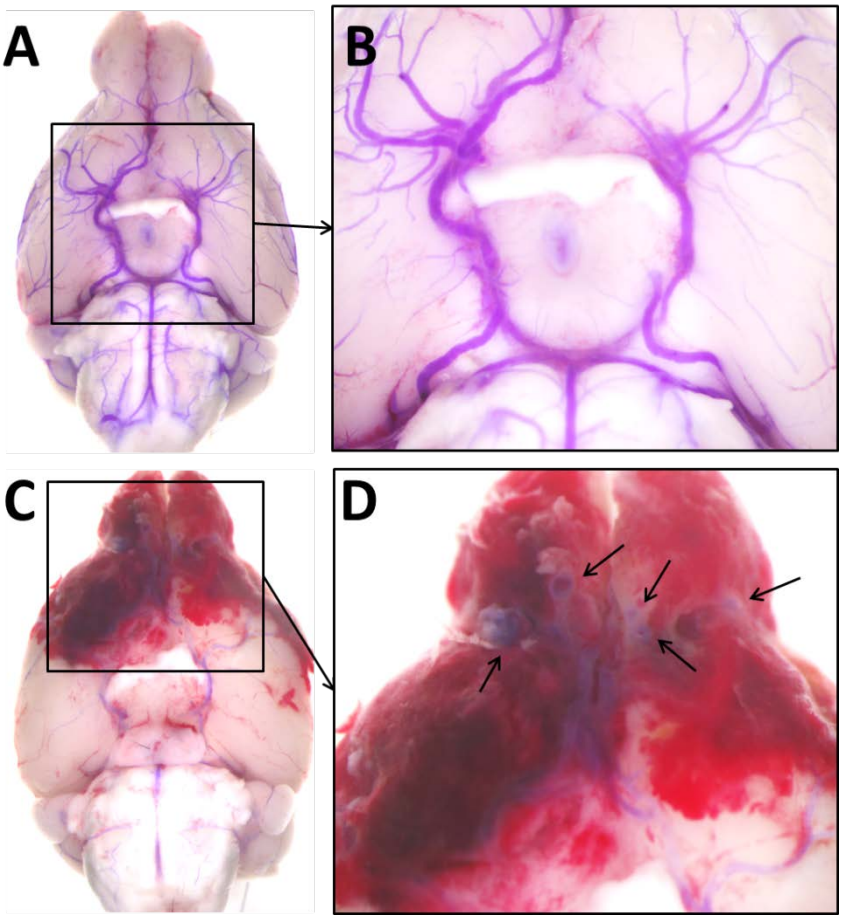


Figure S1: Illustration of cerebral aneurysms and SAH A) Cerebral arteries in a non-transgenic mouse. B) Higher magnification of the same cerebral arteries in A showing no aneurysms. C) Cerebral arteries in a S-P457L mouse with SAH and multiple intracranial aneurysms localized to anterior cerebral and olfactory arteries. D) Higher magnification of the same aneurysms with arrows indicating their location.

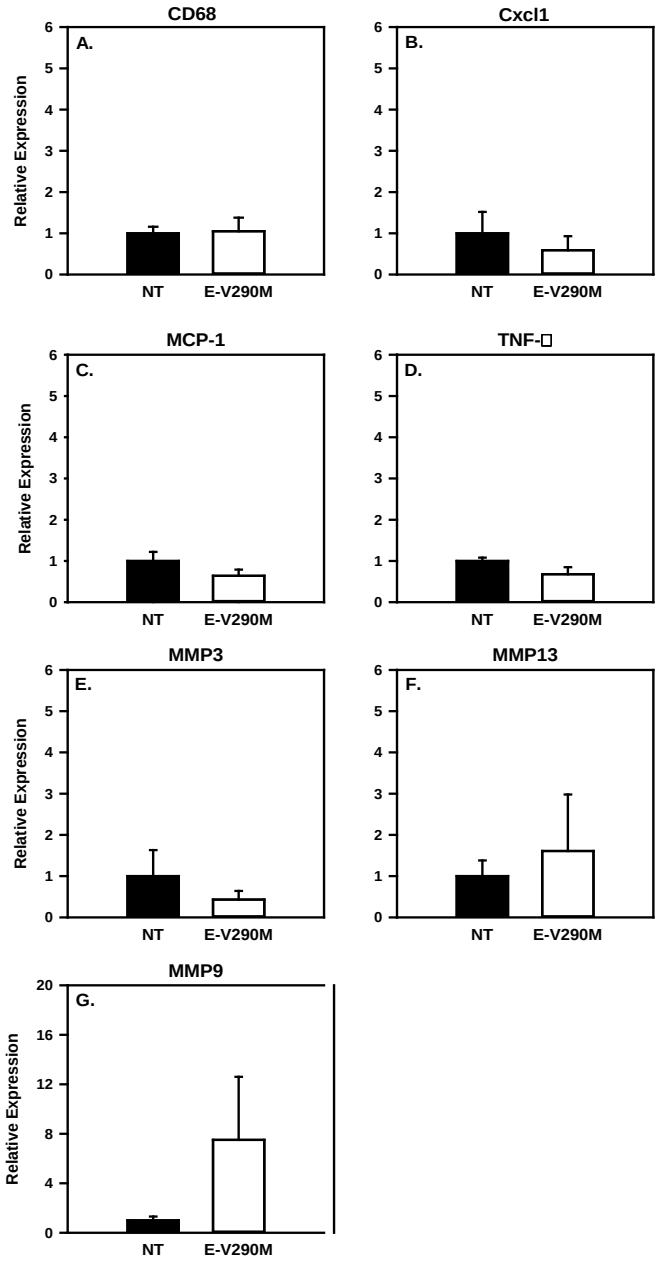
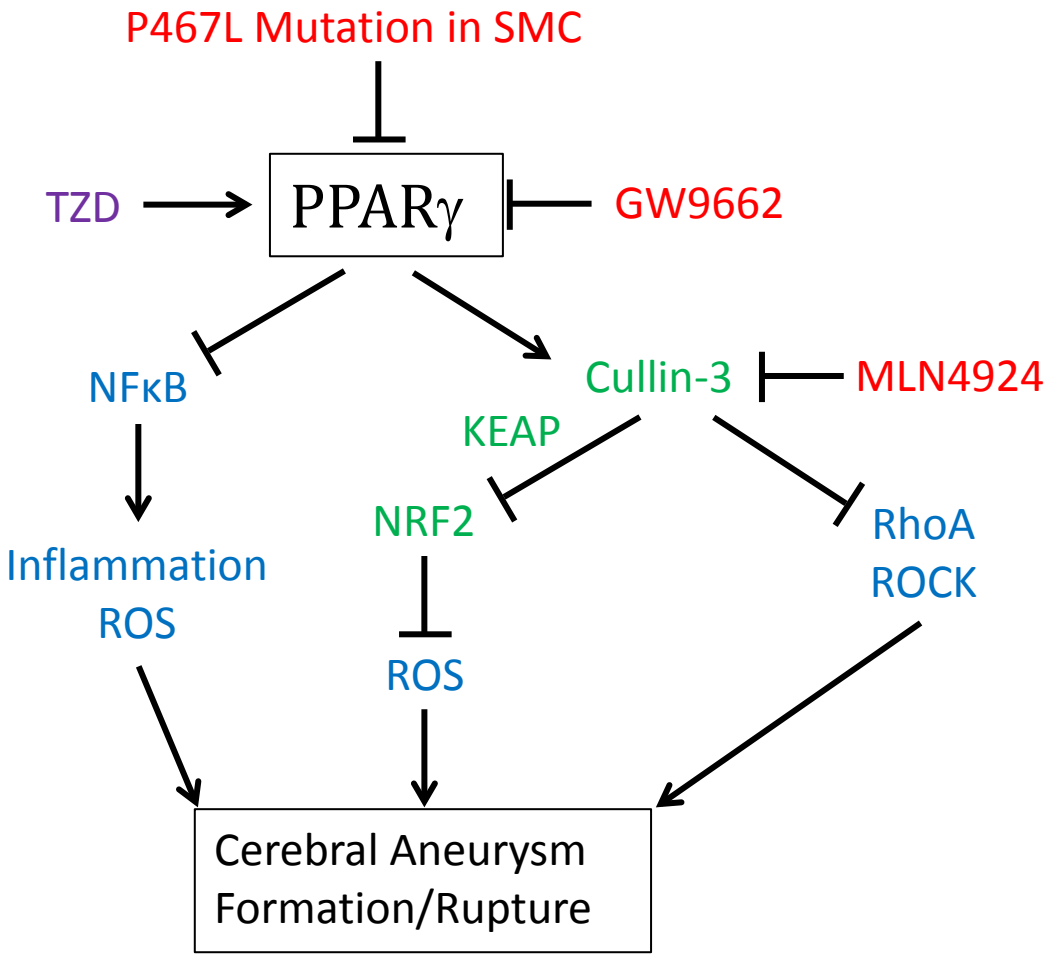


Figure S2: Gene expression of selected inflammatory markers: comparison of gene expression of CD68, Cxc1, MCP-1, TBF-α, MMP 3, MMP 13, and MMP 9 in cerebral arteries in E-V290M versus their non-transgenic littermates. No statistical difference. E-V290M, n=6; NT, n=6.



Increased Aneurysm Formation or Rupture

Decreased Aneurysm Formation or Rupture

Increased Expression or Activity

Decreased Expression or Activity

Figure S3: Schematic figure describing a potential molecular mechanism by which expression of SMC-PPARγ DN increases formation and rupture of cerebral aneurysms.