Objective(s): To evaluate the role of the neuronal cue Semaphorin4D (Sema4D) in thoracic aortic aneurysm (TAA). TAA is a complex life-threatening disease resulting from weakening of the arterial wall and rupture. Although studies have consistently evidenced chronic enzymatic degradation by matrix metalloproteinases (MMPs) and abundant microvessels in the dilated wall, the role of angiogenesis in the pathogenesis of TAA is poorly understood. We have previously uncovered instrumental roles for neuronal guidance cues in the vasculature. Sema4D, a member of this superfamily was previously described to promote tumor-associated angiogenesis. Here, we hypothesized that Sema4D could impact TAA by promoting neo-angiogenesis.

Methods: Aortic samples were collected from patients admitted for TAA repair and control tissues were from organ donors. Transcriptomic profiles were analyzed by RNA-sequencing (RNA-Seq) followed by pathway analysis (DAVID Bioinformatics Resource). Transcripts were validated by quantitative-PCR. Immunofluorescence staining detected and simultaneously located the proteins in specific cellular subsets in damaged sections of the tissue. Through bio-layer interferometry, the affinity between Sema4D and its receptor, PlexinB1 was measured. Macrophages (Mø) and vascular smooth muscle cells (VSMCs) were stimulated with recombinant MMP14 and lysates were immunoblotted to detect cleaved variants of Sema4D.

Results: RNA-Seq revealed increased expression of Sema4D mRNA in diseased aorta validated by quantitative-PCR. Sema4D peaked in diseased aortas of size >6 cm. Distinct patterns of Sema4D were observed in microvessels harbored in the adventitia. Likewise, neovessels accumulated in TAA but not in controls. Sema4D was present in VSMCs (aSMA) and endothelial cells (CD31) only in neovessels and in Mø (CD68) at sites of degradation. Sema4D colocalized with MMP14, an MMP with established roles in TAA. MMP14 was increased in RNA-Seq and positively correlated with the size of diseased tissue. Stimulation of Mø with recombinant MMP14 induced the secretion of a truncated variant of Sema4D, that was reversed in the presence of MMP14 inhibitor suggesting that MMP14 could cleave the extracellular domain of Sema4D thereby generating a soluble ligand. Full length and truncated Sema4D were detected in TAA tissues. Recombinant Sema4D binds with great affinity to its receptor PlexinB1 thereby inducing expression of pro-angiogenic factors in TAA.

Conclusions: Our data identified Sema4D as a novel causative cue that could contribute to TAA by promoting angiogenesis. Devising anti-Sema4D strategies might show therapeutic promise to curb TAA.