Background: VEGF is a well-established stimulator of vascular permeability and angiogenesis. VEGF-induced vascular permeability is an important characteristic of many diseases. Thrombospondin-1 (TSP-1) is a potent angiogenic inhibitor. Therapeutics based on the antiangiogenic domain of TSP-1, designated the three TSP-1 type 1 repeats (3TSR), have shown promising antiangiogenic and anti-tumor efficacy. We performed this study to characterize the regulatory effects of TSP-1 on VEGF-induced vascular permeability and VEGF receptor 2 (VEGFR2) signaling.

Design: Basal-level and VEGF-induced permeability were evaluated in TSP-1 null and wild-type mice using the Miles assay. Lung extracts from TSP-1 null mice and 3TSR-treated FVB mice were used as control in the above experiments. VEGFR2 phosphorylation, including time course and dose response, was further characterized in vitro using 3TSR-treated human dermal microvascular endothelial cells (HDMEC) and vascular endothelial cells isolated from TSP-1 null mice. The vascular morphology was studied in TSP-1 null and wild-type mice using electron microscopy.

Result: Systemic treatment of wild-type mice with 3TSR significantly decreased VEGF-induced permeability (p<0.01, Fig. 1A). VEGF-stimulated VEGFR2 phosphorylation was also significantly decreased in lung extracts from 3TSR-treated mice (p<0.05, Fig. 1B). Moreover, 3TSR significantly decreased VEGF-stimulated VEGFR2 phosphorylation in HDMEC in culture. Surprisingly, VEGF-induced permeability was significantly decreased in TSP-1 null mice (p<0.01) as compared to the wild-type control mice (Fig. 2A). In addition, systemic treatment of TSP-1 null and wild-type mice with VEGF produced lower levels of VEGFR2 phosphorylation in lung extracts of TSP-1 null mice (Fig. 2B). Vascular endothelial cells from TSP-1 null mice also showed significantly decreased VEGFR2 phosphorylation upon VEGF treatment. Whereas the magnitude of the response to VEGF is reduced in TSP-1 null endothelial cells as compared to their wild-type counterparts, the time course (A) and dose response (B) were comparable.

Conclusion: VEGF-induced vascular permeability and VEGFR2 phosphorylation display a biphasic response to TSP-1 concentration in tissues and isolated endothelial cells.