

**THROMBOSPONDIN-1-INDUCED VSMC MIGRATION IS DEPENDENT UPON THE HYALURONIC ACID RECEPTOR CD44**

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**Objective:** Thrombospondin-1 (TSP-1), an extracellular matrix protein, is an acute phase reactant that induces vascular smooth muscle (VSMC) migration and proliferation in areas of vascular injury, and is also upregulated in VSMCs exposed to hyperglycemia. We have previously shown that TSP-1 increases the expression of genes (HAS2, TGF $\beta$ 2 and UGDH) that induce hyaluronic acid (Hya) synthesis in VSMCs. Hya is expressed in atherosclerotic plaque and is linked to the progression of atherosclerosis and intimal hyperplasia. Hypothesis: TSP-1 induced migration is dependent upon the chemotactic molecule, Hya.

**Methods:** Bovine aortic VSMCs were used (passages three to five). A modified Boyden chemotaxis assay was used to assess migration. TSP-1 (20  $\mu$ g/mL), Hya (1, 5, 10  $\mu$ g/mL) or serum free medium (SFM, negative control) were the chemoattractants. VSMCs (50,000 cells per well) were seeded into the upper chamber in the presence or absence of a neutralizing antibody to the Hya receptor CD44 (10  $\mu$ g/mL). Migration proceeded for four hours. Results were recorded as the total cells migrated/5 fields (400x) and analyzed by t-test.  $P < 0.05$  was considered significant.

**Results:** Hya in a dose-dependent manner and TSP-1 independently induced VSMC migration at the concentrations studied. TSP-1-induced VSMC migration was attenuated by the presence of a CD44 neutralizing antibody. Migration in response to TSP-1 in untreated cells was significantly higher than SFM. When the CD44 neutralizing antibody was added, migration was still elevated as compared to SFM ( $P < 0.05$ ); however, there was a 77% decrease in migration as compared to TSP-1 ( $P < 0.05$ ).

**Conclusions:** Findings suggest a functional link between Hya and TSP-1 for VSMC migration. While some studies have shown a role for TSP-1 in Hya metabolism, this is the first report of a Hya receptor playing a role in TSP-1-induced VSMC migration. CD44 should be investigated further as a potential therapeutic target in TSP-1-induced vascular processes.