

## Loss of the Smooth-Muscle-Cell-Angiotensin II-Sensitive (SAS) lncRNA Triggers VSMC Hypertrophy and Cell Cycle Arrest: Application to Hypertension and Aortic Stiffness

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**Introduction:** The reactivation of the cell cycle and increase in proliferation rate (hyperplasia) is a common response of vascular smooth muscle cells (VSMC) to modifications of their environment. VSMC can also increase the mass on the remodeled vessel wall by enlarging their size and becoming hypertrophic. That process has been observed in the aorta of hypertensive mice and response to Angiotensin-II (Ang-II). Hypertrophy is usually accompanied by an increased number of binucleated cells, defects in cell division mechanisms, and senescence. However, the molecular mechanisms favoring VSMC hypertrophy vs. hyperplasia in response to Ang-II and their repercussion on SMC phenotype are not fully understood.

**Methods:** We performed RNAseq analysis on an irreversible epigenetically dedifferentiated VSMC line. Among more than 40 differentially expressed long-non-coding-RNAs (lncRNA), we characterized the novel lncRNA-SAS (Smooth-muscle-cell-Angiotensin II-sensitive). We tested the expression of SAS by RT-qPCR in VSMC in response to dedifferentiation factors such as PDGFB-B and Ang-II. Using GapmeR inhibitor technology, we transiently silenced SAS expression in VSMC and tested proliferation, cell cycle, and mitochondrial function changes.

**Results:** SAS is preferentially expressed in VSMC-rich tissues, including the aorta, in humans and mice. Yet, the functional relevance of this lncRNA on VSMC function has never been investigated. We observed SAS expression was significantly lost in response to Ang-II treatment in VSMC compared to other dedifferentiation treatments. Gapmer-mediated knockdown of SAS potently reduces proliferation and migration in aortic and renal artery-derived VSMC. Interestingly, SAS knockdown led to VSMC hypertrophy and increased number of binucleated cells, suggesting a defect in cell division and cytokinesis. Decreased SAS expression arrested the VSMC cell cycle and promoted senescence (higher number of  $\beta$ -Gal+ cells), recapitulating the phenotype observed in Ang-II treated VSMC.

**Conclusions:** Altogether, our results show that SAS is a potent regulator of VSMC morphology and is required for proper cell division. Ongoing experiments test the role of SAS in the development of systemic hypertension and aortic stiffness-associated vascular remodeling.