Ischemic stroke is associated with reduced sclerostin expression in human atherosclerotic plaques

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**Objectives:** Vascular calcification (VC) is a hallmark of atherosclerosis and commonly found in aging population, increasing the risk of myocardial infarction (MI), stroke and mortality. Our data shows that Wnt/\(\beta\)-catenin signaling pathway and its main inhibitor, sclerostin (Scl, Sost) could be central to this process. However, Scl inhibition with romosozumab has been associated with a higher incidence of major adverse cardiovascular events (MACE). This increases in MACE call into question the safety of romosozumab use, particularly in patients with cerebrovascular and cardiovascular history. However, whether Scl expression levels in atherosclerotic plaques are associated with clinical ischemic events remains unknown. Here we explored the association between Scl expression in human atherosclerosis plaques and the extent of VC, VSMCs phenotypic switch to osteoblast-like cells (OBL), and its correlation to clinical cerebrovascular events.

**Methods:** Carotid plaque specimens of symptomatic (ischemic stroke) and asymptomatic patients undergoing endarterectomy for severe carotid stenosis were co-stained with VSMCs specific marker (Myh11), OBL marker (RUNX2), microcalcification sensitive fluorescent imaging probes (OsteoSense) and Scl and used for quantification analyses.

**Results:** scRNA seq data showed that Sost is upregulated by VSMCs in the aortic region protected against VC, suggesting an inhibitory role of Sost on VSMCs differentiation into OBL. Carotid plaque specimens from asymptomatic patients exhibited a significantly higher percentage of VSMCs expressing Scl (Myh11+Scl+) in parallel with a greater Scl+ area versus symptomatic patients. A negative correlation was observed between the percentage of Myh11+Scl+ cells and the extent of microcalcification (OsteoSense) as well as between the percentage of total Scl+ cells and induction of VSMCs phenotype switch to osteoblast-like cells (quantified as RUNX positive area).

**Conclusion:** In atherosclerotic plaques of patients with ischemic stroke, total and VSMCs -specific Scl expression levels were reduced as compared to asymptomatic patients. Furthermore, reduction in Scl levels was positively associated with the extent of microcalcification, and VSMCs phenotypic switch to OBL. The presented findings suggest that sclerostin expression in human atherosclerotic plaques could contribute to plaque stabilization and be protective against ischemic stroke, explaining why Scl inhibition with romosozumab resulted in a higher incidence of MACE.

**Figure 1**

Bar graph represents median with interquartile range of the percentage of Myh11+Scl+, and Scl+ positive area in asymptomatic and symptomatic carotid artery disease patients, respectively. Mann-Whitney test. **\(p < 0.01\), ****\(p < 0.0001\).
Negative correlation between the percentage of Myh11+ ScI+ cells with the area of vascular micro calcification (OsteoSense positive areas) and ScI+ cells and VMSCs phenotypic switch to OBL in atherosclerotic plaques of carotid artery disease patients.