Novel cardiovascular risk biomarkers in carotid atherogenesis

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“When compared with controls, SOST was able to reduce matrix degradation and inflammatory cell infiltration, thus resulting by the suppression of the Wnt/ β -catenin responsive genes”

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The identification and validation of biomarkers of atherosclerotic plaque progression and vulnerability represent an urgent need in preventive cardiology. Among different classes of biomarkers, the study of bone mediators might be a very promising field of research. On the one hand, they can contribute to determine the degree of calcification and then the number of interfaces between rigid and distensible portions of the plaque exposed to the shear stress forces of the blood flow. In addition, these molecules are also emerging as mediators of plaque inflammation. Especially the axis involving the RANKL and OPG has been demonstrated to influence plaque stability by interfering with the complex balance between anti- and pro-inflammatory molecules [1]. More specifically, RANKL dose-dependently induces neutrophil degranulation *in vitro*, in addition to positively correlating with serum levels of neutrophil proteases in subjects with coronary calcifications [2]. Conversely, the role of two other mediators of atherosclerotic plaque calcification, such as osteopontin OPN and SOST, still remains unclear.

OPN is a secreted protein that plays a role in both physiological and pathological processes. An increase in the circulating levels of OPN has been observed in many acute and chronic inflammatory diseases [3,4]. Within the vascular system, OPN appears to be an important regulator of vascular calcification, being associated with mineralized deposits in both human and mouse models. OPN is highly expressed in human and murine atherosclerotic lesions, where it co-localizes with macrophages and foam cells. Not surprisingly OPN was recently demonstrated as a strong macrophage activator, acting as a promoter of their migration, prolonged survival and foam cell generation [5]. The OPN network also includes a potential upregulation of other proinflammatory mediators involved in the atherosclerotic process, (i.e., angiotensin II and oxidized low-density lipoproteins).

Furthermore, there is accumulating evidence that OPN may be weakened by treatments reducing atherosclerosis [6]. Despite this growing body of results, whether OPN may be useful for a better cardiovascular (CV) risk stratification has not yet been established. Our research group recently reported that serum levels of OPN predicted the occurrence of adverse CV events in patients with severe carotid atherosclerosis undergoing endarterectomy [7]. Such data confirmed previous high-powered clinical trials. Zwakenberg and colleagues recently identified serum OPN as a biomarker strongly associated with CV risk in a cohort of patients with Type 2 diabetes [8]. Nevertheless, those observations raise some complexity about OPN system, starting from its main source of production. Different research groups reported that OPN is also largely secreted by macrophages recruited into the dysfunctional adipose tissue [9,10]. In this regard, a role of aging in determining visceral adipose tissue dysfunction with associated increase in OPN production and release has been described [10]. It is therefore conceivable that both aging as well as some obesity phenotypes (i.e., those associated with higher cardio-metabolic risk) could share pathophysiological signs of dysfunctional adipose tissue, including the upregulated synthesis of OPN. More recently, different mediators have been suggested as a further link between dysfunctional adipose tissue and development of carotid atherosclerosis.

Among the most promising molecules, especially pigment epithelium-derived factor and lipopolysaccharide-binding protein are emerging as potential markers of obesity-related insulin resistance [11,12].

According with those evidences, we also reported that circulating levels of OPN significantly correlated with histopathological features of plaque vulnerability, such as an increased intraplaque content of MMP-9, neutrophils and proinflammatory M1 macrophage subset [7]. Still some questions about the real biological activity of OPN within atherosclerotic plaques remain unanswered. Intraplaque OPN may actively promote neutrophil migration and then their recruitment from the blood stream [13]. Similarly, a modulatory role of OPN on monocyte/macrophage polarization has also been described [14]. Although many efforts are required to clarify the underlying molecular mechanisms, OPN gene expression upregulation has been associated with higher uptake of (18)F-FDG in a PET study on murine atherosclerosis [15]. The recent development of an OPN-specific MRI probe is expected to potentially improve current knowledge about the role of OPN in atherosclerosis [16].

As a product of the *Sost* gene, SOST is a 190-aminoacids glycoprotein acting as a negative regulator of bone formation. More specifically, the binding of sclerostin to the low-density lipoprotein receptor-related proteins-5 and -6, inhibits the Wnt/ β -catenin pathway. As a result, SOST was shown to suppress the transcription of anabolic genes involved in osteoblast/osteocyte activity. However, the *Sost* gene is widespread expressed in the body, with markedly upregulated levels within the heart, aorta, kidney and lung [17]. Several studies demonstrated a relevant role for Wnt/ β -catenin in atherosclerosis, through a complex signaling pathway that also can control the downstream expression of OPG, OPN and several MMPs. It has been then hypothesized that SOST may also have a role in atherosclerosis. However, the association between SOST and arterial wall calcification remains unclear. Similarly, the contribution of SOST to atherosclerotic plaque development and progression has not yet been established. In the early stages of the disease, both negative and positive correlations have been reported with carotid intima media thickness and arterial stiffness, respectively [18,19]. Clinical studies also showed a positive association between circulating SOST levels and atherosclerotic burden [20] and plaque vulnerability [21], but the underlying mechanisms are still unknown. Experimental studies reported some paradoxical anti-inflammatory properties of SOST. Indeed, both *Sost* transgenic mice and recombinant sclerostin administration were able to inhibit aortic aneurysm formation and atherosclerotic plaque development in *ApoE*^{-/-} mice [22]. When compared with controls, SOST was able to reduce matrix degradation and inflammatory cell infiltration, thus resulting by the suppression of the Wnt/ β -catenin responsive genes (i.e., *OPG*, *OPN* and *MMP-9*). These results highlight an apparent contrast between clinical and experimental findings. In addition, this aspect strongly emphasizes a gap of knowledge in the molecular pathways triggered by SOST in atherosclerotic inflammation. Wnt signaling has been largely described as a regulator of the dysmetabolic milieu underlying atherosclerosis. Wnt signaling induces endothelial cell proliferation, survival, as well as the enhancement of monocyte adhesion and transendothelial migration [23]. Wnt signaling activation has also been implicated in the proinflammatory macrophage polarization and foam cell generation as well as in the smooth muscle cell migration and proliferation [23]. Although surprisingly, the positive association observed between atherosclerotic burden and serum levels of SOST (a Wnt signaling pathway inhibitor) might be a defensive mechanism to delay atherosclerotic progression. However, further studies are needed to address many questions about the role of SOST in atherosclerosis. As first, it is mandatory to identify whether SOST is locally synthesized with atherosclerotic plaques and which cell/tissue could be its main source of production. Furthermore, the identification of target cells will pave the way for investigating potential therapeutic approaches, also considering that two anti-SOST antibodies (i.e., romosozumab and blosozumab) have been recently approved for clinical use against osteoporosis. In conclusion, the role of those mediators in carotid atherosclerosis and its acute ischemic complications, such as ischemic stroke, is unclear and requires additional clinical and basic research studies. Conversely, these molecules may have a role as predictors of successful bariatric/metabolic surgery [24]. Additionally, the investigation of selective treatments and pathophysiological pathways triggered by these two molecules might provide in the next future promising therapeutic strategies to reduce carotid atherogenesis.

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