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Modified desmin pre-amyloid oligomers are increased in experimental heart failure

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Objectives: Heart Failure (HF) is the major cause of hospitalization in the US but the molecular mechanisms underlying its development are unclear to date. The increased formation of preamyloid oligomers (PAOs), similar to those observed in Alzheimer's disease, have been reported in several different models of HF. We first reported that the induction of desmin phosphorylation at serines (S) 27 and 31 associate with increased cardiac PAOs deposition in experimental, non-genetic HF. We now show that modified desmin is a likely candidate for the seed initiating the nucleation of toxic PAOs in the heart and isolated cardiac cells.

Materials and methods: We subjected mice to transverse aortic constriction (TAC) for 4 weeks (FS% = 29.3 ± 2.6, P = 0.0001) and transduced neonatal rat ventricular myocytes (NRVMs) using lentiviral vectors carrying alanine (A) or phospho-mimetic aspartate (D) desmin double mutants at S27 and S31, fused with GFP. We analyzed the formation of PAOs in cardiac protein extracts by western blot analysis combined with infrared detection, which enabled the contemporary measurement of PAOs and desmin. We also monitored the effects of phospho-mimetic desmin expression in NRVMs by live imaging.

Results: Co-staining for both desmin and PAOs in TAC mice and NRVMs confirmed the co-migration (by molecular weight) of PAOs with modified desmin, along with their increase in experimental HF (≈3-fold, P = 0.023 and ≈2-fold, P = 0.038, respectively). Cells expressing the doubly phospho-mimetic mutant, which we believe is the physiological form, displayed a “healthier” phenotype as documented by the number of contracting cells (P = 0.041) and localization of GFP desmin at the Z-bands (P = 0.0027). On the contrary the expression of mono-phosphomimetic mutant (S27A, S31D) induced increased desmin aggregation (P = 0.0014).

Conclusions: These data strongly suggest that modified desmin constitutes the seed initiating the formation of cardiac PAOs in non-genetic HF. The increased levels of toxic desmin PAOs in the heart could, therefore, represent a novel mechanism of organ dysfunction in HF, in the absence of genetic mutations.

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Role of catestatin as such or slowly released by fibronectin-coated pharmacologically-active-microcarriers (Fn-Pam) in limiting hypoxic-induced cell death

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Objectives: Catestatin (CST), a 21-amino acid derivate of Chromogranin A, exerts several biological functions, including inhibition of catecholamine release and cardioprotective role. Moreover positive effect of CST on monocyte migration in vitro and the induction of angiogenesis, arteriogenesis and vasculogenesis in the mouse hind limb ischemia model have been demonstrated. Collateral arteries may provide a biological bypass for occluded atherosclerotic vessels, increasing blood flow to ischemic tissue. In such a prospective, CST is a very promising agent for revascularization purposes, in “NO-OPTION” patients. However, proteins have a very short half-life after administration and must be conveniently protected. FN-PAMs, biodegradable and biocompatible polymeric microspheres, have ideal characteristic for this purpose: besides to convey peptides and allow in situ prolonged/controlled delivery, they may also convey cells on their biomimetic surface and may favor their survival and engraftment after cell transplantation. In this study, we show that CST can be incorporated within FN-PAM and aim to demonstrate that CST may be released in a slowly/prolonged manner by FN-PAM. We also aim to demonstrate that CST released by FN-PAM may reduce cell death under different stress conditions.

Materials and methods: CST has to be precipitated to ensure its stability upon subsequent encapsulation. Protein precipitate is formed from aqueous solution by the addition of a watermiscible organic solvent. PLGA-P188-PLGA (triblock) copolymeric microspheres are prepared using solid/oil/water emulsion solvent evaporation-extraction technique. PAMs are coated with Fibronectin and characterized by Immunofluorescence (confocal microscopy). Mesenchymal stem cells (MSC) are exposed to hypoxia (72 h in 1–2%O₂) and reoxygenation (6 h in 21% O₂) in a hypoxic chamber with or without CST, FN-PAMs or CST-FN-PAMs. The protective effects of treatments are detected by MTT assay.

Results: To define the optimum condition of nanoprecipitation we used an experimental design, modifying parameters influencing protein precipitation: ionic strength, mixing and centrifugation time. Nanoprecipitation of CST was found to be 72%. Controlled release of CST from CST-FNPAM greatly limits hypoxic MSC death and enhances MSC survival in post-hypoxic environment.

Conclusions: FN-PAMs are successfully formulated with CST. By an experimental design, we found optimal conditions to obtain a

good CTS nanoprecipitation yield. MSC readily adhere to the FN-PAM and CST-FN-PAMs reduce MSC death enhancing survival in post-hypoxic environment. Data suggest that CST-FN-PAMs are promising tools for therapeutic purpose.

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Study of Notch signaling modulation by shear stress by using an ex vivo cone-and-plate system

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Objectives: The link between endothelial shear stress (SS) patterns and localization of atherosclerotic plaques is well established. The atherosclerotic lesions form in specific regions of the arterial tree characterized by oscillatory SS (OSS), while regions exposed to laminar SS (LSS) are not susceptible to plaques onset. In vitro and in vivo studies have shown that endothelial cells subjected to OSS are predisposed to apoptosis and other dysfunctions, events that underlie plaques formation. The Notch pathway modulates endothelial cells apoptosis and we demonstrated that several Notch pathway components have lower expression levels in regions of mice aorta subjected to OSS compared to LSS. Moreover, we have shown that ivabradine, a heart rate reducing agent which may exert a protective activity by modifying the OSS on the endothelium, induces a less atherogenic gene expression profile in the endothelium of aortic arch of ApoE-deficient mice and modulates the Notch pathway. This study aims to better define the modulation of Notch signaling by SS by using an ex vivo culture system (cone-and-plate system).

Materials and methods: Human Umbilical Vein Endothelial Cells (HUVECs) were exposed to LSS at 50 dyn/cm² (200 rpm) by using smooth cones to OSS at 12.5 dyn/cm² (50 rpm) by using grooved cones for 24 h. After 24 h, at least five random images were taken for each 100-mm dish using a phase contrast microscope to monitor the cells status. We measured mRNA levels of several Notch components (Hey1, Hey2, Hes1, Notch1, Notch2, Notch4, Jagged-1 and Jagged-2) and we also evaluated by immunofluorescence protein levels of Notch receptors (Notch1 and Notch4).

Results: HUVECs exposed to LSS were elongated with the major axis aligned in the direction of flow, whereas those exposed to OSS were round in shape with a not well-defined axis direction, structures that resemble those we found in regions of mice aorta exposed to different SS patterns. A significant increase of many Notch components (Hey1, Hey2, Notch1, Notch2 and Jagged-1) mRNAs levels was obtained in HUVECs exposed to LSS compared to OSS. These data were also supported by immunofluorescence analysis in which we found that in OSS treated cells there was a reduction of Notch1 and Notch4 protein levels compared to LSS treated cells.

Conclusions: These data show a differential Notch regulation by different SS conditions. It remains to be better established whether downregulation of Notch signaling by OSS could be involved in atherosclerotic plaques onset and/or progression.

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Myostatin participate to abdominal aortic atherosclerosis and aneurysm development through VSMCs dysfunction and monocyte activation

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Objectives: Myostatin (Mstn) is a TGF- β family member involved in skeletal muscle growth inhibition and heart fibrosis. Despite the finding that loss of Mstn decreases atherosclerosis in LDL⁻mice, its role in vascular remodeling is still unknown. We sought to verify whether Mstn is operative aortic wall deterioration during development of atherosclerosis and abdominal aortic aneurysm (AAA).

Materials and methods: We first evaluated Mstn expression in samples of human abdominal aorta with increasing severity of atherosclerotic lesions, from healthy vessels up to AAA. Then, we investigated the in vitro effects of Mstn on vascular smooth muscle cell (VSMC) and monocyte biology.

Results: In aortic specimens, Mstn mRNA was dramatically increased in AAA (N = 7) compared to healthy vessels (N = 4) or early atherosclerotic lesions (N = 6) ($p < 0.05$); Mstn immunostaining increased with the severity of the atherosclerotic lesion (normal aortas, N = 4; late atherosclerotic lesions, N = 7; AAA, N = 9), was associated to a reduced positivity for Smoothelin (Smtn), a protein with contractile function in VSMCs ($p < 0.05$), and co-localized with α -SMA and CD45 immunoreactivity, suggesting that Mstn is predominantly expressed in VSMCs and leukocytes. Treatment of A7R5 VSMCs with Mstn (500 ng/ml) modulated the gene expression of MCP-1 and CCR2 (upregulated after 4 h) and of Smoothelin (downregulated after 48 h), increased the migratory rate and decreased the cell proliferation. In THP-1 monocytes, Mstn increased the MCP1-dependent chemotaxis ($p < 0.01$). In PBMNCs, MCP-1 increased Mstn mRNA; in turn, Mstn upregulated α -SMA and MCP-1 mRNA, caused overexpression and cortical distribution of-actin and acted as a chemoattractant dose dependently, with the highest rate at 500 nM, indicating a differentiation into an activated phenotype.

Conclusion: Mstn is overexpressed in atherosclerotic lesions and AAAs at sites of leukocyte infiltration and phenotype-switching VSMCs. Mstn causes loss of cell function in VSMCs and triggers a feed-forward inflammatory loop leading to recruitment of fibroblast-like monocytes. Therefore, Mstn sustains a chronic inflammatory milieu that elicits activation of dystrophic events, resulting in aortic wall deterioration.

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Perifollicular vascularization: Cocultures and tricultures approaches to study the cross-talk between microvascular endothelial cells, follicle papilla cells and keratinocytes

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Objectives: The perifollicular vasculature undergoes hair follicle (HF) cycle dependent expansion and degeneration. Multiple soluble factors derived from follicle dermal papilla cells (FDPCs) may act on