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Plasma exosomes after PCI in non-diabetic STEMI patients fed barley beta-D-glucan-enriched pasta reduce oxidative stress-induced endothelial cell senescence

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Senescent endothelial cells delay endothelialization, which increases restenosis risk in acute coronary syndrome with ST segment elevation (STEMI) patients following primary percutaneous coronary intervention (PCI). Normocaloric diet supplemented with barley beta-D-glucan (BBG)-enriched pasta protects the heart, but it is not yet clear whether this is due to the ability of this dietary combination to release exosomes that prevent endothelial aging following PCI.

In non-diabetic anterior STEMI patients (mean age 57 years, 8 women) who underwent PCI, we isolated plasma exosomes (pEXOs) before (T0) and after 3 months (T1) of normocaloric diet supplemented with 100 g of pasta containing 3 g of BBG (STEMI-BBG, $n = 19$) or without supplementation (STEMI-C, $n = 18$). Nanoparticle tracking analysis (NTA) determined pEXOs size and levels. Senescence-associated β-galactosidase activity was used to identify senescent human umbilical vein endothelial cells (HUVECs) induced by hydrogen peroxide (H2O2; 100 μmol/L for 24 h). HUVECs were treated with pEXOs (10^7particles/mL) for 24 h without and with H2O2. Dihydroethidium staining was used to detect oxygen free radicals (ROS) in HUVECs.

Significantly increased levels of pEXOs were observed at T1 in the STEMI-BBG group, but not in the STEMI-C group. NTA revealed that large pEXOs contribute to this increase, but not the other exosomal subpopulations. T0 and T1 pEXOs isolated from all patients promoted premature endothelial senescence in the absence of ROS elevation. In contrast, T1 pEXOs isolated from STEMI-BBG patients significantly prevented worsening of endothelial senescence induced by exogenous H2O2 via reducing oxidative stress ($p < 0.05$), but not T1 pEXOs from STEMI-C group.

Our findings suggest that pEXOs derived from reperfused non-diabetic STEMI patients exert pro-senescent signaling on HUVECs. However, pEXOs isolated from reperfused STEMI patients consuming BBG-enriched pasta exhibit a unique ability to mitigate the endothelial senescence induced by H2O2. This differential impact could be attributed to distinct transcriptional landscapes associated with the respective microenvironments.

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Dynamic co-culture of endothelial and smooth muscle cells as a platform for pathophysiological investigations of vascular calcifications

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Vascular calcification (VC) is a cardiovascular condition where calcium salt deposits occur within the vessel wall by vascular smooth muscle cells (VSMCs). In vitro models used for investigating vascular calcification typically involve VSMC monocultures under static conditions. However, given the significant role that endothelial cells (ECs) and VSMCs play in vascular function, both in physiological and pathological conditions, the aim of the current study was to create a dynamic co-culture of ECs and VSMCs that could better replicate the in vivo vascular microenvironment.

The presented work utilized a double-flow bioreactor to facilitate cellular interactions and emulate blood flow dynamics. To induce VSMC calcification, the cells were placed in a calcification medium composed of high glucose DMEM supplemented with 1.9 mM NaH_2PO_4/Na_2HPO_4 (1:1) for 7 days. The study assessed calcification, cell viability, inflammation, and molecular markers (SIRT-1, mTOR-1, TGFβ1, and Elastin IV). Results revealed that the dynamic model could replicate VSMC calcification and an inflammatory environment. Additionally, the regulation of effectors responsible for VSMC calcified phenotypic displayed an opposing trend to that seen in static monoculture, highlighting the significance of ECs-VSMCs communication in controlling cell behavior. Therefore, this model provided information that was not obtainable with standard cell monoculture, demonstrating its usefulness in exploring the pathophysiologic mechanisms behind vascular calcification because of its enhanced ability to imitate human vascular tissue.

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The new role of Tryptophan 2,3- dioxygenase in modulating human endothelial cell and endothelial precursor functions

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Tryptophan-2,3-dioxygenase (TDO) and Indoleamine-2,3-dioxygenase (IDO1) are the main enzymes involved in tryptophan (Trp) catabolism to kynurenine (Kyn) via the kynurenine pathway (KP), which is known to have a role in the immune regulation. An increased plasma Kyn/Trp ratio has been found in numerous diseases, including cardiovascular diseases. Most Trp is catabolized by IDO1 and its activity has been associated with worse cardiovascular outcomes in patients with coronary artery disease. TDO is mainly expressed in the liver in physiological conditions and recently, TDO-expressing cells were identified as pericytes in numerous types of tumors and in inflammatory pulmonary lesions, suggesting its pro-angiogenic role. Since TDO and IDO1 involvement in angiogenesis is still unknown, we aimed to characterize their role in human umbilical venular endothelial cells (HUVECs) and in human endothelial colony-forming cell function (ECFCs). TDO and IDO1 expression was evaluated by real-time PCR and immunofluorescence. ELISA assay was performed to assess KP activity. Cell proliferation was evaluated by MTT test and in vitro angiogenesis was studied by Geltrex 3D capillary morphogenesis. TDO and IDO1 were expressed by both HUVECs and ECFCs. To

evaluate IDO1 and TDO function, cells were treated with epacadostat and 680C91, selective inhibitors for IDO1 and TDO, respectively. TDO inhibition significantly impaired HUVEC proliferation and 3D-tube formation in response to VEGF. VEGF stimulated Akt phosphorylation which was prevented by 680C91. Conversely, ERK1/2 phosphorylation was unaffected by TDO inhibition. Kyn production was increased in response to VEGF and was prevented by the TDO inhibitor. ECFC capillary-like structures were also prevented by 680C91, while IDO1 inhibition was devoid of any effect. Our data demonstrated that HUVECs and ECFCs express the two enzymes of the KP. TDO is involved in *in vitro* proliferation and capillary morphogenesis, suggesting a putative pathophysiological role in angiogenesis in addition to its well-known immune modulatory effect.

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Macrophages participate in doxorubicin-induced cardiac damage

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The functional contribution of inflammatory cells in the setup of heart failure in response to anthracycline, specifically in response to Doxorubicin (Dox), is recently becoming of growing interest. Therefore, the study aims to evaluate the role of macrophages in cardiac damage in response to doxorubicin. In vivo mice C57BL/6 were treated with one intraperitoneal injection of Dox (20 mg/kg) and followed up for 5 days by cardiac ultrasound. Moreover, we tested the impact of Dox in macrophage-depleted mice by using Clodrosome Liposomes to evaluate the development of cardiotoxicity. In vitro murine cardiomyoblasts were directly treated with Dox (D-Dox) or with conditioned medium from cultured murine macrophages treated with Dox (M-Dox) and in both conditions, cell death and mitochondrial phenotype were evaluated.

In response to Dox, macrophages infiltration preceded cardiac damage. The depletion of macrophages in mice prevents cardiac damage suggesting a key role of these cells in promoting cardiotoxicity. To evaluate the crosstalk activation between macrophages and cardiac cells in response to Dox, we compared the effects of D-Dox and M-Dox in vitro.

Both effects lead to cell death but were significantly higher in M-Dox treated cells. These events were linked to p53-induced alterations of mitochondria morphology, function, and autophagy. We identify a mechanistic role of catecholamines released by Dox-activated macrophages that lead to mitochondrial apoptosis of cardiac cells trough β-AR stimulation. Our data suggest that the crosstalk between macrophages and cardiomyocytes is determinant in cardiac damage in response to Doxorubicin.

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An X-ray computed μ-tomography analysis for the characterization of 3D-heart shape in a model of cardiac plasticity

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The understanding of the crucial interplay between heart form and function through the development of new non-invasive methodologies for 3D morphological analysis has always aroused great interest in the cardio-scientists community. The X-ray computed μ- tomography (μ-CT) is a non-destructive 3D imaging technique utilizing X-rays to study the internal structures of materials, objects, or organisms. In this study, μ-CT has been used to investigate the 3D structural organization of the goldfish (Carassius auratus) heart, an emerging natural model for evaluating fundamental aspects of the coordinated physiological mechanisms that maintain cardiac steady-state. Ex-vivo isolated hearts were blocked in diastole, fixed in PFA, and stained in Lugol's solution. After dehydration in graded ethanol, the X-ray projections at different angles (steps 0.1°) were acquired. Mathematical algorithms (Feldkamp-Davis-Kress back-projection algorithm) were used to reconstruct the heart, thus obtaining its cross-sectional images. Using Fiji and Avizo software, 3D images of the sample were obtained and used for the analysis. An essential phase of the data analysis was the identification of various regions obtained with innovative segmentation algorithms. Preliminary investigation provides information about the heart's shape, curvature, symmetry, and chamber structural organization. Atrial and ventricular myocardial arrangement, compact vs trabecular myocardial component, inner atrial and ventricular chamber volumes, and bulbar structure have been studied. The data analyses performed have given a quantitative characterization of the 3D morphological differences between the various heart regions, evaluating the size of the cavities to the tissues that make up the heart. Our work provided the first non-invasive reconstruction of the 3D shape of the goldfish heart. It is of absolute importance to go deep inside the relationship between form and function and the associated physical constraints that shape the hemodynamic response of the goldfish heart, particularly when challenged by various environmental stimuli.

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Modulation of purinergic signaling in endothelial cells by tumor microenvironment

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