Topic 10 – Angiogenesis, microcirculation, growth factors, progenitor cells – B

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0232

Small, medium but not large arteries are involved in digital ulcers associated with systemic sclerosis

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Background: Digital ulcers (DU) are a burden in systemic sclerosis (SSc). Microangiopathy is a cardinal feature of SSc that plays a critical role in the development of DU. However, whether injury of medium or large vessels also contributes to DU in SSc is unknown.

Methods:To measure concomitantly in SSc patients with and without active DU i) the Augmentation Index of the reflected wave (Aix_75) by radial applanation tonometry, an index of small and medium arterial function, II) the aortic pulse wave velocity (PWV), a marker of large vessel injury (aortic stiffness).

Results: 63 consecutive SSc patients were included (49 females, aged 57±12 years, disease duration 9.7±7.1 years), including 10 (15.9%) with active DU.

Patients with active DU versus those without had increased Aix_75 (35% [28-38] versus 28% [20-34], p=0.041) whereas no difference existed in PWV (7,0m/s [6.7-10.1] versus 7,6m/s [6.8-8.7], p=0.887), in systolic, diastolic, as well as aortic pulse pressure (p=0.126, 0.592, and 0.161 respectively).

By multivariate analysis, DU remained independently associated with Aix_75 (p=0.020).

Using Aix_75 as a longitudinal variable, and when compared to patients in the low tertile, patients having Aix_75 in the highest tertile had ten-fold more DU (OR=10.23; 95% CI 1.12 to 93.34, p=0.039).

Conclusion: The presence of DU is independently associated with Aix_75 whereas there is no relation with PWV. These data suggest that small and medium arteries are involved in the occurrence of DU whether large vessel stiffness does not contribute. Whether Aix_75 is predictive of further DU remained to be studied.

0383

In vitro 3D model of in vitro angiogenesis using human endothelial cells and pericytes

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Human tissue is three-dimensional, and requires convective transport of nutrients and waste through capillary networks to meet metabolic demands Angiogenesis is the formation of new blood vessels from the existing vasculature. It is a multi-step process that include: degradation of the basement membrane, proliferation and migration (sprouting) of endothelial cells (EC) into the extracellular matrix, alignment of EC into cords, branching, lumen formation, anastomosis, and formation of a new basement membrane. The literatture in 3D in vitro models using endothelial cells is wide, using various types of EC (essentially Human Umbilical Vein Endothelial Cells), but blood vessels are composed of two interacting cells types: endothelial cells form the inner of the vessel wall, and mural cells that wrap the first ones. Pericytes are the mural cells of microvessels. They serve as scaffolding, and they communicate with endothelial cells by direct physical contacts and paracrine signaling pathways. Presently, there are no three-dimensional in vitro models of 3D Matrices which contain human pericyte-coated capillaries. Therefore, we aim at including pericytes in a 3D vascular morphogenesis assay in order to create a 3D in vitro model more close to physiologic conditions. We'll show and discuss our first analyzes and results, the goal of which is to provide new *in vitro* tools in order to better understand vascular biology, for later studies of endothelial cells-pericytes interactions, extracellular matrix-pericytes interactions, and eventually, further elucidate the role of pericytes in the microvasculature.

0304

Treprostinil indirectly regulates endothelial colony forming cell angiogenic properties by increasing VEGF-A produced by mesenchymal stem cells

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Prostacyclin therapy has markedly improved the outcome of patients with pulmonary hypertension (PH). Endothelial dysfunction is a key feature of PH, so the aim of our study was to determine how treprostinil contributes to the angiogenic functions of endothelial progenitors (ECFC). Treprostinil did not modify clonogenic properties nor endothelial differentiation potential from cord blood stem cells. Treprostinil treatment significantly increased the vessel-forming ability of ECFC combined with mesenchymal stem cells (MSC) in Matrigel implanted in nude mice. Silencing or blocking VEGF-A in MSC blocked the pro-angiogenic effect of treprostinil in vitro and in vivo. Clinical relevance was confirmed by the high level of VEGF-A detected in plasma from patients with pediatric pulmonary hypertension who had been treated with treprostinil. Our results suggest that VEGF-A level in patients could be a surrogate biomarker of treprostinil efficacy

0024

Angiogenesis potentialized by highly sulfated fucoidan: role of the chemokines and the proteoglycans

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Fucoidans are natural sulfated polysaccharides used as glycosaminoglycans (GAG) mimetics. Both GAGs and chemokines are important to regulate angiogenesis and wound healing. Fucoidans interacts with pro-angiogenic chemokines, such as RANTES and SDF-1, leads to revascularization and increases endothelial cell migration. However this pro-angiogenic activity remains unclear and depends of the type of fucoidan. Innovative vascular therapies based on GAG mimetic should be developed.

Upstream of developing a tissue engineering therapy, we propose to understand the beneficial effect of fucoidans on angiogenesis by a structure-function study. We hypothesize that the size and sulfation level could regulate the chemokines affinity and modulate its beneficial properties.

We purified and characterized 5 fractions of fucoidans according to their sulfation rate. We tested their affinity to chemokines (Surface Plasmon Resonance), the effect on endothelial cells (HUVEC) migration (Boyden chamber) and their pro-angiogenic properties (Microvascular network formation). We also analyzed the effect of fucoidans on endogen proteoglycans and GAG expression (qRT-PCR, FACS).

The structural analysis of fucoidans resulted in fractions (5-27kDa) composed of fucose, sulfate and uronic acid. The most sulfated fraction (5kDa with the ratio sulfate/fucose at 1.87) presented high affinity to biotinylated-SDF-1 and RANTES. Furthermore, this 5kDa fucoidan significantly increased HUVEC migration and microvascular network formation compared to other fractions.

The 5 kDa fucoidan shows the highest pro-angiogenic effects on HUVEC. Sulfate-rich 5kDa fucoidan confirmed our hypothesis than small and highly sulfated fucoidan is attractive candidate to develop therapies based on revascularization. We now focus to develop regenerative cell therapy of ischemic heart based on the injection of progenitors cells coupled with pro-angiogenic fucoidan and chemokines.

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