Microvascular Angina: Diagnosis and Treatment Particularities

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MicroRNA-216a: a cardiac-specific post-transcriptional regulator of capillary rarefaction associated with heart failure

P. A. Da Costa Martins1, R. Jun1, B. Duggy2, N. Batsch1, L. De Windt3
1University of Maastricht, Maastricht, Netherlands; 2Maastricht University, CARIM, Department of Cardiology, Maastricht, Netherlands

Background: While cardiomyocytes have been the main subject of extensive research in the past, the role of other cardiac cell types in the pathogenesis of heart failure received considerable less attention. Multiple other cell types, including fibroblasts, endothelial cells and vascular smooth muscle cells make up the heart and increasing evidence suggests that these cell types contribute to myocardial health and disease. Angiogenesis is an important adaptive mechanism of the myocardium to conditions of sustained increased demand. A select number of microRNAs were found to participate in post-myocardial angiogenesis but to date, it is unclear, however, if microRNAs regulate cardiac angiogenesis in the non-ischemic failing myocardium, which represents a clinically more relevant question.

Purpose: Establish that non-coding RNAs are main orchestrators of vascular integrity and hemostasis in the heart and changes in expression of the non-coding RNA content in different cardiac cell types underlie defects in neovascularization and progression to heart failure.

Results: To identify cardiac angiomiRs we recently completed a functional, genome-wide microRNA knockdown screen in cultured mouse cardiac microvascular endothelial cells. In this setup, we performed the scratch assay by fully automated scratch induction and image capturing and identified several candidate microRNA miR-216a, one of those candidates affecting endothelial cell migration, is consistently downregulated in a mouse model of cardiac pressure overload induced by transverse aortic constriction. We generated mice with a somatic gene deletion of miR-216a (miR-216a-/-). Abrogation of miR-216a induced dramatic effects on cardiac morphology and function in addition to spontaneous development of pathological cardiac remodeling at baseline, miR-216a-/- mice are also extremely sensitive to cardiac stress and die 1-2 weeks after pressure overload or develop severe cardiac phenotypes 3-4 weeks after myocardial infarction. Interestingly, the defects observed correlate with vascular defects including capillary rarefaction and reduced angiogenic activity. By combining photoacoustics with high-frequency ultrasound we observed different levels of hypoxia throughout the cardiac tissue confirming lower oxygenation of the miR-216a-/- hearts. The observed effects of miR-216 modulation seem to be related to altered autophagic activity in cardiac endothelial cells.

Conclusions: We identified a new angiogenesis-related gene regulatory network where miR-216a underlies the vascular changes associated with pathological cardiac remodeling and/or heart failure.

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Divergent effects of pre- and post-conditioning on microvascular function

S. Vitale1, M. Bertoni1, M.C. Marchetti2, G. Ciliberti1, S. Coiro1, C. Zuchi1, G. Migliorati1, I. Tritto1, C. Riccardi2, G. Ambrosio1
1University of Perugia, Department of Medicine, Division of Pharmacology, Perugia, Italy; 2University of Perugia, Department of Medicine, Division of Cardiology, Perugia, Italy

Background: A short period of ischemia/reperfusion (I/R) that occurs before a prolonged ischemia (preconditioning; preC) or at reflow (postconditioning; postC) reduces tissue necrosis through activation of regulatory kinases cascades, also reciprocally related, including the GPCR-PKG-PKC cascade and the NOS pathway. In this setting, an important role is played by nitric oxide (NO), which is also the main mediator of endothelium-dependent vasodilation. PreC may also protect microcirculation against I/R injury, while the impact of postC is less clear, and mechanism underlying these effects are not completely elucidated.

Purpose: To study the influence of preC and postC on angiogenesis by specifically evaluating leukocyte recruitment and vasodilating reserve, and to correlate these effects with activation of regulatory kinases.

Methods: In rats, cremaster muscle was prepared for in vivo videomicroscopy leaving vascular and neural connections intact. Animals were divided in 4 groups: 1- controls, in which muscle was subjected to 90 min of I and 90 min of R; 2- preC, in which preC was induced before I by 5 min of I and 10 min of R; 3- postC, in which postC was induced at R with 5 cycles of 10 seconds R and 5 seconds I; 4- sham, observed after surgery for an equivalent period of time. Leukocyte recruitment was monitored during I/R at the end of R, vasodilating endothelium-dependent and -independent reserve was assessed by local superfusion with 10-4 M acetylcholine and 10-5 M sodium nitroprusside. Phosphorylation of ERK, Akt, eNOS, PKCα and GSK3β was measured by Western Blotting on muscle homogenates obtained at the end of experiment in a second set of experiments in which, to avoid possible confounding effects of acetylcholine and sodium nitroprusside on kinase activation, rats were divided in 4 groups as described above but vasodilating challenge was not performed.

Results: I/R activated leukocyte recruitment (data not shown) and reduced vasodilating reserve, both endothelium-dependent and independent. Both preC and postC activated kinases and eNOS in a similar degree, and reduced leukocyte recruitment. However, only preC preserved vasodilating reserve (both endothelium-dependent and independent) (*p<0.05 vs I/R, $p<0.05$ vs sham).

Conclusions: Taken together, our data suggest that during ischemia/reperfusion leukocyte recruitment is mostly mediated by reperfusion injury and can be prevented by both preC and postC. Loss of vasodilating reserve seems to be mostly secondary to ischemic injury, on which postC cannot act, involving damage of vascular myocytes.

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Tissue factor variants induce monocyte mobilization and transdifferentiation into endothelial-like cells that promote angiogenesis

G. Arens1, S. Espinosa1, E. Pensa1, J. Crespo1, V. Y. Bogdanov1, L. Badimon1
1Barcelona Cardiovascular Research Center (CSIC-ICCV), 08036 Barcelona, Spain.
2University of Cincinnati College of Medicine, Division of Hematology/Oncology, Cincinnati, United States of America

Background/Introduction: Monocytes (Mo), the triggers of the innate immune response, also contribute to the angiogenic processes. Monocytic cells enhance neovascularization by releasing proangiogenic mediators and/or transdifferentiating into endothelial-like cells (ECL) cells. However, the mechanisms involved are not fully understood. Recently, we have reported that Mo–microvascular endothelial cells (mECs) crosstalk is mediated by TNF-α/FD2, which induces mEC–tissue factor (TF) expression and promotes angiogenesis.

Purpose: Here, we analyzed whether TF might exert angiogenic effects, not only by mEC–TF signaling, but also by mobilization and differentiation of monocytes into the ECL phenotype.

Methods: In vitro angiotube formation was investigated using human mECs and human Mo-subsets. TF expression in mECs was determined by qRT-PCR and immunoblotting. Full length TF (fTF) and alternatively spliced TF (sTF) were overexpressed in mECs, and their supernatants were added to
Mo cultures. CD16 positivity and expression of vascular endothelial cell (VEC) markers in Mo were analyzed by FACS. In in vivo studies, TF overexpressing mECs were injected into mice following induced hindlimb ischemia. Blood flow recovery was analyzed by MRI and changes in expression of VEC markers were analyzed in circulating Mo by FACS.

**Results:** Mo-mECs interaction induced TF signaling and secretion. Supernatants from TF-overexpressing mECs (TF\,+\,+mECs-CM) induced Mo expansion and transdifferentiation to ECLs expressing VEC markers. Over-expression of both TF forms, flTF and asTF, exerted similar effects. Specifically, CD16\,+\,monocytes exposed to TF\,+\,mECs-CM developed tube-like structures in 3D cultures and showed an increased expression, with respect to controls treated with mECs-CM, of VE-cadherin (16.5 ± 1.5 vs 8.6 ± 1.25), vWF (58.7 ± 5.25 vs 38.3 ± 3.45), and eNOS (13.3 ± 2.25 vs 6.5 ± 1.5). This effect was significantly blocked by anti-JT1 integrin antibody (4.4 ± 0.75 vs 16.5 ± 1.5). In in vivo studies, injection of TF-overexpressing mECs into ischemic hindlimbs of mice increased the pool of circulating repair-associated Mo (Ly6Clow Mo) (of total Mo, 28 ± 3.5% were Ly6Clow at day 1 and 50 ± 2.5% at day 5) with a higher expression of VEC markers (% expression of VE-cadherin in Ly6Clow 4.0 ± 0.65 vs 13.5 ± 0.5). Finally, injection of TF\,+\,mECs increased the number of macrophages surrounding collaterals in the hindlimbs with respect to control treatment.

**Conclusion:** These results indicate that TF expression in mECs induces them to release factor(s) that drive CD16\,+\,Mo to polarize into CD16\,+\,Mo, and further transdifferentiate into ECL, favoring microvessel formation. Our results show a TF-mediated positive feedback between endothelial cells and monocytes that stimulate Mo differentiation and induce angiogenesis.