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

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0221

MicroRNA-21 coordinates human multipotent cardiovascular progenitors therapeutic potential and post-ischemic revascularization

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Published clinical trials in patients with ischemic diseases show limited benefit of adult stem cell-based therapy, likely due to their restricted plasticity and commitment towards vascular cell lineage. Here, we have uncovered the potent regenerative ability of MesP1/SSEA-1-expressing cardiovascular progenitors enriched from human embryonic stem cells (hESC). Injection of only 10^4 hESC-derived SSEA-1$^+$/MesP1$^+$ cells, or their progeny obtained after treatment with VEGF-A or PDGF-BB, was effective enough to enhance post-ischemic revascularization in immunodeficient mice with critical limb ischemia (CLI). However, the rate of incorporation of hESC-derived SSEA-1$^+$/MesP1$^+$ cells and their derivatives in ischemic tissues was modest. Alternatively, these cells possessed a unique miR-21 signature that inhibited PTEN thereby activating HIF-1α and the systemic release of VEGF-A. Targeting Dicer or miR-21 limited cell survival *in vivo* and inhibited their pro-angiogenic capacities both in the Matrigel model and in mice with CLI. Interestingly, we observed an impaired post-ischemic angiogenesis in miR-21 deficient mice suggesting an unrestricted role of miR-21 in this regenerative environment. Notably, amongst the inflammatory cell population, miR-21 was highly expressed in circulating and infiltrated monocytes where it targeted PTEN/HIF-1α/VEGF-A signaling. As a result, miR-21 deficient mice displayed an impaired number of infiltrated monocytes and a defective angiogenic phenotype that could be partially restored by retransplantation of bone marrow-derived cells from wild-type littermates. Hence, hESC-derived SSEA-1$^+$/MesP1$^+$ cells progenitor cells are powerful key integrators of therapeutic angiogenesis in ischemic milieu and miR-21 is instrumental in this process as well as in the orchestration of post-ischemic vessel growth.

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Proepicardial prokineticin receptor –1 (PKR1) as a developmental link between heart and kidney

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Background: Prokineticin receptor-1 (PKR1), signals play critical roles in heart and kidney functions. In particular, the systemic mutation of this receptor results in thinning of the myocardium and hypoplastic kidney. However, the molecular and cellular mechanisms controlled by PKR1 signaling in this process are unclear.

Methods and Results: Here, we analyze a tissue-restricted mutations of the PKR1 gene in the proepicardial lineages (Gata5 and Wt1), and we show that PKR1 signaling in the proepicardium and its derivatives is required for proper cardiac and renal morphogenesis. Neonatal mutant mice display impaired proliferation of epicardial-derived cells in their heart and kidneys. Moreover, we detect defective coronary and renal arteriogenesis associated with PKR1 deficiency. Epicardial development is dramatically impaired in mutant mice, including failed expansion of the subepicardial space, blunted invasion of the myocardium, and impaired differentiation of epicardium-derived cells into coronary endothelial and smooth muscle cells. Abnormal mitochondria, lipid accumulation in mutant cardiomyocytes leads to lower contractile response to dobutamine. Impaired proliferation was observed in both Gata5 and WT1 but apoptosis was observed only WT1 lieage. Adult mutant hearts had abnormal rhythmicity and impaired systolic functions. Hypoplastic kidneys at the neonatal mutants were accompanied with deficient glomerular angiogenesis. Outgrown cell from kidney explants had a defective vasculogenic cell differentiation. Atrophy and dilated glomerular structure, abnormal mitochondria, lipid deposition and apoptosis were observed in the adult mutant kidney.

Conclusions: Our findings provide a mechanistic insight into the roles of PKR1 signaling in heart and kidney disorders controlling the maturation of epicardial-derived cell and differentiation in a cell autonomous fashion and affecting cellular communications in a paracrine fashion. Our mouse models recapitulate the complex human heart-kidney disorders.

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Vascular remodeling of the endocardium following cardiac infarction occured by arteriogenesis and angiogenesis

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Coronary vasculature is required to maintain cardiomyocyte survival, via delivery of oxygen and nutrients, and consequently myocardial architecture and cardiac function. Ischemic heart disease following myocardial infarction causes irreversible cell loss and scaring and is a major cause of morbidity and mortality. Revascularization of injured, ischemic and regenerating organs is essential to restore organ function and requires the formation of new vessels by the mechanisms of vasculogenesis, angiogenesis or arteriogenesis. With the objective of studying vascular remodeling during myocardial infarction (MI), we have performed permanent left coronary ligation on *Connexin40-GFP* (*Cx40$^{GFP/+}$*) mice. *Cx40* encodes a gap junction protein and is expressed in endothelial cells of large vessels. In the heart, *Cx40-GFP* expression is detected in coronary arteries but not in veins, capillaries or endocardium. After two weeks of ligation, MI was detected in left ventricle by echocardiography and anatomical examination of these hearts revealed the presence of an extensive network of GFP-positive vasculature within the infarct area. These vessels follow a tortuous route in the remaining ventricular wall and some communicate with the left ventricular lumen forming a crater covered with GFP and VEGF-R2 positive endothelial cells at the endocardial surface. To determine whether these vessels result from neo-vascularization or coronary artery remodeling, we carried out genetic lineage tracing of coronary endothelial cells using an inducible *Cx40-cre* allele. Our results show that GFP positive endothelial cells forming the endocardial carters are not always derived from pre-existing coronary arteries, suggesting that endocardium may also contribute to the generation of new vessels during vascular remodeling in the adult heart by angiogenesis.

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Non-peptidic prokineticin receptor 1 agonist as a novel cardioprotective therapeutic

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Objective: Prokineticins are potent angiogenic peptides that bind to two G protein-coupled receptors to initiate their biological effects. We previously

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have shown that prokineticin receptor-1 (PKR1) signaling contributes to cardiomyocyte survival or repair in myocardial infarction. Here, we discovered the first non-peptidic PKR1 agonists and examined their effects in mice model of heart diseases.

Methods and results: Herein we identify a selective PKR1 agonist both *in vitro* and *in vivo*, utilizing GPCR structure-based virtual screening approach. High Throughput Docking was carried out by GOLD using homology model of PKR1. Asinex gold collection 3D chemical databese (250,000 compounds) was screened by the docking protocol. We provided a strategy with a high potential for *in silico* identifying one agonist hit. We present here IS20, the first synthetic PKR1 agonist that induces angiogenesis in the presence of PKR1 on the endothelial cells seeded on matrigel. IS20 reduced doxorubicin cytotoxicity in H9C2 cells. IS20 promotes mouse epicardial progenitor cell differentiation into endothelial cells. *In vivo* IS20 activates Akt in mice heart. IS20 treatment of mice after coronary ligation reduces mortality by 30%.

Conclusion: This study identifies a non-peptidic PKR1 agonist as therapeutic target holding promise for treatment of heart diseases.

0044

Specific activation of the kallikrein-kinin system in hindlimb ischemia in diabetic mice

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Lack of post-ischemic revascularization is a major complication in diabetic patients. The kallikrein-kinin system (KKS) could play a role in post-ischemic neovascularization in non-diabetic conditions. The aim of this study was to potentialized the post-ischemic neovascularization process in diabetic mice *via* specific activation of bradykinin receptors (B1R or B2R) of KKS.

Hindlimb ischemia was induced in normo or hyperglycemic mice by ligation of the right femoral artery. Mice were then treated or not with specific B1R or B2R agonists (720nmol/kg.d^{-1}). Fourteen days after ischemia, neovascularization was analyzed using angiography, capillary density analysis, foot blood perfusion measurement and biochemistry. At the end of treatment, vascular and capillary density and skin foot blood flow were significantly decreased in diabetic condition. In contrast, treatment of diabetic mice with the B1R or B2R agonist restored post-ischemic neovascularization process to normal levels of vascular density (+45% for B1R, $p<0.01$ and 40% for B2R $p<0.01$), capillary density (+64% for B1R, $p<0.01$ and 66% for B2R, $p<0.01$) and foot blood perfusion (40% for B1R; $p<0.01$ and 30% for B2R, $p<0.01$) when compared with untreated diabetic mice. These beneficial effects were associated with an increase in macrophages infiltration in ischemic tissue and an increase in VEGF protein levels in gastrocnemius muscle. Moreover, in diabetic mice, 7 days of treatment with B2R agonist, but not B1R agonist, significantly increased MCP-1 mRNA expression in muscle (+95%, $p<0,01$), MCP-1 level in plasma (+18.5%, $p<0.05$) and number of circulating monocytes.

KKS modulation *via* B1R or B2R agonist can restore post-ischemic neovascularization in diabetic mice and may represent a new therapeutic strategy in this context.

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Red wine polyphenol compounds favor neovascularisation through estrogen receptor α independent mechanism in mice

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Red wine polyphenols compounds (RWPC) are known to protect against deleterious effects of cardiac and cerebral ischemia. However, they exert different effect depending on the dose on post-ischemic neovascularisation. Low dose (0.2mg/kg/d) promotes angiogenesis, whereas high dose (20mg/kg/d) has anti-angiogenic property. The vascular effect of RWPC is mediated through the activation of a redox-sensitive pathway, mitochondrial biogenesis and the activation of α isoform of the estrogen receptor (ERα). Here we evaluate the implication of ERα on angiogenic properties of RWPC. Using ovariectomized mice lacking ERα treated with high dose of RWPC after hindlimb ischemia, we examined blood flow reperfusion, vascular density, nitric oxide (NO) production, expression and activation of proteins involved in angiogenic process (eNOS, cav-1, VEGF) and muscle energy sensing network proteins (Sirt-1, AMPK and PGC-1). High dose of RWPC treatment reduced both blood flow and vascular density in muscles of mice expressing ERα. These effects were associated with reduced NO production resulting from diminished activity of eNOS. Surprisingly, high dose of RWPC increased blood flow and capillary density in mice lacking ERα. This effect is accompanied with increased NO pathway and production as well as VEGF expression. Interestingly, RWPC was able to activate Sirt-1, AMPK and PGC-1 in hindlimb from both strains. Altogether, the results highlight a pro-angiogenic property of RWPC via an ERα-independent mechanism that is associated with an up-regulation of energy sensing network proteins of the Sirt-1, PGC-1 network. This study depicts a novel way by which polyphenols may represent a therapeutic approach to treat pathologies associated with failed vascularisation.