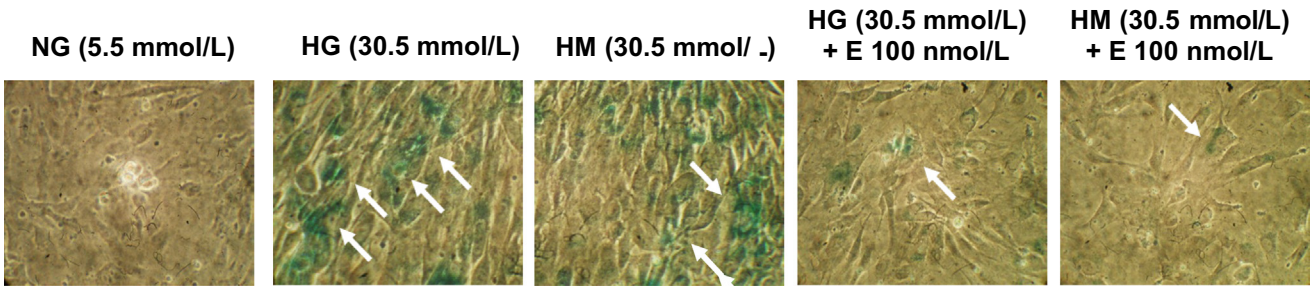

Fig. 1. β-gal staining of cardiac stromal cells under normal glucose (NG), high glucose (HG) and high mannitol (HM) in the presence or absence of Empagliflozin (E).

senescence, and attenuated the effects of HG and HM on the expression of pAKT and P-p38.

Conclusions

E counteracts the pro-senescent effect of HG on CSC by increasing CSp yield and inducing a peculiar gene expression program, leading to pro-survival molecular signatures. These direct pleiotropic effects may help explaining E unexpected benefits on cardiac function in patients with diabetes, as observed in the EMPA-REG trial.

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Rejuvenated extracellular vescicles from telomerase/myocardin co-expressing mesenchymal cells promote revascularization and tissue repair in murine myocardial infarction

Vanessa Doria[a], Damiana Pieragostino[b], Claudia Rossi[b], Simone Guarnieri[b], Csilla T. Nagy[c], Zoltán Giricz[c], Peter Ferdinandy[c], Piero Del Boccio[b], Maria Addolorata Mariggiò[b], Yong-Jian Geng[d], Rosalinda Madonna[d,e,*], Raffaele De Caterina[e]

[a]*Center of Aging Sciences and Translational Medicine - CESI-Met and Institute of Cardiology, "G. D'Annunzio" University, Chieti-Pescara, Chieti, Italy*
[b]*Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy*
[c]*Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary*
[d]*Department of Internal Medicine, University of Texas Medical School in Houston, Houston, Texas*
[e]*Institute of Cardiology, University of Pisa, Pisa, Italy*
*Corresponding author.
E-mail address: rosalinda.madonna@unipi.it (R. Madonna)

Background and aim

Cell therapies are hampered by poor survival and growth of grafts. We aimed at testing whether forced co-expression of the anti-senescence telomerase reverse transcriptase (TERT) and the anti-apoptotic myocardin (MYOCD) improves post-infarct revascularization and tissue repair by adipose tissue-derived mesenchymal stromal cells (AT- MSCs). Specifically, we sought to answer 3 questions: (1) Are rejuvenated aged AT-MSCs (rAT- MSCs) more beneficial than wild-type aged AT-MSCs when transplanted into a murine model of cardiac ischemic injury? (2) Are the effects of the rAT-MSCs mediated via paracrine signaling (i.e., via the secretome)? and (3) Are the effects of the rAT-MSC secretome due to the extracellular vescicle (EV) fraction?

Methods and results

We transplanted AT-MSCs engineered to overexpress MYOCD and TERT in a murine model of acute myocardial infarction (AMI). We characterized in vitro and in vivo paracrine effects of AT-MSCs. When transplanted into infarcted hearts of C57BL/6 mice, "rejuvenated" AT-MSCs overexpressing TERT and MYOCD (rAT-MSCs) decreased scar tissue formation and the intrascar CD3 and B220 lymphocyte infiltration, and increased arteriolar density as well as ejection fraction compared with saline or mock-transduced AT-MSCs. These effects were accompanied by increased numbers of Ki-67$^+$ and CD117$^+$ cells, and the expression of cardiac actin and β-myosin heavy chain within the infarcted hearts. Both rAT-MSCs and their conditioned medium (CM) stimulated intracellular Ca^{2+} flux in cardiospheres (CSps). CSp-derived cells had increased survival when preconditioned with CM or EV fraction from rAT-MSCs and then exposed to simulated ischemia/reperfusion. Proteomic analysis of rAT-MSCs-EV fraction predicted the activation of vascular development and the inhibition of immune cell trafficking. Elevated concentrations of miR-320a, miR-150-5p and miR-126-3p associated with regulation of apoptosis and vasculogenesis were confirmed in rAT-MSCs-EV fraction.

Conclusions

"Rejuvenated" EV from rAT-MSCs overexpressing TERT and MYOCD promote persistence of transplanted aged AT-MSCs and enhance arteriolar density in a murine model of AMI. EV enriched fraction is the active component of the paracrine secretion by rAT-MSCs, with pro-angiogenic and pro-survival activities.

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Improved gap junction-mediated cellular communication between cardiomyoblasts and non myocyte cells treated with Apelin-13

Angela Rita Elia, Ambra Iannuzzi, Emanuela Vitale, Daniele Garelli, Lelio Sciulli, Claudia Giachino, Raffaella Rastaldo

Department of Clinical and Biological Sciences, University of Turin, Regione Gonzole 10, 10043 Orbassano, Turin, Italy

Apelin-13 (Ap) is an endogenous peptide acting on the APJ receptor. It affects various cardiovascular functions, such as cardiac

development, myocardial inotropy, angiogenesis and vasomotor tone in healthy and failing hearts.

Gap junctions are essential for a proper transmission of the impulse in myocardium, which prevents the occurrence of arrhythmias. Notably, 50–60% of deaths in patients with ischemic cardiomyopathy have been attributed to an arrhythmic cause and several studies reported that stem cell therapy is proarrhythmic.

Silencing of Ap expression in mesenchymal stem cells decreases the protein level of Connexin-43 (CX43), the important isoform involved in gap junctions, suggesting that Ap regulates protein expression and gap junction function, though the involved mechanism is still unknown.

We recently showed the presence of APJ receptor on the membrane of cardiac non myocytic cells (NMCs) isolated from adult rat hearts. Thus, this study aimed to investigate whether and how the administration of Ap can affect the expression of CX43 and the formation of gap junctions between NMCs and cardiomyoblasts (H9C2 cellline).

Western Blot analysis revealed a significant increase of CX43 protein level in NMCs treated with 100nM Ap for 48h with respect to non-treated cells ($p<0.05$). These data were confirmed ($p<0.05$) by IF analysis.

The influence of Ap on gap junction function was evidenced by cell-transfer assays using Calcein- AM, a gap junction permeant dye. Ap-treated NMCs were more efficient in transferring the dye to H9C2 with respect to non-treated cells ($p<0.05$); this effect was reversed by the use of the gap junction inhibitor carbenoxolone.

Real-time PCR revealed that the molecular mechanism by which Ap increased CX43 protein was not dependent on gene expression modulation. Rather, it could be explained by a previously undescribed role of Ap in autophagy inhibition in these cells. In fact, Ap-treated NMCs upregulated Akt and mTOR phosphorylation and displayed modulation of the major autophagy marker LC3, showing decreased LC3II/LC3I ratio. These results suggested an early inhibition of the autophagy process before the autophagosome formation and were supported by treating the cells with the early autophagy inhibitor3-MA.

In conclusion, the *in vitro* treatment of NMCs with Ap seems to inhibit autophagy via the PI3K-Akt- mTOR pathway, thus leading to enhancement of CX43 surface protein and gap junction formation between NMCs and cardiomyoblasts. This effect could be very important in heart repair because it can facilitate cell-cell communication and reduce the occurrence of arrhythmias after myocardial infarction.

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Venous pulse wave velocity

L. Ermini[a], L. Pastore[b], C. De Benedictis[b], C. Ferraresi[b], S. Roatta[a]

[a]*Dept. Neuroscience Sect. Physiology, University of Turin, Italy*
[b]*Dept. Mechanical Engineering, Polytechnic of Turin, Italy*

Central venous pressure and volume status are relevant parameters for the characterization of the patient's haemodynamic condition and for the management of fluid therapy however, their invasive assessment is affected by various risks and complications while non-invasive approaches provide only imprecise and subjective indications. Aim of the present study is to explore the possibility to assess changes in venous pressure from changes in the *venous* pulse wave velocity (vPWV). In 9 healthy subjects, pressure pulses were generated artificially in the veins by a PC-driven rapid inflation of a pneumatic cuff (300mmHg in <1sec) placed around a foot. Passage of the pulse wave in the superficial femoral vein distally to the inguinal ligament was detected by Doppler flowmeter and the latency from the pressure stimulus was measured. The vPWV was then calculated as the ratio between traveling distance and latency. Changes in leg venous pressure were obtained by raising the trunk of the subject from the initial supine position by 30 and 60 deg. In each position 15 pressure pulses were delivered every 30 s, at the end-expiratory phase for vPWV assessment. Venous pressure in the leg was non-invasively estimated by assessing the point of collapse of the jugular or axillary vein. The vPWV increased from 1.64 ± 0.06 (supine) to 2.13 ± 0.26 (60 deg) (Student's *t*- test, $p<.01$) and exhibited a very strong correlation with leg venous pressure (overall $r=0.76$). Differences in vPWV among the three positions were statistically significant also on an individual basis in 8/9 subjects (ANOVA + Tukey's HSD post-hoc, $p<.01$). These preliminary results show that vPWV may be easily assessed in healthy subjects and may constitute a good non-invasive indicator of venous pressurechanges.

Keywords: Pulse wave velocity, Central venous pressure, Echo-Doppler

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Erythro-myeloid progenitors contribute endothelial cells independently of KIT

Alessandro Fantin[a,b], Alice Plein[b], Laura Denti[b], Christiana Ruhrberg[b]

[a]*Department of Biosciences, University of Milan, via Celoria 26, 20133 Milano, Italy*
[b]*UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London, EC1V 9EL, UK*

The earliest blood vessels in the mammalian embryo are formed when endothelial cells (ECs) differentiate from angioblasts and coalesce into tubular networks. Thereafter, new ECs are thought to arise solely by proliferation of pre-existing ECs. By combining genetic lineage tracing with expression studies, flow cytometry and gene targeting in mouse embryos, we show that the KIT-positive erythro-myeloid progenitors (EMPs), which are derived from the yolk sac hemogenic endothelium and are the earliest precursors of erythrocytes and macrophages, additionally give rise to ECs that are recruited into pre-existing vasculature. Whereas a first wave of yolk sac-resident EMPs contributes ECs to the yolk sac endothelium, a second wave of EMPs, after colonising the embryo proper, contributes ECs to intraembryonic endothelium. Despite KIT being required for hemogenic endothelial cell specification and function, including erythroid development, the EC contribution by EMPs was not affected by the lack of KIT, but by the expression of the HOXA transcription factor family. By demonstrating that EMPs constitute a hitherto unrecognised and non- redundant source of ECs that persist into adulthood, we reveal that embryonic blood vascular endothelium expands in a dual mechanism that involves both the proliferation of pre-existing ECs and the incorporation of EMP-derived ECs in a HOXA transcription factor-dependent but KIT- independent manner.

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