Impact of heart failure on cardiac stem cells

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Background: Cardiac Stem Cells (CSCs) expanded in vitro from explanted failing hearts (E-) are characterized, with respect to those obtained from healthy donors (D-), by: reduced proliferation and migratory capabilities, shorter telomeres, and a larger fraction of cells showing both telomereassociated damage foci and senescence markers. Since the Autophagy-Lysosome Pathway (ALP) plays a pivotal role in cellular homeostasis by controlling both cellular clearance and response to nutrients, defects on ALP may be associated to aging and heart failure progression.
Aims: to monitor the efficiency of ALP in senescent CSCs isolated from patients with heart failure and to develop a drug-based strategy able to boost ALP, eventually contrasting senescence.
Methods and Results: 14 D-CSC and 20 E-CSC, obtained from healthy and failing human hearts, respectively, were compared in terms of cell surface immunophenotype and senescence marker expression. Although the two groups of cells shared a similar immunophenotype, E-CSC showed a significant enrichment in the fraction of senescent cells (p16+, γH2A.X+ Ki67-). Microarray analysis and Real Time PCR were performed to investigate transcriptional profile (3 D-CSC vs 3 E-CSC) and expression profile of 380 microRNAs (4 D-CSC vs 4 E-CSC), identifying 423 genes down-regulated in E-CSC ($p<0.05$), 23 of which are involved in ALP, in addition to 7 differently expressed miRNAs ($p<0.05$), that are able to target genes linked to ALP and cell senescence regulation. Consistently, the lysosomal compartment of 7 D- and 5 E-CSC was monitored by FACS analysis after staining with lysotracker and acridine orange and E-CSC displayed lysosomes less functional than the D-CSC ones. To study in depth this element, confocal microscopy analysis was performed to evaluate the lysosomal presence of not degradable lipofuscin and of the endomembrane damage marker Galectin 3, together with the amount of nuclear active Transciption Factor EB (TFEB). Noteworthy E-CSC showed a higher abundance of lysosomal lipofuscin and Galectin 3, coupled with a reduced TFEB activation. Given the link between mTOR and senescence and the pivotal role played by mTORC1 in controlling autophagy and TFEB activation, we evaluated mTORC1 activity by western blot analysis. In particular attention was focused on pS6K, Akt in parallel with the autophagic markers Atg3, Atg7, LC3II, p62. Altogether results demonstrated that E-CSC were characterized by an enhanced activity of mTORC1 and an arrest in autophagic degradation. Moving from these elements we develop a three days drug treatment of E-CSC with 10nM Rapamycin (TORC1 inhibitor). This pharmacologic strategy was able to reduce mTORC1 activity, to potentiate the lysosomal functionality, to improve the autophagic flux and to reduce the fraction of senescent cells.
Conclusions: this study demonstrated that E-CSC are characterized by a blunted ALP. The pharmacologic inhibition of TORC1, on one hand, reactivated the pathway and, on the other hand, contrasted senescence offering promising perspectives to improve E-CSC cardiac regenerative efficiency.