DECODING HUMAN CARDIAC STEM CELLS REGENERATIVE POTENTIAL IN ACUTE MYOCARDIAL INFARCTION

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Acute Myocardial Infarction (AMI) remains a leading cause of death worldwide. After AMI, clinical restauration of blood flow aggravates tissue damage (Ischemia/Reperfusion, I/R injury), critically decreasing the number of viable cardiomyocytes (CMs). Human myocardium harbors a population of endogenous cardiac stem/progenitor cells (CSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine molecular crosstalk between CSCs and CMs in stress. Transplantation of CSCs is currently being tested in several clinical trials, and although some improvements have been reported regarding decrease of the infarcted area, it is still not enough to show benefit over pharmacological standard-of-care.

Our work aims at combining the development of relevant I/R *in vitro* human cell models with implementation of advanced mass spectrometry (MS)-based proteomic tools to further characterize hCSC and unveil associated regenerative mechanisms upon AMI. hCSCs employed in the phase I/II clinical trial CARE-MI (NCT02439398) were used (allogeneic therapy).

Different strategies were explored to recapitulate both phases of I/R injury in the human adult heart, including: the use of human adult/mature cells, 3D culture system and stirred-tank bioreactor technology. Firstly, we developed a transwell co-culture cell based I/R model, with human CSCs and human induced pluripotent stem cell derived CMs (hiPSC-CMs). Following this work, and aiming at further improving the relevance of the I/R injury *in vitro* setup, 3D hiPSC-CM aggregate cultures and bioreactors were combined, allowing the control/monitoring of environmental parameters such as pH and dissolved oxygen, critical in the context of I/R physiology.

Important features of I/R injury were successfully captured in the two models, including hiPSC-CM death upon reperfusion, disruption of cell ultra-structure organization, as well as increased release of angiogenic and inflammatory cytokines, consistent with the described pathophysiology of AMI. hCSCs response to I/R was further probed using whole proteome analysis (including quantitative SWATH methodology), allowing us to propose new pathways in the hCSCs-mediated regenerative process along the different phases of I/R injury through the identification of more than 3800 proteins and quantification of 714 proteins. Our data shows that our AMI-setup up-regulates hCSC proteins associated with several pro-migratory, proliferation and stress response-related pathways. Moreover, our results reinforce the idea that paracrine-mediated mechanisms are a central response in hCSC activation, with the enrichment of several paracrine signaling and pro-angiogenic pathways. We also show for the first time increased CXCL6 secretion by hCSCs upon injury, suggesting a relevant role of this angiogenic cytokine in hCSC mediated myocardial regeneration.

Overall, multiple strategies were used to develop novel and robust I/R injury *in vitro* models, recapitulating several features of the human adult myocardium. The systems established allowed to better characterize hCSC mechanisms of action in response to AMI contexts. The knowledge generated has the potential to be used in the development of novel strategies excelling endogenous and transplanted hCSCs regenerative potential.