Abstract: P494

Applying proteomic tools to disclose human cardiac stem cells regenerative potential in ischemia/reperfusion injury

Authors:
M Sebastiao¹, M Paiva¹, I Reis¹, M Sousa¹, I Palacios², M Serra¹, P Gomes-Alves¹, PM Alves¹, ¹iBET, Instituto de Biologia Experimental e Tecnológica - Oeiras - Portugal, ²Coretherapix, Tigenix Group - Madrid - Spain,

Topic(s):
Ischemia, Infarction, Cardioprotection

Citation:
Cardiovascular Research (2018) 114 (Supplement 1), S120

Funding Acknowledgements:
The authors acknowledge CARDIOSTEM (MITP-TB/ECE/0013/2013); FCT (PTDC/BBB-BIO/1414), (SFRH/BPD/52339/2013);iNOVA for Health (UID/Multi/04462/2013).

After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is responsible for a critical decrease in the number of viable cardiomyocytes (hCMs). Human myocardium harbors a population of endogenous cardiac stem/progenitor cells (hCSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine molecular crosstalk between hCSCs and hCMs in stress.

Clinical trials involving transplantation of hCSCs into the infarcted myocardium have demonstrated the potential of these cells. Although some improvements have been reported regarding increase in viable myocardium and improved tissue contractility, extensive data indicates that transplanted cells do not survive in the myocardium and this has led to the postulation of a paracrine mechanism for the observed beneficial effects.

Using the same cells currently employed in the allogeneic hCSCs transplantation clinical trial CARE-MI (EUDRA 2013-001358-81), our work aims at setting up an in vitro human I/R injury model using by using 3D culture approached combined with stirred tank bioreactor technology, in order to better decipher the mechanisms of action of hCSCs upon AMI using proteomic tools.

By using aggregates of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) and a microcarrier based culture of hCSCs, injury setup was performed in bioreactors, allowing paracrine crosstalk between the two cell types via a reciprocal medium exchange via a perfusion system. Ischemia was mimicked by substituting growth media by Ischemia Mimetic Solution and placing the cells at 0% O2 for 5 hours. In the reperfusion step, cells were placed back in their physiological culture conditions (3% O2). The effect of I/R injury in hCSCs was accessed by quantitative total proteome analysis (using LC-MS) at different time points. Growth factor secretion, cells’ viability, as well as hCSCs proliferation was also monitored in this co-culture system. Our first experiments in monolayer static systems showed that this model is able to recapitulate important features of I/R, including hCSC proliferation activation and a protective effect of hCSCs in hiPSC-CMs survival. Upon injury, proteome of hCSCs was enriched in proteins related to cell cycle regulation inhibition, proliferation through EGF signaling, glutathione-mediated reactive oxygen species detoxification and paracrine signalling pathways.

This system will allow further understanding on the molecular landscape of the myocardium during AMI, namely regarding hCSCs regenerative response and hCMs survival. The knowledge generated in this work will hopefully potentiate the development of novel molecular and cell-based therapies for myocardium regeneration.