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HUMAN CARDIAC STEM CELLS FOR ALLOGENEIC CELL THERAPIES: INTEGRATING BIOPROCESS DEVELOPMENT AND 'OMICS CHARACTERIZATION TOOLS

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide. Myocardial infarction (AMI) severely affects patients' heart muscle and microvasculature, critically decreasing the number of functional cardiomyocytes (CM). Stem cell and protein based therapies became promising cardiac repair strategies since it was found that under pathological stress, resident cardiac stem cells (CSC) of the adult myocardium are activated by growth factors (GF) secreted by the surviving CM. Consequently, an auto/paracrine loop is triggered to maintain GF production, which enhances CSC activation and differentiation into new CM, endothelial and smooth muscle cells contributing to the repair of damaged myocardium [1]. Since this repopulation of the myocardium is neither robust nor durable enough to have significant beneficial physiological/anatomical impact in severe and acute myocardial losses, local administration of GF has been shown to be efficient in enhancing CSC activation, improving cardiac output post-AMI through the formation of new vascularized and functional autologous myocardium [2].

The CARE-MI project and allied clinical trials aim at developing broadly available and clinically applicable treatments for ischemic heart diseases by exploiting hCSC biology and the molecular mechanisms responsible for their activation and differentiation in situ. For attaining an efficient therapeutic approach, a rational therapy design must be pursued, requiring not only a robust hCSC production platform, for the creation of "off-the-shelf" allogeneic cell banks, but also the identification and delivery of the appropriate cocktail of growth factors.

On average 1-2x109 injected cells per patient are required for a major regenerative response on injured myocardial tissue. Microcarrier systems have been widely explored for stem cell expansion, and were recently identified as the most effective (based on time and price) cell culture technology for implementation of cell expansion platforms for allogeneic therapies [3, 4].

We have optimized and validated a robust, scalable and controlled bioprocess for the expansion of hCSC on microcarriers. Gene expression microarray and MS-based approaches have been employed to compare the transcriptome, proteome and secretome of hCSC cultured in standard static and stirred microcarrier-based systems. Importantly, the results show that cells retain their phenotype and maintain similar 'omics' profiles to hCSC cultured under traditional monolayer systems.

Moreover, a comprehensive description of hCSC receptome was pursued, enabling further correlation of secretome data with receptor expression to unravel signaling pathways beneath myocardial regeneration. We have exploited and improved a proteomics workflow overcoming the inability demonstrated by other technologies applied in receptor identification, mainly due to the transmembrane nature, high hydrophobic character and relative low concentration of these proteins. This approach was based on enrichment of hCSC plasma membrane fraction and addition of pre-fractionation steps prior to MS analysis. More than 3000 proteins were identified including more than 150 plasma membrane receptors.

This work reveals not only a robust system for hCSC production supporting allogeneic cell therapies but also strongly contributes to depict the hCSC receptome and secretome, which could be translated into the design of a more rational and efficient GF cocktail towards a generic treatment available at all times to produce completely autologous myocardial regeneration.

References: [1] Beltrami AP et al, Cell 2003, 114:763-776; [2] Ellison GM et al, J Am Coll Cardiol 2011, 58:977-986; [3] Serra M et al, Trends Biotechnol 2012, 30:350-9; [4] Simaria AS et al, Biotech Bioeng 2014, 11:69-83.

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