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Deriving clinically compliant mescenchymal stem cells (MSC) from differentiated human ESCs and elucidating MSC paracrine proteome

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Adult tissue-derived mesenchymal stem cells (MSCs) have demonstrated therapeutic efficacy in treating diseases or repairing damaged tissues through mechanisms thought to be mediated by either cell replacement or secretion of paracrine factors. Characterized, self-renewing human ESCs could potentially be an invariable source of consistently uniform MSCs for therapeutic applications. Here we describe a clinically relevant and reproducible manner of generating identical batches of hESC-derived MSC (hESC-MSC) cultures that circumvents exposure to virus, mouse cells, or serum. Trypsinization and propagation of HuES9 or H1 hESCs in feederand serum-free selection media generated three polyclonal, karyotypically stable, and phenotypically MSC-like cultures that do not express pluripotency-associated markers but displayed MSC-like surface antigens and gene expression profile. They differentiate into adipocytes, osteocytes, and chondrocytes in vitro. Gene expression and fluorescence-activated cell sorter analysis identified CD105 and CD24 as highly expressed antigens on hESC-MSCs and hESCs, respectively. CD105+CD24–monoclonal isolates have a typical MSC gene expression profiles and were identical to each other with a highly correlated gene expression profile ($r^2 > .90$). We have developed a protocol to reproducibly generate clinically compliant and identical hESC-MSC cultures. To evaluate the therapeutic potential of MSC paracrine proteome, a chemically defined serum-free culture media was conditioned by hESC-MSCs. hESC-MSC secrected proteome was analyzed by multidimensional protein identification technology (MuDPIT) and cytokine antibody array analysis. The results revealed the presence of 201 unique gene products. 86-88% of these gene products have detectable transcript levels by microarray or qRTPCR assays. Computational analysis predicted that these gene products will significantly drive three major groups of biological processes: metabolism, defense response, and tissue differentiation including vascularization, hematopoiesis and skeletal development. It also predicted that the 201 gene products activate important signalling pathways in cardiovascular biology, bone development and hematopoiesis such as Jak-STAT, MAPK, Tolllike receptor, TGF-β signalling and mTOR signaling pathways. This study identified a large number of MSC secretary products that have the potential to act as paracrine modulators of tissue repair and replacement in diseases of the cardiovascular, hematopoietic and skeletal tissues. Moreover, it suggests that hESC-derived MSC conditioned medium has the potency to treat a variety of diseases in humans without cell transplantation.

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