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Study of cardiomyogenic potential of human Amniotic Fluid Stem Cells

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It has been shown that Amniotic fluid stem cells (AFSCs) have characteristics intermediate between pluripotent embryonic and lineage-restricted adult stem cells, and are non-tumorigenic and low immunogenic. Moreover, they are obtained without destroying human embryos, so that preventing most of the ethical and social controversy. Human AFSCs express some genes specific of both embryonic (OCT3/4, NANOG, c-MYC) and primordial germ stem cells (Fragilis, Stella, c-KIT). We have demonstrated that hAFSCs form in vitro embryoid bodies (EBs) and express markers of three germ layers. Studies reported the ability of hAFSCs to differentiate in vitro into adipocytes and osteocytes, but only few data are available on their cardiomyogenic potential. Aim of this study is to analyze hAFSCs differentiation through the cardiac pathway. Embryonic Bodies (EBs) were obtained from hAFSCs cultured in presence of ascorbic acid and 5-aza-2'-deoxycytidine. Cardiomyogenic potential of hAFSCs and EBs was explored by WB and immunoflorescent analyses of specific markers. Simultaneous quantitative detection and cellular localization analysis of cardiomyogenic markers were conducted with an ImageStream multispectral imaging flow cytometer (Amnis-Seattle, WA) equipped with IDEAS statistical software. We evidenced that both AFSCs and EBs at early stage express Nkx2.5, a transcription factor expressed by cardiomyocytes precursor cells. Moreover, ImageStream imaging cytometer analysis evidenced that EBs formation was accompanied by an up-regulation of Nkx2.5 expression (36.54±1.83% and 64.68±3.23% positive cells in hAFSC and EBs respectively, $p<.005$) and by a significant nuclear translocation (12.98±0.64% and 37.98±1.9% nuclear positive cells in hAFSC and EBs respectively, $p<.005$). Microscopical analysis evidenced inside the EBs cells positive for the presence of cardiac α-Myosin heavy chain protein structurally organized in oriented filaments. Moreover, we detected cells expressing Connexin43. These results evidenced that EBs obtained from hAFSCs cultured in permissive conditions terminally differentiate into cardiomyocytes and suggest them as possible model to study the cardiac differentiation.

Key words

Stem cells; Myocardial differentiation.