Functionally differentiated cardiomyocites derived from human amniotic fluid-derived stem cells

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Purpose. Human amniotic fluid-derived stem cells (hAFSC) are multipotent stem cells sharing characteristics of both embryonic and adult stem cells. It has been already reported that hAFSC can differentiate toward cardiac lineage though the embryonic body (EB) formation, but the 3D structure and cellular heterogeneity of EB represent an important limitation. Aim of this study was to fully differentiate the hAFSC overcoming the EB limitations.

Methods. hAFSC were obtained from normal amniocentesis. Cells cultured in monolayer were exposed sequentially to Ascorbic Acid, 5-Azacytidine, BMP4, Activ-inA, VEGF up to 20 days. Differentiation was evaluated monitoring by Western Blot, immunofluorescent and cytometric analyses the expression of CD90, as mesenchymal stem cell marker, and of Nkx2.5, Gata4, sarcomeric α-actinin (αSA), α cardiac myosin heavy chain (αMHC), cardiac T-troponin (TnT)and Connexin43 as cardiac markers.

Results. During the differentiation cultures cells underwent a progressive decrease of CD90 accompanied by the induction cardiac markers. After 15 days we evidenced that almost the entire cell population was positive for αMHC, αSA cTnT and Connexin 43 expressions (Table I); moreover, even if the % of Gata4+ and Nkx2.5+ cells did not varied during the culture, a significant increase of Nkx2.5 nuclear translocation (9.1±0.9% vs 18.0±1.8% Nkx2.5 nuclear positive cells in hAFSC and differentiated cells respectively, p<.005, analysis by ImageStream) was detected. Some small beating foci (about 8-10% of the plate) were also observed.

Conclusion. We demonstrate that hAFSC can fully differentiate into myocytes giving rise to a homogenous population with cardiac-specific molecular and functional properties.

Table 1. Cell Positivity (%) and Mean Fluorescence Intensity of hAFSC before and after differentiation

	hAFSC		Differentiated hAFSC	
	Cell Positivity (%)	MFI	Cell Positivity (%)	MFI
CD90	99.3±0.5	141.6±45.2	4.5±2.5*	3.2±2.8*
GATA4	82.5±7.4	21.9±1.6	95.7±3.1	24.3±0.6
Nkx2.5	84.5±6.5	14.2±4.7	98.7±0.3	26.7±6.6
αMHC	3.7±5.4	27.9±6.5	98.7±1.1*	155.6±15.6*
αSA	0.3±1.2	5.9±9.2	89.5±5.4*	236.0±10.0*
cTnT	0.1±0.1	0.8±0.4	95.4±3.1*	66.1±3.5*
Connexin 43	43.5±8.1	4.9±1.5	95.5±4.2*	134.4±15.8*

Data are expressed as mean±SD * p<0.05 relative to hAFSC