## Functional Properties of Cardiomyocytes Derived from Human Cardiopoietic Amniotic Fluids

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Human amniotic fluid (hAF) cells share characteristics of both embryonic and adult stem cells. They proliferate rapidly and can differentiate into cells of all embryonic germ layers but do not form teratomas. Embryoid-bodies obtained from hAF have cardiac differentiation potential, but terminal differentiation to cardiomyocytes (CMs) has not yet been described. Our purpose was to promote cardiac differentiation in hAFcells. Cells were exposed to inducing factors for up to 15 days. Only the subset of hAF cells expressing the multipotency markers SSEA4, OCT4 and CD90 (CardiopoieticAF cells) responded to the differentiation process by increasing the expression of the cardiac transcription factors Nkx2.5 and GATA4, sarcomeric proteins (cTnT,  $\alpha$ -MHC,  $\alpha$ -SA), Connexin43 and atrial and ventricular markers. Furthermore, up to 90% of differentiated cells were positive for the calcium pumps CACNA1C and SERCA2a, with approximately 30% of CardiopoieticAF-derived CM-like cells responding to caffeine or adrenergic stimulation. Some spontaneous beating foci were also observed. In conclusion, we demonstrated that CardiopoieticAF cells can differentiate into a population of CM-like cells, characterized by cardiac-specific molecular, structural, and functional properties, that are useful for the development of in vitro models of genetic cardiac disorders, for drug discovery and testing, and for the regenerative medicine.

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