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Reprogramming of human keratinocytes into functional cardiomyocytes

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Purpose. Induced pluripotent stem cells (iPS) can be generated by patients introducing transcription factors that are highly expressed in embryonic stem (ES) cells into somatic cells. However, the various iPS cell lines are characterized by different properties such as differentiation efficiency and potential safety hazards. Among several readily available primary human somatic cell types, keratinocytes can be isolated easily from human skin or hair follicle, and therefore represent an attractive cell source for reprogramming.

The aims of the present study were: 1. To assess the cardiomyogenic potential of human keratynocytes-derived iPS. 2. To increase the differentiation efficiency in order to obtain a homogeneous population of beating cardiomyocytes, also overcoming the limitation of embryonic body formation.

Methods. Established iPS cell line obtained from human keratinocytes was cultured in monolayer and exposed sequentially to Ascorbic Acid, 5-Azacytidine, BMP4, ActivinA, VEGF up to 20 days. Differentiation was evaluated monitoring the expression of Nkx2.5, Gata4, sarcomeric α-actinin, α cardiac myosin heavy chain, cardiac T-troponin and Connexin43 and β-adrenoceptors as cardiac markers, by Western Blot, immunofluorescent and cytometric analyses. An ImageStream analysis for a simultaneous quantitative and morphological evaluation was also performed.

Results. During the differentiation induction, iPS became positive to all the analyzed cardiac markers. In particular, we observed a rapid and significant increase of Nkx2.5 and Gata4 expression (14.7±0.7% vs 43.2±2.1% Nkx2.5$^+$ and 21.7±1.2 vs 65.9±3.6% Gata4$^+$ in iPS and 15 days after the induction respectively, $p<.005$, analysis by ImageStream), with a significant nuclear translocation (nuclear$^+$ cells:7.2±0.8% vs 18.8±2.1% of the Nkx2.5$^+$ cells in iPS and 15 days after the induction respectively, $p<.005$, analysis by ImageStream). The differentiation process was accompanied by a modulation of β_1- and β_2-adrenoceptors expression. Foci of spontaneous contraction were observed after only 5 days from the induction and after 15 days the beating cells represented about the 60-90% of the entire population. Moreover, differentiated cardiomyocytes responded to adrenergic stimulation by increasing the contraction rate.

Conclusion. iPS obtained from hKeratinocytes efficiently differentiate into cardiomyocytes giving rise to a homogenous population with cardiac-specific molecular and functional properties.