Effects of cardiotrophin-1 on differentiation and maturation of rat bone marrow mesenchymal stem cells induced with 5-azacytidine in vitro.


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BACKGROUND: Cardiotrophin-1 (CT-1) is a cytokine involved in the growth and survival of cardiac cells that stimulates cardiomyogenesis in pluripotent murine embryonic stem (ES) cells. But it is not known whether CT-1 is responsible for the fate of differentiated bone marrow mesenchymal stem cells (BMMSCs). METHODS: We investigated the effects of CT-1 on differentiation and maturation of BMMSCs in vitro induced with 5-azacytidine. BMMSCs isolated from femur of rats were induced by CT-1 only, by 5-azacytidine with or without CT-1, and an untreated control group was also set. After 4 weeks of induced culturing, we observed the levels of alpha-cardiac actin and troponin-I by immunohistochemical staining, the ultrastructure of induce-cultured BMMSCs, and the expression of GATA4, Nkx2.5, beta-myosin heavy chain (beta-MHC) and alpha-cardiac actin mRNA by real time RT-PCR analysis. RESULTS: Differentiated BMMSCs treated by 5-azacytidine and CT-1 distinctly showed formations of myofilaments and myotube-like structures-morphological characteristics of myocyte like cells, and spontaneous contraction of a few cells was observed. The protein levels of alpha-cardiac actin and troponin-T were significantly higher than control. Furthermore, mRNA expression of GATA4, Nkx2.5, alpha-cardiac actin and beta-MHC was increased remarkably. CONCLUSIONS: This study suggests that induced culturing of BMMSCs in the presence of 5-azacytidine combined with CT-1 can enhance cardiomyocytic characteristics. CT-1 upregulates the expression of GATA4, Nkx2-5, alpha-cardiac actin and beta-MHC mRNA, and rapidly promotes the differentiation and maturation of cardiomyocyte-like cells differentiated from BMMSCs induced with 5-azacytidine.