P-1 Cardiomyogenic Potential of Human Umbilical Cord Stem Cells

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Objectives: Recently, cardiomyocyte differentiation has become an intriguing target not only for basic research but also for application to regenerative medicine. It has been reported using various methods in cells including embryonic stem cells (ESC), mesenchymal stem cells (MSC), and cardiac stem cells. ESC and cardiac stem cells has ethical and isolation problem respectively. Therefore, investigation to differentiate into cardiomyocytes with MSC has been expanded. Several groups differentiated into cardiomyocytes using unspecific DNA methyltransferase inhibitor 5-azacytidine. Cells were commonly cultivated in complete medium for 2 or 4 weeks or more weeks after treated 5-azacytidine for 24 h. In the present study, After 24 h exposure to 5-azacytidine in human umbilical cord stem cells (hUCSC) change of expression of cardiac progenitor cell-specific genes and cardiomyocyte-specific genes examined at 1 and 4 weeks. Also After 24 h exposure to 5-azacytidine cells were cultivated in complete medium supplement with BMP (bone morphogenic protein), FGF (fibroblast growth factor) and Wnt inhibitor for appropriate cardiac differentiation.

Methods: Isolation and culture of stem cells hUCSC were obtained from human umbilical cord by digesting with 0.5% collagenase for 20~24h at 37℃. Isolated cell was maintained at 37℃/5% CO_2 in DMEM medium containing 10% FBS. Cardiomyogenic differentiation of hUCSC hUCSC were cultivated in serum-free DMEM containing 10 μM 5-azacytidine for 24 h. Then cells were cultivated in DMEM containing 10% FBS in the presence or absence of BMP-2/BMP-4, FGF-4/FGF-8/FGF-10 and Wnt inhibitor for 4 weeks. RT-PCR Before or after differentiation, total RNA was extracted from cells and then subjected to RT-PCR analyses.

Results: Expression of Islet1 (Isl1), myocyte enhancer factor 2C (MEF2C) and α-cardiac actin (α-CA) genes was increased in cells that cultivated in DMEM containing 10% FBS for 1 week after exposure to 5-azacytidine for 24 h. But significantly decreased at 4 weeks. Expression of troponin I (TnI) also was increased at 1 week but not decreased 4 weeks. Expression of troponin T (TnT) was not changed at 1 week but significantly decreased at 4 weeks. Expression of cardiac myosin light chian-1 (Cmlc-1) and β-myosin heavy chain (β-MHC) was not expressed regardless of treatment of 5-azacytidine. When cells were cultured with BMP-2 added FGF-4 or FGF-8 or FGF-10 after exposure to 5-azacytidine for 24 h, cells more increased expression of Cmlc1, TnT, TnI and Kv4.3 genes than non-treated cells. When treated with BMP-4 instead of BMP-2 cells more increased expression of Cmlc1, α-CA, TnI and Kv4.3 genes. Therefore, It is suggested that BMP-2 promote TnT gene expression and BMP-4 is effective on increase of α-CA gene expression.

Conclusion: In conclusion, these results suggest that hUCSC could differentiate into the cardiomyocyte and might be used as potentially therapeutical cells for repairing damaged myocardium.