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Assessment of regional cardiac function of chronic ischemic myocardium in rats by strain-rate imaging

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Heart failure model using small animals such as rat is useful for investigating pathophysiology and treatment, but the assessment of regional cardiac function remains unestablished. We hypothesized that the strain rate (SR) imaging may allow to assessing regional cardiac function in rats. To assess it, we created left coronary artery stenosis in rats at 10 weeks of age ($n=6$, ischemic group). Three months after creation of coronary stenosis or sham surgery ($n=10$, control group), color tissue Doppler imaging by echo was performed. ROI was set in the left ventricular (LV) anterior wall (risk area) and posterior wall (non-risk area) in short-axis view. The SR imaging was performed using by Echopac (GE Vingmed), and peak systolic and diastolic SRs were obtained. In cardiac catheterization, +and-LVdp/dt decreased by 30% and by 40%, respectively, in the ischemic group than the control. The peak systolic and diastolic SRs in the risk area decreased by 62% and by 92% in the ischemic group than control (systolic SR; 0.80 ± 0.19 vs 2.10 ± 0.27 $p < 0.01$, diastolic SR; -1.2 ± 0.4 vs -2.3 ± 0.3 , $p < 0.05$). But in the non-risk area the SRs tended to increase in the ischemic group than control. The peak systolic SR had a positive linear correlation with LV ejection fraction ($r=0.60$, $p < 0.05$). In conclusion, application of the SR imaging to small animals, for the first time, revealed that detection of systolic and diastolic dysfunction in the ischemic areas.

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Promoter-Targeted Selection and Isolation of Smooth Muscle Progenitor Cells From the Bone Marrow Stromal Cells

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[Background] A recent report demonstrated that bone marrow hematopoietic stem cells differentiate into smooth muscle cells (SMCs). On the other hand, there have been no studies closely following cell development of smooth muscle lineage cells among bone marrow stromal cells (BMSCs). [Methods and Results] To investigate the possible existence of smooth muscle progenitors among BMSCs, we tried to detect and follow the in vitro differentiation of such a cell type by employing a promoter-sorting method with a human SM22 α promoter (-480 bp) /green fluorescent protein (GFP) construct. The construct was transfected to adhesion cells that appeared 5 days after the seeding of mononuclear cells from bone marrow. GFP was first detectable 5 days after the transfection in a cell population [Ad (G) cells], that expressed PDGF- β & but neither mature (calponin) nor immature (SMemb) SMC-specific proteins at that time. However, the cells were eventually grown into individual clones that expressed SMC-specific proteins (α -smooth muscle actin, calponin, and SM-1), suggesting that Ad (G) cells have partly at least progenitor properties. [Conclusions] We demonstrated the presence of putative smooth muscle progenitors and followed their differentiation into SMCs among BMSCs. The sorting system reported here has the potential to provide large amounts of BM-SC-derived SMCs of potential use in cell and gene therapy.

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Efficient Cardiomyogenic Differentiation of Embryonic Stem Cell by FGF-2 and BMP-2

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Objective Despite the pluripotency of embryonic stem (ES) cells, the specific control of their cardiomyogenic differentiation remains difficult. We investigated whether growth factors may efficiently enhance the in vitro cardiac differentiation of ES cells. **Methods and Results** Recombinant growth factors at various concentrations or their inhibitors were added on various schedules during the cardiomyogenic differentiation of ES cells. Cardiomyogenic differentiation was assessed by mRNA and protein expressions of several cardiomyocyte-specific genes such as Nkx2.5, α -myosin heavy chain (α MHC) and actinin. Screening of four growth factors of basic fibroblast growth factor (FGF-2), bone morphogenetic protein-2 (BMP-2), transforming growth factors- β and activin revealed that FGF-2 and/or BMP-2 efficiently enhanced the cardiomyogenic differentiation, but only when they were added at the optimal concentration (1.0 ng/ml in FGF-2 and 0.2 ng/ml in BMP-2; relatively lower than expected in both cases) for the first 3 days while embryoid bodies were being formed. Finally, combination of FGF-2 and BMP-2 on the optimized protocol significantly increased ES cell-derived cardiomyocytes (36.2 ± 5.5 % in FGF-2/BMP-2 vs 20.1 ± 6.3 % in control, $p < 0.01$, $n=10$). On the other hand, inhibition of FGF-2 and/or BMP-2 drastically suppressed the cardiomyogenic differentiation. **Conclusion** FGF-2 and BMP-2 play a crucial role in the early cardiomyogenesis. The present results greatly contribute to developing cell transplantation therapy and developmental studies on the heart.

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Embryonic Pulmonary Vascular Development is Regulated by Balanced Expression of VEGF Receptors, Flk-1 and Flt-1

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[Backgrounds]: In spite of the recent advance in cardiovascular surgery, the prognosis of certain complicated congenital heart diseases is still poor. They are known to have impaired development of pulmonary vasculature. Understanding normal development of pulmonary vasculature during embryogenesis is essential for proper treatment of impaired pulmonary vasculature. A VEGF

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Adult Cardiac Sca-1 Positive Cells Differentiate into Beating Cardiomyocytes

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Although recent reports have suggested that there are the cells expressing stem cell markers in the adult heart, it remains to be clarified whether these cells have the characteristics of stem cells such as abilities of differentiating into various types of cells including mature cardiomyocytes. We here demonstrate that Sca-1 positive (Sca-1+) cells in adult hearts have some features of stem cells. Sca-1+ cells were isolated from adult murine hearts by the Magnetic Cell Sorting system and cultured on gelatin-coated dishes. In enriched Sca-1+ cells, ~40 % of the cells expressed CD45, and ~10 % of the cells expressed CD34 and c-kit. When cultured with the differentiation medium, Sca-1+ cells expressed genes of cardiac transcription factors and contractile proteins, and showed sarcomeric structure and spontaneous beating. Some of Sca-1+/CD45+ cells expressed MF20, but none of Sca-1+/CD45+ cells expressed MF20, suggesting that cardiac stem cells are in Sca-1+/CD45- population. When cultured with appropriate conditions, some of Sca-1+ cells expressed von Willebrand factor, smooth muscle cell actin and alkaline phosphatase and were stained with Oil-Red O, suggesting that cardiac Sca-1+ cells could differentiate into various type of cells as well as cardiomyocytes. These results suggest that the Sca-1+ cells in the adult murine heart have a potential as stem cells and may contribute to the regeneration of injured hearts.