行政院國家科學委員會專題研究計畫　成果報告

比較出生後不同時期心臟基因的表現

計畫類別：個別型計畫
計畫編號：NSC91-2314-B-002-228-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：國立臺灣大學醫學院外科

計畫主持人：林芳郁

計畫參與人員：陳益祥醫師,俞松良博士,蔡敏惠小姐

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 92 年 10 月 31 日

中文摘要

在產後心臟的發育期間控制細胞增生及分化的分子機制了解依舊有限。在這研究中,我們用 cDNA 微陣列調查在老鼠心肌衰弱發育期間有差異表達的基因。微陣列(6,144 個基因)以呈色系統用以研究老鼠從 0 天,初生 7 天, 14 天, 到成年老鼠心肌中差異表現的基因。根據產後老鼠心肌細胞的成熟度,有 66 個基因被抑制並且有 51 個基因被活化。這些基因包括與細胞週期,生長激素,訊息傳遞,新陳代謝,細胞骨架,細胞外間質,和一些未知功能的基因。微陣列的資料提供心肌發育期間基因表現模式的一個全視野的輪廓。這個資訊更進一步探索心肌發育的遺傳控制和對各種心肌衰弱等疾病中分子機制將很有助益。

關鍵詞：心肌細胞、出生後發育、基因表現、微陣列

Abstract

The molecular mechanisms controlling the cardiomyocyte proliferation and differentiation during postnatal development are poorly understood. In this study, we investigate the differentially expressed genes during mouse myocardial development in a genome-wide scale by cDNA microarray technique. The mouse cDNA microarray (6,144 genes) with colorimetric detection system was used to identify the differentially expressed genes in the mouse myocardium from 0-day neonatal, 7-day, 14-day postnatal, to adult ICR mice. According to the maturity of postnatal mice, the mRNA expression levels of 66 genes are down-regulated and 51 genes are up-regulated. Our data demonstrated that these genes related to cell cycle, growth factors, signal transduction, metabolic enzymes, cytoskeletone/extracellular matrix proteins, and some expressed sequence tag genes may play the roles in the postnatal myocardial growth. Identification of the differentially expressed genes between neonatal (synthetic) and adult (contractile) myocytes by microarray provides a global profiling of gene expression pattern during myocardial development. This information will be very helpful for further exploring the genetic control of cardiomyocyte proliferation and understanding the molecular mechanisms of cardiomyocytes loss and regeneration in various myocardial diseases.

Keywords-cardiomyocyte; postnatal development; gene expression; microarray.

Introduction

Cardiac muscle cells exhibit two related but distinct modes of growth that are highly regulated during development and disease. Cardiomyocytes rapidly proliferate during the fetal life but exit the cell cycle soon after birth, following which the predominant form of growth shifts from hyperplastic to hypertrophic.[1] During postnatal development, cardiomyocyte growth in man, mouse, and rat undergo this shift, such that further increases in myocardial mass are not typically accompanied by cardiomyocyte proliferation.[2] In human beings, the capability to undergo mitosis and hyperplasia is lost three to six months postnatally.[3] Previous studies also suggested that most cardiomyocytes in rat and mouse heart gradually cease to undergo DNA replication, which is a prerequisite for proliferation, within the first two weeks after birth.[4] For this reason, further response to growth, injury, and increased workload is restricted to increase in the cell mass of existing myocytes, mainly

by hypertrophy, not hyperplasia, in mature cardiomyocytes.[5] If we can understand the molecular mechanisms on how mitosis is blocked in cardiomyocytes during postnatal, it may facilitate the development of new therapies for cardiovascular injury or diseases, based on enhancing the regeneration of the adult myocardium. To achieve this goal, identification and characterization of key molecules participated in newborn hearts are essential. Systematic studies of gene expression patterns by using cDNA microarray provide a powerful approach to molecular dissection of cells and tissues by comparing expression levels of thousands of genes at a time.[6-8] In this study, we initiated a project to profile the gene expression in various postnatal developmental states (0-day neonatal, 7-day, 14-day postnatal, and adult) of the cardiovascular system using the mice cDNA microarray (6,144 genes, including known regulatory genes and expressed sequence tags, mouse ESTs) with colorimetric detection system.[9]

Results

The array signal intensities of day 0 neonatal myocardium were compared with the intensities of subsequent samples (day 7, day 14, and adult). According to the maturity of postnatal mice (0-, 7-, 14-day old and adult), 66 genes that correlated negatively and 51 genes that correlated positively with the development of cardiomyocytes were identified. These genes were grouped into 9 categories on the basis of their cellular functions. These categories included cell cycle regulators, growth factors or apoptotic factors, signal transduction molecules, transcription factors, channels, cytoskeletone/extracellular matrix, stress response proteins, metabolic enzymes, and anonymous genes correlating negatively or positively with terminal differentiation of cardiomyocytes, marked as EST. To confirm the results of the microarray studies, northern-blotting analysis was performed. Seven descending expression of genes (PNA, cyclin B1, IGF-II, MEK4/SEK1, ARL-6, S-adenosylmethionine decarboxylase 3, and HMGCoA synthase) and three ascending expression genes (neuronal protein 3, TB2-like protein, and ceruloplasmin,) were selected base on the microarray data. Figure 5 shows the mRNA levels of these seven descending genes were down-regulated during the postnatal development of cardiomyocytes, whereas the three ascending genes were up-regulated after birth. To demonstrate the protein expression of identified gene was also consistent with the microarray analysis, six antibodies (cyclin B1, PCNA, IGF-II, MEK4/SEK1, and Hsp70), were used to carry out immunohistochemical analysis across the differentiation stages of cardiomyocytes after delivery. The protein levels of PCNA, cyclin B1, IGF-II, and MEK4/SEK1 were down-regulated during the development of cardiomyocytes from neonatal (day 0) to adult, whereas Hsp70 was up-regulated. The immunostaining of PCNA and cyclin B1 were highly expressed in the cell nuclear, whereas the IGF-II, MEK4/SEK1, and Hsp70 were stained in the cell membrane and cytosol. Additionally, we found that cyclin B1 was more predominantly expressed in the atrium than in the ventricle tissues.

Discussion

In this study, we demonstrate the differential expression profile and dynamic expression of numerous gene transcripts in myocardium from neonatal to adult mouse by mice cDNA microarray. According to the results from microarray analysis, some of these genes were decreased after

delivery and some of the putative genes were increased. Among these differently expressed genes, PCNA, Ki67, MEK4/SEK1, BMP-1, IGF, ARL-family, ceruloplasmine, and electron transfer flavoprotein- precursor have previously been reported to be associated with postnatal development, differentiation, or regeneration in myocardium, skeletal muscle, brain, or other tissues. Postnatal development of cardiomyocytes into contractile myotubes is associated with the permanent withdrawal from the cell cycle. The molecular mechanisms responsible for cardiomyocyte terminal differentiation, in particular, the cell cycle regulation, were almost unknown. Recently, some studies have shown that the activity of cyclin-dependent kinases (CDKs) 2, 4, and 6 were down-regulated in day two postnatal cardiomyocytes. The cyclin D1, D2, and D3, and cdk2 were detected in newborn heart, but not in adulthood. Interestingly, several genes previously shown to be involved in the regulation of cell cycle progression were also found in our analysis to be differentially expressed in the neonatal and adult hearts. In the category of cell cycle regulators, we found molecules such as cyclin B1 were down-regulated in the mRNA and protein levels during the development of heart after birth. The other cell cycle regulators, cyclin D1, PCNA, cdk7, cdc2, and cdc25 are only found in neonatal, but almost undetectable after day-14 postnatally to adults. In contrast, the inhibitors of cell cycle progression, p18 and p57, are up-regulated after birth and to reach the peak at day 14 and day 7 postnatally.

Previous reports indicated that cyclin B1/cdc2 complex played an important role in the S-G2 phase of cell cycle. Interestingly, the gene expression levels of cyclin D1 and cdc2 were also found to be down-regulated during terminal differentiation in skeletal myoblasts in vitro. Additionally, the degradation of cyclin B1 and the cells exit the M phase might connect with binuclear or tetranuclear formation in Drosophila spermatids. We suggest that the down-regulation of cyclin B1 might be related with the binuclear formation of cardiomyocytes at day 7 to day 14 during the postnatal development. PCNA is synthesized in the early G1 and S phase of the cell cycle and also known as cyclins or polymerase associated protein. The Ki-67 protein is present during cell cycle progression and was used as an excellent marker for determining cell proliferation. PCNA and Ki67 are both highly expressed in the neonatal, but decreased to undetectable level in the adulthood. These results suggest that down-regulation of both PCNA and Ki67 might involve in the regulation of cardiac cell cycle arrest. Insulin like growth factor family has been studied in many tissues and suggested to involve in the development and regeneration (skeletal muscle myofiber and spinal motoneuron). In the present study, IGF-II was found to reduce after birth, whereas the IGF receptors did not change, indicating that IGF-II might be related with the proliferative activity of cardiomyocytes. The other growth factor, pleiotrophin, a family of mitogenic and angiogenic heparin-binding growth and differentiation factors,was also down-regulated during the differentiation of cardiomyocytes postnatally. These results suggest that IGF-II and pleiotrophin might have the potential for the therapy to induce the cardiomyocyte regeneration in the adult myocardium during cardiac diseases resulting in cardiomyocyte loss. Several previous reports indicated that ceruloplasmin, the major carrier of copper, plays a regulatory role in the copper storage and transport during development, and also suggested that ceruloplasmin protects myocardiual tissue from oxygen free radicals-induced injury in the adult heart. In the present data, ceruloplasmin is up-regulated during

the postnatal myocardial development. The microarray results also showed that glutathione S-transferase,glutathione-S-transferase and the other cardioprotective proteins, Hsp 70, were up-regulated during the myocardial development after delivery. These results indicate that the free radical scavengers and protective proteins are important in preventing adult cardiomyocyte from injury, when its ability of proliferation and regeneration are reduced.

References

1. MacLellan WR, Schneider MD. Genetic dissection of cardiac growth control pathways. Ann Rev Physiol. 2000; 62:289-319.
2. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res. 1998; 83(1):15-26.
3. Tam SKC, Gu W, Mahdavi V, et al. Cardiac myocyte terminal differentiation-potential for cardiac regeneration. Ann New York Acad Sci. 1995; 752: 72-79.
4. Soonpaa MH, Field LJ: Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. Am J Physiol. 1997; 272: H220-H226.
5. Johnatty SE, Dyck JR, Michael LH, et al. Identification of genes regulated during mechanical load-induced cardiac hypertrophy. J Mol Cell Cardiol. 2000; 32(5): 805-815.
6. Chen JJW, Wu R, Yang PC, et al. Profiling expression patterns and isolating differentiatially expressed genes by cDNA microarray system with colorimetry detection. Genomics. 1998; 51: 313-324.
7. Abdellatif M. Leading the way using microarray: a more comprehensive approach for discovery of gene expression patterns. Circulation. 2000; 86: 919-920.
8. Lockhart DJ, Winzeler EA. Genomics, gene expression and DNA arrays. Nature. 2000; 405: 827-835.
9. Hong TM, Yang PC, Peck K, et al., Profiling the down stream genes of tumor suppressor PTEN in lung cancer cells by cDNA microarray. Am J Respir Cell Mol Biol. 2000; 23: 355-363.