Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 368 (2008) 887–892
BBRC
www.elsevier.com/locate/ybbrc

Mouse embryonic stem cell-derived cardiomyocytes express functional adrenoceptors

Hongtao Yang [a,1], Yongzhen Zhang [a,1], Zhiqiang Liu [b], Ping Chen [b], Kangtao Ma [b], Chunyan Zhou [b,*]

[a] Department of Cardiology, Peking University Third Hospital, Beijing 100083, China
[b] Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Peking University, Xue Yuan Road No. 38, Beijing 100083, China

Received 21 January 2008
Available online 13 February 2008

Abstract

The cardiogenic capacity of embryonic stem (ES) cells has been well-investigated. However, little is known about the development of adrenoceptor (AR) systems during the process of ES cell differentiation, which are critically important in cardiac physiology and pharmacology. In this present study, we investigated the expression profile of adrenoceptor subtypes, β-adrenergic modulation of muscarinic receptors and adrenoceptor-related signaling in cardiomyocytes derived from ES cells (ESCMs).

Reverse transcription-polymerase chain reaction revealed that undifferentiated mouse ES cells expressed α_{1A}-, α_{1B}-, α_{1D}- and β_2-AR mRNA. However, β_1-AR was only expressed after vitamin C induction. The expressions of α_{1A}-, α_{1D}- and β_1-ARs increased significantly while α_{1B}- and β_2-ARs showed no significant change during the differentiation process. Furthermore, we detected the expression of tyrosine hydroxylase. Both α_1-AR and β-AR could activate extracellular responsive kinase in ESCMs. Isoprenaline could inhibit the expression of M_2 muscarinic receptor protein. CGP20712A, a β_1-AR antagonist, up-regulated the expression of M_2 muscarinic receptor while ICI118551, a β_2-AR antagonist, showed no effect.

These results indicated that functional adrenoceptors and tyrosine hydroxylase, a critical enzyme in catecholamine biosynthesis, were differentially expressed in ESCMs. Adrenoceptor-related signaling pathways and β-adrenergic modulation of muscarinic receptors were established during differentiation.
© 2008 Elsevier Inc. All rights reserved.

Keywords: Embryonic stem cells; Cardiomyocytes; Adrenoceptors; Muscarinic receptor; Tyrosine hydroxylase; Extracellular responsive kinase

Embryonic stem (ES) cells have the capacity for multilineage differentiation including differentiation into cardiomyocytes. Cardiomyocytes derived from embryonic stem cells (ESCMs) have been well categorized in terms of their surface markers, gene expression profiles, survival rate and the ability to restore damaged heart function [1]. However, the development of adrenoceptor (AR) systems in these cells is poorly understood. Adrenoceptors play crucial roles in cardiac function and their characteristics have been studied in neonatal and mature cardiomyocytes. As functional cardiomyocytes, ESCMs must have developed functioning adrenoceptors and this development during the process of ESCM differentiation needs to be fully investigated.

Cardiomyocytes mainly express three α_1-AR subtypes (α_{1A}, α_{1B}, α_{1D}) and two β-AR subtypes (β_1 and β_2). The β-AR system is perhaps the most important external regulatory mechanism for acute changes in heart rate and force and can also mediate longer term alternations in structure through hypertrophy [2,3]. The α_1-AR system also participates in many physiological and pathological processes such as cardiac contraction, control of vascular tone and cardiac hypertrophy [4]. The adrenoceptor-related extracellular responsive kinase (ERK) pathway plays a crucial role

* Corresponding author. Fax: +86 10 82802417.
E-mail address: chunyanzhou@bjmu.edu.cn (C. Zhou).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.02.014

in adult heart hypertrophy [5], but whether adrenoceptor-related ERK activation exists in ESCMs and differs from that in adult cardiomyocytes remains unknown. Therefore, a detailed study of adrenoceptor subtype-dependent ERK activation in ESCMs was undertaken. Previous studies revealed that β-adrenoceptors emerge earlier in embryonic life than muscarinic receptors. In the developing heart, β-AR input appears to be important in establishing the competence of nonadrenergic signals by influencing the expression and function of signaling proteins other than β-AR itself [6]. The actions of the muscarinic receptor system directly oppose those of the β-adrenoceptor system. The development of the β-adrenergic system may act as a negative modulator of the muscarinic receptor system [7]. Investigating whether β-adrenergic modulation of muscarinic receptors exists in the development of ESCMs may provide an explanation for the cross-talk between receptors with opposing actions.

Epinephrine and norepinephrine, the primary peripheral catecholamines, are neurotransmitters and hormones that can influence cardiac performance, beginning shortly after the heart starts to beat in developing vertebrate embryos. The embryonic heart may have the capacity to produce catecholamines at early developmental stages that precede production of catecholamines in the adrenal gland and cardiac sympathetic nerves [8]. Tyrosine hydroxylase (TH) is the first restricted enzyme in the process of catecholamines biosynthesis. The expression of TH is important for embryonic cardiac differentiation and maintaining survival of ESCMs.

In the present study, we investigated the expression pattern of five main adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1D}, β_1 and β_2), adrenoceptor subtype-dependent ERK activation and β-adrenergic modulation of M_2 muscarinic receptor expression in ESCMs.

Materials and methods

Materials. All agonists and antagonists, mitomycin C, β-mercaptoethanol, vitamin C and anti-mouse α-actinin monoclonal antibody were purchased from Sigma Chemical (St. Louis, MO, USA). Phospho-p44/p42 MAP Kinase (Thr202/Tyr204) antibody and p44/p42 MAP kinase (ERK1/2) antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Leukemia inhibitory factor (LIF), anti-mouse tyrosine hydroxylase monoclonal antibody and rabbit anti-mouse M_2 muscarinic acetylcholine receptor affinity purified polyclonal antibody were purchased from Chemicon (Temecula, CA, USA). Horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit and goat anti-mouse) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Hyclone (Logan, UT, USA). ES cells of mouse line M13 were obtained from Xue's Laboratory, Life Science Center of Peking University.

ES cell culture. ES cells of mouse line M13 were propagated on a confluent layer of mitomycin C-treated ICR (Institute of Cancer Research) mouse embryonic fibroblasts (MEF, from Beijing Animal Administration Center) in high glucose (4.5 g/L) DMEM, supplemented with 15% batch-tested fetal bovine serum, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM β-mercaptoethanol and 500 U/mL LIF [9,10]. Prior to induction of differentiation, the MEF feeder cells were removed from ES cell cultures by attachment of fibroblasts onto 0.1% gelatin-coated plastic during 45 min of incubation for at least two passages in the presence of 1000 U/mL LIF [1].

Induction of cardiomyocyte differentiation via embryoid body formation. ES cells were cultivated into embryo-like clusters (embryoid bodies, EBs) by culturing 1500–2000 M13 cells in each drop of 20 μL of differentiation medium, which consisted of DMEM, supplemented with 15% batch-tested FCS, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM β-mercaptoethanol, 10^{-4} mol/L vitamin C and without LIF [9,10]. After two days, EBs formed in hanging drops were grown for a further five days in suspension culture (200–250 EBs/10 cm Petri dish). At differentiation day 7, EBs were plated out onto 0.1% gelatin-coated surface: (a) Two EB/10 mm glass cover slip in 1 mL of differentiation medium in each well of a 24-well-plate; (b) 2 cm plate in 2 mL differentiation medium. ESCMs were allowed to differentiate further in adherent cultures and examined daily. The time that ESCMs began to beat spontaneously and the number of beating EBs were recorded. The number of beating EBs in 24-well-plates was recorded at different differentiation time points. EBs grown on 2 cm plates were used for Western blot analysis or immunocytochemistry.

Immunocytochemistry. The adherent EBs in 0.1% gelatin-coated 2 cm plate were used for immunocytochemistry at differentiation day 14. The EBs were washed three times with PBS, fixed with 4% polyoxymethylene for 15 min at room temperature and permeabilized with PBST (phosphate buffered saline with 0.2% Triton X-100). The samples were incubated overnight at 40 °C with anti-mouse α-actinin monoclonal antibody (1:200 dilutions), washed three times with PBST and then incubated with TRITC-conjugated secondary antibody (mouse IgG antibody-TRITC, 1:200 dilutions) for 60 min. The nuclei were stained with 1 μg/mL hoechst33342 for 5 min and visualized microscopically with a ×20 objective (Leica systems).

RT-PCR analysis. Total RNA was prepared from ESCMs at six time points (0d, 3d, 7d, 14d, 21d, 28d) using Trizol Reagent. The expressions of five main adrenoceptor subtypes (α_{1A}, α_{1B}, α_{1D}, β_1 and β_2) were assessed by RT-PCR. GAPDH was used as an internal control. PCR were performed for 30–40 cycles, with each cycle consisting of denaturation at 94 °C, annealing at 56 to 64 °C, and amplification at 72 °C for 1 min each [11]. Primers for the RT-PCR are listed in Table 1. Before semi-quantitative analysis, the linear range of the PCR cycles was measured for each receptor and the appropriate number of PCR cycles was determined. PCR products were subjected to electrophoresis on 2% agarose gels, scanned and semi-quantitated using Image-Quant software.

Western blot analysis. The adherent EBs in 0.1% gelatin-coated 2 cm plate were used for Western blot analysis. Total protein of ESCMs was prepared at six time points (0d, 3d, 7d, 14d, 21d, 28d) to detect the expression of tyrosine hydroxylase. ESCMs at differentiation day 14 were stimulated with adrenergic receptor agonist or antagonist to detect phosphorylation of ERK1/2. From differentiation day 10 to day 13, ESCMs were stimulated with β-adrenergic agonist or antagonist for 96 h and then total protein of ESCMs was prepared for Western blot analysis to investigate β-adrenergic modulation of M_2 muscarinic receptor with anti-M_2 muscarinic acetylcholine receptor antibody.

Statistical analysis. Values are presented as mean ± SEM. The significance of differences was determined by one-way ANOVA. The accepted level of significance was $P < 0.05$.

Results

ES cells differentiated into cardiomyocytes after induction

ES cells were propagated on feeder cells for two days. Cardiomyocyte differentiation was initiated by inducing EB formation from undifferentiated ES cells. After five days suspension in culture, EBs were allowed to differentiate further in adherent cultures. Spontaneously contracting

Download English Version:

https://daneshyari.com/en/article/1936129

Download Persian Version:

https://daneshyari.com/article/1936129

Daneshyari.com