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Mitogen-activated protein kinase p38 modulates pacemaker ion channels differentiation in P19-derived pluripotent cells

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Abstract

Signal regulators during early cardiogenetic differentiation for the cellular automaticity are largely unknown. Our investigations were designed to clarify the role of transcription factors and their modulators in P19-derived cardiomyocytes to the expression of cardiac pacemaker ion channels. Transcription factors Csx/Nkx2.5 and GATA4 but not MEF2C were markedly inhibited by p38 MAP kinase inhibition in a distinct manner; expression but not phosphorylation of GATA4 was reduced by inhibition of p38 MAP kinase actions. In the presence of an ERK1/2,5 inhibitor PD98059 or a JNK MAP kinase inhibitor SP600125, P19 cells successfully differentiated into cardiomyocytes displaying spontaneous beatings with expression of three types of pacemaker ion channels. We demonstrate that acquisition of cellular automaticity and the expression of pacemaker ion channels are regulated by the transcription factors, Csx/Nkx2.5 and GATA4, through intracellular signals including p38 MAP kinase in the process of P19-derived pluripotent cells differentiation into cardiomyocytes.

Keywords: Csx/Nkx2.5, GATA4, MEF2C, p38MAP kinase, Cardiogenesis, P19CL6

Background

The generation of pacemaker ionic channels is regarded as one of the most important factors responsible for the spontaneous beating of myocytes. To date, many studies have demonstrated several ionic mechanisms for the generation of slow diastolic depolarization in action potentials at the sino-atrial node in the heart [1, 2], including the L-type Ca^{2+} channel current ($I_{Ca.L}$), the T-type Ca^{2+} channel current ($I_{Ca.T}$) [3], the hyperpolarization-activated inward current (I_f) [4], the rapidly activating delayed rectifier K^+ current (I_{Kr}) [5], and the sustained inward current (I_{st}) [6].

In mammalian embryonal differentiation, morphogenesis of the cardiovascular system is initiated in 20 gestational days in humans and 7.5 gestational days in mice, followed by the formation of a simple tubular heart and generation of spontaneous beating [7]. The generation of specific contractile proteins and ion channel proteins in earlier cardiac differentiation is triggered by cardiac-specific gene expression. It has recently been shown that the expression of cardiac genes is regulated by several specific transcription factors during embryonal heart differentiation [8, 9]. Csx/Nkx2.5, GATA4, and MEF2C are considered to be cardiac-specific transcription factors that play the critical roles in the early development of the heart, and serve as useful molecular markers to identify cardiac inductive signals from other tissues or germ layers [10].

The homeobox gene Csx/Nkx2.5, a vertebrate homologue of *Drosophila tinman*, is one of the earliest known markers of mesoderm that give rise to myocardium, and its expression persists throughout the myocardium in the fully formed heart [7, 8]. GATA4 serves as

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