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# Cardiac specific transcription factor Csx/Nkx2.5 regulates transient-outward $K^+$ channel expression in pluripotent P19 cell-derived cardiomyocytes

Tomoko Uchino[1,2], Ming-Qi Zheng[1,3], Yan Wang[1] and Katsushige Ono[1*]

**Abstract**

The homeobox-containing gene Csx/Nkx2.5 codes several cardiac transcription factors and plays a critical role in early cardiogenesis. We investigated the effect of Csx/Nkx2.5 on the expression of cardiac ion channels using P19-derived cardiomyocytes. P19CL6 cells and P19CL6 cells with Csx/Nkx2.5 overexpression (P19CL6-Csx cells) were induced to differentiate into cardiomyocytes by treatment with dimethyl sulfoxide. Action potentials and membrane currents were measured by whole cell patch clamp at different differentiation stage: the early stage (1–5 days after beating had begun) and the late stage (10–15 days after beating). Expression of Csx/Nkx2.5 mRNA was increased as the differentiation stages advanced in both P19CL6 and P19CL6-Csx cells. In action potential configuration, maximal diastolic potentials in P19CL6-Csx cells exhibited more hyperpolarized potential (−64.2 mV) than those in P19CL6 cells (−54.8 mV, $p < 0.01$) in the early stage. In P19CL6 cells, among 6 different voltage-gated and ligand-operated $K^+$ channels expressed during the early stage, the transient-outward $K^+$ channel was most predominant. By overexpression of Csx/Nkx2.5, developmental decrease in the transient-outward $K^+$ channel was suppressed. Homeobox-containing gene Csx/Nkx2.5 modifies the amount of distinct ionic channels, during differentiation periods, predominantly changing the expression of the transient-outward $K^+$ channel.

**Keywords:** Potassium channel, Csx/Nkx2.5, Cardiomyocytes, Transient outward current, Cardiogenesis, Pluripotency, P19CL6, Homeobox

## Introduction

A homeobox-containing gene Csx/Nkx2.5 is one of the cardiac-enriched transcription factors found by Komuro and Izumo [1]. Targeted disruption of murine Csx/Nkx2.5 results in embryonic lethality due to abnormal looping morphogenesis of the primary heart tube [2]. Recently, many different human Csx/Nkx2.5 mutations have been reported in patients with cardiac malformation such as atrial septal defects, atrioventricular conduction delays, ventricular septal defects, tetralogy of Fallot, and tricuspid valve abnormalities [3, 4]. These reports suggest that the main role of Csx/Nkx2.5 includes regulation of cardiac morphological differentiation. Moreover, its ability to protect the heart from stress has also been reported [5], suggesting that Csx/Nkx2.5 may have various effects on differentiation of the heart.

Establishment of an in vitro cardiomyocyte differentiation system has allowed us to study the function of ion channels in very early stages of differentiation. P19 embryonal carcinoma cells are a pluripotent cell line which can differentiate into cardiomyocytes after

*Correspondence: ono@oita-u.ac.jp
[1] Department of Pathophysiology, Oita University School of Medicine, Oita, Japan
Full list of author information is available at the end of the article