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論　文　内　容　の　要　旨

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論文題名	Fate Mapping of Trps1-Daughter Cells during Mice Development (マウス発生過程でのTrps1娘細胞の運命検索)

論文内容の要旨

Introduction

Tricho-rhino-phalangeal syndrome (TRPS) is a rare syndrome characterized by craniofacial and skeletal deformities. Abnormal hair growth and joint problems are the most diagnostic features of TRPS. Recent reports showed relatively high population of the TRPS patients exhibit congenital heart defects. Cardiac defects observed ranged from minor to severe anomalies. This study was designed to understand the etiology of broad range anomalies observed in TRPS patients through fate mapping study during the mice development.

Material and Methods

I. Generation of a novel transgenic mice strain (Trps1-Cre)

Comparison of the TRPS1 genomic sequence of human and mouse was performed using the genome VISTA software. This analysis indicated that the 15kbp sequence (-5 to +10kbp) around the transcriptional start site is highly conserved between the two species. Using mouse genomic DNA as a template, 4kb murine TRPS1 proximal promoter was cloned and sub cloned in front of Cre cDNA sequence (Trps1-Cre). Transgene was released from the vector and gel purified for injection. Several stable mouse lines were established.

II. Visualization of the Cre activity

Cre-dependent R26R reporter female (B6.129S4Gt (ROSA) 26sortm1Sor/J: R26R) obtained from Jackson Laboratory was used to detect the Cre activity. The litters generated from crossing of Trp1-Cre male mice with R26R+/+ female was genotyped and Trps1-Cre; R26R+/- mice were used for the assay (Soriano, 1999). After genotyping, whole mount and sectional X-gal staining was performed for detection of LacZ activity.

III. Immunohistochemistry

Immunohistochemistry for SOX9 was applied to transverse and sagittal heart sections of embryos at embryonic day 13.5.

IV. In situ hybridization histochemistry

In situ hybridization histochemistry was carried out to heart sections at embryonic day 13.3, 15.5 and postnatal day 2.

V. Real time polymerase chain reaction (qPCR)

The right atria and left atria were collected, and the ventricular wall was cut to separate the other cardiac regions into right ventricle, left ventricle, interventricular septum, pulmonary artery and ascending aorta (PA+AO). Total RNA was purified, and then cDNA was prepared. The qPCR was performed using Bio-Rad Miniopticon. Trps1 relative expression was calculated by ΔΔct method using Hprt1 as normalizing gene.

Results and Conclusion

Trps1-daughter cells were visualized during embryonic and postnatal stages. X-gal

labeled cells were observed in the appendicular joints, dermal papilla of hair follicle and endocardial cushion of the early embryonic stage. Later, extensive staining was observed in valves (atrioventricular, aortic, and pulmonary) aortic sinus, atrial walls, ventricular walls and also in the interventricular septum.

In situ hybridization analysis identified restricted expression in endocardial cushions of outflow tract and in the leaflets of all mature cardiac valves. Relative Trps1 expression of the pulmonary and aortic vessels regions (PA+AO) had almost double quantity of Trps1 compared to the other heart regions.

These observations indicate that the sequence examined contain skeletal cell, hair follicle and endocardium enhancer of Trps1. Further Trps1-daughter cells fate mapping results partially explain why TRPS patients exhibit broad range of congenital cardiac defects although Trps1 mRNA expression was restricted. This novel Trps1-Cre mouse will be a useful strain to achieve Cre-mediated recombination in Trps1 expressing (or expressed) cells during cardiac and chondrocyte development.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は毛髪、顎顔面、骨格などの発生異常を示す遺伝性疾患である毛髪鼻指骨症候群(TRPS)の責任遺伝子 *TRPS1* 発現細胞の系譜を発生過程で追跡したものである。その結果 Trps1 娘細胞は異常が知られている部位に加え、心臓原基にも認められた。心臓原基での *Trps1* 遺伝子の発現は心内膜隆起に限局していたものの、Trps1 娘細胞はその遺伝子発現部位よりもはるかに広範囲に認められた。本研究結果は TRPS で見られる表現型の起こる原因解明に新たな所見を加えるものであり、博士(学術)の学位論文として価値のあるものと認める。