学 位 論 文 の 要 旨

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学 位 論 文 名　Identification of *Stim1* as a candidate gene for exaggerated sympathetic response to stress in the stroke-prone spontaneously hypertensive rat.

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論 文 内 容 の 要 旨

INTRODUCTION

The sympathetic nervous system (SNS) has been implied to play a key role in the pathogenesis of hypertension. The stroke-prone spontaneously hypertensive rats (SHRSP) are known to be vulnerable to various types of stress, which might contribute to the pathogenesis of severe hypertension and stroke observed in this strain, and thus the genetic factors underlying this may provide us important clues to understand the pathogenesis of hypertension in humans. Previously, by using a congenic strain (called SPwch1.72) constructed between SHRSP/Izm and the normotensive Wistar-Kyoto rat/Izm (WKY/Izm), we showed that a 1.8-Mbp fragment on chromosome 1 (Chr1) of SHRSP harbored a responsible gene (or genes) for the exaggerated sympathetic response to stress. To further narrow down the candidate region, in this study, another congenic strain (SPwch1.71) harboring a smaller fragment on Chr1 including two functional candidate genes, *Phox2a* and *Ship2*, was generated.

Physiological evaluation of the stress responses in this new congenic strain (SPwch1.71) indicated that the region harboring *Phox2a* and *Ship2* was not likely to have causative roles in the exaggerated stress response in SHRSP/Izm. In spite of that, further attempt to explore causative genes in the remaining candidate region succeeded to identify another strong candidate gene, the stromal interaction molecule 1 (*Stim1*).

MATERIALS AND METHODS

All the animal procedures were approved by the ethics committee for animal research in Shimane University. Sympathetic response to cold and restraint stress was compared among SHRSP/Izm, SPwch1.71, SPwch1.72 and WKY/Izm by three different methods [urinary norepinephrine (NE) excretion, blood pressure (BP) measurement by the telemetry system and the power spectral analysis on heart rate (HR) variability]. Briefly, restraint stress was imposed by placing rats for 3 h in a stainless-steel holder adjusted to the rat's body size. As for cold stress, a rat was placed in a cage kept at 4°C for 3 h (in the telemetry experiments) or for 6 h (in the collection of urine samples). Urinary NE was measured by HPLC. The power spectral analysis was done on HR variability under restraint stress using the telemetry for ECG. The ratio between the low frequency (LF; 0.04–1.0Hz) and the high frequency component (HF; 1.0–3.0Hz) was used as an indicator of the relative sympathetic activity. The LF/HF ratio was recorded for 30 s in every 10 min throughout the experiment, and the change in LF/HF (ΔLF/HF) was calculated as the difference between the averaged LF/HF during the periods with and without the stresses. BP and HR changes under restraint and cold stress were monitored with the telemetry system for BP (Data Science Inc, St. Paul, MN). BP and HR were monitored for 10 s in every 10 min during the experiment. The change in BP (ΔBP) and in HR (ΔHR) was calculated as the difference between the averaged BPs during the periods wth and without the stresses.

After the whole genome sequencing, sequence variations between SHRSP/Izm and WKY/Izm in the target chromosomal fragments were explored using SAMtools.

Gene and protein expression analyses were performed by quantitative reverse transcription PCR (RT-PCR) and Western blot, respectively, using the tissue of the brainstem.

Inter-strain differences were tested either by Student's t-test or by ANOVA with Dunnett's post-hoc test. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

BP of SPwch1.71 and 1.72 did not differ significantly from that of SHRSP/Izm when measured by the telemetry. When BP change under the cold and restraint stress was monitored by the telemetry, the response was similar between SHRSP/Izm and SPwch1.71, while SPwch1.72 showed a blunted response. In accordance with this observation, increase in urinary NE excretion under the cold stress did not differ significantly between SPwch1.71 and SHRSP/Izm, while it significantly reduced in SPwch1.72 when compared with SHRSP/Izm. Further, a power spectral

analysis on HR variation indicated that ⊿LF/HF under the restraint stress was comparable between SHRSP/Izm and SPwch1.71, whereas a significant reduction was observed in SPwch1.72.

Collectively, the results of these physiological experiments indicated that the region covered by SPwch1.71 did not contribute to the difference in the sympathetic stress response between SHRSP/Izm and WKY/Izm, and could be excluded from the candidate region. We thus narrowed down the candidate region between *Trpc2* and *Olr111* as the maximal estimation, which harbored 12 candidate genes.

Sequence analysis of the 12 potential candidate genes in this region identified a nonsense mutation in the stromal interaction molecule 1 (*Stim1*) gene of SHRSP/Izm, which was shared among 4 substrains of SHRSP. A western blot analysis confirmed a truncated form of STIM1 in SHRSP/Izm. In addition, the analysis revealed that the protein level of STIM1 in the brainstem was significantly lower in SHRSP/Izm when compared with WKY/Izm. STIM1 plays a key role in the cellular Ca^{2+} dynamics through interacting with ORAI1 and/or the transient receptor potential cation channel 1 (TRPC1). Of note, the C-terminus lysine residues (K684, K685), which were lost in the truncated form in SHRSP/Izm, were reported to be essential in the interaction of STIM1 with the TRPC1. The truncatied STIM1 may be causally related to the exaggerated response of SNS in SHRSP through abnormal regulation of TRPC1.

CONCLUSION

In conclusion, we found that *Stim1* is the best candidate in terms of the gene function as well as of the potential significance of the sequence variations identified in it. To obtain conclusive evidence for pathological roles of the truncated STIM1, it is essential to clarify effects of the truncation on the cellular calcium dynamics. Further studies on the role of STIM1 in the regulation of SNS are warranted.