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Osaka University
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

電位依存性ニトロマニオンチャネルVSP/Hv1は免疫細胞に発現し、活性酸素類生産など様々な生理機能に寄与することを知られている。しかし、これらの知見は主にマウスを用いたin vitroのものであった。本論文提出者は、ゼブラフィッシュを実験動物に使用することでこの問題点の克服を試みた。まず、論文提出者はin vitroの実験系で、ゼブラフィッシュVSP（Dr-Hv1）のニトロマニオンチャネルとしての活性を確認した。次に、VSPの免疫系細胞特に好中球における発現が種を超えて保存されていることを、分子生物学的手法、電気生理学的手法を駆使することで確認した。更に、CRISPR/Cas法によりVSP欠損ゼブラフィッシュ系統の作製を行い、食食細胞でのDr-Hv1の役割をin vivoで解析する系を構築した。また、候補者はこの研究過程で、マウスとゼブラフィッシュVSP間の亜鉛感受性の差異が、両者の血清中の亜鉛濃度の違いと対応することも明らかにし、今後の比較生理学的研究の発展に繋がる可能性を示した。これらの研究は候補者自身の高度な知識の習得と技術の工夫のために公正に行われ、分子生物学分野での波及効果を十分に与える内容であり、博士（医学）の学位授与に値すると判断された。
| 氏 名 | RATANAYOTA Adisorn |
|-------|---------------------|
| 論文題名 | Molecular and functional characterization of the voltage-gated proton channel in zebrafish neutrophils (ゼブラフィッシュの好中球における電位依存性プロトンチャネルの分子及び機能の解析) |

**論文内容の要旨**

**目的(Purpose)**

Voltage-gated proton channel (Hv1/VSOP) is the membrane protein that mediates the rapid flow of protons across the cell membrane. Thus far, most researchers have used mammalian model for studying the function of Hv1. However, the mammalian model has limitations in performing *in vivo* experiments. This study, therefore, aims to establish the *in vivo* platform for functional analysis of Hv1 by using zebrafish, which are transparent during development, as an alternative animal model.

**方法ならびに成績(Methods/Results)**

Since zebrafish Hv1 protein (DrHv1) has never been studied, I started by exploring its biophysical properties using the heterologous expression system. Patch-clamp recording of DrHv1-expressing HEK293T cells confirmed the voltage-dependent proton conductance of DrHv1 with differences in voltage-threshold and activation kinetics as compared to mouse Hv1. Further analysis indicated that DrHv1 preserves all essential biophysical properties of voltage-gated proton channel, except that it is relatively resistant to extracellular Zn²⁺, a potent inhibitor of mammalian Hv1. Notably, the serum Zn²⁺ concentration is significantly higher in zebrafish than in mouse. This evidence raises the possibility that Zn²⁺ sensitivity was acquired following a change in serum Zn²⁺ concentration during the evolution.

Moving from the *in vitro* analysis to the *in vivo* model, I revealed the expression of DrHv1 in various organs of zebrafish. The RT-PCR results indicated that DrHv1 is highly expressed in the kidney which is the primary hematopoietic organ. Subsequent electrophysiologica l recording confirmed the functional expression of DrHv1 in zebrafish neutrophils, providing the first evidence for the conserved role of Hv1 in phagocytes of non-mammalian vertebrates.

I applied CRISPR-Cas9 technology to generate DrHv1⁻/⁻ zebrafish for comparative functional analysis of Hv1. The gRNA targeting DrHv1-encoding gene was co-injected with Cas9 endonuclease into the fertilized one-cell stage wild-type zebrafish embryos. Sequences analysis revealed frameshift mutation and premature stop codon in DrHv1-encoding gene, causing early termination of DrHv1 translation. Patch-clamp recording confirmed no proton current in neutrophils derived from DrHv1⁻/⁻ zebrafish, indicating successful CRISPR-Cas9-mediated mutagenesis. I also provided evidence proving that DrHv1⁻/⁻ zebrafish are available as the *in vivo* platform for comprehensive investigation of Hv1.

**総括(Conclusion)**

This study has explored for the first time that zebrafish Hv1 protein is the voltage-gated proton channel. The expression of DrHv1 in zebrafish neutrophils indicates the conserved role of Hv1 in non-mammalian phagocytic cells. CRISPR-Cas9-mediated genome editing of DrHv1-encoding gene also shows successful outcomes. The study of DrHv1 spotlights the biological variation in diverse animal species and illustrates the power of zebrafish as the excellent *in vivo* model for studying the physiological function of Hv1 in the living animal.