論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	WU JI
論文題名 Title	EHBP1L1, an apicobasal polarity regulator, is critical for nuclear polarization during enucleation of erythroblasts （上皮極性を制御するEHBP1L1は赤芽球が脱核する時の核の極性化に必須である）

論文内容の要旨

〔目　的(Purpose)〕

Cell polarity is a fundamental feature of cells characterized by the asymmetric distribution of organelles and molecules and plays important roles in many physiological events. In erythroblasts, nuclei become condensed and asymmetrically positioned during differentiation and are eventually extruded, which produces reticulocytes and pyrenocytes. Previous studies have shown that membrane trafficking is a crucial role that regulates the dynamic process of enucleation. However, the detailed molecular mechanisms underlying the role of membrane trafficking in erythroblast enucleation remain unknown.

Our subsequent study identified EH domain-binding protein 1 like 1 (EHBP1L1) as a Rab8 effector protein that regulates epithelial apical transport. EHBP1L1 knockout mice died of anemia after birth, suggesting an important role of EHBP1L1 in erythropoiesis. Therefore, this study aims to elucidate the role of EHBP1L1 in erythropoiesis, especially erythroblast enucleation, and to contribute to the understanding of the erythropoiesis mechanism, the treatment of blood diseases and the development of artificial hematopoiesis.

〔方法ならびに成績(Methods/Results)〕

In this study, we first generated systemic EHBP1L1 -/- mice through the CRISPR/Cas9 system and found that EHBP1L1 deletion resulted in lethal anemia, manifested by failure of erythroblast enucleation and hemolysis. By using imaging flow cytometry, we analyzed the involvement of EHBP1L1 in enucleation and found that EHBP1L1 regulates erythroblast nuclear polarization. We then investigated the molecular mechanisms in vitro using the erythroblast cell line MEDEP-BRC5. Based on shRNA-mediated gene knockdown and chemical inhibitor treatment, we found that EHBP1L1 and its associated proteins Rab10, Bin1, and dynamin 2 are also critical for enucleation. Through morphological analysis of peripheral blood erythrocytes and detection of hemolysis indicators, we also found that EHBP1L1 is critical for maintaining the normal morphology and structural stability of erythrocytes. Through hematopoietic stem cell transplantation experiments in mice, we further confirmed at the in vivo level that EHBP1L1 deletion causes abnormal erythroblast enucleation and hemolysis.

Furthermore, nuclei in skeletal muscle fibers of Ehbp1l1-/- mice are not restricted to the periphery, as observed in patients with centronuclear myopathy with mutations in Bin1 or dynamin 2 genes, suggesting that EHBP1L1 is also involved in the nuclear polarization of skeletal muscle cells.

〔総　括(Conclusion)〕

In this study, we found that EHBP1L1 promotes nuclear polarization and subsequent enucleation of erythroblasts in coordination with Rab10, Bin1, and dynamin. EHBP1L1 maintains the proper morphology and structural stability of erythrocytes. Thus, EHBP1L1 is a novel regulator of late stages of erythropoiesis. Loss of EHBP1L1 also causes abnormal central positioning of nuclei and mitochondria in skeletal muscle cell. These results indicate that EHBP1L1 plays an important and universal role in multiple cell polarity systems.

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

細胞極性は、細胞小器官と分子の非対称分布を特徴とする細胞の基本的な特徴であり、多くの生理学的現象において重要な役割を果たす。赤血球が生成される際、分化中の赤芽球では核が非対称に配置された後、最終的に押し出される（脱核という）。膜輸送が脱核を調節に重要なことが知られているが、膜輸送の詳細な分子機構は不明である。

この研究では、EHBP1L1がRab10、Bin1、ダイナミンと連携して核極性化とそれに続く脱核を促進すること、及び赤血球の適切な形態と構造安定性に維持に重要なことを見出した。さらに、EHBP1L1の欠損は骨格筋細胞の核の極性化の異常も引き起こし、ヒトの中心核ミオパチーと同様の表現型を示した。これらの結果は、EHBP1L1 が複数の細胞種における極性化の分子機構において重要かつ普遍的な役割を果たしていることを示したものであり、博士（医学）の学位授与に値する。