様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Platelet activation plays a repulsive role of lymphatic to blood vessels in mouse peripheral tissues

研究課題名（英文）Platelet activation plays a repulsive role of lymphatic to blood vessels in mouse peripheral tissues

研究代表者

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研究成果の概要（和文）：血管とリンパ管の秩序だった高次構造と組織内分布は動的平衡状態にある。われわれは、血小板活性化シグナルに重要なホスホリパーゼCγ2のノックアウト（Plcg2-/-）マウスを解析し、末梢組織中に血管とリンパ管の異常吻合がランダムに形成されることを明らかにした。異常吻合部位にはリンパ管内皮細胞から血管内に伸びる糸状仮足様の突起が認められた。

研究成果の学術的意義や社会的意義
We confirmed platelets function to keep lymph-blood partitioning in mouse peripheral tissues after LECs migrate out of the jugular veins. It indicate a novel role for platelets in partitioning blood and lymphatic vascular compartments by promoting LEC retraction in mouse peripheral tissues.

研究成果の概要（英文）：Lymphatic vessels are established as a vasculature separate from blood vessels in peripheral tissues.In this study, we analyzed Phospholipase Cγ2 (Plcg2) knockout mice, which lack platelet activation and show blood-filled lymphatic vasculature. We detected lymph-blood misconnections in embryonic back skin of Plcg2 knockout mice by fluorescent angiography, indicating the role of platelets in the maintenance of lymph-blood partitioning during lymphatic vessel elongation. We also performed time-lapse analysis and found a retraction of LEC protrusion in vitro. These effects were inhibited by an inhibitor of TGF-β signals, while TGF-β induced a retraction of LEC protrusion. In vivo analysis indicates the role of TGF-β in keeping lymphatic vessels separate from blood vessels. These results indicate a novel role for platelets in partitioning blood and lymphatic vascular compartments by promoting LEC retraction in mouse peripheral tissues.

研究分野：細胞病態学

キーワード：lymphatic　development　mouse

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Lymphatic vascular system was considered as a passive drainage system responsible for removal of fluid, lipids, and immune cells from tissues. Lymphatic endothelial cells (LECs) were initiated by the expression of transcription factor Prox1 and Sox18, in a subpopulation of venous endothelial cells. Recent study demonstrated that platelets have an unexpected and critical role in separation of blood and lymphatic system in embryonic early stage. A critical clue was found in podoplanin/platelet C−type lectin−like receptor 2 (CLEC−2) interaction, which mediate platelets aggregation and blood/lymphatic separation in mice. Activated CLEC−2 dimer initiates Syk and Slp−76 signaling, mice deficient in podoplanin/CLEC−2, or Syk and Slp76 exhibited blood−filled lymphatics during embryonic development and died shortly after birth. Phospholipase Cγ2 (Plcg2) was activated by Syk and Slp−76, which is also required for blood−lymph separation. How platelets initiate and maintain lymphatic vessels separate from blood vessels during lymphatic sprouting remains unknown. Moreover, apoptosis stimulating protein of p53 (Aspp1) was identified as an endothelial−specific gene and aberrant isolated islands were detected in Aspp1$^{-/-}$ embryonic back skin during lymphangiogenesis. Interestingly, we detected abnormal blood−filled lymphatic islands in Aspp1, Plcg2 doubleknockout (Aspp1$^{-/-}$, Plcg2$^{-/-}$) embryonic back skin in mice. Based on these finding, we thus hypothesized that abnormal blood−lymph connections may formed during lymphatic vessels development in the peripheral tissue.

２．研究の目的

- To clarify the role of platelets in separation of blood and lymphatic systems in Plcg2$^{-/-}$ embryos in the back skin
- To clarify the cellular events of blood−lymphatic interactions.
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３．研究の方法

Note: We maintain all the transgenic mouse lines required for the future experiments mentioned below.

Phenotypic analysis in Aspp1$^{-/-}$, Plcg2$^{-/-}$ double knockout embryos

In order to investigate the embryonic phenotype of aberrant blood−filled lymphatic islands in Aspp1$^{-/-}$, Plcg2$^{-/-}$ embryos, we will perform a time−course analysis from E12.5 till E15.5, to understand how these blood−filled lymphatic islands were formed. For this purpose, we will perform flat−mount fluorescent confocal microscopy after immunostaining for the pan−endothelial cell marker PECAM−1, the erythroid cell marker TER−119, the lymphatic marker Lyve−1 or VEGFR3. We will try to find the genesis and developmental of the abnormal blood−filled lymphatic islands in embryonic peripheral tissue. We will also analyze the developmental of lymphovenous junctions, in order to investigate whether blood−filled lymphatic vessels formation was caused by fluid back flow or not.

Fluorescent angiography injection in Aspp1$^{-/-}$, Plcg2$^{-/-}$ embryos

To investigate embryonic blood flow and distribution of abnormal blood−lymph connection in Aspp1$^{-/-}$, Plcg2$^{-/-}$ embryos, we will perform fluorescent angiography by injection of isolectin GS−IB4 conjugated with Alex Fluor 488 at E13.5 or E14.5. If blood−lymph connections happen in the peripheral tissue, we should detect isolectin GS−IB4 fluorescent signals in lymphatic vessels or abnormal lymphatic islands.

In vitro ES cells differentiation analysis using Aspp1^{+}/ Plcg2^{-}, Aspp1^{-}/ Plcg2^{-} ES cells.

Our study suggests that platelets play a crucial role in separation of blood and lymphatic vessels in peripheral tissue. To analyze the role of platelets in separation of blood and lymphatic endothelial cells, we will generate Aspp1^{+}/ Plcg2^{-}, Aspp1^{-}/ Plcg2^{-} ES cells and stimulate these cells with platelets or control factors during Aspp1/Plcg2 ES cells differentiation.

4. 研究の成果

In this study, we hypothesized that platelets function to keep lymph−blood partitioning in mouse peripheral tissues after LECs migrate out of the jugular veins. We detected lymph−blood misconnection sites in embryonic skin of Plcg2$^{-/-}$ mice and analyzed the effect of activated platelets on LECs. We also provide *in vitro* and *in vivo* evidence that TGF−β plays an important role in lymph−blood partitioning. These results indicate a novel role for platelets in partitioning blood and lymphatic vascular compartments by promoting LEC retraction in mouse peripheral tissues.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Liu Xinyi
２．発表標題 Platelet activation blocks misconnection of lymphatic to blood vessels in mouse peripheral tissues
３．学会等名 The 16th Korea-Japan Joint Symposium on Vascular Biology（国際学会）
４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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