【Grant-in-Aid for Scientific Research (S)】

Broad Section I

Title of Project： **Elucidating the fundamental mechanism of platelet biogenesis and its medical application**

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Research Project Number： 21H05047 Researcher number： 50286986
Term of Project： FY2021-2025 Budget Allocation ： 145,300 Thousand Yen
Keyword：platelet, megakaryocyte, bioreactor, lipid membrane, turbulence

【Purpose and Background of the Research】

Platelets are essential for homeostasis of the body such as hemostasis, physiologically active substance transport, vascular integrity maintenance, and innate immune defense against bacterial infection including MRSA. Preventive transfusion is performed repeatedly for the patients with bleeding predisposition. Meanwhile, based on supply difficulty due to aging societies, extremely short expiration date, and pandemic conditions including COVID19, we have been developing a system to produce artificial platelets from human iPSC-derived megakaryocyte (MK) cell lines and found that turbulence promotes platelet production. However, the molecular mechanism of its production is not clear. In this research proposal, we address two questions: (1) what is the mechanism by which turbulent stimulation controls MK maturation and what is the cause of spatiotemporal MK heterogeneity? (2) what is the molecular mechanism of MK membrane reconstitution and cleavage during platelet production? Through this, we will ultimately realize artificial platelet production with unprecedented efficiency (Fig. 1).

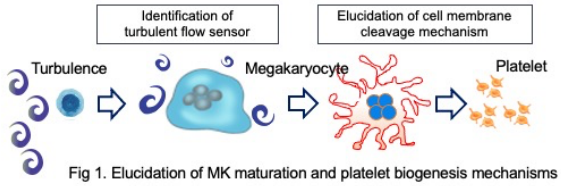


Fig 1. Elucidation of MK maturation and platelet biogenesis mechanisms

【Research Methods】

(1) Elucidating the mechanism of turbulence-controlled MK maturation and spatiotemporal maturation heterogeneity (verifying multiple potential receptors)

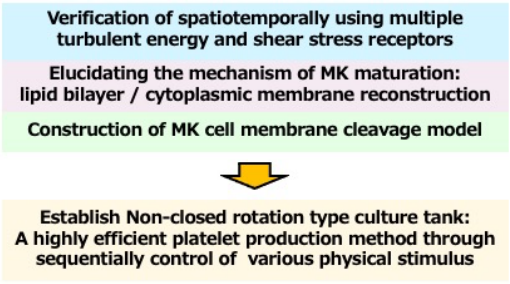
We found that turbulence with optimum values of turbulent energy and shear stress is essential for MK maturation and lipid membrane cleavage, and from preliminary verification, turbulence that varies with the temporal transition of the maturation process. Since we obtained results strongly suggesting the existence of a flow sensor, we evaluated the maturity of MKs associated with suppression of expression of multiple candidate molecules / structures. We will consider the molecular mechanism of the maturation process and the reason why it becomes heterogeneous from cell to cell.

(2) Identification of molecules responsible for cleavage of MK cell membrane and construction of a membrane cleavage model upon platelets production

The membrane deformability and cleaving activities of proteins in MKs are verified using an artificial lipid membrane (liposome) to narrow down candidates. Then, preparation of MK-derived lipid membrane will be optimized and applied to evaluate in vitro lipid membrane cleavage by the candidate proteins and identify effectors.

(3) Development of new culture system

We will establish a highly efficient platelet production method by realizing non-closed rotation-type culture tanks.



(Fig. 2: Below)

【Expected Research Achievements and Scientific Significance】

The meanings of multiple platelet production systems in vivo that occur with pathophysiological conditions and age differences will be reconsidered. Through in vitro reconstitution of experimental design, it is expected to understand multiple platelet production mechanisms as part of the lipid membrane remodeling mechanism. It will also lead to the development of new in vitro manufacturing methods.

【Publications Relevant to the Project】

- Nishimura T, Oyama T, Hu HT et al. Filopodium-derived vesicles produced by MIM enhance the migration of recipient cells. *Dev Cell* 56(6):842-559, 2021.
- Seo H, Chen SJ, Hashimoto H et al, A beta1-tubulin-based MK maturation reporter system identifies novel drugs that promote platelet production. *Blood Adv* 2(17):2262-2272, 2018.
- Ito Y, Nakamura S, Sugimoto N, et al. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. *Cell* 174(3):636-648, 2018.

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