様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　元 年　 ５ 月 １４ 日現在

機関番号：１２６０１
研究種目：基盤研究(C)（一般）
研究期間：2016～2018
課題番号：１６Ｋ０９８２１

研究課題名（和文）Fibrinolytic factors control MSC expansion thereby enhancing hematopoiesis

研究課題名（英文）Fibrinolytic factors control MSC expansion thereby enhancing hematopoiesis

研究代表者
Ｈｅｉｓｓｉｇ　Ｂｅａｔｅ（Heissig, Beate）

東京大学・医科学研究所・准教授

研究者番号：３０３７２９３１

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：組織プラスミノーゲンアクチベーター（tPA）は脳卒中患者の治療に使用される酵素です。間葉系幹細胞は、腫瘍床を含む様々な組織の幹細胞を増殖させることができます。我々は、tPAが骨髄および腫瘍細胞床内の間葉系幹細胞を増殖させることができることを示す。我々は、ｔＰＡレベルを制御することが造血幹細胞の増殖および腫瘍細胞の増殖を調節し得ることを提案する。

研究成果の学術的意義や社会的意義
骨髄および腫瘍細胞ニッチにおけるＭＳＣの動員または拡大を調節することにおける線維素溶解因子の重要な役割を強調している。造血幹細胞ならびに癌幹細胞の増殖におけるＭＳＣの役割を実証する研究と一緒に、我々は、線維素溶解因子が臨床現場での造血幹細胞の増殖に有用であり得ることを提案する。 ＭＳＣを動員するそれらの能力のために癌患者に与えられる線維素溶解因子は、腫瘍細胞増殖を支持するかもしれない。

研究成果の概要（英文）：Tissue plasminogen activator (tPA) is an enzyme used for the treatment of stroke patients. Mesenchymal stem cells can expand stem cells in various tissues, including the tumor bed. We show that tPA can expand mesenchymal stem cells within the bone marrow and the tumor cell bed. We propose that controlling tPA levels can regulate hematopoietic stem cell expansion and tumor cell growth.

研究分野：造血

キーワード：間葉系幹細胞

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

Mesenchymal stromal cells (MSCs) constitute a crucial subset of BM stromal cells. They are plastic-adherent multipotent cells, characterized by their ability to give rise to colony-forming unit-fibroblasts (CFU-Fs) and to differentiate into osteogenic, adipogenic, and chondrogenic lineages. Aside from the BM, MSCs exist in various connective tissues such as fat, muscle, skin, and placenta. They make up 0.001% to 0.01% of human adult BM cells and can be localized in perivascular spaces. Plasmin is generated by cleavage of the proenzyme plasminogen (Plg) by urokinase-type plasminogen activator (uPA) or tissue-type plasminogen activator (tPA). Activation of plasmin or MMPs cannot only accelerate local ECM degradation but can also lead to the activation/inactivation of chemo-/cytokines, receptors, and other proteases.

２．研究の目的

The purpose of the study was to examine the potential of fibrinolytic factors like tPA for the expansion of mesenchymal stem cells

３．研究の方法

We examined the effect of tPA on the expansion of mesenchymal stem cells using mice. Recombinant tPA was given to wildtype mice to show its effects on MSC expansion. In addition, mice deficient in tPA or its downstream target plasminogen (Plg) were used to demonstrate how lack of these fibrinolytic factors affects the mesenchymal stem cell content in vivo. In vitro co-culture systems were implemented to demonstrate the mechanism of mesenchymal stem cell expansion.

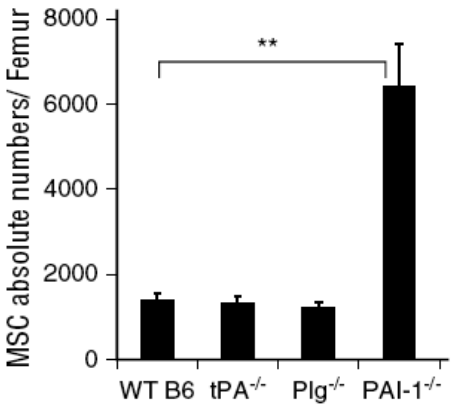
４．研究成果

Here, we observed that by activating plasminogen and matrix metalloproteinase-9, tPA expands murine bone marrow–derived $CD45^{-}TER119^{-}Sca\text{-}1^{+}PDGFR\alpha^{+}$ mesenchymal stromal cells ($P\alpha S$-MSCs) in vivo through a crosstalk between $P\alpha S$-MSCs and endothelial cells. We show that deletion of the endogenous inhibitor of tPA, PAI-1 enahnces MSC expansion within the BM. Mechanistically, tPA induces the release of Kit ligand from $P\alpha S$-MSCs, which activates $c\text{-}Kit^{+}$ endothelial cells to secrete MSC growth factors: platelet-derived growth factor-BB (PDGF-BB) and fibroblast growth factor 2 (FGF2). In synergy, FGF2 and PDGF-BB upregulate PDGFRα expression in $P\alpha S$-MSCs, which ultimately leads to $P\alpha S$-MSC expansion (Dhahri et al. Blood 2016; Reference 1).

In a related study, we demonstrate that tumor derived tPA enhances the recruitment of MSC within the growing melanoma tumor in vivo (Salama et al. FASEB 2019; Reference 2).

Figure 1. Tissue-type plasminogen activator expands $CD45^{-}Ter119^{-}Sca\text{-}1^{+}PDGFR\alpha^{+}$ MSCs in mice. C57BL/6 (WT B6) mice were injected daily intraperitoneally with a serpine-resistant recombinant tissue tPA on day 0 and day 1. MSCs were identified using the following marker profile: $CD45^{-}Ter119^{-}Sca\text{-}1^{+}PDGFR\alpha^{+}$ ($P\alpha S$-MSCs) by FACS. (A) Absolute numbers of $P\alpha S$-MSCs per femur in WT B6 and $tPA^{-/-}$, $Plg^{-/-}$, and $PAI\text{-}1^{-/-}$ BM cells under steady state as determined by FACS (n = 3-4/group). (B)

Figure 1.



５．主な発表論文等

〔雑誌論文〕（計　3　件）

1. Dhahri D, Sato-Kusubata K, Ohki-Koizumi M, Nishida C, Tashiro Y, Munakata S, *et al.* Fibrinolytic crosstalk with endothelial cells expands murine mesenchymal

stromal cells. Blood **2016**;128:1063-75
2. Eiamboonsert S, Salama Y, Watarai H, Dhahri D, Tsuda Y, Okada Y, Hattori K, and Heissig Beate. The role of plasmin in the pathogenesis of murine multiple myeloma. Biochem Biophys Res Commun. 2017; 488:387-392,

3. Salama Y, Lin SY, Dhahri D, Hattori K, Heissig B. The fibrinolytic factor tPA drives LRP1-mediated melanoma growth and metastasis. FASEB J **2019**;33:3465-80

〔学会発表〕（計　4　件）

1. Heissig B. Organizer, Speaker and Chair at the Niche cells for tissue regeneration. Joint Workshop between Norway and Japan in Regenerative Medicine, June 4th, 2018, Norwegian Embassy Tokyo.
2. Heissig B. Dual or half career: trapped between success and support. European Research Nights; Tokyo, March 7th, 2018.
3. Heissig B. The niche/microenvrionment shapes the fate of researchers and stem cells. European Research Day, Nov 25 2016. Delegation of the European Union to Japan, Tokyo.
4. Heissig B. Fibrinolytic factors in stem cell regulation and cancer. The 91th of Stem Cell Biology and Regenerative Medicine Forum. Canada-Japan Joint Research Program on “Epigenetics of Stem Cells” Hematopoiesis in Health and Disease, Tokyo, Oct. 17th 2016.

〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等 http://stemcell-u-tokyo.

６．研究組織

(1)研究分担者
研究分担者氏名：

ローマ字氏名：

所属研究機関名：

部局名：

職名：

研究者番号（8 桁）：

(2)研究協力者

研究協力者氏名：服部 浩一

ローマ字氏名：Koichi Hattori