様　式　C－１９、F－１９－１、Z－１９　（共通）

科研費
KAKENHI

# 科学研究費助成事業　　研究成果報告書

令和　５ 年　６ 月　５ 日現在

機関番号：１７４０１

研究種目：若手研究

研究期間：2021～2022

課題番号：２１Ｋ１６２７２

研究課題名（和文）Enhancer based fine-tuning of Runx family for the regulation of HSC division during bone marrow recovery

研究課題名（英文）Enhancer based fine-tuning of Runx family for the regulation of HSC division during bone marrow recovery

研究代表者

Ｓｈａｈ・Ａｄｉｌ・Ｉｓｈｔｉｙａｑ　Ａｈｍａｄ (Ahmad, Shah Adil Ishtiyaq)

熊本大学・国際先端医学研究機構・客員助教

研究者番号：８０８９３８３３

交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：骨髄再生の造血幹細胞を用いて、造血幹細胞の分化に関わるRUNX ファイリーのエンハンサーの候補領域を同定した。実際にその領域では、活性化エンハンサーマークとして知られるH3K27アセチル化の修飾を確認した。特に私が同定したRunx3のエンハンサー領域は、幹細胞が試験管内で分化する時も使用している可能性が示唆された。従って、本研究では造血幹細胞の分化制御に関与しうるRunx3のエンハンサー領域を同定した。

研究成果の学術的意義や社会的意義
The achievement of my research may contribute to understanding the mechanism for the determination of HSC fate under proliferation conditions. So, this achievement may also contribute to the development for ex vivo expansion of HSCs.

研究成果の概要（英文）：I compared chromatin accessibility of Runx enhancers between self-renewal and differentiation types of HSCs after 5-FU administration by ATA-seq, and identified candidate regions of novel metabolic-epigenetic functional axis targeting RUNX enhancer sequences. Moreover, I confirm that these regions have H3K27 acetylation by CUT&Tag system. In addition, Runx3, which is related to differentiation, was highly expressed in EPCR Low HSCs, compared to EPCR High HSCs that is most primitive HSCs. Consistent with this results, the chromatin accessibility of enhancer regions that I identify by using HSCs obtained from 5-FU-treated mice was enhanced when EPCR high HSCs differentiate into EPCR low HSCs in vitro. These data suggest that I identify the enhancer regions of Runx3 that is important for the regulation of expression level in HSCs and this enhancer regions is involved in the differentiation of HSCs.

研究分野：Hematology

キーワード：stem cells

※科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属します。

## １．研究開始当初の背景

Hematopoietic stem cells (HSCs) are a unique rare population of cells which possess the ability to generate all other blood cells under proper cellular and environmental condition. HSCs have long been at the center of interest to understand the regulation of hematopoiesis and bone marrow (BM) regeneration following chemotherapy. Despite continuous efforts from many researchers several aspects of these regulatory networks are still not resolved completely, especially regarding the switching between self-renew and differentiation division of HSCs during regeneration. We have studied the early stage of bone marrow regeneration following chemotherapy induced myeloablation and suggest that epigenetic status in critical factor for hematopoiesis is changed during born marrow regeneration. In the proposed study, I would like to focus on Runx-family of transcription factors that have been reported to have important role for the regulation of hematopoiesis. They are also considered as tumor suppressor genes as their dysregulation through mutations have been associated with leukemias. A recent study showed that overexpression of Runx3 suppressed Runx1 in transgenic model mouse (Yokomizo-Nakano et al., 2020). Runx1 was previously reported to enrich the hematopoiesis-specific transcription factors during the onset of hematopoiesis through epigenetic modification (Lichtinger et al., 2012). Physical binding of Runx1 to CHD7 – a chromatin modelling protein, has also been shown to have important role in hematopoiesis by a recent study (Hsu et al., 2020). Although Runx3 is highly expressed in HSPCs, previous study with hematopoietic deletion of Runx3 suggested a similar function to Runx1 (Wang et al., 2013). However, a recent study showed that Runx3 promotes cytotoxic T lymphocyte production by promoting the accessibility of the cis-regulatory landscape (Wang et al., 2018). All these reports confirm a critical role of Runx-family in hematopoiesis, while the intricate and complex regulatory mechanism still remain elusive.

Cellular metabolism plays a crucial role in regulating HSC maintenance and different metabolic signatures have been reported to represent specific developmental or cellular stage of HSCs (Nakamura-Ishizu et al., 2020). Our group has been studying the metabolic features of HSCs at steady state and during stress recovery following chemotherapy induced by 5-fluorouracil (5-FU) treatment. In a recent study, we observed that reductive Glutamine (Gln) pathway plays a key role in regulating HSC division during stress recovery. As we checked the dynamics of bone marrow cell population immediately after 5-FU administration, we identified two distinct phases – early phase (days 3-7) and late phase (days 9-12). We used Lineage-EPCR+CD150+CD48- (L-ESLAM) markers to identify HSC population in the regenerating bone marrow. Early phase exhibits the highest rate of HSC recovery, while at late phase CD48+ or CD150- progenitor cell production speeds up. Mitochondrial metabolism at these two phases correlates with previously reported metabolic features during HSC division – early phase HSCs showing high mitochondrial membrane potential (MMP) and high intracellular $Ca^{2+}$, and both parameters decreasing in late phase (Umemoto et al., 2018). Early phase HSCs also showed higher engraftment potential compared to late phase. These findings indicate the involvement of mitochondrial status to determine HSC divisional fate during regeneration. However, enhancing the MMP in ex vivo culture was not sufficient to maintain high frequency of HSCs, which suggested the requirement of additional unknown factors.

As we compared the gene expression pattern at different stages, we observed that the cytoplasmic pathway generating Acetyl CoA was suppressed in the early phase of regeneration, especially by the reduced expression of isocitrate dehydrogenase (Idh1). In vitro inhibition of Idh1 and Acly respectively by hLDL and BMS303141 reduced CD48+ progenitor cell fraction. Interestingly, inhibition of histone acetyltransferase (HAT) by A-485 also showed similar output from HSC culture. Inhibition of Idh1 and Acly (by hLDL and BMS303141) also showed reduced global histone acetylation on H3K27Ac staining. When we checked BM cell population 10 days after 5-FU treatment, L-ESLAM cells showed reduced acetylation compared to CD48+ progenitors, indicating acetylation induced chromatin accessibility regulation for HSC division. ATAC-seq data revealed that early phase HSCs show markedly reduced chromatin accessibility compared to steady state, with higher number of closed sites. Compared to early phase, CD48 locus showed increased accessibility and increased H3K27Ac in the late phase, which supports the CD48+

progenitor production. As we searched further, we located 314 enhancers opening in the late phase which are also active in multi-potent progenitors (MPPs) in comparison to HSCs. Among these enhancers, Runx1, Runx2 and Runx3 stand out notably as many previous studies reported the importance of Runx family members for the regulation of hematopoiesis (Figure 3). We also observed that Runx binding motif was significantly enriched within accessibility changed regions in MPPs.

In light of the above background, I hypothesize that epigenetic regulation of Runx family members is a major factor to determine HSC divisional fate during bone marrow regeneration. Two key questions sum-up the proposed study. First question is how exactly the Runx family members are regulated in different divisional stage or cell population. We have already shown the metabolic-epigenetic regulatory axis, but it is necessary to clarify the role of each enhancer and the magnitude of their cellular influence. My second question is the cellular amount of Runx molecules and any change of their relative abundance during the course of HSC division. Although many previous studies reported Runx1 as an essential factor for hematopoiesis with Runx2 and Runx3 also having collaborative or compensatory role, their precise role is not yet resolved. The second question will also clarify the individual role of Runx molecules for HSC maintenance. In our preliminary analysis, we already observed that Runx molecules are upregulated in the late-phase of BM regeneration compared to the early-phase, with Runx3 showing the highest magnitude of upregulation. We also observed increased Runx in progenitor cell population compared to HSCs, which complies with our hypothesis.

## ２．研究の目的

The purpose of this project is to unravel the epigenetic regulation of Runx family proteins during bone marrow regeneration and to specify the function of Runx proteins for the regulation of HSC division. Although chemotherapy induced stress is a well-known process, the regulation of HSC division during regeneration is not resolved yet. This project is based on unpublished research finding from our lab and it proposes a novel concept to understand HSC maintenance. By addressing the enhancer based Runx regulation during bone marrow regeneration this project will shed light on how the HSC divisional fate is regulated, which remains a major question impeding the understanding of HSC biology.

## ３．研究の方法

To identify the enhancer regions that are important to regulate RUNX, I performed ATAC-seq and CUT&Tag for H3K27 acetylation. As samples, I used HSCs in the early (stem cell type) and late phase (differentiation type) during hematopoietic regeneration post 5-FU administration. Alternatively, I used EPCR high (stem cell type) or low HSCs (differentiation type)in the steady state.

## ４．研究成果

I compared chromatin accessibility of Runx enhancers between self-renewal and differentiation types of HSCs after 5-FU administration by ATA-seq, and identified candidate regions of novel metabolic-epigenetic functional axis targeting RUNX enhancer sequences. Moreover, I confirm that these regions have H3K27 acetylation by CUT&Tag system. In addition, Runx3, which is related to differentiation, was highly expressed in EPCR Low HSCs, compared to EPCR High HSCs that is most primitive HSCs. Consistent with this results, the chromatin accessibility of enhancer regions that I identify by using HSCs obtained from 5-FU-treated mice was enhanced when EPCR high HSCs differentiate into EPCR low HSCs in vitro. These data suggest that I identify the enhancer regions of Runx3 that is important for the regulation of expression level in HSCs and this enhancer regions is involved in the differentiation of HSCs.

## ５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

－

## ６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
| --- | --- | --- | --- |

## ７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

## ８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
| --- | --- |