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研究課題名（和文）A Crosstalk Between Inflammation and Epigenetics in Regulating HSC Fitness

研究課題名（英文）A Crosstalk Between Inflammation and Epigenetics in Regulating HSC Fitness

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研究成果の概要（和文）：造血幹細胞の動的な機能は、静止と活性化の可逆的なバランスに依存している。細胞分裂を停止し、再び休止期に入る仕組みや、このためのシグナル伝達経路は、必ずしも明白ではない。私たちは、炎症シグナルが、MYD88やSETD2の分解に機能するE3ユビキチンリガーゼSPOPを介して、造血幹細胞が制御されていることを見出した。定常状態では、細胞質内のSPOPがMYD88の分解を促進し、H3K36me2を減少させて、造血幹細胞の疲弊を防いでいた。一方、炎症ストレス下では、SPOPは核内に移動してSETD2を分解することで造血幹細胞を増殖させた。

研究成果の学術的意義や社会的意義
Our study has provided a deeper understanding of HSC homeostasis via the crosstalk between inflammation and epigenetic regulations. It should be able provide new therapeutic strategy for preventing HSCs from exhaustion by inactivation of MYD88-dependent inflammatory activation in the future.

研究成果の概要（英文）：The functionality of hematopoietic stem cell (HSC) compartment hinges on the limited reversible balance between quiescence and activation. The identity of cell-intrinsic mechanisms required to terminate activation and re-enter dormancy, and the signaling pathways that mediate this transition, remain unresolved key questions. Based on our data, we have proposed that A crosstalk between inflammation and epigenetics controls HSC fitness through nucleocytoplasmic shuttling of an E3 ubiquitin ligase Speckle-type POZ protein (SPOP) functioning in MYD88 or SETD2 degradation. We observed: (1) In steady state, cytoplasmic SPOP drove MYD88 degradation to decrease H3K36me2 and prevent HSC exhaustion; (2) In response to inflammation, SPOP translocated into the nucleus and subsequently degraded SETD2 to promote expansion of HSC.

研究分野：血液内科学

キーワード：Hematopoietic stem cells　Inflammation　Toll-like receptor　Transformation　Regeneration　Histone methylation

１．研究開始当初の背景

Hematopoietic stem cells (HSCs) sustain hematopoiesis throughout our entire lives. HSCs exit dormancy to restore homeostasis in response to stresses, such as acute inflammation, and must return to a quiescent state to maintain the HSCs' pool (Matatall KA, Cell Rep. 2016). Direct sensing of inflammatory signals, including pathogen-associated molecular patterns (such as Toll-like receptor (TLR) ligands coupling MYD88-IRFs/NF-kB cascade), triggers transcriptional and epigenetic networks that facilitate the switch from steady-state to emergency/stress hematopoiesis (Nagai Y, Immunity 2006). Importantly, HSCs need to re-enter quiescence to prevent HSC exhaustion, which could lead to bone marrow failure (Pietras EM, Blood 2017). HSCs' quiescence is controlled by regulatory networks, including histone modifications (e.g. H3K36) and the ubiquitin proteasome system (e.g. SPOP) (Cabezas-Wallscheid N, CSC 2014). However, the key questions of epigenetics in maintaining the integrity in HSC fitness under stresses to be resolved by this work are: (1) What are the key mechanisms driving HSC activation to proliferate and divert to differentiation in response to inflammation? (2) What are the key regulators that sense sufficient blood cell production and trigger HSC re-entry to quiescence? (3) How to prevent HSC exhaustion under stress?

２．研究の目的

We have uncovered a critical role of methyltransferase SET Domain Containing 2 (SETD2) in regulating quiescence, proliferation and differentiation of adult HSCs via maintenance of H3K36me3 (Zhou Y, Haematologica 2018). Importantly, SETD2's stability is regulated by an E3 ubiquitin ligase Speckle-type POZ protein (SPOP) (Zhu K, Nucleic Acids Res. 2017). Thus, SPOP is likely to be a negative regulator for HSC quiescence, and inhibition of SPOP may maintain HSC quiescence. However, mice with hematopoietic-specific deletion of Spop (Mx1-Cre/Spopflf) develop lethal neutrophilia with reduced red blood cell numbers and hemoglobin (Guillamot M, Nat Immunol. 2019), which phenocopied our published Setd2 deficient mouse model (Mx1-Cre/Setd2f/f and Vav1-Cre/Setd2f/f). Additionally, hematopoietic stem and progenitor cells (HSPCs), either with Spop- or Setd2-deficiency, share a common transcriptional signature in emergency hematopoiesis identified by RNA-seq analysis. Two hundred and twenty-eight common genes with abnormal expression are identified from both Spop- and Setd2-deficient HSPCs. Within these genes, we found that the common enrichment of upregulated genes in myeloid cells mediated immunity and activation, and cell migration, while the common enrichment of downregulated genes in T-cell mediated immunity and cell cycle control, suggesting that a common immune signal pathway is involved in the SPOP-SETD2-H3K36me3 axis to control HSC fitness. The MYD88-dependent immune signal pathway has been demonstrated to sustainably activate in HSPCs with Spop-deficiency. To determine whether the activation of MYD88-

dependent immune signaling is essential to control HSC fitness in the context of SPOP and SETD2, administrations of lipopolysaccharides (LPS) and poly(I:C) to trigger MYD88 activation, were performed in Vav1-Cre/Spopf/f (SpopΔ/Δ) and Vav1-Cre/Setd2f/f (Setd2Δ/Δ) mice, based on published protocols (Takizawa H, CSC 2017). Both SpopΔ/Δ and Setd2Δ/Δ HSCs, upon inflammatory stresses, showed more severe phenotypes of reduced self-renewal and quiescence, compared to those HSCs without inflammatory stresses. Activation of the MYD88-dependent cascades can increase histone methyltransferase ASH1L expression to accumulate H3K36me2. As we found that the loss of SETD2 increases the ratio of H3K36me2/H3K36me3, it also leads to a loss of stem cell identity and an increase of differentiation towards progenitors.

Based on these findings, we hypothesized that a crosstalk between MYD88-dependent inflammation resolution and the H3K36 epigenetic circuit controls HSC fitness through nucleocytoplasmic shuttling of SPOP functioning in MYD88 or SETD2 degradation, and targeting of SPOP-mediated MYD88-dependent inflammation and H3K36me2/me3 imbalance can maintain HSC stemness.

３．研究の方法

To determine the immune signals of Myd88 downstream in regulating Ash1l/H3K36me2 levels, LPS and poly(I:C) administrations were performed in SpopΔ/Δ mice. The activations of Ash1l-related immune signaling and altered levels of Setd2, H3K36me2 and me3 was revealed by co-immunoprecipitation. Indeed, phospho-mimicking mutations resulted in ablation of SPOP interactions with MYD88. The binding efficacy between SPOP and MYD88, MYD88 phosphorylation, ASH1L expression, and H3K36 methylations was analyzed with or without LPS. We found cytoplasmic SPOP triggered MYD88 degradation to decrease ASH1L-H3K36me2, while SPOP dissociated with MYD88 to activate downstream MYD88 cascade to promote HSCs proliferation upon inflammation. To determine the alteration of direct interactions between Spop and Myd88 or Setd2 in mouse HSPCs, age- and gender-matched *Spop2*$^{f/f}$, Setd2$^{f/f}$ *and Setd2*$^{\Delta/\Delta}$ mice were injected with LPS and poly(I:C). To explore clinical translation potential for maintaining HSC stemness through chemically manipulating SPOP and MYD88, pharmacological inhibitions of SPOP (SPOP-IN-6b, *Guo ZQ, Cancer Cell. 2016*) and MYD88 inhibitor peptides was administrated in C57BL/6J mice prior to or together with LPS or poly(I:C) injection. We observed that targeting of SPOP-mediated MYD88-dependent inflammation appeared to restore H3K36me2/me3 balance and maintain HSC stemness.

We have applied our findings on role of inflammation on malignant transformation. Aberrant innate immune signaling in myelodysplastic syndrome (MDS) hematopoietic stem/progenitor cells has been implicated as a driver of the development of MDS. We demonstrated that a prior stimulation with bacterial and viral products followed by loss of the Tet2 gene facilitated the development of MDS via up-regulating the target genes of the Elf1

transcription factor and remodeling the epigenome in hematopoietic stem cells in a manner that was dependent on Polo-like kinases (Plk) downstream of Tlr3/4-Trif signaling, but did not increase genomic mutations. The pharmacological inhibition of Plk function or the knockdown of Elf1 expression was sufficient to prevent the epigenetic remodeling in HSCs and diminish the enhanced clonogenicity and the impaired erythropoiesis. Therefore, prior infection stress and the acquisition of a driver mutation remodeled the transcriptional and epigenetic landscapes and cellular functions in HSCs via the Trif-Plk-Elf1 axis, which promoted the development of MDS (Yokomizo-Nakano T, et al. J Exp Med 2023).

４．研究成果

Our proposed study has provided a deeper understanding of HSC homeostasis regulated through the crosstalk between inflammation and epigenetic regulations. By targeting of the key HSC regulators identified from this project, it should be able provide new therapeutic strategy for preventing HSCs from exhaustion by inactivation of MYD88-dependent inflammatory activation in the future.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Yokomizo-Nakano Takako、Hamashima Ai、Kubota Sho、Bai Jie、Sorin Supannika、Sun Yuqi、Kikuchi Kenta、Iimori Mihoko、Morii Mariko、Kanai Akinori、Iwama Atsushi、Huang Gang、Kurotaki Daisuke、Takizawa Hitoshi、Matsui Hirotaka、Sashida Goro	４．巻 220
２．論文標題 Exposure to microbial products followed by loss of Tet2 promotes myelodysplastic syndrome via remodeling HSCs	５．発行年 2023年
３．雑誌名 Journal of Experimental Medicine	６．最初と最後の頁 e20220962
掲載論文のDOI（デジタルオブジェクト識別子） 10.1084/jem.20220962	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Sho Kubota, Yuqi Sun, Jie Bai, Takako Yokomizo-Nakano, Mariko Morii, Takako Ideue, Motomi Osato, Terumasa Umemoto, Kimi Araki, Goro Sashida
２．発表標題 HMGA2 maintains hematopoietic stem cell via pleiotropic regulation of the transcription in stress conditions
３．学会等名 63rd ASH Annual Meeting and Exposition(国際学会)（国際学会）
４．発表年 2021年

１．発表者名 Trisomy 8 remodels chromatin and activates transcription of Runx1-target genes in hematopoietic stem cell
２．発表標題 Jie Bai, Kimi Araki, Yasuhiro Kazuki, Sho Kubota, Kenta Kikuchi, Ai Hamashima, Mihoko Iimori, Minetaro Ogawa, Daisuke Kurotaki, Mitsuo Oshimura, Goro Sashida
３．学会等名 65th ASH Annual Meeting and Exposition(国際学会)（国際学会）
４．発表年 2023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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研究協力者	白　潔 (Bai Jie)		

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			
米国	シンシナティ小児病院	テキサス大学サンアントニオ		