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研究課題名（和文）Studying the role of clonal hematopoiesis in mouse solid tumors using a native-tissue relevant context model

研究課題名（英文）Studying the role of clonal hematopoiesis in mouse solid tumors using a native-tissue relevant context model

研究代表者

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研究成果の概要（和文）：クローン性造血（CH）は、固形癌の25%以上で予後と関連していることが確認されている。大腸癌(CR)におけるクローン性造血の役割を明らかにするために、CRオルガノイド細胞をTet2(CHの共通変異遺伝子)KOマウスに多数注入した。VAVCre（全細胞KO）およびCD4（T細胞）マウスでは、CRの肝転移腫瘍負荷（TB）およびTexが減少した。つまり、Tet2欠損T細胞はCRの発生を抑制した。

研究成果の学術的意義や社会的意義
We clarified the roles of clonal hematopoiesis in a colorectal cancer (CRC) liver metastasis model which is native-relevant. Our findings may bring attention about CH in the prognosis and treatment of CRC in the era of precision medicine.

研究成果の概要（英文）：Immune cells with somatic mutations (IMsm) are infiltrated into tumor tissues in cancer patients with clonal hematopoiesis (CH). To examine the roles of IMsm in colorectal liver metastasis (CLM), colon cancer organoid cells (CCOC) were transplanted into spleens of various Tet2 conditional knockout mice: VAVCre (Tet2 deletion in all blood cells), LysMCre (myeloid), CD19Cre (B), and CD4Cre (T). We found that CML tumor burden of VAVCre and CD4Cre were lower than those of control. Differentially expressed gene analysis for WTA and immunofluorescence staining showed that PDCD1, TIM3, TIGIT, and LAG3, encoding inhibitory receptors were repressed in CD8+ cells sorted from VAVCre and CD4Cre livers comparing to control. In conclusion, deficiency of Tet2 in hematopoietic cells led to decrease of exhausted CD8+ T cells and suppressed CLM.

研究分野：Cancer immunity

キーワード：Clonal hematopoiesis　Colorectal cancer　TET2

１．研究開始当初の背景

Clonal hematopoiesis (CH) has been observed in over 20% of patients diagnosed with solid cancers, and it has been associated with a poor prognosis in most cases, with the exception of colorectal cancer (CRC) patients who tend to have a favorable prognosis. Among the various gene mutations detected in CH, *TET2* mutations are particularly prevalent. Previous research has demonstrated that the impact of *TET2*-mutated CH immune cells depends on the specific types of cancer tissues involved. Specifically, studies have shown that Tet2-KO myeloid cells can inhibit the progression of melanoma, while promoting the growth of hepatoma and lung cancers.

２．研究の目的

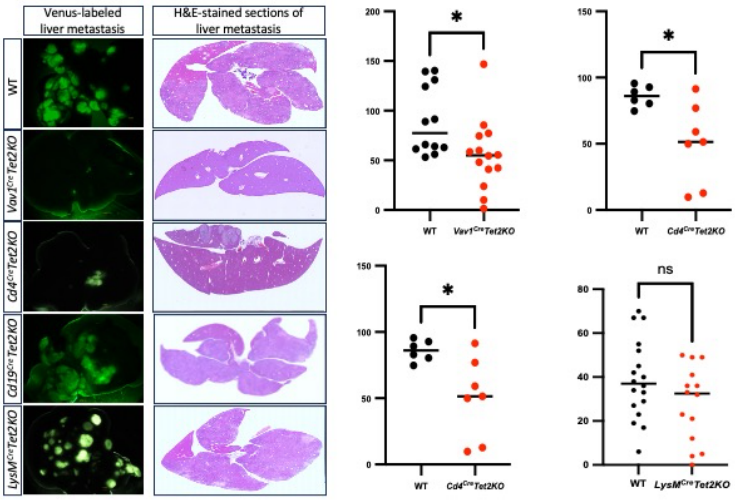
We clarified the roles of *TET2*-mutated CH immune cells on CRC progression.

３．研究の方法

We transplanted CRC organoid cells into the spleens of different types of *Tet2* conditional knockout mice: *Vav1Cre* (with *Tet2* gene deletion in all hematopoietic cells), *LysMCre* (myeloid cells), *Cd19Cre* (B lymphocytes), and *Cd4Cre* (T lymphocytes). After a period of 30 days, we collected livers for analysis. The extent of liver metastasis tumor burden (LMTB) was determined by counting the number of tumor foci on 10 hematoxylin-stained slides, with an interval of 80 μm between each slide. We utilized immunohistochemistry (IHC) and flow cytometry (FC) to analyze immune cells. The number of positive cells was automatically counted in 10 fields at 20x magnification. Furthermore, we performed whole transcriptome analysis (WTA) on sorted CD4+, CD8+, CD11b+, and CD19+ cells, respectively. We also perform scRNA sequencing of CD3 cells to identify the significant cell cluster and target molecules.

４．研究成果

Figure 1: Colorectal liver metastasis tumor burden

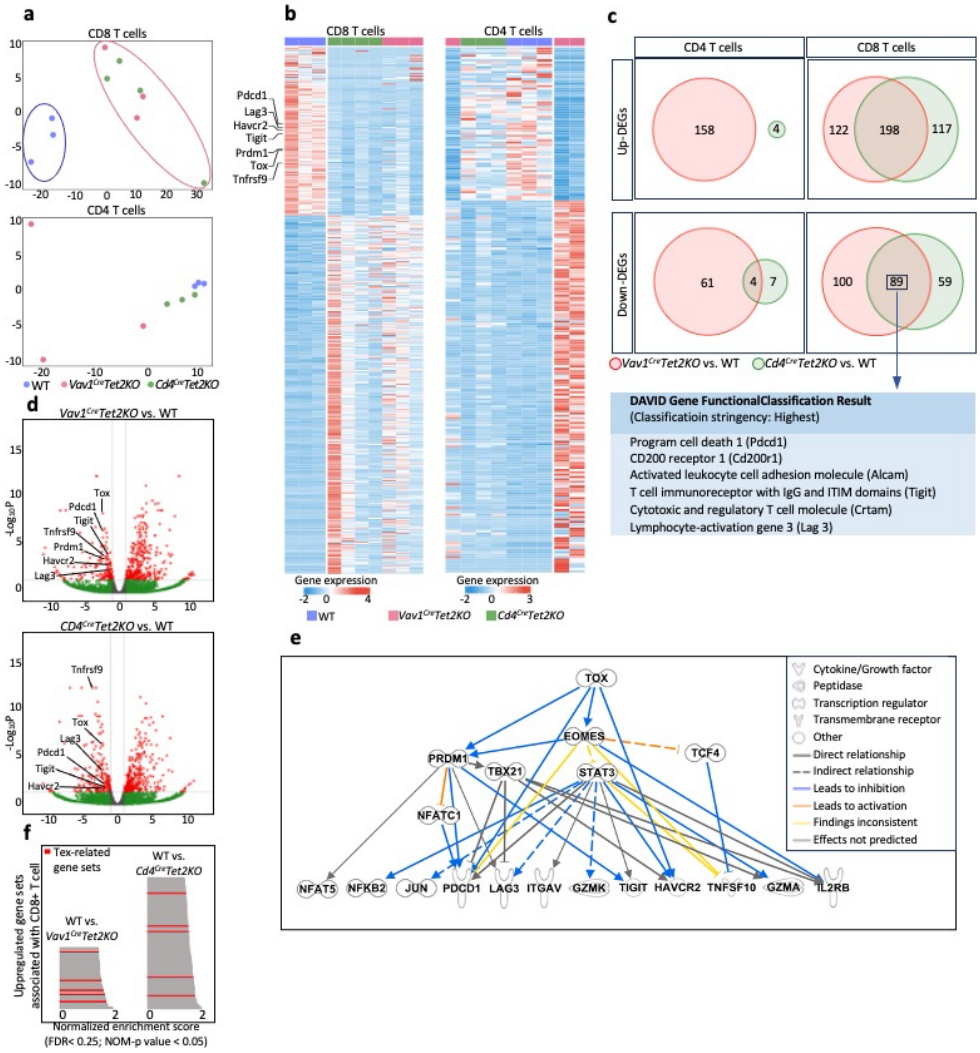


LMTB was significantly lower in *Vav1Cre* and *Cd4Cre* mice compared to the control mice (*Vav1Cre* vs. control: 55.65±35.62 vs. 89.93±34.57 foci/1000 mm², $p<0.05$; *Cd4Cre* vs. control: 50.19±30.39 vs. 85.98±7.92 foci/1000 mm², $p<0.05$). However, the LMTB of *Cd19Cre* and *LysMCre* mice was comparable to that of control mice (Figure 1).

The differential gene expression of WTA of CD4+ showed no difference while those of CD8+ cells revealed that 198 genes up-regulated, while 89 genes down-regulated in both of *Vav1Cre* and *Cd4Cre* mice compared the control mice (Figure 2a, b). Among the down-regulated genes, a gene group consisting of *Pdcd1, Tigit, Lag3,* and *Havcr2*, which encode inhibitory molecules associated with exhausted T cells exhibited enrichment (Enrichment score =3.77) based on the analysis using DAVID (Figure 2c, d). Ingenuity Pathway Analysis further indicated that these molecules were downregulated by the

upstream regulator TOX (Figure 2e). Furthermore, T cell exhaustion signaling pathway were downregulated in both *Vav1Cre* and *Cd4Cre mice* by using gene set enrichment analysis (Figure 2f).

Figure 2: Whole transcript analysis of tumor- infiltrating CD4 and CD8 cells



Tumor-infiltrating CD8 T cells (WT, n=1803; Vav1Cre, n=2184) were clustered and identified as nine T cell clusters, including Ifngr2+ stem-like CD8 T cells (Ifngr2+ TSCL), Ly6c+ stem-like CD8 T cells (Ly6c+ TSCL), Ifit+ stem-like CD8 T cells (Ifit+ TSCL), highly expressed Tox CD8 T cells (Tox^{hi} CD8 T), early exhausted T cells (Early Tex), fully exhausted T cells (fully Tex), CD8 effector T cells (CD8 Teff), CD8 NK-like T cells (CD8 NKLT), and Foxp3+Cd25+ CD8 regulatory T cells (CD8 Treg) (Figure 3 a). Stack bar plot showed that highly expressed Tox CD8 cells were higher in WT compare with those of Vav1Cre (Figure 3 b).

Figure 3: ScRNA sequencing analysis of tumor-infiltrating CD8 cells

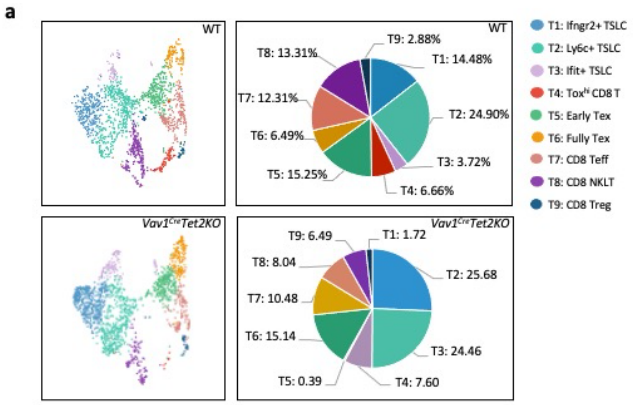
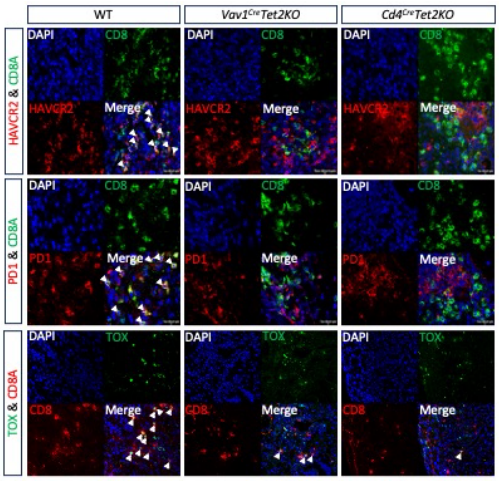
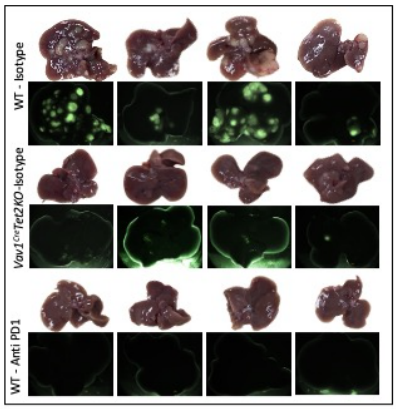


Figure 4: IF staining of exhausted markers



The immunofluorescence results demonstrated that the number of CD8-T cells expressing PDCD1 and HAVCR2 was decreased in *Vav1Cre* mice and *Cd4Cre* compared to the control mice (*Vav1Cre* and *Cd4Cre* vs control; 5.91±2.50 and 12.86±5.3 vs. 35.15±8.21 cells/100 CD8-T cells, $p<0.01$ for PDCD1; 4.51±4.06 and 13.65±7.84 vs. 24.51±9.94 cells/100 CD8-T cells, $p<0.01$ for HAVCR2). Moreover, the number of CD8-T cells expressing TOX was also significantly decreased in *Vav1Cre* mice and *Cd4Cre* compared to the control mice (*Vav1Cre* and *Cd4Cre* vs control; 5.19±7.48 and 11.31±14.43 vs. 35.15±16.50 cells/100 CD8-T cells; $p<0.01$) (Figure 4).

Figure 4: anti-PD1 antibody treatment results



To demonstrate the role of exhausted T cells in CRC liver metastasis, we used anti PD1 antibody to treat the WT mice. We found that both PD1-treated and *Vav1Cre* mice had lower TLMB than isotype-treated mice (Figure 5).

The depletion of *Tet2* gene in hematopoietic cells resulted in a reduction of exhausted CD8+ T cells and suppressed the liver metastasis of CRC organoid cells. These findings provide insights into the favorable prognosis observed in CRC patients with CH. Considering the high prevalence of CH in cancer patients, our results suggest that studying the roles of CH-immune cells could shed new light on understanding the cancer microenvironment from a novel perspective.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　3件）

１．著者名 Fujisawa M, Nguyen TB, Abe Y , Suehara Y, Fukumoto K, Suma S, Makashima K, Kaneko C, Nguyen YTM, Usuki K, Narita K, Matsue K, Nakamura N, Ishikawa S, Miura F, Ito T, Suzuki A, Suzuki Y, Mizuno S, Takahashi S, Chiba S, and Sakata-Yanigimoto M	４．巻 140 (18)
２．論文標題 Clonal germinal center B cells function as a niche for T-cell lymphoma	５．発行年 2022年
３．雑誌名 Blood	６．最初と最後の頁 1937-1950
掲載論文のDOI（デジタルオブジェクト識別子） 10.1182/blood.2022015451.	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

１．著者名 Abe Y, Sakata-Yanagimoto M, Fujisawa M, Miyoshi H, Suehara Y, Hattori K, Kusakabe M, Sakamoto T, Nishikii H, Nguyen TB, Yoshida C, Tabata R, Terao T, Owada Y, Enomoto T, Bando H, Nakayama M, Ohshima K, Usuki K, Oda T, Matsue K, and Chiba S	４．巻 24
２．論文標題 A single-cell atlas of non-haematopoietic cells in human lymph nodes and lymphoma reveals a landscape of stromal remodelling	５．発行年 2022年
３．雑誌名 Nature Cell Biology	６．最初と最後の頁 565-578
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41556-022-00866-3	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

１．著者名 Nguyen YTM, Fujisawa M, Nguyen TB, Suehara Y, Sakamoto T, Ryota M, Yoshiaki A, Fukumoto K, Hattori K, Noguchi M, Daisuke M, Chiba S, and Sakata-Yanagimoto M	４．巻 112(12)
２．論文標題 Tet2 deficiency in immune cells exacerbates tumor progression by increasing angiogenesis in a lung cancer model	５．発行年 2021年
３．雑誌名 Cancer Science	６．最初と最後の頁 4931-4943
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/cas.15165	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5件（うち招待講演　0件／うち国際学会　3件）

１．発表者名 Nguyen YTM, Fujisawa M, Nguyen TB, Suehara Y, Sakamoto T, Ryota M, Yoshiaki A, Fukumoto K, Hattori K, Noguchi M, Daisuke M, Chiba S, and Sakata-Yanagimoto M
２．発表標題 S100a8/S100a9-Emmprin-Vegfa Axis Initiated by Tet2-deficient Immune Cells Exacerbates Lung Cancer Progression through Promotion of Angiogenesis
３．学会等名 63rd American Society of Hematology Annual Meeting & Exposition（国際学会）
４．発表年 2021年

１．発表者名
Fujisawa M, Nguyen TB, Abe Y, Suehara Y, Fukumoto K, Suma S, Usuki K, Narita K, Matsue K, Nakamura N, Suzuki A, Suzuki Y, Chiba S, and Sakata-Yanagimoto M

２．発表標題
TET2-mutated germinal center B-cells drive tumorigeneis through CD40-CD40LG signaling in angioimmunoblastic T-cell lymphoma

３．学会等名
63rd American Society of Hematology Annual Meeting & Exposition（国際学会）

４．発表年
2021年

１．発表者名
Nguyen TB, Le ANT, Duan YJ, Nguyen YTM, Nakayama M, Oshima M, Sasaki Y, Sakamoto T, Fujisawa M, Chiba S and Sakata-Yanagimoto M

２．発表標題
The roles of immune cells derived from clonal hematopoiesis in colorectal liver metastasis

３．学会等名
第82回日本癌学会学術総会

４．発表年
2023年

１．発表者名
Nguyen TB, Le ANT, Duan YJ, Nguyen YTM, Nakayama M, Oshima M, Sasaki Y, Sakamoto T, Fujisawa M, Chiba S and Sakata-Yanagimoto M

２．発表標題
The roles of immune cells derived from clonal hematopoiesis in colon cancer metastasis

３．学会等名
第85回日本血液学会学術集会

４．発表年
2023年

１．発表者名
Nguyen TB, Le ANT, Duan YJ, Nguyen YTM, Nakayama M, Oshima M, Sasaki Y, Sakamoto T, Fujisawa M, Chiba S and Sakata-Yanagimoto M

２．発表標題
The roles of immune cells derived from clonal hematopoiesis in colorectal cancer metastasis

３．学会等名
65th American Society of Hematology Annual Meeting and Exposition（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

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （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