（報告様式４）

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I. 基本情報

事　業　名：（日本語）次世代がん医療創生研究事業
（英　語）Project for Cancer Research and Therapeutic Evolution

研究開発課題名：（日本語）血液がんにおける腫瘍細胞と微小環境との相互作用の分子メカニズムに基づく治療標的の照準化
（英　語）Taking aim at therapeutic targets in blood cancers focusing on molecular mechanisms that arise from the interaction between tumor cells and microenvironmental cells

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分担研究開発課題名：（日本語）血液がんにおける腫瘍細胞と微小環境との相互作用の分子メカニズムに基づく治療標的の同定
（英　語）Identification of therapeutic targets in blood cancers focusing on molecular mechanisms that arise from the interaction between tumor cells and microenvironmental cells

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分担研究開発課題名：（日本語）血液がん解析のためのモデルマウス作製
（英　語）Establishment of model mice for analysis of blood cancers

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II. 成果の概要（総括研究報告）

高橋智教授（筑波大学 医学医療系解剖学発生学）らのグループらとともに、血管免疫芽球性 T 細胞リンパ腫（AITL）および急性骨髄性白血病（AML）の治療標的を見出し、その中で臨床的に有用な治療標的を同定することを目指して研究を行った。治療標的発見に向け、腫瘍細胞内のシグナルとともに、腫瘍細胞と腫瘍微少環境細胞との相互作用シグナルにも注目した。

この目的のため、まず、AITL のモデルマウスの作製を試みた。具体的には、AITL の 70%で *TET2* 遺伝子の機能低下型変異と *RHOA* 遺伝子の G17V 変異が認められることに注目し、*Tet2* 遺伝子を血液リンパ系細胞で欠失し、T 細胞で G17V 変異 RHOA を発現するマウス（Tet2/RHOA マウス）を作製した。この系統のマウスは、大多数の個体が脾腫とリンパ節腫大を呈して死亡すること、腫大した脾臓やリンパ節では濾胞性ヘルパーT 細胞が浸潤していること、腫大リンパ節の組織学的特徴が AITL に類似していることを見出した。すなわち、AITL モデルマウス作製に成功した。一方、腫瘍発症には微少環境細胞でも *Tet2* 遺伝子が欠失する必要があることを示した。したがって、AITL をモデルとして、腫瘍細胞と腫瘍微少環境細胞との相互作用シグナルを解析する研究材料を作出した。

一方、AITL 腫瘍細胞内における治療標的も同定した。すなわち、G17V 変異 RHOA が特異的に VAV1 に結合し、VAV1 のチロシンリン酸化亢進を介して T 細胞受容体（TCR）シグナルを増強することを見出していた。本年度の研究では、*RHOA* 変異陰性の AITL の相当数で、*VAV1* 遺伝子自身に変異あるいは融合遺伝子形成が生じていることを見出した。さらに、これら変異/融合 VAV1 の機能を解析し、AITL の病態形成には G17V 変異 RHOA-VAV1 経路シグナルの亢進が重要な役割を果たしていることを明らかにした。また、G17V 変異 RHOA-VAV1 経路シグナルを抑制する薬剤が AITL の治療薬となる可能性を見出した。

さらに、T 細胞で野生型および変異/融合 VAV1 を発現するマウスも作製し、脾臓に構造異常を生じていることを明らかにした。

AML についても、患者検体の解析とマウスモデルを組み合わせて骨髄微少環境の研究に取り組んだ。患者検体の解析では、骨髄細胞からストローマ細胞を分取し、シングルセルで発現解析が可能であることを明らかにした。一方、ヒト AML 関連融合遺伝子をマウス骨髄細胞に導入し同系マウスに移植する系において、レシピエントマウスとして NOTCH シグナルが作動しない遺伝子改変マウスを用いることにより、移植後より早期に死亡することを見出した。すなわち、AML においても腫瘍細胞と微少環境細胞の相互作用が治療標的になることを明らかにした。

Together with the group led by Professor Satoru Takahashi (Department of Anatomy and Embryology, Faculty of Medicine, University of Tsukuba), we performed research aiming at discovering therapeutic targets and amongst them identifying clinically available ones in angioimmunoblastic T-cell lymphoma (AITL) and acute myelogenous leukemia (AML).

For these purposes, we first intended to create a mouse model for AITL. Specifically, based on the fact that both loss-of-function mutations in *TET2* and G17V *RHOA*

mutations are simultaneously found in 70% of AITL, we created mice both with *Tet2* deletion in the blood cells and lymphocytes, and with G17V-mutant RHOA expression in T cells (Tet2/RHOA mice). We found that majority of Tet2/RHOA mice died with splenomegaly and lymphadenopathy, that follicular helper T cells were infiltrated in the enlarged spleen and lymph nodes, and that the histological features of swollen lymph nodes were similar to those of AITL. Therefore, we succeeded in creating the AITL model mice. We also found that the *Tet2* gene needs to be deleted in the microenvironmental cells for the tumor development. Thus, with AITL as a model, we succeeded in creating a research material to analyze signaling occurring in the interaction between the tumor cells and environmental cells.

In another approach, we identified a therapeutic target within the tumor cells in AITL. Specifically, we had discovered that G17V-mutant RHOA specifically binds to VAV1 and enhances tyrosine phosphorylation of VAV1, which then enhances T-cell receptor (TCR) signaling. By the study performed in this fiscal year, we found that a significant proportion of AITL that are negative for *RHOA* mutations have mutations or a fusion gene formation in the *VAV1* gene. Furthermore, through the functional analysis of these mutant/fusion VAV1 proteins, we clarified the presence of a pathway consisting of G17V-mutant RHOA and VAV1. Enhancement of this pathway signaling should play a significant role in the development and pathophysiology of AITL. We further discovered a possibility that inhibitors of the G17V-mutant RHOA – VAV1 pathway could be therapeutics for AITL.

We also created mice expressing wild-type and mutant/fusion VAV1 in T cells, and found that there are structural abnormalities in the spleen of these mice.

Regarding AML, we analyzed the bone marrow microenvironment with a series of patient samples and a mouse model. In the analysis of patient samples, we isolated stromal cells and enabled expression analysis with single cells. In the mouse system, we introduced a human AML-related fusion gene into mouse bone marrow cells and transplanted these cells into syngenic mice. When genetically modified mice with blunted NOTCH signaling were used as recipients, these mice died of AML earlier than control after the transplantation. Thus, we indicated that the interaction between AML cells and bone marrow microenvironmental cells could be a therapeutic target.

III. 成果の外部への発表

（1）学会誌・雑誌等における論文一覧（国内誌　3　件、国際誌　30　件）

1. Pierini A*, Nishikii H*, Baker J, Kimura T, Kwon H-S, Pan Y, Chen Y, Alvarez M, Strober W, Velardi A, Shizuru JA, Wu JY, Chiba S, Negrin RS. Foxp3+ regulatory T cells maintain the bone marrow microenvironment for B cell lymphopoiesis. ***Nat Commun.***, in press (*equal contribution)

2. Shichita T, Ito M, Morita R, Komai K, Noguchi Y, Ooboshi H, Koshida R, Takahashi S, Kodama T, Yoshimura A. MAFB prevents excess inflammation after ischemic stroke by accelerating clearance of damage signals through MSR1. ***Nat Med***. 2017, Apr 10. doi: 10.1038/nm.4312. [Epub ahead of print]
3. Ku CJ, Sekiguchi JM, Panwar B, Guan Y, Takahashi S, Yoh K, Maillard I, Hosoya T, Engel JD. GATA3 abundance is a critical determinant of T cell receptor beta allelic exclusion. ***Mol Cell Biol***. 2017, Mar 20. pii: MCB.00052-17. doi: 10.1128/MCB.00052-17. [Epub ahead of print]
4. Nguyen TB , Sakata-Yanagimoto M, Asabe Y, Matsubara D, Kano J, Yoshida K, Shiraishi Y, Chiba K, Tanaka H, Miyano S, Izutsu K, Nakamura N, Takeuchi K, Miyoshi H, Ohshima K, Minowa T, Ogawa S, Noguchi M, Chiba S. Identification of cell-type-specific mutations in nodal T-cell lymphomas. ***Blood Cancer J***. 2017, 7(1):e516.
5. Yagishita Y, Uruno A, Fukutomi T, Saito R, Saigusa D, Pi J, Fukamizu A, Sugiyama F, Takahashi S, Yamamoto M. Nrf2 Improves Leptin and Insulin Resistance Provoked by Hypothalamic Oxidative Stress. ***Cell Rep***. 2017, 18(8):2030-44.
6. Tokue M, Ikami K, Mizuno S, Takagi C, Miyagi A, Takada R, Noda C, Kitadate Y, Hara K, Mizuguchi H, Sato T, Taketo MM, Sugiyama F, Ogawa T, Kobayashi S, Ueno N, Takahashi S, Takada S, Yoshida S. SHISA6 Confers Resistance to Differentiation-Promoting Wnt/β-Catenin Signaling in Mouse Spermatogenic Stem Cells. ***Stem Cell Rep***. 2017, 8(3):561-75.
7. Morita H, Yamaguchi A, Shiba D, Shirakawa M, Takahashi S. Impact of a simulated gravity load for atmospheric reentry, 10 g for 2 min, on conscious mice. ***J Physiol Sci***. 2017, Feb 9. doi: 10.1007/s12576-017-0526-z. [Epub ahead of print]
8. Makishima H, Yoshizato T, Yoshida K, Sekeres MA, Radivoyevitch T, Suzuki H, Przychodzen B, Nagata Y, Meggendorfer M, Sanada M, Okuno Y, Hirsch C, Kuzmanovic T, Sato Y, Sato-Otsubo A, LaFramboise T, Hosono N, Shiraishi Y, Chiba K, Haferlach C, Kern W, Tanaka H, Shiozawa Y, Gómez-Seguí I, Husseinzadeh HD, Thota S, Guinta KM, Dienes B, Nakamaki T, Miyawaki S, Sauntharajah Y, Chiba S, Miyano S, Shih LY, Haferlach T, Ogawa S, Maciejewski JP. Dynamics of clonal evolution in myelodysplastic syndromes. ***Nat Genet***. 2017, 49(2):204-12.
9. Nishizawa S, Sakata-Yanagimoto M, Hattori K, Muto H, Nguyen T, Izutsu K, Yoshida K, Ogawa S, Nakamura N, Chiba S. BCL6 locus is hypermethylated in angioimmunoblastic T-cell lymphoma. ***Int J Hematol***. 2017, 105(4):465-9.
10. Chiba S. Dysregulation of TET2 in hematologic malignancies. ***Int J Hematol***. 2017, 105(1):17-22.
11. Koshida R, Oishi H, Hamada M, Takei Y, Takahashi S. MafB is required for development of the hindbrain choroid plexus. ***Biochem Biophys Res Commun***. 2017, 483(1):288-93.
12. Ogawa Y, Irukayama-Tomobe Y, Murakoshi N, Kiyama M, Ishikawa Y, Hosokawa N, Tominaga H, Uchida S, Kimura S, Kanuka M, Morita M, Hamada M, Takahashi S, Hayashi Y, Yanagisawa M. Peripherally administered orexin improves survival of mice with endotoxin shock. ***Elife***. 2016, 5. pii: e21055.

13. Inoue A, Matsumoto I, Tanaka Y, Umeda N, Takai C, Kawaguchi H, Ebe H, Yoshida H, Matsumoto Y, Segawa S, Takahashi S, Sumida T. TIARP attenuates autoantibody-mediated arthritis via the suppression of neutrophil migration by reducing CXCL2/CXCR2 and IL-6 expression. ***Sci Rep***. 2016, 6:38684.
14. Nishikii H, Kim BS, Yokoyama Y, Chen Y, Baker J, Pierini A, Alvarez M, Mavers M, Maas-Bauer K, Pan Y, Chiba S, Negrin RS. DR3 signaling modulates the function of Foxp3+ regulatory T cells and the severity of acute graft-versus-host disease. ***Blood***. 2016, 128(24):2846-58.
15. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, Ma J, Nakane S, Tomita J, Ikkyu A, Kakizaki M, Hotta-Hirashima N, Kanno S, Komiya H, Asano F, Honda T, Kim SJ, Harano K, Muramoto H, Yonezawa T, Mizuno S, Miyazaki S, Connor L, Kumar V, Miura I, Suzuki T, Watanabe A, Abe M, Sugiyama F, Takahashi S, Sakimura K, Hayashi Y, Liu Q, Kume K, Wakana S, Takahashi JS, Yanagisawa M. Forward-genetics analysis of sleep in randomly mutagenized mice. ***Nature***. 2016, 539(7629):378-83.
16. Hagihara M, Iriyama N, Yoshida C, Wakita H, Chiba S, Okamoto S, Kawakami K, Takezako N, Kumagai T, Inokuchi K, Ohyashiki K, Taguchi J, Yano S, Igarashi T, Kouzai Y, Morita S, Sakamoto J, Sakamaki H. Association of pleural effusion with an early molecular response in patients with newly diagnosed chronic-phase chronic myeloid leukemia receiving dasatinib: Results of a D-First study. ***Oncol Rep***. 2016, 36(5):2976-82.
17. Huang CW, Hong TW, Wang YJ, Chen KC, Pei JC, Chuang TY, Lai WS, Tsai SH, Chu R, Chen WC, Sheen LY, Takahashi S, Ding ST, Shen TL. Ophiocordyceps formosana improves hyperglycemia and depression-like behavior in an STZ-induced diabetic mouse model. ***BMC Complement Altern Med***. 2016, 16(1):310.
18. Ninomiya H, Obara N, Chiba S, Usuki K, Nishiwaki K, Matsumura I, Shichishima T, Okamoto S, Nishimura JI, Ohyashiki K, Nakao S, Ando K, Kanda Y, Kawaguchi T, Nakakuma H, Harada D, Akiyama H, Kinoshita T, Ozawa K, Omine M, Kanakura Y. Interim analysis of post-marketing surveillance of eculizumab for paroxysmal nocturnal hemoglobinuria in Japan. ***Int J Hematol***. 2016, 104(5):548-58.
19. Nagao R, Kikuti YY, Carreras J, Kikuchi T, Miyaoka M, Matsushita H, Kojima M, Ando K, Sakata-Yanagimoto M, Chiba S, Nakamura N. Clinicopathologic analysis of angioimmunoblastic T-cell lymphoma with or without RHOA G17V mutation using formalin-fixed paraffin-embedded sections. ***Am J Surg Pathol***. 2016, 40(8):1041-50.
20. Tran MT, Hamada M, Nakamura M, Jeon H, Kamei R, Tsunakawa Y, Kulathunga K, Lin YY, Fujisawa K, Kudo T, Takahashi S. MafB deficiency accelerates the development of obesity in mice. ***FEBS Open Bio***. 2016, 6(6):540-7.
21. Khoa le TP, Azami T, Tsukiyama T, Matsushita J, Tsukiyama-Fujii S, Takahashi S, Ema M. Visualization of the Epiblast and Visceral Endodermal Cells Using Fgf5-P2A-Venus BAC Transgenic Mice and Epiblast Stem Cells. ***PLoS One***. 2016, 11(7):e0159246.
22. Nakagawa Y, Oikawa F, Mizuno S, Ohno H, Yagishita Y, Satoh A, Osaki Y, Takei K, Kikuchi T, Han SI, Matsuzaka T, Iwasaki H, Kobayashi K, Yatoh S, Yahagi N, Isaka M, Suzuki H, Sone H, Takahashi S, Yamada N, Shimano H. Hyperlipidemia and hepatitis in liver-specific

CREB3L3 knockout mice generated using a one-step CRISPR/Cas9 system. ***Sci Rep***. 2016, 6:27857.

23. Kataoka K, Shiraishi Y, Takeda Y, Sakata S, Matsumoto M, Nagano S, Maeda T, Nagata Y, Kitanaka A, Mizuno S, Tanaka H, Chiba K, Ito S, Watatani Y, Kakiuchi N, Suzuki H, Yoshizato T, Yoshida K, Sanada M, Itonaga H, Imaizumi Y, Totoki Y, Munakata W, Nakamura H, Hama N, Shide K, Kubuki Y, Hidaka T, Kameda T, Masuda K, Minato N, Kashiwase K, Izutsu K, Takaori-Kondo A, Miyazaki Y, Takahashi S, Shibata T, Kawamoto H, Akatsuka Y, Shimoda K, Takeuchi K, Seya T, Miyano S, Ogawa S. Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. ***Nature***. 2016, 534(7607):402-6.
24. Al-Soudy AS, Nakanishi T, Mizuno S, Hasegawa Y, Shawki HH, Katoh MC, Basha WA, Ibrahim AE, El-Shemy HA, Iseki H, Yoshiki A, Hiromori Y, Nagase H, Takahashi S, Oishi H, Sugiyama F. Germline recombination in a novel Cre transgenic line, Prl3b1-Cre mouse. ***Genesis***. 2016, 54(7):389-97.
25. Miyai M, Hamada M, Moriguchi T, Hiruma J, Kamitani-Kawamoto A, Watanabe H, Hara-Chikuma M, Takahashi K, Takahashi S, Kataoka K. Transcription Factor MafB Coordinates Epidermal Keratinocyte Differentiation. ***J Invest Dermatol***. 2016, 136(9):1848-57.
26. Hasegawa Y, Hoshino Y, Ibrahim AE, Kato K, Daitoku Y, Tanimoto Y, Ikeda Y, Oishi H, Takahashi S, Yoshiki A, Yagami K, Iseki H, Mizuno S, Sugiyama F. Generation of CRISPR/Cas9-mediated bicistronic knock-in ins1-cre driver mice. ***Exp Anim***. 2016, 65(3):319-27.
27. Hattori K, Sakata-Yanagimoto M, Okoshi Y, Goshima Y, Yanagimoto S, Nakamoto-Matsubara R, Sato T, Noguchi M, Takano S, Ishikawa E, Yamamoto T, Matsumura A, Chiba S. MYD88 (L265P) mutation is associated with an unfavourable outcome of primary central nervous system lymphoma. ***Br J Haematol***. 2016, Epub ahead of print on May 10 (doi: 10.1111/bjh.14080.)
28. Sakata-Yanagimoto M, Yokoyama Y, Muto H, Obara N, Kurita N, Kato T, Hasegawa Y, Miyazaki Y, Kurokawa M, Chiba S. A nationwide survey of co-occurrence of malignant lymphomas and myelodysplastic syndromes/myeloproliferative neoplasms. ***Ann Hematol***. 2016, 95(5):829-30.
29. Daassi D, Hamada M, Jeon H, Imamura Y, Nhu Tran MT, Takahashi S. Differential expression patterns of MafB and c-Maf in macrophages in vivo and in vitro. ***Biochem Biophys Res Commun***. 2016, 473(1):118-24.
30. Shawki HH, Kigoshi T, Katoh Y, Matsuda M, Ugboma CM, Takahashi S, Oishi H, Kawashima A. Identification, localization, and functional analysis of the homologues of mouse CABS1 protein in porcine testis. ***Exp Anim***. 2016, 65(3):253-65.

1. 千葉　滋. 骨髄系腫瘍とリンパ系腫瘍における TET2 変異の意義. 臨床血液 57(12):2554-2563, 2016
2. 坂田（柳元）麻実子, 千葉　滋. 血管免疫芽球性 T リンパ腫発症の分子学的な発症機序. 臨床血液 57（8）: 1038-1042, 2016
3. 千葉　滋. 骨髄系腫瘍とリンパ系腫瘍における TET2 変異の意義. 臨床血液 57（6）: 715-722, 2016

（2）学会・シンポジウム等における口頭・ポスター発表

1. Generation of Knock-in mice by CRISPR/Cas9 transfer into fertilized eggs. Invited Speaker, Satoru Takahashi, The 20th US-Japan Cellular and Gene Therapy Conference, FDA White Oak campus, Silver Spring, USA, 2017/03/09, 国外.
2. Mechanisms of lymphomagenesis by mutant RHOA. Invited Speaker, Shigeru Chiba, 2017 US-Japan Symposium on Normal/Malignant Hematopoiesis and Novel Therapies for Hematologic Malignancies, Waikoloca, HI, USA, 2017/02/23, 国外.
3. Recurrent VAV1 Abnormalities in Angioimmunoblastic T Cell Lymphoma. Poster, Daisuke Komori, Mamiko Sakata-Yanagimoto, Sharna Tanzima Nuhat, Kota Fukumoto, Manabu Fujisawa, Shoko Nishizawa, Kosei Matsue, Koji Izutsu, Naoya Nakamura, Kenichi Yoshida, Seishi Ogawa, Shigeru Chiba, The American Society of Hematology 58th Annual Meeting and Exposition, San Diego, CA, USA, 2016/12/05, 国外
4. Generation of genetically modified mice by using CRISPR/Cas9 system. Invited Speaker, Satoru Takahashi, The 13th Nikko International Symposium, Jichi University, Koyama, Tochigi, 2016/10/28, 国内.
5. Combined loss of Tet2 and Tet3 induces AML sensitive to hypomethylating agents. Oral, Koichiro Maie, Mamiko Sakata-Yanagimoto, Motohiko Oshima, Takayasu Kato, Hideharu Muto, Enguerran Mouly, Olivier A. Bernard, Haruhiko Koseki, Atsushi Iwama, Shigeru Chiba, The 78th Annual Meeting of the Japanese Society of Hematology, Yokohama, 2016/10/14, 国内.
6. TET2 mutations: a predisposing factor for myeloid and T-cell malignancies. Oral, Mamiko Sakata-Yanagimoto, Koichiro Maie, Bich Tran Nguyen, Shigeru Chiba, The 75th Annual Meeting of the Japanese Cancer Association, Yokohama, 2016/10/08, 国内.
7. MafB regulates tumor growth through controlling number of tumor-associated macrophages. Invited Speaker, Satoru Takahashi, The 75th Annual Meeting of the Japanese Cancer Association, Yokohama, 2016/10/07, 国内.
8. Integrity of TET dioxygenase activity determines leukemic transformation. Poster, Koichiro Maie, Mamiko Sakata-Yanagimoto, Motohiko Oshima, Yaeko Nakajima, Hirotaka Matsui, Takayasu Kato, Hideharu Muto, Haruhiko Koseki, Atsushi Iwama, Shigeru Chiba, The 75th Annual Meeting of the Japanese Cancer Association, Yokohama, 2016/10/06, 国内.
9. Imaging pancreatic b cell development and diabetes. Invited Speaker, Satoru Takahashi, RIKEN BRC Summer course, Riken BRC, Tsukuba, Ibaraki, 2016/07/25, 国内.
10. Aging and TET Dioxygenases in Leukemo/lymphomagenesis. Invited Speaker, Shigeru Chiba, The 5th JCA-AACR Special Joint Conference, Urayasu, Chiba, 2016/07/14, 国内.
11. Introduction of Laboratory Animal Resource Center, University of Tsukuba. Invited Speaker, Satoru Takahashi, CU-IACUC join symposium, Cairo University, Cairo, Egypt, 2016/04/23, 国外.

（3）「国民との科学・技術対話社会」に対する取り組み

該当なし

（4）特許出願

公開対象なし。