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　２ 年　 ６ 月 １７ 日現在

機関番号：１２１０２
研究種目：若手研究
研究期間：2018～2019
課題番号：１８Ｋ１６０７７

研究課題名（和文）Modeling Angioimmunoblastic T-cell lymphoma in mouse based on its genetic lesions

研究課題名（英文）Modeling Angioimmunoblastic T-cell lymphoma in mouse based on its genetic lesions

研究代表者

ＮＧＵＹＥＮ　ＢＩＣＨ・ＴＲＡＮ（NGUYEN, BICH-TRAN）

筑波大学・医学医療系・研究員

研究者番号：１０８１２９０１

交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：RHOAとTET2の機能喪失型変異の共存、及び Angioimmunoblastic T-cell lymphoma (AITL) 患者におけるVAV1変異が確認されました。AITLの病因を明らかにするために、これらの遺伝的障害に基づいてモデルマウスを作ります。Tet2-/-RHOATgマウスには、T細胞受容体の情報伝達経路の規制緩和により、AITL- likeリンパ腫が発症しました。ダサチニブがAITLの治療に効果的であることがわかりました。p53-/-VAV1Tgマウスには成熟な細胞の腫瘍を含むT-cell neoplasmが発症しました。Myc経路の異常これらの腫瘍で発見されました。

研究成果の学術的意義や社会的意義
The roles of common genetic lesions in human T-cell lymphoma (TCN) were clarified. Using these mice for testing some potential drugs, we have found that dasatinib was effective in mouse TCN treatment. Our results may be useful for TCN treatment and improve the prognosis of this intractable disease.

研究成果の概要（英文）：Genetic analysis has identified the co-exist of TET2 loss-of-function and RHOA mutations, and VAV1 mutations in Angioimmunoblastic T-cell lymphoma (AITL), an aggressive T-cell neoplasm (TCN). To clarify the AITL pathogenesis, we generated mouse models based on these genetic lesions. Mice expressing Tet2 deficiency and RHOA mutant (Tet2-/-RHOATg) and mice expressing p53 deficiency and VAV1 mutant (p53-/- VAV1Tg) were generated. Tet2-/-RHOATg mice developed AITL-like lymphomas due to deregulated T-cell receptor signaling pathway. We found that dasatinib, a multikinase inhibitor, was effective in the treatment of mouse AITL. p53-/- VAV1Tg mice developed TCN mimicked human peripheral T-cell lymphomas while p53-/- developed immature TCN. Enrichment of Myc-related pathways and somatic copy number alterations of Myc locus were found in these tumors. The combination of Tet2 deficiency and RHOA mutant or the one of p53 deficiency and VAV1 mutants led to the development of TCN in mice.

研究分野：Hematology-Oncology

キーワード：AITL　dasatinib　RHOA　TET2　VAV1

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive T-cell neoplasm (TCN). Genetic analysis has identified the co-exist of a *RHOA* hotspot mutation (RHOAG17V) and *TET2* loss-of-function mutations in 70% of AITL patients (Sakata-Yanagimoto et al., 2014). In the other hand, *VAV1* mutations including *VAV1* deletions in C-terminal SH3 domain (V-Del) and fusion of *VAV1* lacking the C-terminal SH3 domain with various partner genes mutations (V-Fus) have been found in 8% of non *RHOA*-mutated patients (Fujisawa et al., 2018). However, the role of these genetic lesions in AITL has not been clarified yet.

２．研究の目的

In order to elucidate the AITL pathogenesis, we modeled AITL in mouse based on these genetic lesions.

３．研究の方法

Mice expressing RHOAG17V and VAV1 mutants (VAV1Tg) under CD2 promoter were generated. VAV1Tg RHOAG17V mice were crossed with MxCreTet2f/f mice and pIpC was given to obtain Tet2 deficiency and RHOAG17V mutant (Tet2-/-RHOAG17V) mice. VAV1Tg mice were crossed with p53-/- mice to get p53 deficiency and VAV1 mutant (p53-/- VAV1Tg) mice. Tumor acquired from tumor-developed mice were analyzed by pathology, flowcytometry and whole transcriptome assays. Tumor cells were transplanted in immunodeficient nude mice and dasatinib or JQ1 were tested in the tumor-engrafted mice.

４．研究成果

Tet2$^{-/-}$G17VRHOA mice had splenomegaly, lymphadenopathy and died around 40 week old. The lymph nodes and the spleens showed diffuse infiltration of tumor cells expressing T_{FH} markers, i.e., CD4, PD1, and ICOS. Whole transcriptome analysis followed by gene-set enrichment analysis revealed enrichment of Il-2/STAT5, Il-6/JAK-STAT3, and TNFα/NFκB signaling pathways in Tet2$^{-/-}$G17VRHOA CD4^{+} spleen cells. The serum concentrations of Il-2, Il-6, and TNFα in Tet2$^{-/-}$G17VRHOA were significantly higher than control at the same age ($p<0.05$). Tumor cells were oligoclonal and transplanted into nude mice. Mice given with dasatinib starting at 10 days after the transplantation showed higher overall survival compared to the vehicle control.

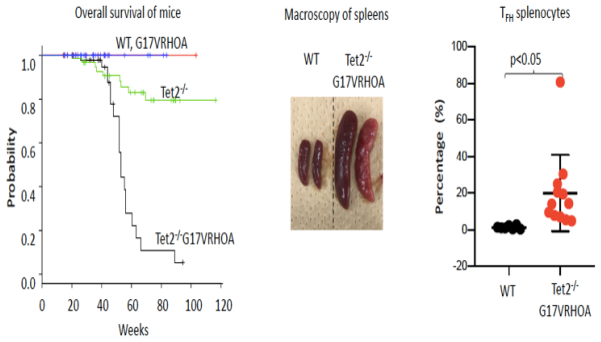
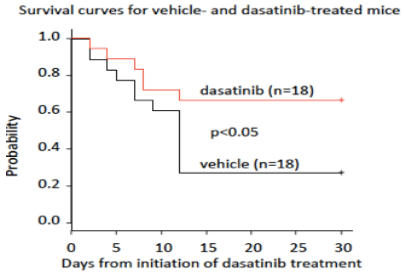


Figure 1: Tet2$^{-/-}$G17VRHOA mice developed AITL-like lymphomas



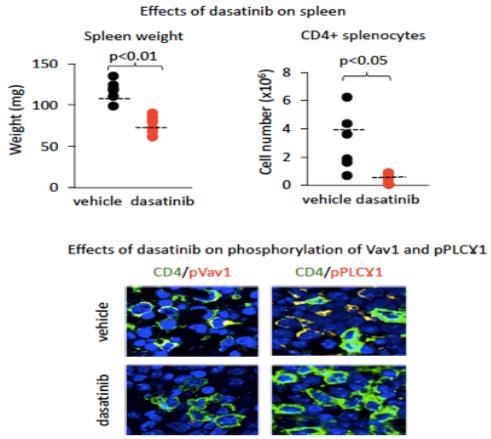


Figure 2: Effect of dasatinib treatment on AITL-like lymphoma- developed mice

No tumors in VAV1Tg mice for up to one year. However, tumors did develop in comparably-aged mice in p53$^{-/-}$ VAV1-Tg and those mice died with shorter latencies than did p53$^{-/-}$ mice. Notably, various TCN with tendency of maturation developed in p53$^{-/-}$ VAV1-Tg mice, while p53$^{-/-}$ mice exhibited only immature TCN. Mature TCN in p53$^{-/-}$ VAV1-Tg mice mimicked human peripheral T-cell lymphoma (PTCL)-GATA3 and exhibited features of type 2

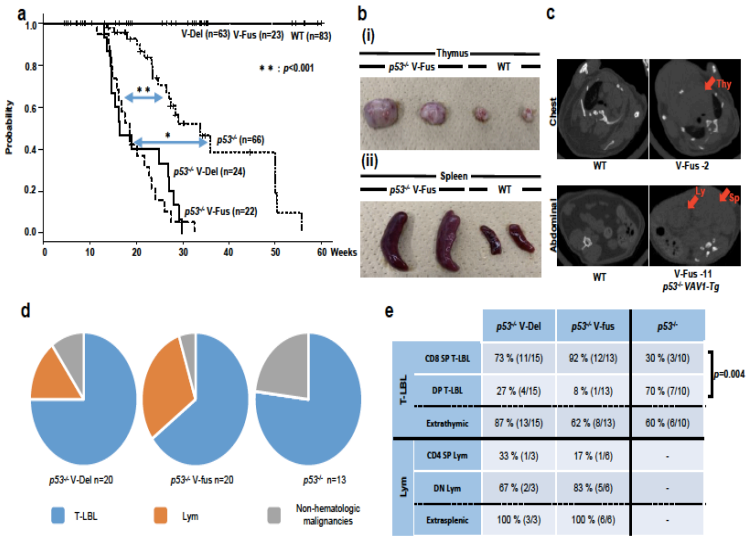


Figure 3: Tumor developments in VAV1Tg, p53$^{-/-}$ VAV1-Tg, and p53$^{-/-}$ mice

T helper (TH2) cells. Phenotypes seen following transplantation of either p53$^{-/-}$ VAV1 or p53$^{-/-}$ cells into nude mice were comparable, indicating cell-autonomous tumor-initiating capacity. Whole transcriptome analysis showed enrichment of multiple Myc-related pathways in TCN from p53$^{-/-}$ VAV1-Tg mice relative to p53$^{-/-}$ or wild-type T cells. Remarkably, focal somatic copy number alterations, including at the Myc locus, were found recurrently in TCN of p53$^{-/-}$ VAV1-Tg mice. Treatment of nude mice transplanted with p53$^{-/-}$ VAV1-Tg tumor cells with JQ1, a bromodomain inhibitor, which targets the Myc pathway, prolonged animal survival.

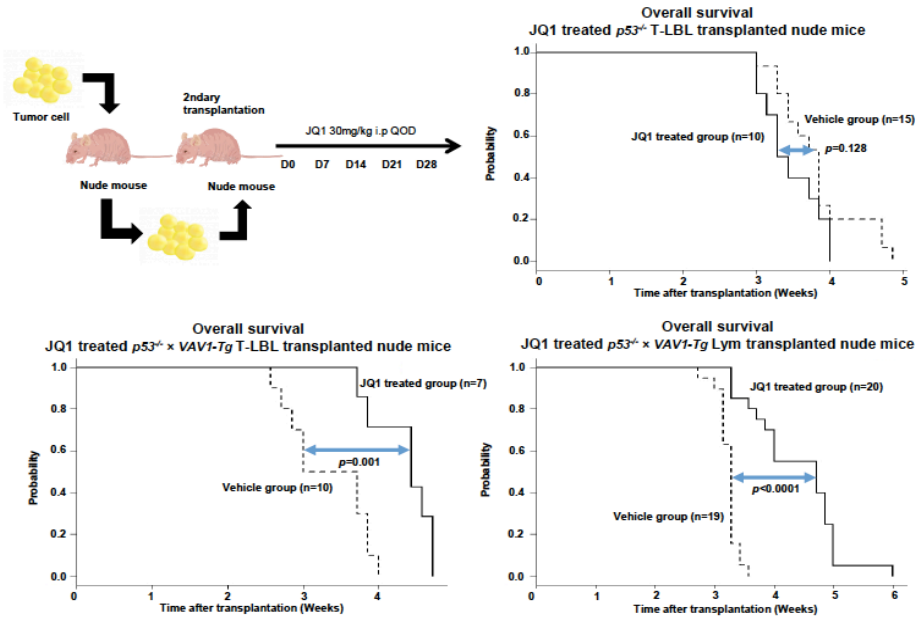


Figure 4: Effects of JQ1 on VAV1-mutant tumor cells

The combination of RHOAG17V mutant and Tet2 deficiency or the one of VAV1 mutants and p53 deficiency led to the development of TCN in mice. These mouse models may be useful to examine novel treatment strategies for TCN.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Sakurako Suma,Mamiko Sakata-Yanagimoto, Tran B. Nguyen, Keiichiro Hattori, Taiki Sato, Masayuki Noguchi,Yasuhito Nannya, Seishi Ogawa, Rei Watanabe, Manabu Fujimoto,Naoya Nakamura. Manabu Kusakabe, Hidekazu Nishikii, Takayasu Kato, Shigeru Chiba	４．巻 108
２．論文標題 Blastic plasmacytoid dendritic cell neoplasm arising from clonal hematopoiesis	５．発行年 2018年
３．雑誌名 International Journal of Hematology	６．最初と最後の頁 447-451
掲載論文のDOI（デジタルオブジェクト識別子） https ://doi.org/10.1007/s1218 5-018-2461-z	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 Tran B. Nguyen, Mamiko Sakata-Yanagimoto, Manabu Fujisawa, Kota Fukumoto, Atsushi Shinagawa, Takuya Suyama, Yuji Sato, Hidekazu Nishikii, Naoshi Obara, Manabu Kusakabe, Shintaro Yanagimoto, Seishi Ogawa, Koichi Ohshima and Shigeru Chiba	４．巻 80(9)
２．論文標題 Dasatinib is an effective treatment for angioimmunoblastic T-cell lymphoma	５．発行年 2020年
３．雑誌名 Cancer Research	６．最初と最後の頁 1875-1884
掲載論文のDOI（デジタルオブジェクト識別子） https ://doi.org/10.1158/0008-5472.CAN-19-2787	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　4件）

１．発表者名 Nguyen Bich Tran
２．発表標題 Effectiveness of Dasatinib in an Angioimmunoblastic T-cell Lymphoma Mouse Model
３．学会等名 Japanese Association of Hematology International Symposium（国際学会）
４．発表年 2018年

１．発表者名 Nguyen Bich Tran
２．発表標題 Effectiveness of Dasatinib in a T-cell Lymphoma Mouse Model
３．学会等名 Japanese Cancer Association Annual Meeting 2018（国際学会）
４．発表年 2018年

１．発表者名
Nguyen Bich Tran

２．発表標題
Effectiveness of Dasatinib in an Angioimmunoblastic T-cell Lymphoma Mouse Model

３．学会等名
Japanese Association of Hematology Annual Meeting 2018（国際学会）

４．発表年
2018年

１．発表者名
Nguyen Bich Tran

２．発表標題
Targeting T-cell Receptor signaling pathway as Angioimmunoblastic T-cell treatment

３．学会等名
Japanese Cancer Association Annual Meeting 2019（国際学会）

４．発表年
2019年

１．発表者名
Kota Fukumoto

２．発表標題
VAV1 Mutations Contributes to the Development of T-Cell Malignancies in Mice

３．学会等名
the 61st ASH Annual Meeting and Exposition, USA

４．発表年
2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

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