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　６ 年　 ６ 月 １４ 日現在

機関番号：１２６０１
研究種目：若手研究
研究期間：2022～2023
課題番号：２２Ｋ１６３１８

研究課題名（和文）Dissecting the tumor suppressive role of Utx in multiple myeloma

研究課題名（英文）Dissecting the tumor suppressive role of Utx in multiple myeloma

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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）：ヒストンデメチラーゼUTXは多発性骨髄腫(MM)患者の1.5～4%で不活化/欠失している。Utx欠損とBraf V600E変異を組み合わせ、骨髄腫マウスモデルを確立した。cIDR ドメインが UTX の腫瘍抑制機能に主に関与し、デメチラーゼ活性は重要ではないことが示された。RNAシークエンスデータから、形質細胞がMM様のトランスクリプトームを徐々に獲得する過程が認められ、UTXの非存在下でエピゲノムの再プログラミングが徐々に進行する可能性が考えられたが、MM特性を獲得した形質細胞クローンが徐々に増幅する可能性もあり、今後のさらなる検証が必要である。

研究成果の学術的意義や社会的意義
We developed a mouse model in which Utx loss and the activating Braf V600E mutation induced mature B-cell malignancies including multiple myeloma. We found that epigenomic reprogramming gradually proceeded and/or plasma cell clones that aquired myeloma-like properties were selected over time.

研究成果の概要（英文）：UTX, an X-linked histone demethylase, is inactivated/deleted in 1.5-4% of multiple myeloma (MM) patients. We established a novel myeloma mouse model in which Utx loss and Braf V600E mutation are combined. We observed a significantly shortened survival of compound mice compared to Cre negative control, single Utx loss or single Braf V600E KI. Add-back of WT or mutant UTX revealed that the cIDR domain is largely responsible for the tumor suppressor function of UTX while its demethylase activity is dispensable. Analysis of our RNA seq data from BM plasma cells from MM mice showed moderate transcriptomic reprogramming of plasma cells towards a myeloma-like transcriptome after a long latency. Our Utx/Braf mutant cells had largely different chromatin accessibility and H3K27ac status from control plasma cells. These data suggest that epigenomic reprogramming gradually proceeds in the absence of UTX and/or plasma cell clones that acquire myeloma-like properties are selected over time.

研究分野：Hematology

キーワード：myeloma　UTX　Braf V600E　epigenetics　mature B cells　mouse model

様　式　Ｃ－１９、Ｆ－１９－１（共通）

## １．研究開始当初の背景

Multiple myeloma (MM) is a neoplastic disease of terminally differentiated plasma cells. In the last decade or so, development of novel therapies for MM has significantly improved the outcome of patients. However, most patients relapse or develop resistance to available therapies (Pawlyn and Davies. Blood. 2019). Dysfunction of the epigenetic modifiers has recently been incriminated in hematologic malignancies. The histone methyltransferase EZH2, an enzymatic component of Polycomb repressive complex 2 (PRC2), is mutated in germinal center (GC) B-cell type diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) (Morin el al. Nature Genetics. 2010). In addition, EZH2 is overexpressed in MM and correlates with disease progression (Kalushkova et al. PLoS One. 2010).

UTX (ubiquitously transcribed tetratricopeptide repeat X chromosome; also known as KDM6A) counteracts the transcriptional repression mediated by PRC2 by actively removing the repressive H3K27me2/3 marks (Brand et al. Cell Stem Cell. 2019). In addition, UTX is a component of MLL3/4 COMPASS complexes (Shilatifard. Annu. Rev. Biochem. 2012) and coordinates the activation of enhancers in concert with p300/CBP histone acetyltransferases (HATs) in a demethylase activity-independent manner (Wang et al. Mol Cell. 2017) (Figure 1).

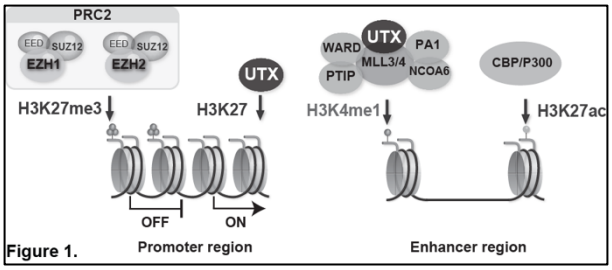


Figure 1.

In MM, inactivating somatic mutations in *UTX* were detected in up to 4% of MM patients (van Haaften et al. Nat Genet. 2009 and Pawlyn et al. Clin Cancer Res. 2016). In addition, mutations in *UTX* were found in 30-40% of human MM cell lines (Ezponda et al. Cell Reports. 2017). Importantly, poor overall survival (OS) was observed in MM patients with a *UTX* mutation or deletion (Pawlyn et al. Clin Cancer Res. 2016). These findings suggest a role as a tumor suppressor. However, the molecular consequences of UTX loss in myelomagenesis have yet to be thoroughly investigated.

## ２．研究の目的

In this study, I aimed to elucidate the tumor suppressor role of the histone demethylase Utx in the pathogenesis of multiple myeloma (MM) using conditional knock-out of *Utx* in a mouse model. To accelerate myelomagenesis, I crossed *Utx* flox and *Braf* $^{V600E}$ mice. Mutations in *KRAS*, *NRAS* and *BRAF* were found in up to 50% of newly diagnosed MM patients (Walker et al. Clin Oncol. 2015 and Blood. 2018). In order to induce conditional gene targeting in germinal center (GC), post-GC B cells and plasma cells (PCs), I crossed the *Utx/Braf* compound mice with *Cγ1Cre* transgenic strains.

## 3. 研究の方法

- Flow Cytometry and Fluorescence-Activated Cell Sorting: surface flow cytometry and cell sorting of mouse BM, spleen, enlarged lymph nodes, tumors, or body

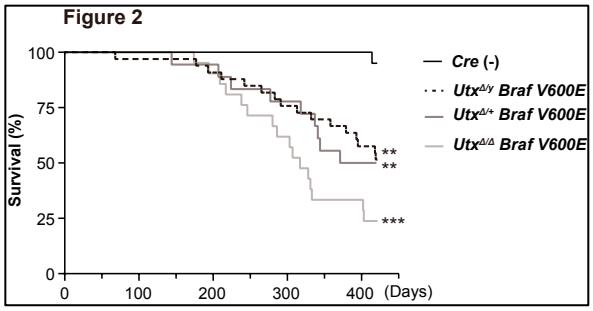
fluids.

- RNA-sequencing (RNA-seq) analysis of plasma cell samples from mice of different genotypes.
- Chromatin immunoprecipitation sequencing (ChIP-seq) for active-H3K27ac and repressive-H3K27me3 modifications.
- Assay for transposase-accessible chromatin with high-throughput sequencing (ATAC seq).
- Gene set enrichment analyses (GSEA).
- Expression of wild-type, enzymatically inactive or mutant *UTX* in *UTX*-null murine MM cells using lentiviral vectors.

## 4．研究成果

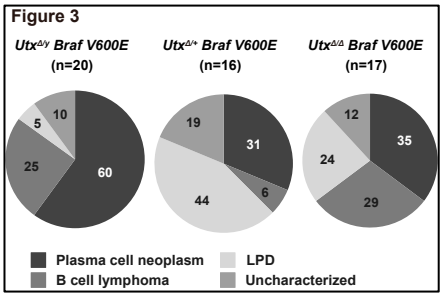
### *Utx* loss cooperates with $Braf^{V600E}$ to induce B-cell neoplasms

We found that concurrent *Utx* loss and $Braf^{V600E}$ expression significantly shortened the survival of mice compared to *Cre* negative control, single *Utx* loss, and single $Braf^{V600E}$ expression (Fig. 2). $Utx^{\Delta/\Delta}Braf^{V600E}$ females succumbed to disease earlier than $Utx^{\Delta/Y}Braf^{V600E}$ males and $Utx^{\Delta/+}Braf^{V600E}$ females. Notably, $Utx^{\Delta/\Delta}Braf^{V600E}$, $Utx^{\Delta/Y}Braf^{V600E}$, and $Utx^{\Delta/+}Braf^{V600E}$ mice displayed heterogeneous phenotypes that included plasma cell neoplasms, B cell lymphoma, and LPD, with plasma cell neoplasms having the highest frequencies in $Utx^{\Delta/\Delta}Braf^{V600E}$ and $Utx^{\Delta/Y}Braf^{V600E}$ mice (Fig.3).

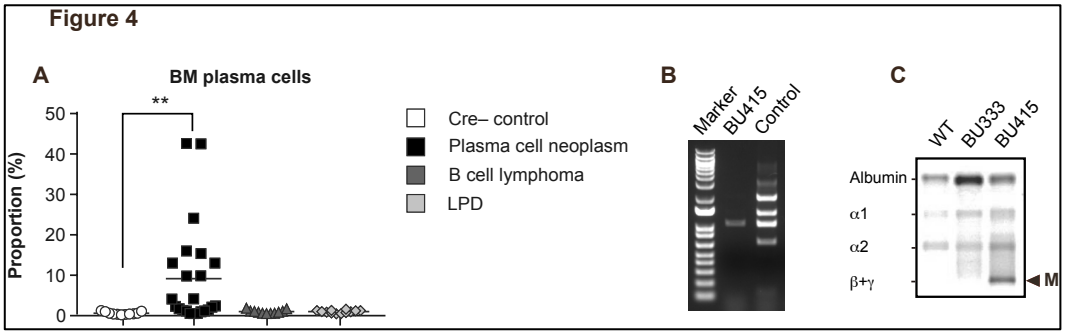



### Development of plasma cell neoplasia in *Utx* insufficient $Braf^{V600E}$ mice

The most frequent neoplasm that developed in *Utx* insufficient $Braf^{V600E}$ mice was plasma cell neoplasm: 60%, 31.3%, and 35.3% in $Utx^{\Delta/Y}Braf^{V600E}$, $Utx^{\Delta/+}Braf^{V600E}$, and $Utx^{\Delta/\Delta}Braf^{V600E}$ mice, respectively (Fig. 3). These mice showed significant increase in the percentage of plasma cells in the BM and spleen (Fig. 4A).



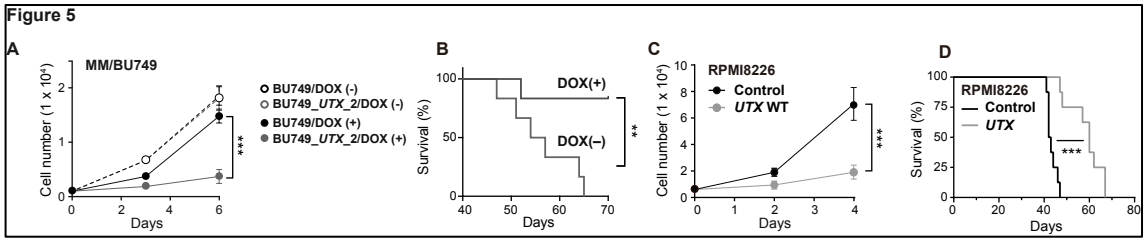

Genomic PCR analysis of purified plasma cells revealed that they were of clonal origin showing a monotonous pattern of rearrangement of the immunoglobulin heavy chain (*Igh*) gene



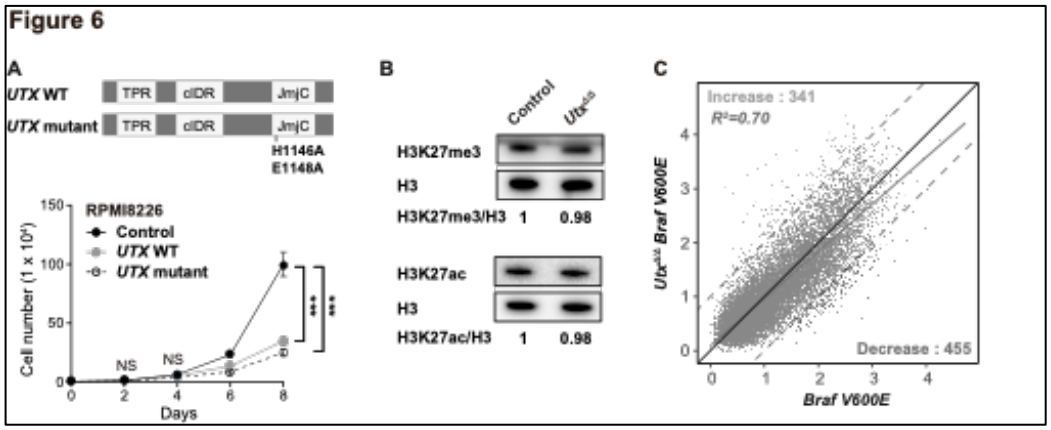

(Fig. 4B). M-spike was detected in serum protein electrophoresis (SPEP) assays in half of the mice with plasma cell neoplasms (Fig. 4C).

## Catalytic activity is dispensable for the tumor suppressor function of UTX in MM

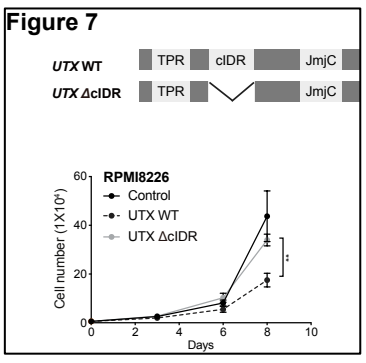
We developed a plasmacytic cell line (MM/BU749) from a moribund $Utx^{\Delta/\Delta}Braf^{V600E}$ mouse (BU-749) with an MM-like disease. We conditionally overexpressed human *UTX* in MM/BU749 cells using a Tet-on lentivirus system. Exogenous *UTX* significantly impaired the proliferation of MM/BU749 in suspension culture in the presence of doxycycline (DOX) in vitro and in vivo (Fig.5A, B). *UTX* add-back in *UTX*-null human MM cell line (RPMI8226) also impaired their growth in vitro and in vivo (Figure 5C, D).




To investigate the role of the demethylase activity of UTX in MM, we transduced RPMI8226 cells with WT and a demethylase-inactive mutant *UTX*. Of note, add-back of H1146A/E1148A mutant impaired the growth of RPMI8226 cells in a manner similar to WT UTX (Fig.6A). We then performed western blot analysis of histone modifications in plasma cells from $Utx^{\Delta/\Delta}$ mice and found no significant changes in both H3K27me3 and H3K27ac levels (Fig. 6B).



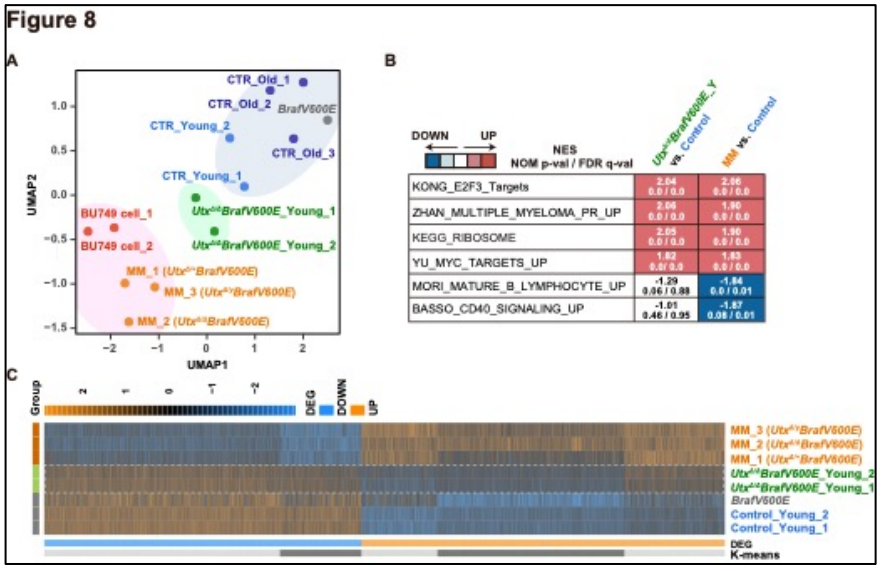

In addition, ChIP-seq analysis did not reveal any significant change in H3K27me3 levels in $Utx^{\Delta/\Delta}Braf^{V600E}$ plasma cells compared to $Braf^{V600E}$ plasma cells (Fig. 6C). These results indicate that UTX exerts a tumor suppressor function in a demethylase activity-independent manner.




Recently, it has been proposed that the tumor suppressor function of UTX largely relies on UTX condensation, higher-order assembles that are mediated by a core intrinsically disordered region (cIDR) (Shi et al. Nature. 2021). We therefore tested the add-back of ΔcIDR UTX mutant in *UTX*-null MM cells. A UTX mutant lacking cIDR mostly failed to suppress the growth of the cells (Fig. 7). These results suggest that the cIDR domain is largely responsible for the tumor suppressor function of UTX in MM.

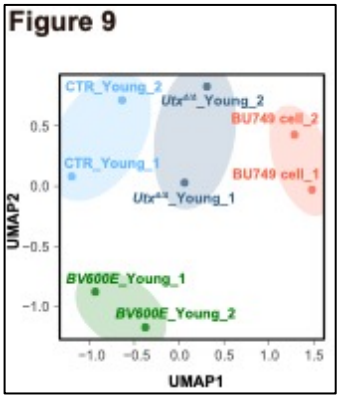
### *Utx* loss with *Braf*$^{V600E}$ induces myeloma-like gene signature in plasma cells

To understand the impact of *UTX* loss on the plasma cell transcriptome, we performed RNA-seq of plasma cells from young (20~25-week-old) and old (40~50-week-old) *Cγ1-Cre* negative control mice, *Braf*$^{V600E}$ mice (31-week-old), young *Utx*$^{Δ/Δ}$*Braf*$^{V600E}$ mice (20~25-week-old), *Utx/Braf*$^{V600E}$ mice with overt MM (MM1-3, 20~50-week-old), and BU749 (*Utx*$^{Δ/Δ}$*Braf*$^{V600E}$) cells. Clustering analysis using UMAP of RNA seq data from BMPCs showed that *Utx* loss together with *Braf*$^{V600E}$ induced moderate transcriptomic reprogramming of plasma cells towards a myeloma-like transcriptome after a long latency (Fig. 8A). Gene set enrichment analysis revealed that genes related to multiple myeloma, *Myc*, cell cycle and ribosomes were positively enriched, while gene sets associated with B lymphocytes and CD40 signal were downregulated in compound mice that developed MM and to a moderate degree in compound mice before overt disease onset (Fig. 8B). K-means clustering showed the gradual upregulation of genes related to multiple myeloma and Myc from normal plasma cells to overt myeloma (Fig. 8C).




### Characteristics of chromatin accessibility in *Utx*-null myeloma cells

To understand the transcriptional networks operating in *Utx*-null myeloma cells, we performed ATAC-seq in plasma cells from young *Cγ1-Cre* negative control, *Braf*$^{V600E}$ and *Utx*$^{Δ/Δ}$ mice (24-27-week-old), and BU749 (*Utx*$^{Δ/Δ}$*Braf*$^{V600E}$) plasma cells. UMAP analysis and hierarchical clustering of ATAC-seq data revealed that BU749 cells had largely different chromatin accessibility from control, *Braf*$^{V600E}$, and *Utx*$^{Δ/Δ}$ plasma cells, while the changes among control, *Braf*$^{V600E}$, and *Utx*$^{Δ/Δ}$ plasma cells were mild (Fig. 9).



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<br>Rizq Ola、Mimura Naoya、Oshima Motohiko、et al. | ４．巻<br>37 |
| ２．論文標題<br>UTX inactivation in germinal center B cells promotes the development of multiple myeloma with extramedullary disease | ５．発行年<br>2023年 |
| ３．雑誌名<br>Leukemia | ６．最初と最後の頁<br>1895～1907 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>10.1038/s41375-023-01928-7 | 査読の有無<br>有 |
| オープンアクセス<br>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<br>該当する |

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　3件）

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| １．発表者名<br>Ola Rizq |
| ２．発表標題<br>Concurrent Utx loss and Braf V600E expression drives myelomagenesis in mice |
| ３．学会等名<br>The 47th Annual Meeting of the Japanese Society of Myeloma（国際学会） |
| ４．発表年<br>2022年 |

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| １．発表者名<br>Ola Rizq |
| ２．発表標題<br>Utx insufficiency cooperates with BrafV600E mutation in a mouse model of human multiple myeloma |
| ３．学会等名<br>The 81st Annual Meeting of the Japanese Cancer Association（国際学会） |
| ４．発表年<br>2022年 |

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|---|
| １．発表者名<br>Ola Rizq |
| ２．発表標題<br>Chromatin and transcriptomic remodeling by Utx loss and Braf V600E mutation induces myeloma-like disease in mice |
| ３．学会等名<br>The 48th Annual Meeting of the Japanese Society of Myeloma（国際学会） |
| ４．発表年<br>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<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 |
|---|---|