The EZH2 inhibitor tazemetostat upregulates the expression of CCL17/TARC in B-cell lymphoma and enhances T-cell recruitment(Abstract_要旨)

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論文題目	The EZH2 inhibitor tazemetostat upregulates the expression of CCL17/TARC in B-cell lymphoma and enhances T-cell recruitment（EZH2 阻害剤 tazemetostat は、B 細胞リンパ腫における CCL17/TARC の発現を上昇させ、T 細胞の遊走を促進する）		

（論文内容の要旨）

The histone methyltransferase enhancer of zeste homologue 2 (EZH2) is a component of the polycomb group complex and is involved in repressing gene expression through trimethylation of histone H3 on lysine 27 (H3K27me3). An inhibitor of EZH2, tazemetostat, has been developed for the treatment of B-cell lymphoma and is approved for the treatment of Follicular lymphoma (FL) with EZH2-activating mutations. However, clinical studies have shown that it is also effective to some extent of FL without EZH2 mutations and lymphomas of other histologic subtypes.

To elucidate the biological effects of tazemetostat that are shared among B-cell lymphomas, genes targeted by tazemetostat in B-cell lymphoma cells were screened by gene microarray analysis and quantitative reverse-transcription PCR. The expression of chemokine (C-C motif) ligand 17 (CCL17) was shown to be significantly upregulated in all eleven B-cell lymphoma lines examined. CCL17 is a chemokine that is physiologically expressed in antigen-presenting cells, and induces trafficking of CCR4-positive T cells. It is also well known as a hallmark of Hodgkin/Reed-Sternberg (H/RS) cells in Hodgkin lymphoma (HL) and is responsible for the T-cell rich microenvironment of HL. Although the gene expression patterns are greatly different between H/RS cells and B cells, H/RS cells are considered to be mostly originated from germinal center B cells based on the immunoglobulin gene analysis. To examine the hypothesis that tazemetostat may have a function of altering the gene expression pattern of B-cell lymphomas to those typical of H/RS cells, gene set enrichment analysis (GSEA) was performed, and a positive correlation was found between the genes upregulated by tazemetostat in five FL cell lines and those reported to be overexpressed in H/RS cells. Chromatin immunoprecipitation (ChIP)-quantitative PCR results showed that the *CCL17* promoter region was enriched in repressive histone modification H3K27me3, and tazemetostat induced H3K27 demethylation and activated gene transcription. CCL17 secretion of B-cell lymphoma lines was also increased in parallel with the concentration of tazemetostat, which was further enhanced by CpG costimulation.

To explore whether enhancement of CCL17 production in B-cell lymphoma with tazemetostat treatment can promote chemotaxis of T cells, an in vitro transwell T-cell migration assay was performed. The results showed that the number of migrated T cells increased when the lower chamber was filled with supernatant from B-cell lymphoma cells treated with higher tazemetostat dose. In contrast, cell migration was suppressed when T cells were pretreated with CCR4-blocking antibody, indicating that T-cell migration was mainly driven by CCL17. Evaluation of migrated CD4+, CD8+ and Treg-cell subsets using an MTT assay and flow cytometry demonstrated that migrated cells contained much larger numbers of nonregulatory CD4+ and CD8+ T cells than regulatory T cells. T cells that migrated toward the supernatant of FL cells treated with tazemetostat produced higher levels of IFN-γ against FL cells, and IFN-γ production was further enhanced when PD-1 blocking antibody was added. These results suggested that T cells attracted to CCL17 produced by B-cell lymphoma can exert cytotoxicity against lymphoma cells, and the reaction is further enhanced by PD-1 blockade.

To clarify whether CCL17 can be expressed and involved in the regulation of immune microenvironment in human B-cell lymphomas, publicly available human lymphoma databases were analyzed, and CCL17 gene expression was shown to be inversely correlated with the EZH2 activation signature and significantly paralleled the CD4+ and CD8+ T-cell–rich signature in FL and germinal center B-cell–like diffuse large B-cell lymphoma.

This study indicates that tazemetostat can potentially activate anti-lymphoma response by upregulating CCL17 expression in B-cell lymphoma cells and promote T-cell recruitment, which provides a rationale for its combination with immunotherapy.

（論文審査の結果の要旨）

ヒストンメチル基転移酵素である EZH2 の阻害薬である tazemetostat は EZH2 変異陽性の濾胞性リンパ腫(FL)に対し承認されているが、臨床試験では他病型にも治療効果が示唆されており、その機序は十分解明されていない。本研究では B 細胞リンパ腫細胞株を用いたスクリーニングにより、同薬が CCL17 の発現を誘導することを見出した。CCL17 はホジキンリンパ腫(HL)で高発現するケモカインであり、FL 細胞株で tazemetostat により上昇する遺伝子群と、HL で高発現が報告されている 83 遺伝子群を比較したところ両群間で正の相関を認め、同薬は FL において HL に類似する遺伝子発現をもたらすことが示唆された。また tazemetostat は CCL17 のプロモータ領域の H3K27me3 の脱メチル化により遺伝子発現を誘導し、CCL17 分泌を惹起し、T 細胞遊走を促すことが示された。CCL17 のレセプターである CCR4 は主に Th2 細胞と制御性 T 細胞に発現するが、遊走した T 細胞はリンパ腫細胞に対する IFN-γ産生能を有し、抗腫瘍免疫を担いうると考えられた。ヒトの胚中心 B 細胞型リンパ腫の公開データベースを用いた解析により、これらの実験結果に矛盾しない結果が得られた。

以上の研究は B 細胞リンパ腫の病態および EZH2 阻害薬の奏効機序の解明に貢献し、今後の悪性リンパ腫の治療戦略の改善に寄与するところが多い。

したがって、本論文は博士（医学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 5 年 1 月 20 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降