論文内容の要旨

論文題目 Transient inhibition of NF-κB by DHMEQ induces cell death of primary effusion lymphoma without induction of HHV-8 reactivation

（DHMEQ は Primary effusion lymphoma の NF-kB 活性を一過性に阻害し HHV-8 の再活性を誘導せずに細胞死を誘導する）

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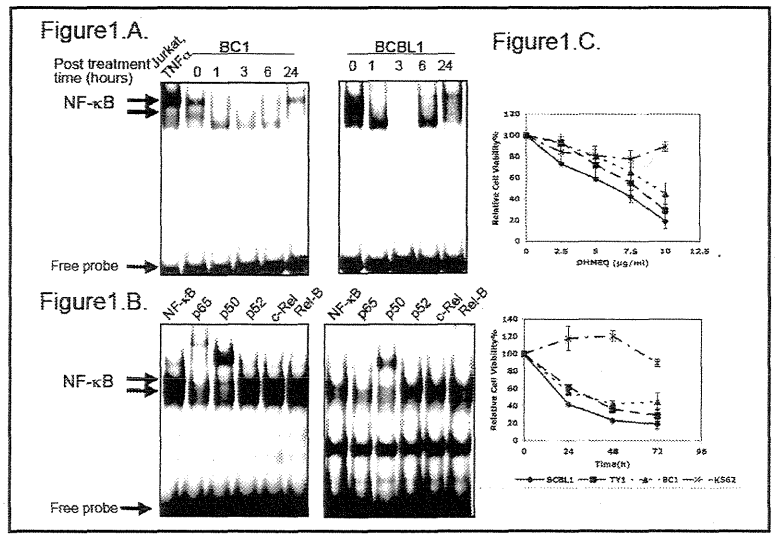
Primary effusion lymphoma (PEL) is a subtype of non-Hodgkin lymphoma that is caused by Human Herpes Virus 8 (HHV-8) infection. PEL is likely to be seen in immunocompromised individuals, such as AIDS patients. HHV-8 induces constitutive NF-κB activation that characterizes the tumor cells of PEL. PEL is resistant against conventional protocols of chemotherapy. DHMEQ, an NF-κB inhibitor, has been effective on various tumor cells, for example adult T-cell leukemia (ATL) and Hodgkin's lymphoma, which have constitutively activated NF-κB. Thus, in search for a new therapeutic modality of PEL based on the idea of molecular targeting, we examined the effect of DHMEQ on



PEL cells. We confirmed NF-κB activation and presence of p50 and p65 as its sub-components in PEL cell lines with EMSA and supershift assays (Fig. 1 A and B). DHMEQ quickly and transiently abrogated NF-κB activation in PEL cells (Fig. 1 A), which leads to reduction in the viability of the PEL cells in a dose dependent manner, that examined by WST-8 assays (Fig. 1C). Results of Annexin V staining, TUNEL assay, and fluorescent microscopic detection of cleaved caspases indicated that the reduction in the cell viability was due to the apoptosis induction through activation of both mitochondrial and membrane pathways (Fig. 2 A to C).

Figure 2.A.

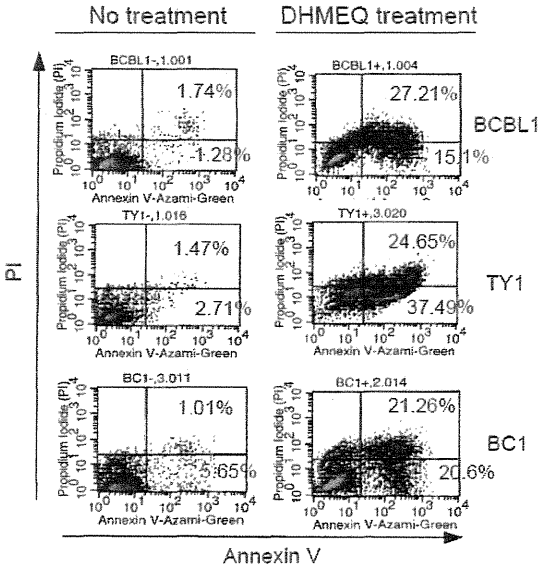


Figure 2.B.

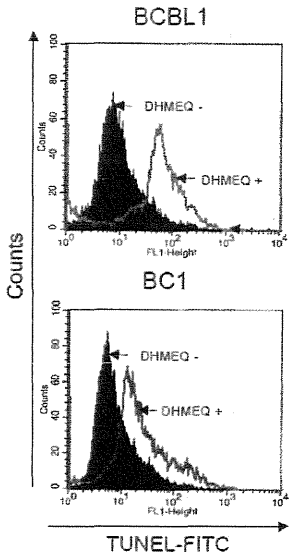
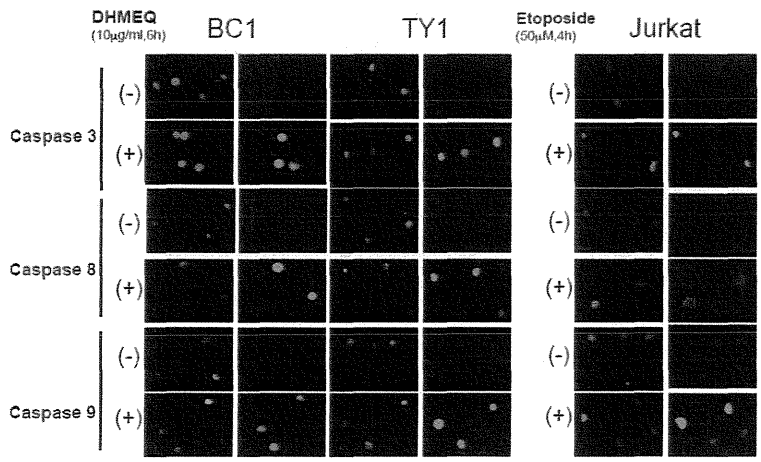
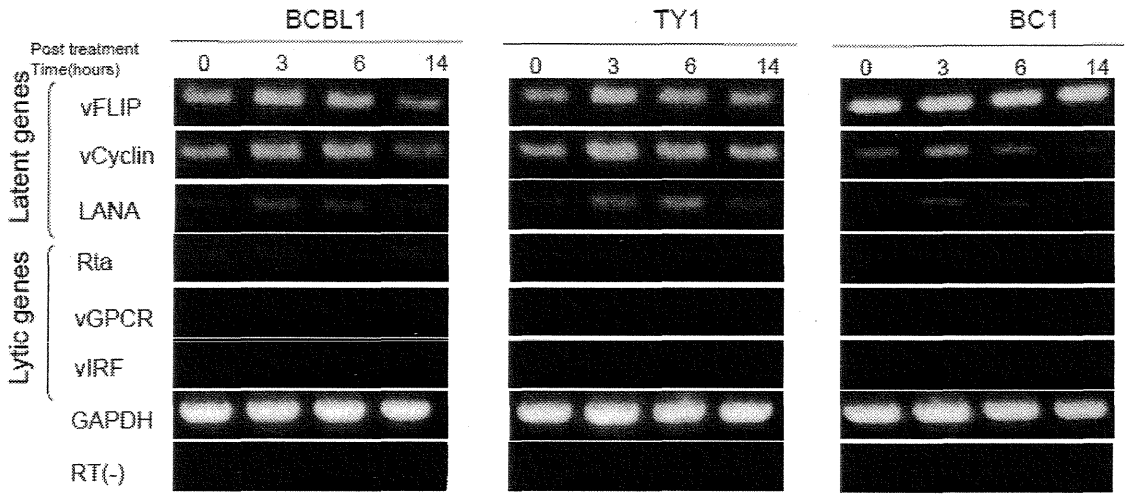


Figure 2.C.



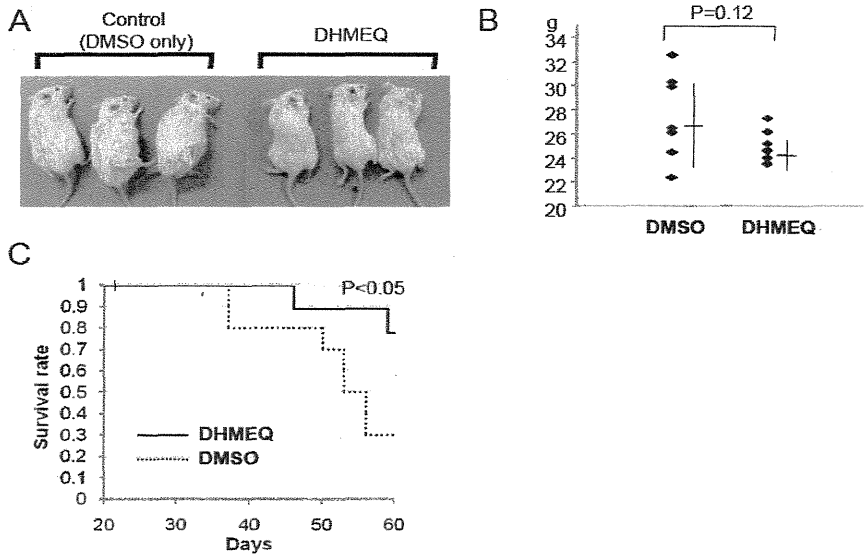
To study effects of DHMEQ treatment on whole genome, we performed DNA array analysis using Agilent array system. DHMEQ resulted in down-regulation of NF-κB target genes, such as IL6, Myc, CCR5, Bcl-xL and NF-κB1, as well as up-regulation of pro-apoptotic, stress responses, and negative regulators of cell proliferation genes. Next we tested whether HHV-8 lytic genes are activated by DHMEQ. RT-PCR analysis of selected HHV-8 genes expression pattern did not show transition from latent phase to lytic phase and virus replication (Figure 3).

Figure 3. No evidence for reactivation of HHV-8



To examine the effect of NF-kB inhibition in a xenograft model, we next tested effects of DHMEQ on TY-1 cells inoculated in SCID mice. Administration of DHEMQ in SCID mice reduced the gross appearance of tumor size in treated group, which was evident by comparing the body weights of the treated an un-treated groupds, although the difference was not statistically significant (Figure 4. A-B). On the other hand, DHMEQ treatment significantly increased the survival rate (Cox-Mantel test; $p<0.05$) (Figure 4 C).

Fig. 4. Results of the xenograft model



Taken together, our data demonstrated that DHMEQ is able to transiently abrogate the NF-κB activation and initiates the apoptosis cascade irreversibly without activation of HHV-8 replication. In addition, DHMEQ rescued the xenografted mice. Therefore, we suggest DHMEQ as a promising candidate for molecular target therapy for the PEL.