INDUCTION OF IMPDH2 AND NUCLEOLAR HYPERTROPHY ARE REQUIRED FOR GROWTH TRANSFORMATION OF RESTING B CELLS BY EBV

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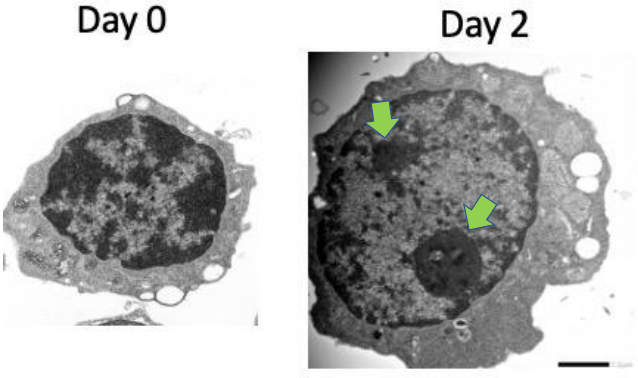
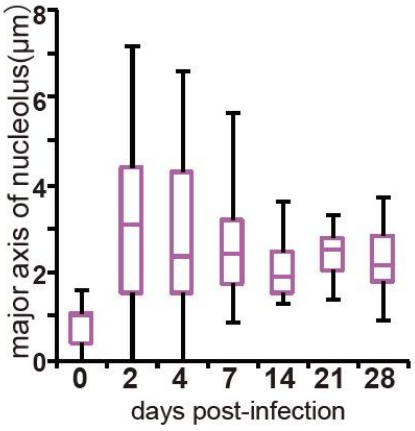
Growth transformation of primary B cells *in vitro* by Epstein-Barr virus (EBV) represents initial step of oncogenesis of post-transplant lymphoproliferative disorder (PTLD). We first carried out electron microscopic analysis and immunostaining of primary B cells infected with wild-type EBV. Sizes of cell and nucleus were enlarged as reported previously, and interestingly, we noticed that the size of nucleolus was remarkably increased by 2 days after infection. According to the recent paper [1], hypertrophy of nucleoli, which is brought about by induction of IMPDH2 gene, is required for efficient growth promotion in cancers. Indeed, our RNAseq analysis [2] revealed that the IMPDH2 gene was markedly induced, peaking at day 2. By using knockout virus of EBNA2 or LMP1, we found that EBNA2 but not LMP1 was responsible for the induction of IMPDH2 gene. Inhibitor experiment showed that MYC was also involved in the induction of IMPDH2. We also found that IMPDH2 overexpression caused increased production of GTP, pre-rRNA, and pre-tRNA levels in infected cells. Inhibition of IMPDH2 by mycophenolic acid (MPA) perfectly blocked growth transformation of primary B cells by EBV, which was associated with smaller sizes of nucleolus, nucleus, and cell. A pro-drug of MPA, Mycophenolate Mofetil (MMF) has already been approved as an immunosuppressant, and thus we tested it in mice xenograft model. Oral administration of MMF significantly decreased the tumor size, peripheral blood viral DNA level, and improved the survival of the mice. Taken together, EBV induces expression of IMPDH2 in the EBNA2/MYC-dependent manner, which causes hypertrophy of nucleoli, nuclei, and cells, and efficient growth promotion. Our results provide basic evidence that MMF suppresses PTLD when used as an immunosuppressant.

[1] Kofuji et al., 2019. Nat Cell Biol. 21:1003-14
[2] Yanagi et al., 2021. Virology. 557:44-54





Nucleolar hypertrophy after EBV infection of primary B cells. (left) Electron microscopic observation of primary B cells infected with EBV. Note that size of nucleolus increased, as well as nucleus and cell sizes. Arrowheads indicate enlarged nucleoli. (right) Quantification of major axis of nucleolus after infection.