ANTI-PROLIFERATIVE EFFECTS OF COMBINED TREATMENT WITH TERT INHIBITOR AND CHEMOTHERAPEUTIC AGENTS IN EBV-POSITIVE MALIGNANT B CELLS XENOGRAFTED IN ZEBRAFISH

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INTRODUCTION: Epstein–Barr virus (EBV)-associated malignancies, as well as lymphoblastoid cell lines (LCLs), obtained in vitro by EBV infection of B cells, maintain their ability to grow indefinitely through inappropriate activation of telomere specific reverse transcriptase (TERT), the catalytic component of telomerase. In addition to its canonical role in stabilizing telomeres, TERT may promote EBV-driven tumorigenesis through extra-telomeric functions. On this ground, our previous studies demonstrated that high levels of TERT expression in LCLs prevent the activation of EBV lytic cycle, which is instead triggered by TERT silencing, through the NOTCH2/BATF pathway, resulting in death of infected cells [1-3]. Interestingly, short-term TERT inhibition by BIBR1532 (BIBR) causes cell cycle arrest and apoptosis in LCL partly by inducing telomere length-independent activation of the DNA damage response in vitro and in xenograft model [4,5]. Importantly, TERT inhibition also sensitizes EBV-positive tumor cells to antiviral therapy and enhances the pro-apoptotic effects of chemotherapeutic agents in vitro [2,4]. Here, we investigate the possible application of TERT inhibitors in combination with antineoplastic drugs to counteract EBV-positive tumor growth *in vivo.*
METHODS: EBV-positive malignant B cells were treated with BIBR or DMSO, as control, for 24 hours (h). Cells were then labeled with CM-DiI and injected into the yolk sac of 72h post fertilization zebrafish embryos. The embryos were then placed in medium with or without drugs [2 mM cyclophosphamide (CY) or 5 µM fludarabine (FLU)]. The number of fluorescent cells was monitored in dissociated embryos by flow cytometry at 24, 48 and 72h post treatment (hpt).
RESULTS: BIBR and drug treatment decreased xenografted cells proliferation compared to control cells in untreated embryos, with BIBR showing the higher inhibitory effect at each time point. Importantly, the combination of BIBR and drugs treatment further impaired xenografted cells proliferation, with more than four-time decrease, particularly at 48hpt for CY and 72hpt for FLU.
CONCLUSIONS: TERT inhibition increases susceptibility to antineoplastic drugs, supporting the use of TERT inhibitors in combination with chemotherapeutic agents as an efficient anticancer approach for EBV-associated malignancies.

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