[S3-5] [4/19/2002(Fri) 12:00-12:25/Hall A]

Effects of Histone Deacetylase Inhibitor on Cell Gowth and Viral Gene Expression in the Cells Latently Infected with Epstein-Barr Virus

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Epstein-Barr virus (EBV) is carried by more than 90% of the adult population worldwide as a largely nonpathogenic infection. Primary infection is generally silent, but associated with infectious mononucleosis in adolescence. In vitro infection of B-lymphocytes with EBV results in transformation of infected cells into continuously proliferating lymphoblastoid cell lines (LCLs). EBV latent infection is also strongly associated with the pathogenesis of several human tumors including posttransplant lymphoproliferative disease (PTLD), Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), Hodgkin disease (HD), NK/T-cell lymphoma and stomach cancer. There are three distinct types of EBV latency, characterized by differential expression of EBV latent genes. Latency III (displayed by LCL and PTLD) expresses the full repertoire of 12 latent genes (EBNA-1, EBNA-2, EBNA-3A, -3B, -3C, EBNA-LP, LMP1, LMP2, EBER-1, EBER-2, Bam A RNAs) whereas latency I (as seen in BL) shows a restricted expression of EBNA1 and EBERs. Latency II (noted in most of non-B-lymphoid tumors such as NPC, HD, and NK/T cell lymphoma) expresses EBNA1, EBERs, LMP1, and LMP2. Since all EBNA proteins except EBNA1 efficiently elicit host CTL reactions, the exclusive expression of EBNA1 is believed to play a critical role in helping immune escape of associated tumors from immune surveillance. Such differential expression of latent genes is due to the selective usage and/or repression of EBV latent promoters. All 6 EBNA mRNAs in latency III are made from a giant transcript derived from a common Wp or Cp promoter. However, in latency I and II, the Wp and Cp are hypermethylated and inactive. Instead, a far downstream promoter Qp is active in deriving EBNA1 expression. In latency I, the LMP1 promoter is also inactive and hypermethylated. EBV latency is maintained by repressing expression of a transactivator, BZLF1 (Zta, ZEBRA), responsible for the switching from the latent state to lytic replication cycle. The BZLF promoter is known to be under a negative control of host YY1 factor.

Histone deacetylases (HDACs) is recruited to gene-specific promoters by interaction with

DNA-binding repressors such as YY1 and MeCP2, a 5-methyl cytosine binding protein in CpG methylated DNA. HDACs after recruited to a certain promoter, remove acetyl moieties from acetylated core histones and keep the inactive state of the promoter. Depression by inhibition of HDAC activity with trichostatin A (TSA) therefore alters expression of such genes, thereby leading to G1 arrest, apoptosis, and cell differentiation in cultured cells. Since EBV latency is likely to be regulated by repressors interacting with HDAC, TSA, a reversible HDAC inhibitor, is expected to alter expression of EBV genes and growth properties of latently infected cells. Especially expression of EBNA proteins with CTL epitopes repressed in type I and type II tumor is likely to enhance immunogenicity of these tumors.

This study was designed to investigate the effects of tricostatin A (TSA, a reversible HDAC inhibitor) on cell cycle progression and EBV lytic reactivation in EBV transformed LCLs (SNU-20 and SNU-1103, latency 3) and BL cell line (Akata, latency 1). BJAB (an EBV negative cell line) and EBV negative Akata cells were used as negative control cells. TSA at concentrations higher than 10nM caused cell growth inhibition as well as acetylation of histone proteins. The growth of both EBV positive and negative cells was inhibited by TSA and this seems to be due to G1, G2/M arrest and apoptosis of the cells. The effects of TSA on the expression of cell cycle related proteins were studied by RNase Protection Assay (RPA) and Western blot analysis. In BJAB, the expression of p21 was significantly increased but G1 and G2/M cyclins were not affected by TSA treatment. In LCLs, the level of p21 was unchanged while the expression of all cyclins except cyclin E was reduced by TSA treatment. The level of p57 was significantly increased by TSA in BJAB and LCLs. To analyse the effects of TSA on EBV lytic reactivation, the expression of lytic antigens were assessed by immunofluorescene assay and Western blot after TSA treatment. Expression of EBV lytic antigens were enhanced by TSA in both latency I and latency III type cell lines.

These results suggest that TSA is useful to activate EBV lytic replication cycle and that TSA causes cell cycle arrest in EBV positive cell lines as well as in EBV negative cell lines, supporting the possibility of HDAC inhibitors to be developed as anti-tumor reagents for EBV associated tumors.