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Novel expression and regulation of TIMP-1 in Epstein Barr virus-infected cells and its impact on cell survival

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ABSTRACT

Epstein Barr virus (EBV) uses various strategies to manipulate host cytokine production in favor of the survival of infected B-cells. Microarray and cytokine protein array assays revealed that tissue inhibitor of metalloproteinase-1 (TIMP-1) was significantly up-regulated in EBV-infected primary B cells and maintained in abundance in EBV-immortalized lymphoblastoid cell lines (LCLs). TIMP-1 plays critical roles in extracellular matrix homeostasis and regulates signaling pathways. In this study, we demonstrated that the EBV-encoded immediate early lytic protein, Zta, upregulates mainly TIMP-1 expression by binding to the AP-1 site within the TIMP-1 promoter. Moreover, knockdown of TIMP-1 expression promoted cisplastin and cold shock-induced death of LCLs. This study provides a mechanistic link between EBV-induced TIMP-1 expression and its impact on LCL survival.

Introduction

Epstein–Barr virus (EBV) is a human γ-herpes virus which infects more than 90% of adults worldwide (Young and Rickinson, 2004). However, primary EBV infections usually are asymptomatic. As a human oncogenic virus, EBV infection is strongly associated with many tumors, such as Burkitt's lymphoma (BL), Hodgkin's disease (HD), nasopharyngeal carcinoma (NPC) and gastric carcinoma (Hislop et al., 2007; Kuppers, 2003; Rickinson and Kieff, 2007). *In vitro*, EBV can immortalize primary resting B cells to generate lymphoblastoid cell lines (LCLs) with unlimited proliferative potential (Rickinson and Kieff, 2007). LCLs have a similar EBV gene expression profile to B cells isolated from post-transplant lymphoproliferative disorder (PTLD) (Kuppers, 2003). Thus, LCL lines can be a model to investigate the interplay between EB viral products and immortalization of B cells.

One of the unique characteristics of EBV-associated NPC is that this cancer has a strong ability to metastasize to lymphoid and other tissues (Lo et al., 2004). Our and other previous studies have shown that several EBV products can activate genes facilitating metastasis and invasion, which may be a reason for the high metastasis rate of NPC (Lo et al., 2004; Lu et al., 2003; Luo and Yao, 2013). Among these upregulated genes, matrix metalloproteinases (MMP) constitute a major family. One of the key functions of MMP is to digest the extracellular matrix and basal membrane, which is the critical step in normal tissue remodeling and also in cancer progression (Khokha et al., 2013). Of note, many EBV gene products regulate MMP gene expression. In our and other previous study, both the EBV latent membrane protein 1 (LMP1) and Zta, a viral transactivator, upregulated the expression of MMP1 and MMP9 to promote cell migration and invasion (Lu et al., 2003; Yoshizaki et al., 1998). A report from Chang's lab concluded that latent membrane protein 2A (LMP2A) can promote MMP9 expression *via* its interaction with WW domain-containing oxidoreductase (Lan et al., 2013a). Also, Zta stimulates the expression of MMP3 and MMP9, promoting cell invasion (Lan et al., 2013b). The LMP1-mediated invasive ability is through MMP9 (Murono et al., 2000). In summary, MMPs are the critical mediators of EBV-triggered cell migration and invasion.

Abbreviations: EBV, Epstein–Barr virus; LCLs, lymphoblastoid cell lines; TIMPs, Tissue inhibitors of metalloproteinases; BL, Burkitt's lymphoma; HD, Hodgkin's disease; NPC, nasopharyngeal carcinoma; MMPs, matrix metalloproteinases; PTLD, post-transplant lymphoproliferative disorder

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In addition to cytokines, other secreted proteins also play important roles in the determination of cell fate. The tissue inhibitors of metalloproteinases (TIMP) family is composed of four members (TIMP-1, 2, 3, and 4) (Brew and Nagase, 2010). Glycosylation of TIMP-1 in its N-terminal domain determines protein folding and stability and its shuttling between the cytosol and cell membrane (Caterina et al., 1998). The well known function of TIMPs is to inhibit the activity of matrix metalloproteinases (MMPs) (Stetler-Stevenson, 2008). Evidence suggests that the balance between MMPs and TIMPs plays a critical role in both the development and pathology of the epithelial–mesenchymal transition (EMT), and for homeostasis and remodeling of the extracellular matrix (Liotta et al., 1991; Zheng et al., 2009). However, recent studies have shown that TIMP-1 can function in an MMP-independent way in breast and epithelial cells, by suppressing cell death pathways and promoting cell survival (Jung et al., 2006; Liu et al., 2003, 2005; Tsagaraki et al., 2010). TIMP-1 frequently is over-expressed in several types of cancers. According to previous studies, TIMP-1 is highly expressed in EBV-positive BL, compared to EBV-negative BL cells (Guedez et al., 1998a). TIMP-1 serves as a prognostic marker in multiple myeloma (Guedez and Stetler-Stevenson, 2010). Therefore, dissecting the biological role of TIMP-1 is very important for understanding the immortalization and oncogenesis of EBV-infected cells.

This study aimed to investigate whether EBV can regulate TIMP-1 expression directly, using cDNA microarray and cytokine protein array approaches. We found that TIMP-1 was upregulated in EBV-infected primary B cells. The EBV Zta protein up-regulated TIMP-1 gene expression by binding to the AP-1 site in its promoter. Through treatment with cisplastin or stimulation of cold shock, we demonstrated that TIMP-1 exhibits anti-apoptotic activity. Thus, induction of TIMP-1 expression by EBV might enhance the survival of EBV-infected B cells and promote the development of EBV-associated malignancies.

Results

TIMP-1 was induced in EBV-infected B cells and EBV-immortalized LCLs

To identify the cytokines induced during EBV infection, cytokine antibody arrays were performed with primary B cells infected with or without EBV for 7 days. As shown in Fig. 1A, expression of TIMP-1 increased in the EBV-infected B cells. During the EBV infection of human primary B cells, the expression of viral proteins EBNA1 and Zta indicated that EBV infection is successful (Fig. 1B). So far, the role of TIMP-1 in EBV-infected B cells remains unclear. We first monitored the kinetics of TIMP-1 expression by RT-Q-PCR and ELISA during the process of EBV immortalization (Fig. 1C and D). We found that TIMP-1 transcripts were enhanced over 50 fold at day 3 post-infection and enhanced further from 70 to 160 fold at days 7 to 28 post-infection. Consistently, TIMP-1 protein was found by ELISA to be secreted into the supernatant as early as day 3 post-infection and increased further in the period of days 7 to 28 post-infection (Fig. 1D). The abundant expression of TIMP-1 RNA and proteins was also measured in ten EBV-immortalized LCLs established from different individual donors (Fig. 1E and F). These results showed that TIMP-1 expression was robustly induced during the process of EBV immortalization of primary B cells and the expression of TIMP-1 was maintained constitutively in LCLs.

Expression of TIMP-1 was compared between EBV infection and stimulation by B mitogens

In vivo, B cells can be activated by a variety of agents, including mitogens such as lipopolysaccharide (LPS), IL-4 plus anti-CD40 antibody and poly I:C. Treatment with IL-4 plus anti-CD40 antibody mimics the process of T-cell dependent B cell activation (Banchereau et al., 1991), while other treatments trigger T-cell independent B cell activation (Hoshino et al., 1999; Jiang et al., 2003). However, TIMP-1 expression was only induced by EBV infection but not by treatment with IL4 plus anti-CD40 antibody, LPS or poly I:C (Fig. 2A and B). The efficacies of other treatments were demonstrated by detecting the expression of activation-induced deaminase (AID) for IL4 plus anti-CD40 antibody, IL-8 for LPS and IFN-β for poly I:C (Fig. 2C–E). Therefore, we suggest that specific EBV viral proteins may regulate the expression of TIMP-1 expression.

The EBV lytic protein, Zta, regulates the gene expression of TIMP-1

To determine which EBV products contribute directly to the increased expression of TIMP-1, ectopic expression of various viral proteins was transduced in 293T cells and BJAB cells (Fig. 3A and B). Interestingly, compared to vector control transfectants, ectopically expressed Zta protein induced the expression of TIMP-1 in 293T cells. In BJAB cells, TIMP-1 expression was upregulated higher in Zta-expressing BJAB cells than that in LMP1 or LMP2A-expressing BJAB cells (Fig. 3B). As shown in Fig. 3C, Zta mediated induction of TIMP-1 in a dose-dependent manner. Next, we determined whether TIMP-1 can be induced by Zta in the other EBV-negative Burkitt's lymphoma cell lines, Akata, and Ramos. Consistently, expression of TIMP-1 was also induced in these cell lines (Fig. 3D). A Zta-targeted shRNA was used to confirm that Zta can mediate TIMP-1 expression in LCLs. Knockdown of Zta expression in LCLs led to a significant decrease of TIMP-1 expression (Fig. 3E). Therefore, Zta plays a critical role in inducing and maintaining the expression of TIMP-1 in LCLs and all tested cells types. Additionally, TIMP-1 could be minor induced by other viral proteins, such as LMP1 and LMP2A in BL derived cells.

Zta bound onto the AP1 site in the TIMP-1 promoter

It is known that Zta mimics a transactivator of AP-1 and regulates gene expression by directly binding to AP-1 sites on the promoters and that LMP1 may also influence the gene expression *via* AP-1 sites (Kouzarides et al., 1991; Yoshizaki et al., 1998). Six AP-1 sites were found by analyzing the sequence of the TIMP-1 promoter spanning position −1197 to −77 nt, (Fig. 4A). In order to determine whether Zta or LMP1 functions through the AP-1 site in the TIMP-1 promoter region, a series of luciferase reporters with TIMP-1 promoter fragments spanning position −1197 to −77 nt was constructed (Fig. 4A). The transfection efficiency is about 80%. An approximately 4-fold induction of the TIMP-1 promoter was seen when Zta was expressed, but LMP1 did not have any effect on TIMP-1 promoter activity in BJAB cells (Fig. 4B). To demonstrate further that Zta binds directly to this AP-1 site, a luciferase reporter was constructed with a mutated AP-1 site in the TIMP-1 promoter. Importantly, activity of the luciferase reporter with the mutated AP-1 site was completely abolished in Zta expressing cells (Fig. 4B), indicating that this AP-1 site is required for Zta transactivation. An EMSA was performed in Zta expressing BJAB cells to determine whether Zta can bind directly to the TIMP-1 promoter. Complexes of Zta and DNA were detected in cells transfected with the Zta expressing plasmid (Fig. 4C, lane 3). In contrast, the complexes of Zta and DNA were not detectable in the presence of the vector control (Fig. 4C, lane 2), labeled mutant probe (Fig. 4C, lane 4) and a 64-fold excess of unlabeled wild type probe (Fig. 4C, lane 10). Furthermore, the addition of excess non-labeled mutant oligonucleotides did not affect the interaction of Zta and the wild type probe (Fig. 4C, land 11). Taken together, Zta bind specifically to the AP-1 site of the TIMP-1 promoter in the region −92 to −86 nt.