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Pathologic Significance of EBV Encoded RNA in NPC

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1. Introduction

The EBV-encoded RNAs (EBERs) are the most abundant EBV transcripts (about 10^7 copies per cell) during latent infection by EBV in a variety of cells. Owing to its expression abundance and universal existence in all of the 3 forms of latent infection, EBERs have been under intensive studies since they were discovered by Lernar (Lerner et al., 1981) for the first time. Looking back over the past 30 years, great efforts have been made to unveil the accurate role of EBERs in the latency and transformation process, the definite secondary structure and the signaling pathways they participate in. Despite significant achievements were achieved in these fields, most pioneer work was conducted in lymphoma cells. Bearing this in mind, we explore the similarities between lymphoma and carcinoma to fill the gaps in our knowledge of EBERs' roles in nasopharyngeal carcinoma (NPC). However, it remains to be clarified whether the same scenario accurately applies to the pathological significance of EBERs in NPC.

Epstein-Barr virus (EBV) is consistently detected in NPC from regions of both high and low incidence. In EBV infected cells, there exist some polyribosomal virus-specific RNAs which are the most abundant RNAs (Rymo, 1979). Initial transcription mapping studies by Kieff and colleagues indicated that polyribosomal virus-specific RNA was encoded primarily by the internal repeat region of EBV DNA and, to a lesser extent, by certain other regions of the genome (Orellana & Kieff, 1977; Powell et al., 1979). Making use of cloned restriction endonuclease fragments of EBV, Arrand discovered that the major cytoplasmic RNA in these cells was specified by part of the EcoRI J fragment, which was consistent with Rymo's observation (Arrand & Rymo, 1982). Meanwhile, there were reports that revealed SLE antibodies anti-La, but not the other sera tested, identified two new small RNAs, which corresponded to the most actively transcribed portion of EBV DNA in Rymo's investigation and they were termed EBERs for the first time. In the following 1980's, emphasis were put on the structure, transcription regulation and the function of EBER-La complex. After these preliminary explorations, intensive research was focused on the role of EBERs in the oncogenesis of lymphoma, the involvement of EBERs in the process of lymphoblastoid cell line (LCL) transformation and the potential anti-apoptosis response triggered by EBERs. With these inspiring achievements, some scholars were intrigued by the autocrine growth of several tumor cells and successfully discovered the link between cytokine induction and EBERs in B and T lymphocyte, gastric carcinoma and nasopharyngeal carcinoma in the

following decade. More recently, our knowledge has been deepened by unveiling the TLR3 and RIG-I signaling pathways induced by EBERs, which are responsible for the autocrine growth of lymphomas and some EBV associated pathogenesis (Iwakiri et al., 2005; Samanta et al., 2008). However, the accurate role of EBERs in the pathogenesis of NPC is still obscure. There have been some contradictory reports with respect to the contribution of EBERs to the oncogenesis of NPC and the relationship between EBERs and anti-apoptosis response. What makes these dilemmas more complicated is the existence of EBERs in various stages of NPC. Interestingly, expression of the EBERs seems to be down-regulated during differentiation. Thus examples of NPC that have differing degrees of differentiation lack EBER expression in differentiated areas (Pathmanathan et al., 1995). The EBERs are also not detected in the permissive EBV infection, hairy leukoplakia, and are downregulated during viral replication (Gilligan et al., 1990). Collecting the previous data together, despite that the EBERs have been studied over 3 decades and some observations indicate they may play important roles in the transformation of lymphoma (Yajima et al., 2005) and NPC (Yoshizaki et al., 2007), the exact function of EBERs in NPC are still controversial.

2. Structure, transcription and clinical significance of EBERs

EBERs, the most abundant cytoplasmic RNA species identified in five lymphoid cell lines and a Burkitt lymphoma biopsy, are encoded by the right-hand 1,000 base pairs of the EcoRI J fragment of EBV DNA (Rosa et al., 1981). EBER1 is 166 (167) nucleotides long and EBER2 is 172 ± 1 nucleotides long with the heterogeneity resides at the 3' termini (Fig. 1). Striking similarities are apparent both between the EBERs and the two adenovirus-associated RNAs, VAI and VAII, and between the regions of the two viral genomes that specify these small RNAs (Arrand et al., 1989). The EBER genes are separated by 161 base pairs and are transcribed from the same DNA strand. Both EBER genes carry intragenic transcription control regions A and B boxes which can be transcribed by RNA polymerase III (pol III). However, both EBER1 and 2 contain upstream elements and TATA-like sequences typical of pol Ⅱ promoters including Sp1 and ATF binding sites (Howe & Shu, 1989). Within 1 kilo base EBER region, 10 single base changes which group the strains into two families (1 and 2) have been identified. The EBER1 sequences are completely conserved, two base changes are within EBER2-coding sequence and eight are outside the coding regions (Arrand et al., 1989). EBV has been shown to induce the cellular transcription factors TFIIIB and TFIIIC (leading to induction of general pol III-mediated transcription) and the typical pol II transcription factor ATF-2, that enhance expression of EBER1 and EBER2 (Felton-Edkins et al., 2006), which may account for the low expression of transfected EBERs plasmids in EBV-negative cells (Komano et al., 1999). To elucidate transcription regulation of EBERs more exactly, Thomas J Owen discovered that transient expression of EBNA1 in Ad/AH cells stably expressing the EBERs led to induction of both EBER1 and EBER2 through transcription factors used by EBER genes, including TFIIIC, ATF-2 and c-Myc (Owen et al., 2010). To shed more light on the transcription of EBERs, Hans Helmut Niller analyzed protein binding at the EBER locus of EBV by genomic footprinting electrophoretic mobility shift, reporter gene assay, and chromatin immunoprecipitation in a panel of six B-cell lines. With these methods, 130 base pairs upstream of the EBER1 gene, contains two E-boxes providing a consensus sequence for binding of the transcription factor and oncoprotein c-Myc to the EBV genome. Translocated and deregulated c-myc directly activates and maintains the antiapoptotic functions of the EBER locus in a single EBV-infected B cell

which is undergoing the germinal center (GC) reaction. This single translocated and surviving cell is the founder cell of an endemic BL, which accounts for the oncogenic role of EBV in lymphoma (Niller et al., 2003). What's more, Ferenc Banati found that in vitro methylation blocked binding of the cellular proteins c-Myc and ATF to the 50-region of the EBER-1 gene, which indicated a complicated transcription regulation of EBERs (Banati et al., 2008). With the special transcription elements of EBERs, Choy had devised shRNA plasmid to silence gene expression, which achieves better effect in some cases (Choy et al., 2008).

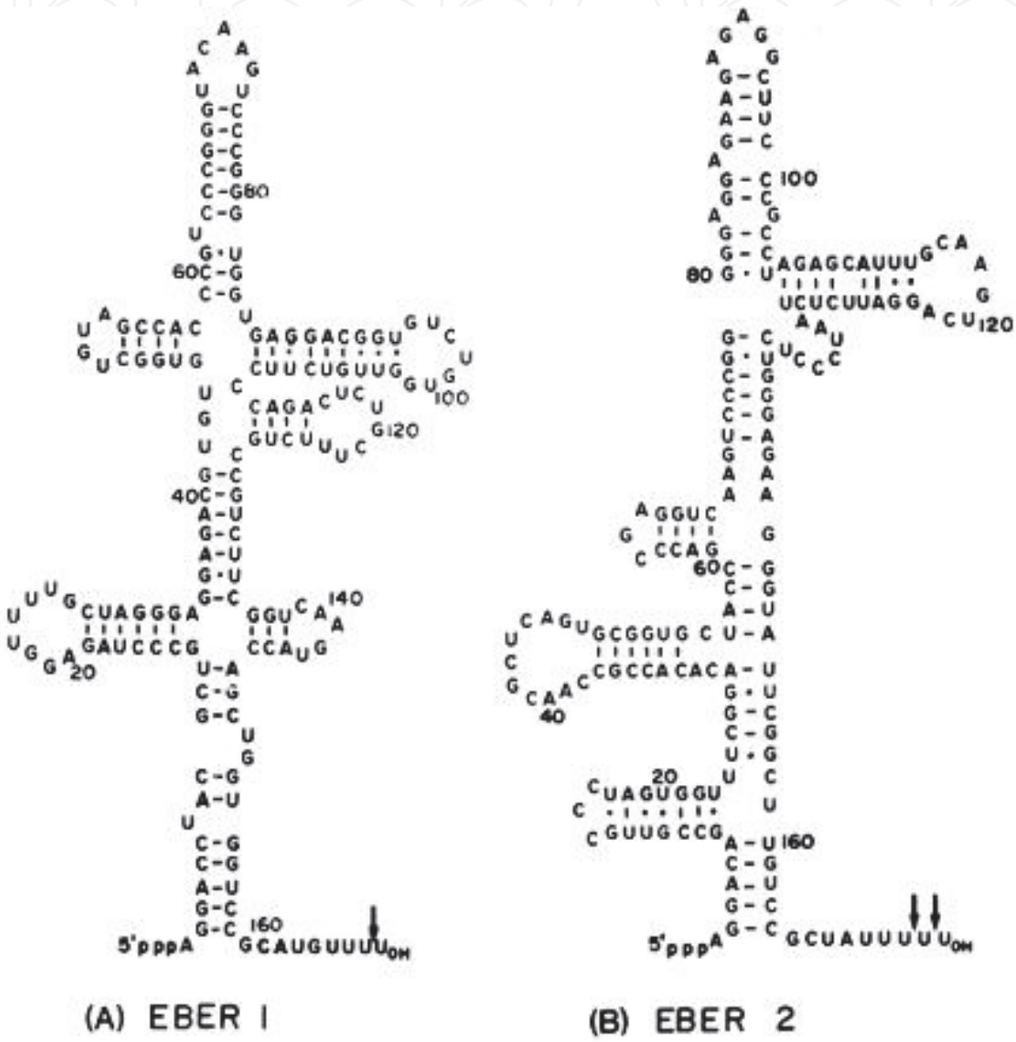

Fig. 1. Potential secondary structures of EBERs. The arrows indicate alternate 3' termini. (A) EBER 1; (B) EBER 2. Adapted from Rosa *et al*. (1981)

EBER in situ hybridization is considered the gold standard for detecting and localizing latent EBV in tissue samples (Ambinder & Mann, 1994). After all, EBER transcripts are consistently expressed in virtually all the EBV positive tumors, and they are likewise expressed in lymphoid tissues taken from patients with infectious mononucleosis, and in the rare infected cell representing normal flora in healthy virus carriers. The only EBV-related lesion that lacks EBERs is oral hairy leukoplakia, a purely lytic infection of oral epithelial

cells. (Gilligan et al., 1990). Recently, researchers have discovered that EBERs could be used as a sensitive marker to monitor NPC cells at various metastatic sites by techniques of in situ hybridization. In cases of metastatic cancer of unknown origin, it is thus reasonable to consider NPC if EBV is present in the tumor cells (Chao et al., 1996). Kimura has established a novel flow cytometric in situ hybridization assay to detect EBV+ suspension cells using a peptide nucleic acid probe specific for EBERs. With this method, they can not only decide the EBV load but also locate EBV-infected cells, which will be beneficial for diagnosis of Epstein-Barr virus (EBV)–associated diseases and exploration of the pathogenesis of EBV infection (Kimura et al., 2009).

3. Localization and potential function of EBERs involved RNP complexes

EBERs are believed to be confined in the nucleus by ISH according to several early publications (Barletta et al., 1993; Chang et al., 1992; Howe & Steitz, 1986). In contrast, Schwemmle *et al*. traced EBERs localization in interphase and mitotic phase and they discovered both RNAs were found in the cytoplasm as well as in the nuclei of interphase cells. The cytoplasm distribution of the EBERs was similar to that of the double-stranded RNA-dependent protein kinase, to which these RNAs could bind, and the location was coincident with the rough endoplasmic reticulum. Thus a cytoplasmic location for EBER-1 and EBER-2 in interphase cells is consistent with the evidence for a role for these small RNAs in translational control (Schwemmle et al., 1992). Despite this sole publication in accord with the potential function of EBERs involved RNP complexes, a recent report (Fok et al., 2006) indicated that EBERs are confined to the nucleus. They carried out heterokaryon assays and oocyte assays, the outcomes of which indicated EBERs did not shutter out of the nucleus under any circumstances and speculated that the report of cytoplasmic localization of EBERs (Schwemmle et al., 1992) was probably due to the complement between the probe and regions including conserved polymerase III promoter elements A and B. EBER1 was shown to have a half-life of 25-30 hours, and was more stable than RNAs that did undergo shuttling, indicating that rapid cytoplasmic degradation was not responsible for the inability to detect shuttling.

While the accurate localization of EBERs is still controversial, the scenario of the function of EBERs involved RNP complexes is perhaps more complicated. EBERs, which resemble another virus encoded RNA VA RNAs (adenovirus virus-associated RNAs) , were firstly found to be complexed with La. Although there is no striking nucleotide sequence homology between EBERs and VAs, similarities exist in their size, degree of secondary structure, and genomic organization (Bhat & Thimmappaya, 1983). The many shared features of the two RNA molecules enumerated above and the fact that they bind a common antigenic host protein supports the supposition that these RNPs play similar roles in virus-infected cells. A specific role in the splicing of adenovirus messenger RNAs has been proposed for the VA RNAs (Murray & Holliday, 1979). The demonstration of a direct physical association between the VA RNAs and certain adenovirus late messenger RNAs supports this proposal (Mathews, 1980). Thus, EBERs could well perform comparable functions in splicing of EBV messenger RNAs. Furthermore, VA RNAs play an important role in adenovirus replication by rescuing cells from inhibition of protein translation mediated by the cellular kinase PKR, which is induced by interferon and activated by double-stranded RNAs produced during replication of many viruses (Ghadge et al., 1994;

Hovanessian, 1989). Considering the resemblance, Bhat and Thimmappaya successfully proved that EBERs can functionally substitute for the VA RNAs in the lytic growth of Ad5 (Bhat & Thimmappaya, 1983, 1985). What's more, EBERs could directly bind PKR and inhibit its activity, then block phosphorylation of eIF2a, thus resulting in the blockage of inhibition of protein synthesis by eIF2a (Clarke et al., 1991; Sharp et al., 1993). When added to reticulocyte lysates at high concentrations, EBER-1 could prevent inhibition of translation by double-stranded RNA (Clarke et al., 1990). However, EBER-1 enhanced overall protein synthesis in the absence of PKR expression (Laing et al., 2002; Laing et al., 1995). In support of EBERs function regardless of PKR, EBER-deleted recombinant EBV transformed primary B lymphocytes into LCLs, which were indistinguishable from LCLs transformed by wildtype EBV in their proliferation, in latency-associated EBV gene expression, and in their permissiveness for EBV replication cycle gene expression (Swaminathan et al., 1991). Especially, another publication indicated EBERs could support replication of the defective adenovirus in vivo but PKR phosphorylation status wasn't influenced (Wang et al., 2005). This difference is likely a result of distinct subcellular compartmentalization of these two molecules, with the EBERs being exclusively nuclear, while PKR is predominately found in the cytoplasm.

Furthermore, it was speculated EBERs could partly restores resistance to both spontaneous and interferon-induced apoptosis (Komano et al., 1999) and PKR probably act as the mediator of the EBER protective effect against apoptosis despite controversial observation provided by Ruf et al. (Ruf et al., 2000). According to Komano et al., Transfection of the EBER genes into EBV-negative Akata clones restored the capacity for growth in soft agar, tumorigenicity in SCID mice, resistance to apoptotic inducers, and upregulated expression of bcl-2 oncoprotein that were originally retained in parental EBV-positive Akata cells and lost in EBV-negative subclones. To support this hypothesis, researchers have made it clear that when EBV-negative Akata cells transfected with EBERs were analysed, PKR autophosphorylation *in vitro* was inhibited (Nanbo et al., 2002). However, Ruf reported that EBERs did posses a modest ability to protect the cell against interferon-induced apoptosis, but this process was independent of PKR-eIF-2α activation (Ruf et al., 2005). Thus Swaminathan suggested that EBERs might inhibit apoptosis while it was unlikely that inhibition of PKR was the primary mechanism for this effect (Swaminathan, 2010).

EBER-1 also interacts with the ribosomal protein L22, a componenet of the 60S eukaryotic ribosomal subunit unique to eukaryotes (Dobbelstein & Shenk, 1995; Toczyski et al., 1994; Toczyski & Steitz, 1991, 1993). In EBV-infected BL cells, roughly 50% of the cellular pool of L22 is found in association with EBER-1 ribonucleoprotein (RNP) particles, and a substantial fraction of L22 is physically relocalized from nucleoli to the nucleoplasm. Using the recombinant viruses and novel EBER expression vectors, the nuclear redistribution of rpL22 protein by EBER1 in 293 cells was confirmed (Gregorovic et al., 2011). Binding to 28S rRNA likely serves to target L22 to nucleoli, while binding to EBER-1 RNA likely results in sequestration or retention of L22 in the nucleoplasm. In truth, BL cells expressing mutated EBER-1 RNAs incapable of binding to and relocalizing L22 have significantly reduced capacity to enhance cell growth potential relative to BL cells expressing wild-type EBERs (Houmani et al., 2009), which indicated that the EBER1-L22 complex may be beneficial for lymphoma growth.

Intriguingly, to date there has been no investigation with respect to the function of EBERs involved RNP complexes in NPC and whether the same machinery in lymphomas readily applies to NPC remains to be seen.

4. EBERs associated oncogenesis and cell transformation

Comparison of EBV-positive and -negative cell clones revealed that the presence of EBV in Akata cells was required for the cells to be more malignant and apoptosis resistant, which underlined the oncogenic role of EBV in the genesis of BL (Komano et al., 1998; Ruf et al., 1999). Subsequent studies revealed that EBERs were responsible for these phenotypes (Komano et al., 1999; Ruf et al., 2005). Transfection of the EBER genes into EBV-negative Akata clones restored the capacity for growth in soft agar, tumorigenicity in SCID mice, resistance to apoptotic inducers, and upregulated expression of bcl-2 that was originally retained in parental EBV-positive Akata cells but lost in EBV-negative subclones. More recently, a new investigation indicated that in vivo expression of a polymerase III driven non-coding viral EBER-1 construct led to the transgenic mouse more inclined to develop tumor. In Repellin's reports, they provided the first evidence by producing ten transgenic mouse lines expressing EBER1 in the lymphoid compartment, and discovered the transgenic mice developed lymphoid hyperplasia, which in some cases proceeded to B cell malignancy (Repellin et al., 2010).

Because EBERs are expressed in large amounts in latently infected cells and virtually all EBV-associated tumors, it had long been speculated that they may play a vital role in the process of transformation. To support this hypothesis, EBER-negative recombinants generated by Yajima in 2005 provided a quantitative advantage in transformation ability. Transformation assays performed with high titres of recombinant EBV generated from the EBER knockout and knock-in strains revealed that the EBER knock-in recombinant possessed approximately 20-fold more transforming ability than the EBER-negative recombinant. Furthermore, growth of the EBER-negative LCLs was impaired compared with that of the revertants under low serum conditions. In contrast, Swaminathan *et al* demonstrated that EBERs were not essential for the immotalization of B lymphocytes or for the replication of the virus. In their experiments, strains of EBV with deletions of the small RNA (EBER) genes were made by homologous recombination using the EBV P3HR-1 strain, which has undergone deletion of the essential transforming gene that encoded the EBV nuclear antigen, EBNA-2, and a DNA fragment that was wild type at the EBNA-2 locus but from which the EBER genes had been deleted. EBER-deleted recombinants transformed primary B lymphocytes into LCLs, which were indistinguishable from LCLs transformed by wildtype EBV. However, they were not able to produce a large quantity of pure EBER-deleted EBV, which may lead to the false negative outcome. To address this issue more specifically, Wu *et al*. (Wu et al., 2007) demonstrated that the transforming ability of recombinant EBVs expressing EBER2 was as high as that of EBVs expressing both EBER1 and EBER2. In contrast, the transforming ability of recombinant EBVs carrying EBER1 was impaired and was similar to that of EBV lacking both EBER1 and EBER2. Gregorovic *et al*. (Gregorovic et al., 2011)recently reported there was little effect of either EBER deletion on the transformation efficiency. This contrasts with the results of a previous study (Wu et al., 2007) where deletion of EBER2 caused a 50-fold reduction of

transformation efficiency. It should be noted that a different EBV strain background was used in the two experiments.

With respect to the role of EBER in the carcinogenesis of NPC, it was reported that EBERs expression may confer an apoptotic-resistant phenotype in immortalized nasopharyngeal epithelial cells. The EBER-expressing NP69 cells attained a higher growth rate compared to cells transfected with control plasmid (pcDNA3). However, the EBER-expressing NP69 cells did not form colonies in soft agar and were non-tumorigenic in nude mice (Wong et al., 2005). Iwakiri, however, reported that EBV infection induces IGF-1 expression in NPC cell lines, and that the secreted IGF-1 acts as an autocrine growth factor. These findings seem to be operative in vivo, as NPC biopsies consistently express IGF-1 (Iwakiri et al., 2005). Recently, in contrast, there are somewhat contradictive observations from Tomokazu. In their experimets, MDCK cells transfected with EBERs-high-expression vector showed an enhanced growth ability in soft agar compared with the MDCK transfected with EBERs-low-expression vector-transfected or untransfected MDCK cells. However, they did not show the acquisition of any anti-apoptotic potential against either IFN-α or serum deprivation. Introduction of EBERs-low-expression vector into MDCK cells did not show anchor independent growth characteristics (Yoshizaki et al., 2007). The reasons for these contrary outcomes are not clear and whether EBERs could transform cells or even be tumorigenic are still obscure. It may be attributable to the origin of the cell line. For instance, NPC-KT, the parental cell line of EBV-neg-KT, was derived from NPC, whereas MDCK is derived from normal epitheliumand (Yoshizaki et al., 2007).

It has long been believed that both EBER1 and EBER2 play similar roles in the pathologic process. Microarray expression profiling, however, identified genes whose expression correlates with the presence of EBER1 or EBER2 (Gregorovic et al., 2011). To researchers' surprise, although most emphasis has previously been given to EBER1 because it is more abundant than EBER2, the differences in cellular gene expression were greater with EBER2 deletion. The number of genes and degree to which the regulated genes were unique to EBER1 or EBER2 was further analyzed, showing that the greater number of differences in cell gene expression was observed in EBER2 deletion. To look more specifically at some of the cellular genes whose expression correlated with EBER2 expression, the expression values from individual cell lines were derived. In each case, the expression level in parental and revertant was similar, but the expression in the EBER2 deletion was consistently different. Some additional data from an earlier comparison of del-EBER2 and parental LCLs were consistent. LCL gene expression was modified according to the presence of EBER2. The examples include genes involved in receptor function and signaling (CNKRS3, CXCL12, CXCR3, DACT1, GDF15, GPR125, IGF1, and IL12RB2A), cellular adhesion (IGSF4), a transcription factor (TBX15), an RNA binding protein (MEX3A), and a proposed tumor suppressor gene (SASH1). This comprehensive description of EBER2 related genes indicates many facets of biological process related to EBER2. Especially there seems to be a link between EBER2 and lymphoma invasion and metastasis. Hopefully this microarray analysis may lead to new insight into the EBERs' research in EBV related carcinomas.

5. EBERs participate in cytokine secretion pathway through TLR3 and RIG-I

Expression of a variety of cytokines and growth factors is enhanced in several types of EBERs-expressing cells. It was demonstrated that IL-10 induced by EBERs acts as an

autocrine growth factor for BL (Kitagawa et al., 2000). It was found that EBV-positive Akata and Mutu cell clones expressed higher levels of interleukin (IL)-10 than their EBV-negative subclones at the transcription level. Transfection of an individual EBV latent gene into EBV-negative Akata cells revealed that EBERs were responsible for IL-10 induction. Recombinanat IL-10 enabled EBV-negative Akata cells to grow in low (0.1%) serum conditions. Likewise, Iwakiri *et al*. reported infection of EBV-negative gastric carcinoma cell lines with EBV led to expression of a limited number of EBV genes including EBERs and was correlated with increased IGF-1 production, as was transfection of EBERs genes (Iwakiri et al., 2003). Using the recombinant virus, Yang *et al*. (Yang et al., 2004) found that a human T-cell line, MT-2, was susceptible to EBV infection, and succeeded in isolating EBV-infected cell clones with type II EBV latency, which was identical with those seen in EBV-infected T cells in vivo. EBV-positive MT-2 cells expressed higher levels of interleukin (IL)-9 than EBV-negative MT-2 cells at the transcriptional level. It was also demonstrated that EBV-encoded small RNA was responsible for IL-9 expression. Addition of recombinant IL-9 accelerated the growth of MT-2 cells, whereas growth of the EBV-converted MT-2 cells was blocked by treatment with an anti-IL-9 antibody. These results suggest that IL-9 induced by EBV-encoded small RNA acts as an autocrine growth factor for EBV-infected T cells.

Since EBERs are expected to form dsRNA structure, it's believed they activate RIG (Yoneyama et al., 2004), a specific pattern-recognition receptors (PRR) that specifically recognize pathogen-associated molecular patterns (PAMPs) within microbes and induce interferon induction. Recently this hypothesis was experimentally tested by Samanta *et al* (Samanta et al., 2006). According to their observation, transfection of RIG-I plasmid induced IFNs and IFN-stimulated genes (ISGs) in EBV-positive Burkitt's lymphoma (BL) cells, but not in their EBV-negative counterparts or EBER-knockout EBV-infected BL cells. Transfection of EBER plasmid or in vitro synthesized EBERs induced expression of type I IFNs and ISGs in RIG-I-expressing, EBV-negative BL cells, but not in RIG-I-minus counterparts. (Samanta et al., 2006). EBERs are recognized by RIG-I through the RNA helicase domain, and following recognition, RIG-I associates with the adaptor IPS-1 via CARD. IPS-1 is localized to mitochondria and initiates signaling leading to activation of IRF3 and NF-κB to induce type-I IFNs and inflammatory cytokines. Furthermore, they indicated that EBERs induce an anti-inflammatory cytokine IL-10 through RIG-I-mediated IRF-3 but not NF-κB signaling (FIG. 2) (Samanta et al., 2008).

Toll-like receptors (TLRs) constitute distinct families of PRRs that sense nucleic acids derived from viruses and trigger antiviral innate immune responses through activation of signaling cascades via Toll/IL-1 receptor (TIR) domain-containing adaptors (Akira & Takeda, 2004). Iwakiri et al. (Iwakiri et al., 2009) reported that EBERs were released extracellulary and recognized by TLR3, leading to induction of type-I IFN and inflammatory cytokines. A substantial amount of EBER, which was sufficient to induce TLR3 signaling involving IRF3 and NF-B activation, was released from EBV-infected cells. Thus, EBERs can contribute to the pathogenesis of EBV infection through interaction with RIG-I and TLR3.

TLRs and RIG-I as PRRs could trigger the innate immune response, as first line of defense against pathogens and tissue injury. This response i.e. inflammation, is a complex response to infection, trauma, and other conditions of homeostatic imbalance (Nathan, 2002). An acute inflammatory response is usually beneficial, especially in response to microbial

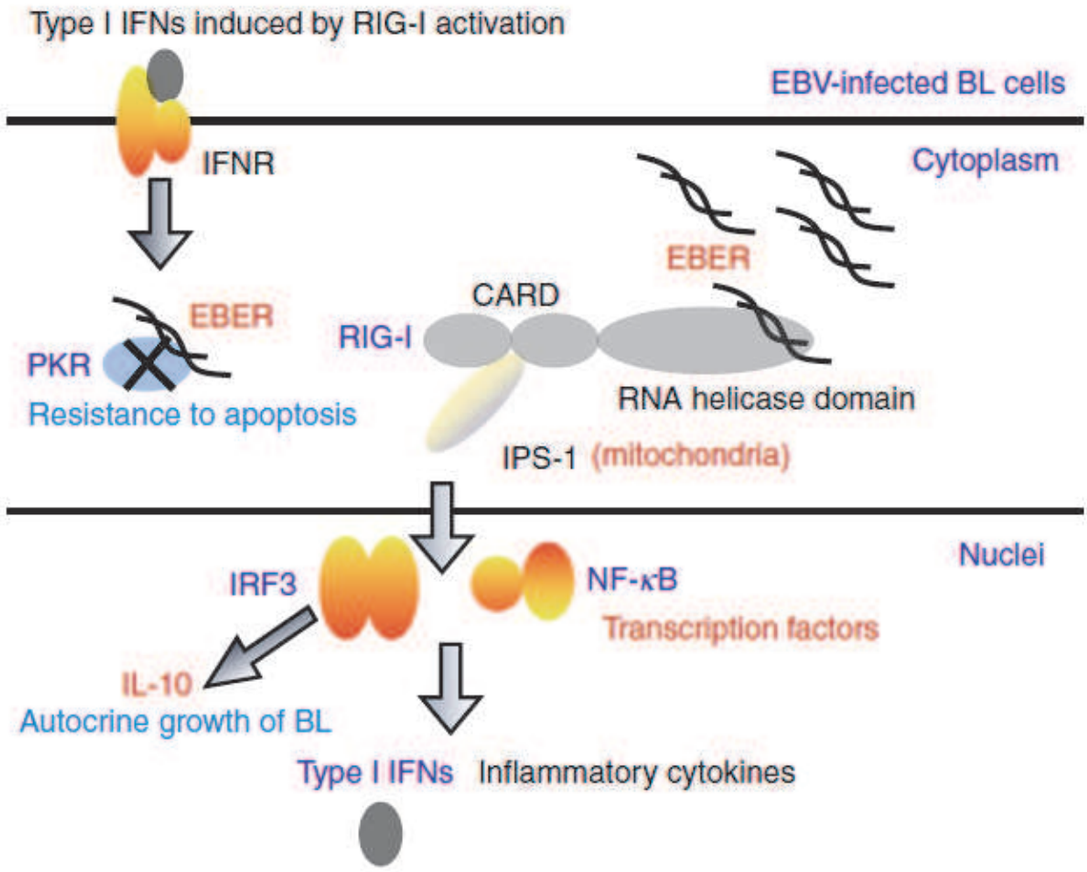

Fig. 2. Modulation of RIG-I signaling by EBERs contributes to EBV-mediated oncogenesis in BL cells. What's more, EBERs bind PKR and inhibit its phosphorylation, which disturb PKR mediated apoptosis. Adapted from Iwakiri & Takada, 2010.

infections and tissue damage. A well-regulated inflammatory response can also be anti-tumorigenic and have a role in tumorsuppression (Mantovani et al., 2008). Chronic inflammation, however, is detrimental and, among other deleterious effects, will frequently predispose cells for an oncogenic transformation. Various mechanisms account for the oncogenic role of chronic inflammation. These include induction of genomic instability, increasing angiogenesis, altering the genomic epigenetic state and increasing cell proliferation. Over-production of reactive oxygen and nitrogen species (RONS), aberrant inflammatory cytokine and chemokine expression, increased (COX-2) and NFκB expression are just some of the molecular factors that contribute to inflammation-induced

carcinogenesis (Schetter et al., 2010). Considering the emerging link between EBERs and TLR and RIG-I in lymphoma and the involvement of PRR in the subsequent inflammation associated cancer, it was intriguing to investigate this potential interaction in other carcinoma. For instance, we demonstrated that EBERs' expression could instantly trigger acute accumulation of inflammation cytokines and this response was impaired in EBERs knock-down NPC cell lines (unpublished data). Furthermore, EBERs induced this inflammation response through TLR3 and RIG-I signaling, as was the scenario in lymphoma. To dissect the exact role of the EBER induced signaling pathway, we have established a positive feedback loop between NF-κB and the EBV encoded oncogenic protein LMP1, which constitute the cascade downstream of EBERs activated NF-κB (Fig. 3).

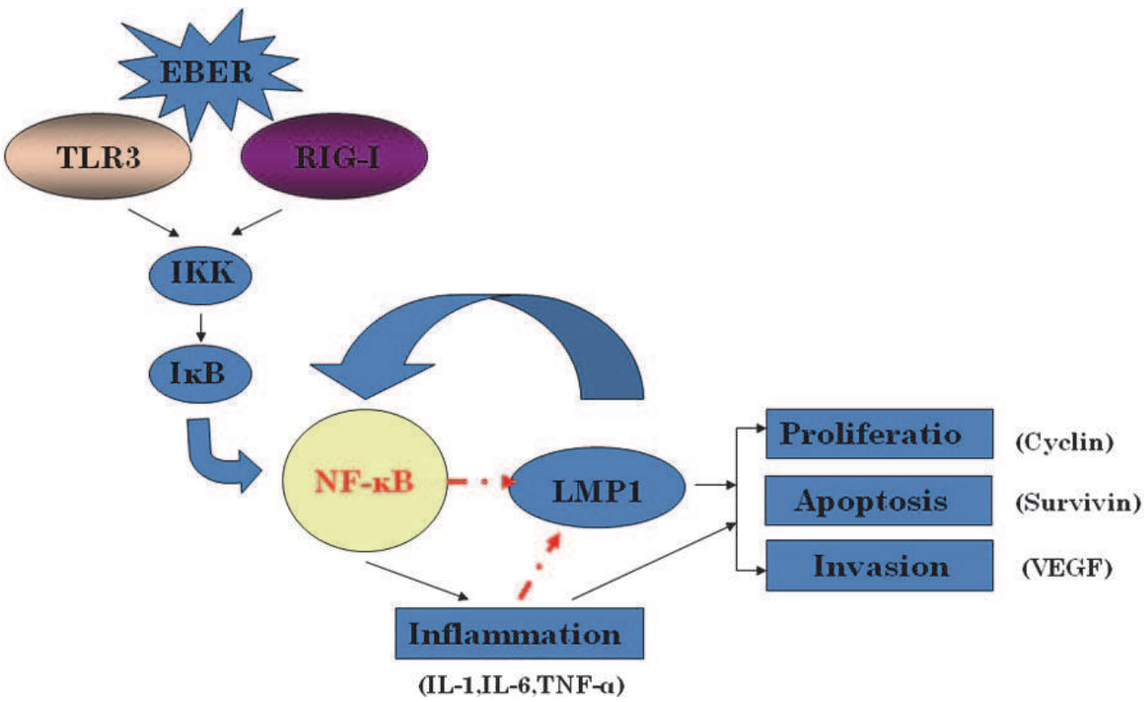

Fig. 3. EBERs induced inflammation response in NPC is intensified by the feedback loop composed of NF-κB and LMP1. Dotted line represents tentative transcription activation. Refer to main text for details.

In light of this model, When nasopharyngeal epithelial cells were infected by EBV, EBERs could initiate NF-κB induced pro-inflammatory cytokines production through PRRs TLR3 and RIG-I. Thus cellular physiological status is shifted in several avenues such as proliferation and apoptosis due to the pro-inflammatory cytokines. Meanwhile, NF-κB could induce oncogenic protein LMP1 transcription, which in turn trigger the NF-κB pathway thus intensify the pro-inflammatory cytokines production. Cooperation between this pro-inflammatory cytokine production and LMP-1 probably potentiate risk of developing NPC.

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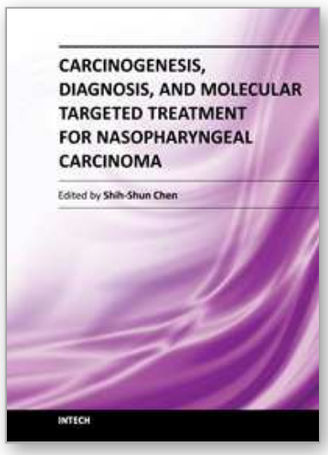

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This book is a comprehensive treatise of the potential risk factors associated with NPC development, the tools employed in the diagnosis and detection of NPC, the concepts behind NPC patients who develop neuro-endocrine abnormalities and ear-related complications after radiotherapy and chemotherapy, the molecular mechanisms leading to NPC carcinogenesis, and the potential therapeutic molecular targets for NPC.

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