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Rb selectively inhibits innate IFN-β production by enhancing deacetylation of IFN-β promoter through HDAC1 and HDAC8

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ABSTRACT

Type I IFN production is tightly controlled by host to generate efficient viral clearance without harmful immunopathology or induction of autoimmune disorders. Epigenetic regulation of type I IFN production in innate immunity and inflammatory disorders remains to be fully understood. Several tumor suppressors have been shown to regulate immune response and inflammation. However, the non-classical functions of tumor suppressors in innate immunity and inflammatory diseases need further identification. Here we report retinoblastoma protein (Rb) deficiency selectively enhanced TLR- and virus-triggered production of IFN-β which thus induced more IFN-α generation in the later phase of innate stimuli, but had no effect on the production of TNF, IL-6 and early phase IFN-α in macrophages. *Rb1*$^{fl/fl}$*Lyz2cre*$^{+}$ Rb-deficient mice exhibited more resistant to lethal virus infection and more effective clearance of influenza virus. Rb selectively bound *Ifnb1* enhancer region, but not the promoter of *Ifna4*, *Tnf* and *Il6*, by interacting with c-Jun, the component of IFN-β enhanceosome. Then Rb recruited HDAC1 and HDAC8 to attenuate acetylation of Histone H3/H4 in *Ifnb1* promoter, resulting in suppression of *Ifnb1* transcription. Therefore, Rb selectively inhibits innate IFN-β production by enhancing deacetylation of *Ifnb1* promoter, exhibiting a previous unknown non-classical role in innate immunity, which also suggests a role of Rb in the regulation of type I IFN production in inflammatory or autoimmune diseases.

1. Introduction

Tumor suppressors, the sensors of multiple forms of cellular stress such as DNA damage, are traditionally known as regulators of cell cycle, apoptosis and carcinogenesis. Since tumor suppressors are involved in carcinogenesis of certain types of cancers induced by chronic infection and inflammation, they might be related to the inflammation process as well. Indeed, accumulating evidence reveals that tumor suppressors not only play roles in the control of tumor development and progression but also in cancer-related inflammation and innate immune responses. For instance, p53 mutant can augment and prolong NF-κB activity and intensify chronic colitis in mice, leading to a high risk of developing invasive colon carcinoma [1]. In addition, p53 can directly repress transcription of *Il4*, *Il6* and *Il12*, and p53-deficient mice are susceptible to autoimmune diseases, suggesting that p53 inhibits autoimmune inflammation [2]. Nevertheless, whether retinoblastoma protein (Rb), the first identified tumor suppressor, functions in innate immune response remains unknown.

Type I interferon (IFN), as the crucial innate defense element against viruses, is rapidly produced to activate an antiviral state when viral invasion is detected by pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and cytosolic DNA sensors [3–5]. Sensing of viral infection by PRRs activates TANK-binding kinase 1 (TBK1) and inhibitor of kappaB kinase epsilon (IKKε) through distinct signaling pathway, leading to the phosphorylation and nuclear translocation of interferon regulatory

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factor 3 (IRF3) and IRF7. These two IRFs then bind to the promoter of *Ifna* and *Ifnb1* gene, and trigger these genes transcription [6]. Insufficient type I IFN production leads to deficient clearance of viruses and contributes to persistent viral infection, while excessive type I IFN production can be harmful to the host and gives rise to inflammatory or autoimmune diseases [7,8]. Thus type I IFN production is tightly controlled by host to generate efficient viral clearance without harmful immunopathology. Regulation of type I IFN production can occur at different levels, from the receptors for viral recognition to the signal adaptors, kinases and transcription factors responsible for IFN production [8–12]. Moreover, increasing attention is paid to the regulation of type I IFN production at the transcriptional level [13–15]. However, the transcriptional regulation of type I IFN production, especially through epigenetic modifications, remains to be fully understood.

Rb, a well-known tumor suppressor, participates in many different cellular processes, including control of cell development and differentiation, regulation of apoptosis and autophagy, and maintenance of chromosomal stability [16]. In immune system, Rb can promote thymic involution and inhibit T cell proliferation [17]. Some studies have suggested that Rb is frequently targeted by viral proteins during DNA or RNA virus infection-related cancer origin and development [18]. The viral oncoprotein E7 expressed by human papillomavirus can attenuate the growth-suppressive properties of Rb, thus stimulating cell proliferation and resistance to apoptosis of virus-infected cells [18]. In addition, virus infection usually induces stressful response of the host cells with changes of tumor suppressors, and the unresolved inflammation induced by viral infection may contribute to carcinogenesis. Therefore, we wonder whether Rb is directly involved in innate immunity and inflammation. Indeed, accumulating evidence shows that *Rb1* gene expression is up-regulated in response to virus and bacterial infection (GSE675, GSE13395, GSE13670) [19–21], and *Rb1* expression also displays significantly change in some autoimmune diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS) (GSE10500, GSE16032, GSE38010) [22–24]. However, the role and the underlying mechanism of Rb in the innate immunity and inflammatory diseases remain largely unknown.

Herein, we report that Rb deficiency can protect mice from RNA and DNA virus infection by selectively producing more IFN-β in the early phase and inducing more type I IFNs in the later phase. Our study demonstrates a new way to negatively regulate IFN-β production by tumor suppressor Rb through epigenetic modification in innate immune responses.

2. Materials and methods

2.1. Mice

The *Rb1*$^{fl/fl}$ mice (B6; 129-*Rb1*^{tm3Tyj}/J), the LysMcre mice (B6; 129P2-*Lyzs*$^{tm1(cre)Ifo}$/J) and the *Ifnαβ*$R^{-/-}$ mice (B6.129S2-*Ifnar1*^{tm1Agt}/Mmjax) were obtained from Jackson Laboratories and bred in specific pathogen-free conditions; 6- to 8-wk-old littermate mice were used in the experiments (body weight and sex balanced). All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Second Military Medical University, Shanghai.

2.2. Reagents and antibodies

LPS (*Escherichia coli* serotype 0111:B4), CpG-ODN and Poly(I:C) were from Sigma-Aldrich which have been previously described [25]. Antibodies to Rb were from Cell Signaling Technology. Antibodies specific to acetyl-histone H3 and acetyl-histone H4 were from Millipore. Histone Deacetylases antibodies HDAC1 and HADC2 were from Abcam, HDAC3 was from Cell Signaling Technology, HDAC8 was from Epigentek. Lamin A/C, GAPDH, β-actin, c-Jun, ATF-2, IRF3 antibodies and phosphospecific antibodies against ERK (Thr202/Tyr204), JNK (Thr183/Tyr185), p65 (Ser536) and IRF-3 (Ser396) were from Cell Signaling Technology.

2.3. Cell culture and pathogens

HEK293T and RAW264.7 cell lines were obtained from American Type Culture Collection. Thioglycollate (Merck) elicited mouse peritoneal macrophages, and bone marrow derive macrophage (BMDM) were prepared as described previously [25]. VSV (Indiana Strain) was propagated and amplified by infection of a monolayer of HEK293T cells. Twenty-four hours after infection, the cells were thawed and refreezed, then clarified and harvested the supernatants containing virus by centrifugation. Viral titer was determined by TCID50 on HEK293T cells. Influenza virus strain A/Puerto Rico/8/1981 H1N1 (PR8) (provided by Dr. H. Yao, Zhejiang University School of Medicine, Hangzhou, China) was propagated in 8- to 10-day-old embryonated chicken eggs. Sendai virus (kindly provided by Dr. Bin Sun, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China) and HSV-1 virus (Kos strain, kindly provided by Dr. Qihan Li, Chinese Academy of Medical Sciences, China) were obtained as indicated.

2.4. Plasmid constructs and transfection

Recombinant vectors encoding mouse *Rb1* (GenBank accession no. NM_009029.2) were amplified from cDNA of mouse peritoneal macrophages, and then cloned into pcDNA3.1 vectors (Invitrogen). The promoter of *Ifnb1* and truncates of *Ifnb1* promoter were cloned into the PGL3-enhance luciferase vector (Promega) to construct promoter luciferase reporter vectors as described previously [25]. All constructs were confirmed by sequencing. Plasmids were transiently transfected into HEK293T cells with jetPEI reagents (Polyplus Transfection) following the manufacturer instructions.

2.5. RNA interference

Mouse primary peritoneal macrophages were prepared and cultured as above overnight, then changed the medium into fresh medium before interference. The cells were transfected with siRNA using INTERFERin reagent (Polyplus Transfection) as described in the manufacturer instruction. The sequences of siRNAs specific for mouse *Rb1* were 5′-GGAGUUUGAUUCCAUUAUA-3′ and 5′-GCAUAUCUCCGACUAAAUA-3′. The sequence of siRNA specific for mouse *c-Jun* was c-Jun-1 5′-GUGCCUACGGCUACAGUAA-3′ or c-Jun-2 5′-CAGCUUCCUGCCUUUGUAA-3′. The sequence of siRNA specific for mouse *Hdac1* and *Hdac8* was 5′-AGUGCUGUGAAGCUUAAUA-3′ and 5′-CGGCAAGUGUCUGAAGUAU-3′ respectively. The sequence of control siRNA was 5′-UUCUCCGAACGUGUCACGU-3′. These siRNA were designed and synthesized by GenePharma Co. (Shanghai, China) or Thermo Scientific. After 48 h, the cells or culture supernatants were harvest for ELISA assay, real time PCR analysis, Western blot or ChIP assay.

2.6. Lentiviral transduction

For lentiviral transduction, mouse primary peritoneal macrophages (2×10^5 well^{-1}) were cultured in 24 well plates overnight. Macrophages were infected with Rb (MOI = 25) or control EGFP (MOI = 25) lentiviruses diluting in Polybrene (GENECHEM CO. Shanghai). After 8–12 h, the medium was changed into fresh DMEM medium containing 10% (vol/vol) fetal bovine serum. The