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Original Article

Toll-like receptor 3 (TLR3) functions as a pivotal target in latent membrane protein 1 (LMP1)-mediated nasopharyngeal carcinoma cell proliferation

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Abstract: Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) activation of NF-κB is pivotal for EBV-infected B lymphocyte survival. Herein, we found that LMP1 markedly rescued the suppressed the proliferation of several nasopharyngeal carcinoma (NPC) cell lines caused by a Toll-like receptor 3 (TLR3) ligand poly (I:C). We profiled the expression alterations of TLR3 and LMP1 within these NPC cell lines in response to poly (I:C) treatment, and found a high correlation between them ws found, suggesting potential involvement of TLR3 in LMP1 signaling. Then, cells deficient in TLR3 were used to assess its role in poly (I:C)-induced inhibition of cell proliferation and LMP1-mediated NF-κB activation. NF-κB p65 activation and the consequent pro-inflammatory responses were unresponsive to poly (I:C) stimulation after TLR3 knockdown (KD), and NOS2 and MMP9 were substantially suppressed in CNE1-745, but nearly normal in LMP1-overexpressed CNE1-LMP1-745 cells. This suggests an alternative pathway that LMP1 may depend on, in terms of NOS2 and MMP9 regulation, whereas an unusual TLR3-dependent expression of c-Myc was identified. Consistently, poly (I:C)-induced retarded growth was reversed by TLR3 silencing, which was especially enhanced in LMP1-overexpressed cells. TLR3 is essential for poly (I:C)-incited NPC cell death, and occupies a critical role in LMP1-mediated NF-κB activation. Our findings provide new insight into the mechanism underlying LMP1-involved EBV-associated pathogenesis of refractory NPC, thereby potentially improving treatment outcome.

Keywords: Nasopharyngeal carcinoma (NPC), EB virus, latent membrane protein 1 (LMP1), TLR3, NF-κB

Introduction

Nasopharyngeal carcinoma (NPC) arises from the nasopharyngeal mucosa and is characterized by distinct geographic distribution [1]. According to recent study, >70% of new NPC cases are in east and Southeast Asia and most of them are in China [2]. Although technological advances in radiotherapy modalities and the extensive applications of systemic therapy have improved the prognosis in the early stages [3], elevating the survival rate of advanced NPC remains challenging due to its considerable propensity for distant metastasis [4, 5]. Therefore, studies have been launched in recent decades to find novel effective strategies for treating this intractable cancer.

Epstein-Barr virus (EBV) infection was deemed the predominant cause of NPC, and the virus can employ a variety of evasion strategies that facilitate immune escape during latency and productive infection [6, 7]. The latent membrane protein 1 (LMP1) is a main oncogene encoded by EBV that initiates multiple signaling pathways, including those of the nuclear factor-κB (NF-κB) family. Particularly, LMP1-mediated constitutive NF-κB activation results in the up-regulation of diverse anti-apoptotic proteins and provides growth signals in latently infected cells [8], thereby contributing to immune evasion.

Toll-like receptors (TLRs) comprise a class of conserved transmembrane pattern-recognition receptors (PRRs) that recognize specific pathogen-associated molecular patterns (PAMPs) and trigger inflammatory responses as well as innate defense programs against the invading microbes by activating transcription factors

such as nuclear factor (NF-κB), while inducing the NF-κB-triggered expression of interferons (IFNs) and cytokines [9, 10]. This promotes the activation of antigen presenting cells and mediates adaptive immune responses and thereby features prominently in host resistance to microbial infections [11]. TLR3 is well-known to function as a receptor for double-stranded RNA, which is fundamental for antiviral responses [12]. Concretely speaking, upon dsRNA binding the sole cytoplasmic adaptor TRIF is recruited to form a complex with TLR3 [13, 14], and this signaling complex can drive the recruitment of several downstream signaling molecules including, particularly, tumor necrosis factor (TNF) receptor-associated factors, further resulting in NF-κB activation [15].

Since LMP1 can act as a constitutively active TNF receptor-associated factor in activation of the NF-κB signaling pathway, we rationally assumed that LMP1 is involved in the viral dsRNA-triggered innate immune response within EBV-associated tumor cells by cooperating or/and interacting with TLR3 to initiate NF-κB-mediated downstream pro-inflammatory and anti-apoptotic signaling transduction. Our results will provide new insight into the molecular mechanistic underpinnings of LMP1 oncoprotein-mediated EBV infection-associated pathogenesis of NPC, which will further lead to novel potential treatment targets, and improvement of treatment.

Materials and methods

Cell culture

The human NPC cell lines CNE1, CNE2, HNE1, HNE2 were kindly presented by Dr Chaonan Qian (Sun Yat-sen University, Guangzhou, China). LMP1-overexpressed cell lines were created by artificially lentivirus transfecting LMP1 cDNA into parental cell lines. Cells were maintained in RPMI1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 1.5 mM of L-Glutamine, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), and grown in a humidified atmosphere of 5% CO_2 at 37°C. To determine the effects of poly (I:C), cells were treated with 50 μg/mL of poly (I:C) (Sigma-Aldrich, Merck KGaA, Germany) or vehicle (sterile H_2O) for different time periods (0, 24 h, 48 h, and 72 h) before cell collection.

Cell proliferation assay

Cells were seeded into 96-well plates at a density of 3×10^4 cells/well in 100 μl RPMI1640 medium and allowed to adhere overnight. The cells were transfected with control (NS) or plasmid DNA, and after 48 h the cells were exposed to 50 *μ*g/ml of poly (I:C) for indicated periods. Cell Counting Kit-8 (CCK8, Dojindo Laboratories Co., Ltd., Kumamoto, Japan) was added into different wells (1:10). Cells were incubated for 3 h at 37°C in the dark, after which the growth curve was generated based on the absorbance at 450 nm. The assays were conducted in triplicate and repeated at least 5 times.

Protein extraction and western blotting analysis

Cells were harvested 48 h after treatment with 50 μg/ml of poly (I:C) by briefly trypsinization, centrifugation at 2000 rpm for 5 min, and washing twice with PBS. Total cell lysates were prepared using M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, Waltham, MA USA) and subjected to centrifugation at 12,000 *rpm* for 10 min to remove cell debris. Protein concentration was measured by BCA method.

For immunoblotting, samples containing equal amounts of protein were separated on 10% SDS-PAGE and transferred to PVDF membranes at 30 mA for 70 min. The membranes were blocked with 5% non-fat milk and then incubated overnight at 4°C with monoclonal primary antibodies against human TLR3 (1:30 diluted, sc-32232, Santa Cruz Biotechnology, Inc., CA, USA), LMP1 (1:50, GM089729, Gene Tech, Inc., Shanghai, China), NOS2 (1:200, sc-8310, Santa Cruz), MMP-9 (1:200, sc-6841, Santa Cruz), c-Myc (1:40, sc-373712, Santa Cruz), and GADPH (1:1000, ab9485, Abcam, Mass, USA), followed by rinsing 3 times with PBST for 15 min, and subsequently incubated at room temperature for 1 hour with HRP-conjugated secondary goat anti-rabbit IgG, rabbit anti-goat IgG, or goat anti-mouse IgG (all of which were diluted at 1:2000, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). The membrane was washed with PBST three times (15 min per time) and then developed using SuperSignal West Pico ECL reagents (Pierce, ThermoFisher Scientific Inc., Waltham, MA, USA). Protein bands were imaged and pro-

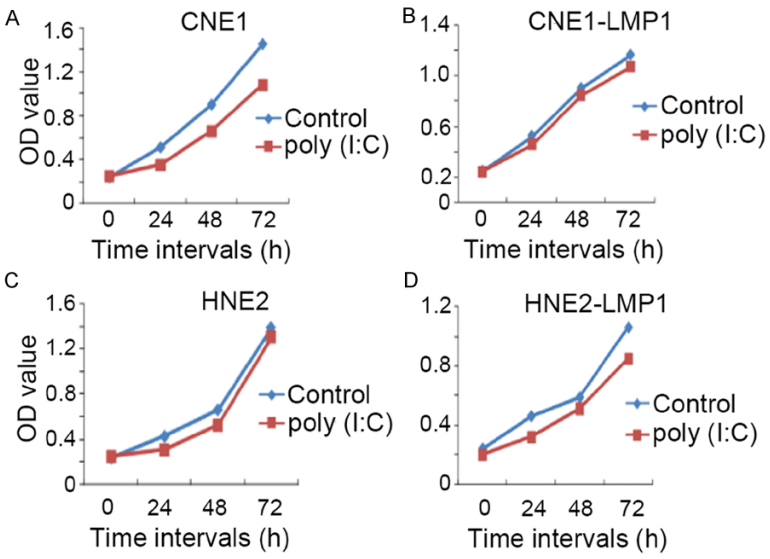

Figure 1. LMP1 rescued the inhibited NPC cell proliferation caused by poly (I:C) treatment. The proliferation rates of CNE1 (A), CNE1-LMP1 (B), HNE2 (C), and HNE2-LMP1 (D) in the presence and absence of 50 μg/mL poly (I:C) at varying time intervals were assessed by CCK-8 assay. Data are presented as the mean ± SD of triplicate determinations.

cessed using a Gel-Pro Analyzer software (Media Cybernetics, Inc., Rockville, MD, USA).

Apoptosis assay by flow cytometry

Apoptosis was determined by flow cytometry using an Annexin V-FITC/PI apoptosis detection kit (Mbchem M3031, purchased from Majorbio Co., Ltd., Shanghai, China) following the manufacturer's instructions. Cells were collected by pelleting and resuspended in Annexin V-binding buffer (Mbchem M3036) at a density of 1×10^6 cells/mL, and stained with 5 μl Annexin V FITC for 15 min at 4°C away from light. The reaction was stopped by the addition of 10 μl propidium iodide (PI), followed by an incubation for 5 min at 4°C in the dark. Early apoptosis was evaluated within 1 hour, whereas late apoptosis was evaluated after 12 hours. Fluorescence intensity was measured by BD FACS Calibur flow cytometer (BD Bioscience, Bedford, MA, USA). All experiments were repeated at least 3 times with similar results. Cells in the early stage of apoptosis were Annexin V-stained, while Annexin V- and PI-positive cells were considered in the late stage of apoptosis.

Transfection

To generate the TLR3 knockdown cell lines, negative control and TLR3 siRNA plasmid constructs were purchased from GenePharma Co., Ltd (Shanghai, China). Cells were cultured in RPMI1640 supplemented with 10% (v/v) FBS containing antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) (Sigma) for 24 h. Afterwards, CNE1 cells and CNE1-LMP1 cells were transfected with either a scrambled siRNA (siRNA-scr, non-targeting control) or TLR3 siRNA plasmids for 8 h using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Cells were then selected in RPMI1640 medium supplemented with 10% (v/v) FBS for another 48 hr prior to the evaluation of transfection and silencing rate by western blot.

Results

LMP1 overexpression rescued the poly (I:C)-induced apoptosis of diverse NPC cell lines

To investigate the role of EB virus-encoded oncogenic protein LMP1 in viral infection-induced apoptosis in NPC cells, we introduced polyinosinic-polycytidylic acid (poly (I:C)), a synthetic analog of viral double stranded RNA (dsRNA), into our experiment. First, series of NPC cell lines were cultured in the presence or absence of 50 μg/ml of poly (I:C) prior to subsequent analysis of the cellular proliferation rate at discrete time points. As shown in **Figure 1A**, the proliferation of CNE1 NPC cells was obviously repressed by the addition of poly (I:C) in a time-dependent manner, as we expected, whereas poly (I:C) failed to make a significant difference in the proliferation rate of another NPC cell line HNE2 (**Figure 1C**). Here we also noticed that the expression of LMP1 in CNE1 was extremely weak, in contrast to the intense expression of LMP1 detected in HNE2 (**Figure 2A**). We assumed that the different observations in each cell line might result from varying LMP1 expression levels, thus we next artificially overexpressed LMP1 in these cell lines. Remarkably, the inhibitory activity of poly (I:C) was dramatically restrained in the CNE1-LMP1 cell line (**Figure 1B**), while LMP1 overexpression

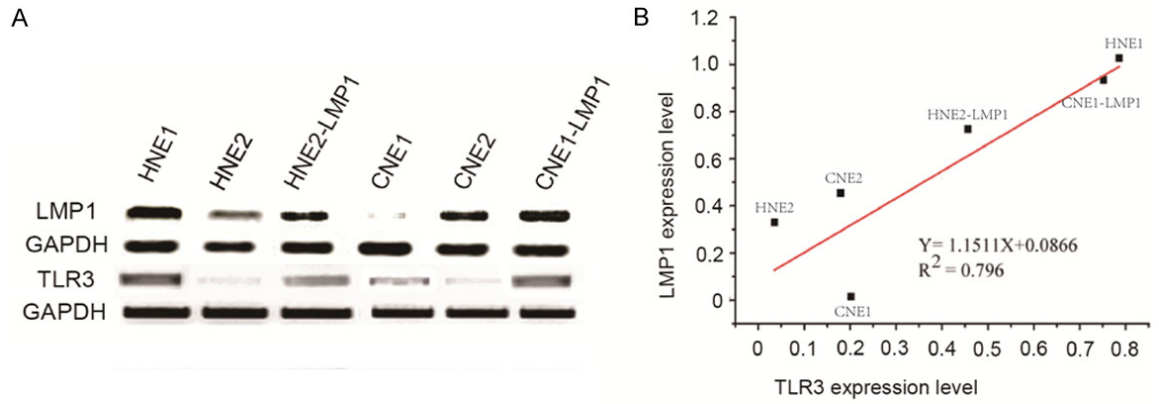

Figure 2. LMP1 promoted TLR3 expression. A. LMP1 overexpression in CNE1 and HNE2 substantially increased TLR3 production. For sake of comparison, proteins blotted to the same piece of X-ray film but in nonadjacent columns were put adjacent, which was displayed by a solid line. B. The expression profiles of diverse NPC cell lines were evaluated by western blot assay. GAPDH was used as an internal control and the expression of LMP1 and TLR3 were normalized to that of GAPDH. Correlation plots demonstrating LMP1 expression plotted against TLR3 in diverse NPC cell lines, and a tight correlation was observed (R^2=0.796).

exerted a fairly small effect on HNE2 cell proliferation (**Figure 1D**). This may be because HNE2 expressed sufficient LMP1 protein *per se*, hence its insensitivity to exogenous LMP1. Taken together, LMP1 repressed the poly (I:C)-induced apoptosis in multiple NPC cell lines, suggesting that LMP1 may function in promoting the onset and progression of NPC. Since poly (I:C) is a ligand choice of toll-like receptor 3 (TLR3) and upon its recognition, TLR3 activates the transcription factor and subsequently triggers the downstream signaling [16], we proposed that LMP1 may facilitate NPC cells' surviving apoptosis by interacting with TLR3, while interfering with the subsequent signaling transduction.

Potential relevance between LMP1 and TLR3 revealed by their expression patterns in different cell lines

To investigate the effect of LMP1 on TLR3 in terms of expression level, NHE1, HNE2, CNE1, and CNE2 NPC cell lines were cultured *in vitro* and subjected to western blot analysis for their expression of LMP1 and TLR3 (**Figure 2A**). Afterwards, correlation analysis for expression patterns was performed, and a positive correlation between LMP1 and TLR3 expression level in each cell line was revealed. LMP1 displayed strong or moderate expression in HNE1, CNE2, and HNE2, whereas a slightly expressed LMP1 was detected in CNE1 (**Figure 2A**). In order to further understand whether LMP1 can regulate TLR3 expression, we overexpressed LMP1 respectively in a poorly-differentiated NPC cell line HNE2 and well-differentiated CNE1. The results demonstrated that TLR3 was greatly increased in LMP1-overexpressed cells, indicating that LMP1 could provoke TLR3 expression in NPC cells, regardless of the differentiation status of NPC cells (**Figure 2B**). Furthermore, this may result in a positive correlation between LMP1 and TLR3 expression in NPC cells.

We thus assumed that EB virus invades hosts and induces the onset of NPC probably through LMP1-mediated TLR3 signaling activation.

LMP1 promotes TLR3 activation as shown by increased NF-κB p65 expression in CNE1

Given that the NF-κB signaling pathway is crucial for inflammatory cytokine production in TLR signaling [17, 18], the expression alterations of TLR3 and NF-κB p65 in response to poly (I:C) and enhanced LMP1 expression in CNE1 cells were analyzed. As shown in **Figure 3A** and **3B**, both exposure to poly (I:C) or/and LMP1 overexpression markedly enhanced the expression level of TRL3 in CNE1 in comparison to the wild type (WT) CNE1 devoid of poly (I:C) stimulation (**Figure 3A** and **3C**). Similar results were observed in the expression of NF-κB p65 protein, with a marked increase in response to stimulation of poly (I:C) or/and LMP1 overexpression (**Figure 3A** and **3D**). However, the two

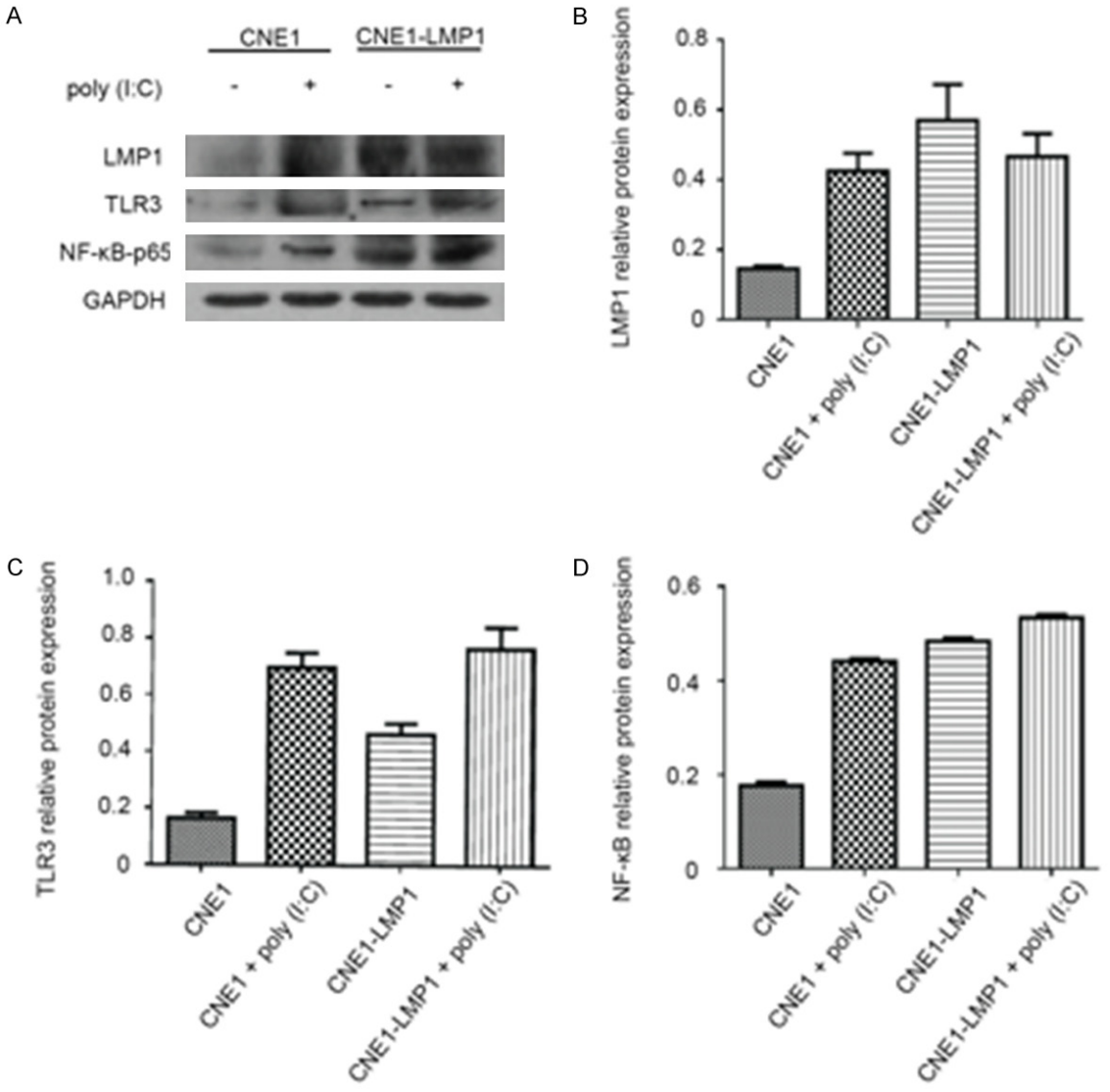

Figure 3. LMP1-transfection and poly (I:C) stimulation were able to induce TLR3 expression and NF-κB p65 activation (A). The immunoblotting assay was carried out to visualize the expression patterns of LMP1, TLR3, and p65 in CNE1 and CNE1-LMP1 cells with or without poly (I:C) stimulation. The expression alterations of LMP1 (B), TLR3 (C), and p65 (D) were presented in a histogram. All experiments were performed in triplicate, and data are presented as the mean ± SD.

stimuli, addition of poly (I:C) and exogenous LMP1, independently drove the expression of TLR3 and NF-κB p65, but not in a synergistic fashion (**Figure 3C** and **3D**). This may be explained by the fact that both exogenous LMP1 overexpression and poly (I:C) stinulation could activate the poly (I:C)-TLR3 signaling to the largest extent, thus the insensitivity of this pathway to further stimulation. These in aggregate, revealed a high correlation between TLR3 and p65 expression, suggesting that LMP1 might activate p65 signaling transduction pathway through cooperating with TLR3.

TLR3 plays a pivotal role in LMP1-mediated NF-κB p65 downstream signaling activation

In order to further characterize the role that TLR3 plays in the LMP1-induced NF-κB p65 activation and subsequent pro-inflammatory signaling events in CNE1 cells, we generated the TLR3 knockdown CNE1-745 and CNE1-LMP1-745 cell lines, in which TLR3 signal was barely detected by using immunoblotting (**Figure 4**). Given that a number of genes involved in the inflammatory response are driven by the transcription factor NF-κB (p50-p65)

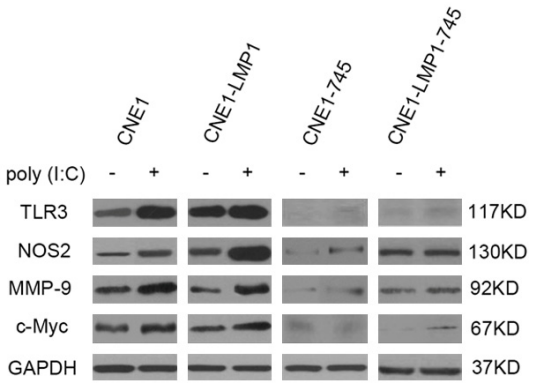

Figure 4. LMP1 rescued the impaired NF-κB p65 activation and subsequent pro-inflammatory gene production in the TLR3-knockdown cells. CNE1-745 and CNE1-LMP1-745 were generated through TLR3 knockdown in WT CNE1 and CNE1-LMP1 cell lines. The expression levels of TLR3 and p65-regulated pro-inflammatory genes including iNOS, MMP-9, and c-Myc in all these cell lines were assessed by immunoblotting assay with corresponding antibodies, with or without pretreatment by 50 μg/mL of poly (I:C) for 16 h. GAPDH was used as an internal control to ensure equal loading.

including inducible nitric oxide synthase (iNOS), matrix metalloproteinase 9 (MMP9), and c-Myc [19], we assessed NF-κB p65 activity in TLR3-deficient cells by testing the expression levels of these genes after exposure to poly (I:C) treatment. First, in cells normally expressing TLR3, stimulation with poly (I:C) promoted the expression of endogenous TLR3, as well as NF-κB p65 downstream genes including NOS2 (iNOS), MMP9, and c-Myc, compared to the vehicle-treated cells (shown in **Figure 4**).

Of note, TLR3 silencing resulted in a dramatic decrease in the inflammatory signature within CNE1 cells. Moreover, poly (I:C) stimulation did not display enhanced NOS2, MMP9, and c-Myc expression when compared to unstimulated cells, indicating a crucial role of TLR3 in the cellular inflammatory response to the exogenous PAMP stimuli. On the other hand, when LMP1 was exacerbated in TLR3 knockdown cells, no significant influence on the overall variation of NOS2 and MMP9 expression was observed, whereas c-Myc production in TLR3 knockdown cell lines was greatly suppressed, regardless of the enhanced LMP1 level. Thus, LMP1 may elevate NOS2 and MMP9 levels by an alternative pathway, which can compensate, at least in part, for the reduced pro-inflammatory gene expression caused by TLR3 deficiency. However, there seems to be no such parallel signaling pathway that LMP1 can resort to, and c-Myc seems to display a TLR3-dependent pattern in terms of the expression level. Notably, TLR3-deficient CNE1-745 CNE1-LMP1-745 cells were unresponsive to poly (I:C) stimulation, both in terms of c-Myc expression and the production of NOS2 and MMP9. These data together suggested that TLR3 plays a crucial role in the LMP1-mediated NF-κB pathway activation.

TLR3 was involved in poly (I:C)-induced growth inhibition

As shown in **Figure 5**, at the very start, incubation with poly (I:C) resulted in the retarded growth of both CNE1 and CNE1-LMP1 cell lines. Of note, following TLR3 silencing, cells were refractory to poly (I:C) incitement in terms of the proliferation rate, indicating that TLR3 features prominently in poly (I:C)-induced inhibition of proliferation. Taken together, these findings indicate that TLR3 is required for poly (I:C)-induced retardation of growth rate.

Discussion

Nasopharyngeal carcinoma (NPC) is a major cause of death in southern China and southeastern Asia. Although chemoradiotherapy has been defined as the standard regimen for locally advanced NPC, many patients are resistant and it is ineffective for those with metastatic NPC. Furthermore, there are a variety of mechanisms in EBV-associated NPC cells that they can exploit to escape from immunologic surveillance [20]. Understanding the mechanisms underlying EBV-mediated NPC cells surviving apoptosis may pave the way to novel therapeutic strategies.

Toll-like receptors (TLRs) are responsible for the host innate immune response against pathogen invasion, by initiating signaling cascades that trigger the production of serious of inflammatory cytokines that can recruit immunocytes to wipe out the infected cells in case of microbe spreading, or enhance the resistance of infected cells [21]. Among TLR family members, TLR3 is a crucial one because it is a functional receptor for dsRNA, a byproduct of replication of viruses, and may activate at least three transcription factor families, namely IRF-3, NF-κB, and AP-1, thereby stimulating protective inflammatory responses. To date, TLR3 is

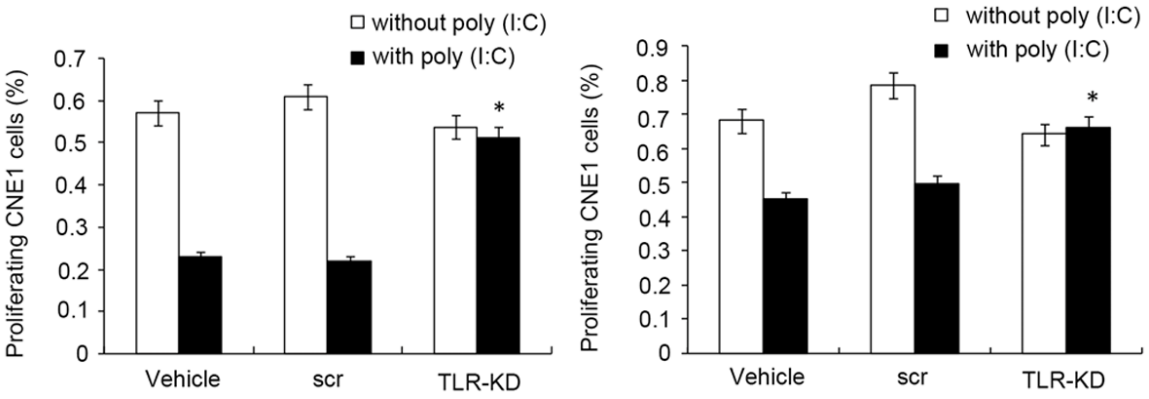

Figure 5. TLR3 silencing counteracted the inhibitory effects of poly (I:C) on the proliferation of CNE1 and CNE1-LMP1 cell lines. Blank: cells without RNA interference; scr: scramble siRNA, another negative control that has the same nucleotide composition, but not the same sequence. *P<0.05 compared to the negative controls in the presence of poly (I:C).

the only TLR member that has been shown to play a non-redundant role for protection against herpes simplex virus (HSV-1) infection in the central nervous system and consequent herpes simplex encephalitis [22]. Meanwhile, TLR3 may be responsible for an unbalanced pro-inflammatory response in hepatocytes that characterizes the pathogenesis of HCV-associated chronic hepatitis and hepatocellular carcinoma [23]. Poly (I:C)-induced TLR3 activation produces an antiviral state that restricts HIV-1 infection in primary human macrophages [18], whereas TLR3 is highly expressed in proximity to infiltrating mononuclear cells in HIV myopathies, suggesting a detrimental role of TLR3 in HIV-1-induced pathogenesis [24].

Particularly, TLR3 could trigger the signaling of both the extrinsic and intrinsic apoptotic pathways by means of diverse molecular mechanisms [25-27]. Besides, an anti-metastatic ability of TLR3 by downregulating the expression of chemokine receptor CXCR4 was reported elsewhere [28, 29]. On the other hand, TLR3 expression is related to tumor aggressiveness such as higher probability of metastasis and recurrence [30, 31]. Thus, the conflicting data make it a challenge to understand the multi-faced contribution of TLR3 in viral infections that involve various and complicated mechanisms, while attracting our attention to delineating more functions of TLR3 associated with the onset and development of carcinoma. Our study investigating the protective versus pathogenic role of TLR3 in EBV infection-associated NPC will provide an important entry point.

In this study, a wide array of human-derived NPC cell lines were treated with poly (I:C), a widely used synthetic mimic of natural viral dsRNA, to research the role of LMP1 coupled with TLR3 play in viral stress-inducible host innate immune responses. We found that poly (I:C) promoted the apoptosis of NPC cells in a time-dependent manner. Moreover, poly (I:C) stimulated TLR3 expression, and meanwhile the p65 transcription factor was activated as well as downstream pro-inflammatory gene production. This suggested that poly (I:C) activates both the apoptosis pathway and NF-κB p65-mediated inflammatory responses by potentially regulating the TLR3 signal pathway, in accordance with the previous study [32].

EBV has numerous mechanisms to confound the antiviral and antitumor actions in order to establish life-long latent infection in B lymphocytes following primary infection [33]. As we all know, LMP1 is essential for EBV-infected B lymphocyte survival and malignant transformation. Our results indicated that LMP1 confers on NPC cells partial resistance to poly (I:C)-induced cell death, which facilitates our assumption that LMP1 primarily acts in EBV immune evasion. However, in the absence of human T lymphocytes, our *in vitro* data seem insufficient to demonstrate that LMP1 functions in NPC tumor cell resistance to the effector mechanisms of cytotoxic lymphocytes in the context of the actions of immune-surveillance. Therefore this assumption remains to be corroborated by *in vivo* experiments with the participation of lymphocytes.

Intriguingly, we observed exogenous LMP1 overexpression promoted TLR3 levels in NPC cell lines CNE1 and HNE2. The oncoprotein LMP1 also facilitated p65 transcription factor activation and elevated the expression levels of pro-inflammatory genes including NOS2, MMP9, and c-Myc. On the other hand, to further determine whether TLR3 plays a role in this process, we assessed the proliferation rate of TLR3-deficient cells and the pro-inflammation-relevant protein expression after poly (I:C) treatment. Cell lines deficient in TLR3 expression (i.e. CNE1-745 and CNE1-LMP-745) failed to respond to poly (I:C) treatment, moreover cells deficient in TLR3, indicative of the crucial role of TLR3 in the poly (I:C)-induced retarded proliferation as well as p65 inflammatory response.

Markedly, the enhanced LMP1 expression partially rescued the impaired pro-inflammatory products compared to CNE1-745 cell line. Since LMP1 could alleviate the inhibited cell proliferation, here we proposed an intriguing assumption: TLR3 could not only enhance the poly (I:C)-incited cell death and drive NF-κB p65 activation and triggered downstream pro-inflammatory response; that LMP1 may interact with TLR3 while restraining the cell death stimulated by poly (I:C), in which TLR3 was implicated. Therefore, there was a compensatory increase in TLR3 expression, and thus an enhanced inflammatory response. In other words, upon poly (I:C) stimulation, LMP1 may alleviate cell death through suppressing one of TLR3's dual-functions, which was compensated by elevated TLR3 expression and thus, the other one function of TLR3, which activated p65 inflammatory pathway, was enhanced.

Inflammation is an essential protective immune response against viral infection, but it can go awry and stimulate the etiopathogenesis of a variety of chronic diseases including carcinoma. It is estimated that chronic inflammation can act as a primary promoting factor involved in sporadic tumorigenesis, accounting for approximately 20% of human cancer [34]. However, due to the complexity and diversity of the mechanisms, the molecular linkage of chronic inflammation to tumor development remains to be unveiled. As a hallmark of inflammation that is usually found in carcinomas [35], NF-κB may serve as a matchmaker in the context of inflammation-associated cancer [36]. Here we proposed that TLR3 is potentially essential for promoting inflammation-associated NPC progression by switching on NF-κB. On the other hand, it played a critical role in poly (I:C)-triggered cellular growth inhibition, but this was targeted and repressed by LMP1. Therefore, TLR3 and LMP1 are candidate targets in the development of novel drugs for NPC treatment.

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Disclosure of conflict of interest

None.

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