Effects and mechanisms of innate immune molecules on inhibiting nasopharyngeal carcinoma

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To the Editor: The nasopharyngeal epithelium is frequently exposed to various harmful carcinogens. When the nasopharyngeal mucosa is damaged, local inflammation is induced.[1] Chronic inflammation of the nasopharyngeal epithelium may eventually evolve into nasopharyngeal carcinoma (NPC). While the mucus secreted by the nasopharyngeal epithelium forms the body's primitive immune protection barrier as it contains a series of proteins and peptides that can sense, bind, degrade and remove various harmful substances. Lactotransferrin (LTF)[2] and bactericidal/permeability-increasing (BPI) protein family members[3] are an important component of the innate immune protection barrier.

There are two BPI protein family members, BPIFA1 (also named SPLUNC1)[4] and BPIFB1 (LPLUNC1),[5] specifically expressed in human nasopharyngeal mucosa and the upper respiratory tract epithelium. They can specifically bind to the cell wall component of Gram-negative bacteria, have bactericidal function and possesses the capacity for endotoxin neutralization. BPIFA1 protein inhibited the growth of bacteria and demonstrated bactericidal effects.[6] An *in vitro* bacterial LPS binding assay further confirmed that BPIFA1 protein could specifically and directly bind to LPS through the BPI domain, thereby eliminating bacteria.[4]

Epstain-Barr virus (EBV) is a verified and important cause of NPC.[7] EBV mainly infects lymphocytes through the interaction of the EBV viral envelope glycoprotein gp350 with the receptor molecule CD21 on the surface of lymphocytes.[8] EBV selectively adheres to the surface of lymphocytes and then invades the lymphocytes.[9] However CD21 is not expressed on the surface of epithelial cells.[10] Zheng *et al*[10] observed the transmission of EBV from lymphocytes to nasopharyngeal epithelial cells through the co-culture of lymphocytes and nasopharyngeal epithelial cells. EBV bound to the CD21 on the lymphocytes, then the lymphocytes transferred EBV to the nasopharyngeal epithelium, which was then infected with EBV.[10] The treatment of EBV-transduced cells with BPIFA1 significantly promoted the apoptosis and lysis of EBV-infected cells, and some EB viruses also showed obvious structural damage.[4] Moreover, BPIFA1 also inhibits the expression of the EBV-encoded tumor gene *LMP1* and promotes the expression of the EBV surface glycoprotein gp350. The expression of gp350 facilitates the recognition of EBV by the human immune system and initiates the complement pathway and antibody-dependent cell-mediated cytotoxicity (ADCC) to eliminate the EB virus.[4] LTF is also highly expressed in the normal nasopharyngeal epithelium. LTF also reduced the efficiency of the infection of lymphocytes by EBV and inhibited the transfer of EBV from lymphocytes to epithelial cells. Furthermore, LTF could bind to CD21 on lymphocytes, thereby blocking the binding site of EBV.[10] This explains the effects of LTF on the prevention of adsorption and entry of EBV into lymphocytes, as well as the prevention of the transfer of EBV from lymphocytes to nasopharyngeal epithelial cells.[10]

LTF, *BIPFA1*, and *BPIFB1* are all significantly downregulated in NPC.[2] In patients with low expression of these innate immune moleculars, the prognosis is significantly worse, which suggests that *LTF*, *BIPFA1*, and *BPIFB1* are important candidate tumor suppressor genes in NPC.[2,11] As nasopharyngeal tissue progresses from mild dysplasia to severe dysplasia and then to NPC, the expression levels of BPIFA1 and BPIFB1 were also gradually reduced. The concentrations of BPIFA1 or BPIFB1 protein in nasopharyngeal secretions of NPC

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patients were also significantly lower than those of normal controls, which suggest that they can serve as molecular markers for early screening of NPC.

High degree of infiltration by inflammatory cells is often observed in biopsy samples of NPC. Epithelial cell proliferation stimulated by nonresolving inflammation is the first step in the malignant transformation of inflammation to cancer.[12] Liao *et al* used LPS to stimulate macrophage cells to mimic an inflammatory state and used the cell culture supernatant of LPS-stimulated macrophages (containing various inflammatory factors) to treat NPC cells. They found that inflammatory factors activated the NF-κB and STAT3 signaling pathways through Toll-like receptors (TLRs) and promoted the proliferation of NPC cells. The activation of TLRs and the NF-κB and STAT3 pathways is the key step in the initiation of the inflammatory response.[13]

EBV also activated the NF-κB pathway through the interaction of its surface glycoprotein gp350 with TLR2 on the surface of macrophages.[13] Moreover, EBV particles, EBV DNA and various encoded products can be recognized by TLR9 on macrophages, which leads to the activation of the NF-κB pathway and then induces the synthesis and release of the pro-inflammatory factors IL-8 and MCP-1. CD14 on the cell surface acts as a co-receptor of TLR9,[14] promotes the transport of EBV to the endosome and enhances the ability of TLR9 to recognize ligands, which promotes inflammatory responses. NPC epithelial cells can also be stimulated by inflammatory factors in the inflammatory microenvironment to activate inflammation-related signaling pathways such as NF-κB and STAT3, secrete pro-inflammatory factors and maintain and amplify local inflammatory responses.[11] Excessive inflammatory reactions, especially in a microenvironment that results from nonresolving inflammation, may lead to DNA breakage, increase the probability of mutation and induce cell proliferation, which may eventually lead to carcinogenesis.[13] Furthermore, inflammatory factors can also promote the growth and metastasis of tumor cells and accelerate tumor progression. In addition, EBV expresses the tumorigenic protein LMP1, which promotes the proliferation of nasopharyngeal epithelium through the regulation of a series of signaling pathways in host cells, including the expression of miR-203 and its target genes *E2F3* and *CCNG1*.[8]

The innate immune molecules expressed by the host cells play an important role in inhibiting the malignant transformation of the nasopharyngeal epithelium. LTF not only reduces the ability of TLR9 to recognize EBV DNA but also inhibits activation of the NF-κB pathway and reduces the EBV-induced inflammatory response.[14] LTF can also regulate the G1/S phase of the cell cycle regulatory network centered on Cyclin/CDK/CKI/pRb to inhibit NPC cell growth and proliferation.[2] Moreover, through the downregulation of the expression of c-Jun (AP1 transcription factor), LTF can inhibit the transcriptional activation of c-Jun by the AKT pathway kinase PDK1, which downregulates the activity of the AKT pathway and inhibits the growth of NPC cells through downstream AKT signaling.[15]

BPIFA1 and BPIFB1 can also significantly inhibit the growth of NPC cells, induce apoptosis of NPC cells and partially reverse their malignant phenotype. BPIFA1 increases the expression of the miR-141 target gene *PTEN* through the downregulation of miR-141 and inhibits the proliferation of NPC cells through the downstream PTEN signaling pathway.[16] Further studies on miR-141/PTEN downstream signaling revealed that, following the upregulation of PTEN expression and the inhibition of PTEN phosphorylation by BPIFA1, on the one hand, BPIFA1 upregulates p27 expression to inhibit cell cycle-associated proteins, which causes cell cycle arrest at G0/G1 phase in NPC cells.[16] On the other hand, BPIFA1 induces apoptosis of NPC cells via the regulation of the Bcl/Bax/Bad signaling pathways. Similar to LTF, BPIFB1 also inhibits the activity of the MAPK signaling pathway, which then downregulate the expression levels of cyclinD1 and CDK4 and Rb phosphorylation. This in turn slows down the G1-S progression in NPC cells and blocks their proliferation.[5] BPIFB1 can promote apoptosis of NPC cells by regulating the expression levels of Bcl-2 and Bax. Furthermore, BPIFB1 can significantly inhibit the activation of the NF-κB pathway after it is induced by inflammatory factors such as IL-6, inhibit the nuclear translocation of NF-κB and STAT3 and reduce the transcriptional activity of NF-κB and STAT3.[11] BPIFB1 can also repress the NPC cell proliferation induced by inflammatory factors through downstream signaling pathways of NF-κB and STAT3.

The inflammatory microenvironment can lead to the occurrence of malignant tumors, and various inflammatory factors can also promote the invasion and metastasis of tumors. However, innate immune molecules can inhibit the invasion and metastasis of NPC in different ways. Deng *et al* found that overexpression of LTF in NPC cells significantly decreased the ability of cells to invade and metastasize and that the number of metastatic lesions was significantly reduced after engraftment of these cells into nude mice.[15] The molecular mechanism involves the binding of LTF to the NPC cytoskeletal protein K18 through which invasion and metastasis of NPC are inhibited via the regulation of 14-3-3 protein and its downstream signaling pathway.[15] LTF can also inhibit PDK1 and then inhibit AKT phosphorylation and membrane translocation, which leads to the inhibition of tumor cell proliferation, invasion and metastasis through the PDK1/AKT pathway. By exploring the upstream regulatory mechanism of *LTF*, it was found that *LTF* is the target gene of miR-214.[17] LTF is negatively correlated with miR-214 expression in NPC biopsy samples, and the expression of miR-214 is significantly higher in NPC metastases than in primary tumors. Inhibition of miR-214 expression can significantly increase the expression of LTF and reduce the invasion and metastatic ability of NPC cells.[17]

Radiotherapy is currently the preferred clinical treatment for NPC. However, some patients experience radiotherapy resistance, local recurrence and distant metastasis, which are the main causes of treatment failure.[18] In NPC patients with similar tumor grades after treatment with the

same dosage of radiation therapy, the progression-free survival (PFS) and overall survival (OS) of patients with positive BPIFB1 expression were significantly higher than those of patients whose tumors did not express BPIFB1.[11] This suggests that BPIFB1 may also play roles in the metastasis and radiosensitivity of NPC. Through 2D and 3D culture models and a nude mouse model of "tail vein injection-lung metastasis," Wei *et al*[3] confirmed that BPIFB1 could significantly inhibit the invasion and metastasis of NPC cells *in vitro*. Two molecules that interact with BPIFB1, VTN, and VIM, were screened and identified by immunoprecipitation combined with protein mass spectrometry.[3] Further studies found that BPIFB1 could downregulate the expression levels of VTN and ITGAV and could inhibit the formation of the VTN/ITGAV complex and the activation of the FAK-Src-ERK (downstream of ITGAV) pathway.[19] This results in the inhibition of NPC cell invasiveness and migration. BPIFB1 can also bind to the mesenchymal marker VIM to inhibit its expression, which leads to the inhibition of the invasion and metastasis of NPC cells, and thus the inhibition of epithelial-mesenchymal transition (EMT). BPIFB1 could also enhance the sensitivity of NPC cells to ionizing radiation. The mechanism of action occurs through the association of BPIFB1 and VTN, which inhibits the activation of the ATM/Chk2 and ATR/Chk1 DNA damage repair pathways, reduces the survival ability of NPC cells after ionizing radiation and promotes cell apoptosis.[18]

The carcinogenic mechanism of EBV is far from clear. In addition to encoding a series of tumorigenic proteins, it has recently been found that EBV can also regulate the expression levels of its own genes and host genes by encoding a series of microRNAs (miRNAs).[20] The regulation of immune and inflammation-related pathways by some EBV-encoded miRNAs has also been reported. However, their relationships with innate immune molecules deserve further exploration. LTF, BPIFA1, and BPIFB1 are innate immune proteins secreted by human nasopharyngeal epithelium. They all exert the effects of resisting pathogenic microbial infections, inhibiting chronic inflammation and arresting the development of tumors. They regulate the proliferation, apoptosis, invasion, and metastasis and radiotherapy resistance of NPC cells through multiple signaling pathways. The signal transduction pathways regulated by these proteins finally converge into several key signaling pathways such as the NF-κB, MAPK, and AKT pathways. However, whether cross-talk exists between these innate immune molecules and their downstream signaling pathways is worthy of further investigation. It is worth exploring not only whether EBV-encoded miRNAs are involved but also whether non-coding RNAs of the host cell itself are involved. These non-coding RNAs include miRNA, long non-coding RNA and circular RNA (circRNA), which have recently become a research hotspot in the biomedical field. In conclusion, comprehensive elucidation of the roles and mechanisms of innate immune molecules has important value for understanding of the pathogenesis of NPC.

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Conflicts of interest

None.

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