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Original Research Article

EBV-positive LMP1-mediated Signal Pathway in Nasopharyngeal Carcinoma

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Abstract: Nasopharyngeal carcinoma (NPC) is one of the main cancers and death factors of otorhinolaryngological malignant tumors. A number of studies have shown that the occurrence and development of NPC is clearly related to Epstein-Barr virus (EBV) infection. Among the few proteins expressed by EBV, latent membrane protein 1 (LMP1) is considered to be a protein with the function of oncogenes, because it can make the epithelium tend toward malignant transformation; therefore LMP1 plays an important role in the occurrence and development of NPC, and is also considered to be the oncogene of EBV. LMP1 can further affect cell migration and apoptosis by participating in multiple intracellular signal transduction pathways, which is closely related to a variety of lymphoid tissue diseases and malignant tumors; nasopharyngeal carcinoma is no exception. This article reviews the signal pathways mediated by LMP1 in NPC.

Keywords: Epstein-Barr virus (EBV); Latent membrane protein 1 (LMP1); Nasopharyngeal Carcinoma (NPC); Signaling pathway

1. NF-κB

There are three pathways of the NF- κ B signal pathway in malignant tumors:

(1) TRAF2 and TRAF3 activate Iκkinase and promote the phosphorylation of IκBα, then separate from NF-κB and hydrolyze immediately , and finally, NF-κB is transferred into the nucleus to function as a transcription factor;

(2) Trad binds to the YDD domain of the LMP1 CTAR2 domain and activates NF-κB. it is also mediated by IκBα phosphorylation pathway;

(3) In the absence of KKγ, LMP1 mediates the hydrolysis of NF-κB2 P100 into active P52, while P52 can be transferred to the nucleus to combine with NF-κB subunit p65 and Re 1B to form active NF-κB, thus regulating the NF-κB signal transduction pathway. In NPC, Liu HD et al.[1] found that the NF-κB pathway was inhibited by the chemical inhibitor Bay11-7082 and the stable or transient expression of IκBα(DNMIκBα) dominant negative mutant, indicating that this site is functional and LMP1-enhanced iEκ activity is partially regulated by this site. It has further been demonstrated that LMP1 promotes the binding of NF-κB subunits p52 and p65 to κNF- κB and κAP-1 motifs in vitro, respectively. Chemical inhibitors targeting NF-κB pathway and dominant negative mutants can weaken the enhanced binding of LMP1. TAO YG et al.[2] showed that LMP1 not only subtly regulates EGFR expression and up-regulates EGFR phosphorylation through its CTAR1 recruit TRAFs molecule, but also trans-activates EGFR promoter activity through the CTAR1-mediated NF- κB signaling pathway. The latest study [3] shows that LMP1 can up-regulate the expression of neurotrophin tyrosine kinase type 2 receptor (NTRK2 or TrkB) to further affect the ability of nasopharyngeal carcinoma cells to resist apoptosis and promote the migration and invasion of nasopharyngeal carcinoma cells.

2. PI3K -Akt/PKB

In malignant tumors, when cells are stimulated by growth factors, Akt/PKB is transferred to the cell membrane and obtains catalytic activity to catalyze its own phosphorylation of S124 and T450. At the same time, PI3K catalyzes its substrate to produce PIP3, PDK-1, and then catalyzes the phosphorylation of T308 and S473 of Akt/PKB in the presence of PIP3, which makes Akt/PKB fully activated and gives full play to its biological function. In nasopharyngeal carcinoma cell lines, Yang CF et al. [4] demonstrated that LMP1 can activate and trigger the phosphatidylinositol 3 kinase / protein kinase B (PI3K/AKT) pathway, and further stimulate the expression of CSC markers in cells, the development of side groups, and tumor formation. This study shows that PI3K/AKT pathway plays a very important role in the induction and maintenance of CSC in nasopharyngeal carcinoma. The PI3K/AKT pathway is also activated by phosphorylase in LMP1-induced CD44 (+ / High) cells. The study also found that LMP1 mainly regulates the expression of human miR-21 through the PI3K/AKT/ FOXO3a signal pathway, and also activates its downstream effector Bcl-2, through the PI3K/Akt signal pathway, which can lead to the occurrence of NPC. The latest studies indicated that cholesteryl ester promoted NPC cell proliferation by activating the PI3K/AKT pathway and inhibition of this pathway in SQLE-over expressed or cholesteryl ester-treated cells resulted in a significant reduction of NPC cell proliferation.

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3. MAPK

MARK mainly consists of three families: p38, JNK and ERK, and many studies around the world have shown that the occurrence of malignant tumors is related to them. When these three pathways were inhibited, the invasion ability of NPC cells was decreased. Some studies [5] have shown that when inhibiting the expression of p38MAPK signal pathway, the activity of MMP-1 will also be affected and further inhibited. Inhibition of the JNK/SP-1 signaling pathway can reduce mMP-2 expression by regulating TPA-induced MMP-9 over expression and the erK1/2 signaling pathway, both of which can inhibit the movement of NPC cells [6].

(1) Raf→MEK→ERK pathway, Mainou et al.[7] showed that ERK1/2 is mainly activated by the interaction between CTAR1 of LMP1 and TRAF2 or TRAF3. Dawson et al. [8] found that LMP1 may bypass Ras and Raf and initiate this signal pathway directly from MEK through the experimental study of human epithelial cell line SCC12F. The latest study [9] found that LMP1 inhibits the phosphorylation of AMPK and its substrates ACC and Raptor by phosphorylating LKB1, at serine 428, thus inhibiting the LKB1-AMPK pathway. The MEK/ERK-MAPK signal pathway activated by the CTAR1 domain is the reason for the inactivation of LKB1-AMPK;

(2) JNK/SAPK： Eliopoulos AG et al. [10] found that stable or transient expression of CTAR2-mediated B95.8 LMP1 prototype in epithelial cells or B-cell-derived cells can activate c-Jun N-terminal kinase (JNK, also known as stress-activated protein kinase, SAPK) pathway);

(3) P38-MAPK :Zhang Z et al. [11] found that EVS (the source of LMP1 positive nasopharyngeal carcinoma cells) can induce the proliferation and invasion of nasopharyngeal carcinoma cells, inhibit cell apoptosis, especially promote radiation resistance, and transduced LMP1 can realize its carcinogenic function by activating the P38MAPK signal in the receptor cells.

4. JAKs/STATs

JAK is a non-receptor tyrosine kinase. Members of the JAK family include JAK1/JAK2/JAK3 and Tyk2. The STAT family is mainly composed of STAT1, STAT2, STAT3, STAT4, STAT5a and so on. They are considered to be important centers for the regulation of many cytokines and growth factors [12]. Vaysberg M et al. [13] found that LMP1 could combine with STAT to initiate the JAK-STAT signaling pathway to promote cell proliferation, activate the MAPK pathway to affect cell invasion and metastasis, and inhibit cell apoptosis by up-regulation of bcl-2 expression. The JAK2/STAT3 signaling pathway is the one most closely associated with NPC metastasis. After activation of this pathway, coX-2 expression can be abnormally increased. Cox-2 cannot only cooperate with VEGF to promote tumor angiogenesis, but also cooperate with MMP-2 to promote tumor angiogenesis and tumor cell metastasis.

5. AP-1

Mutations of LMP1 within and downstream of iEκ, inhibited by chemical inhibitor SP600125, and stable or transient expression of C-Jun (TAM67) dominant negative mutants suggest that this site is partially regulated by functional and LMP1-enhanced iEκ activity. LMP1 promotes the binding of AP-1 family members c-Jun and c-Fos to κAP-1 motifs in vitro, and chemical inhibitors targeting AP-1 pathway and dominant negative mutants can weaken the enhanced binding of LMP1 [1]. Zheng P et al. [14] recent studies have shown that LMP1 can up-regulate the Capn4 promoter through the C-terminal activation region CTAR 1 and CTAR2 domain in a dose-dependent manner to activate AP-1, and that LMP1 promotes actin rearrangement by activating AP-1, Capn4 and LMP1 through ERK/JNK phosphorylation, and ultimately promotes the migration of NPC cells. O'Neil JD et al. [15] found that binding of EBNA1 to the promoters of C-Jun and ATF2 enhances the activity of AP-1 transcription factor in NPC cells. This study also showed that the expression of AP-1 in response to the expression of interleukin8, vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1 increased in response to the expression of EBNA1, thereby promoting the formation of blood vessels.

6. Others

Recent data suggest that LMP1 deoxyribozyme targeting inhibits tumor angiogenesis by increasing radiosensitivity via the JNKs/HIF-1 pathway in NPC. Yang L et al. [16] found that LMP1 increased VEGF expression through the JNK/ C-Jun signaling pathway, and showed that DZ1 inhibited HIF-1/VEGF activity enhanced the radiosensitivity of NPC cells.. Ding RR et al. [17] found that LMP1 mediated the expression of PIM1 through NF-κB, PKC and STAT3 signals, promoted the proliferation of NPC cells, and was involved in the clinical progression of NPC. Fang W et al. [18] found that EB virus-induced latent membrane protein 1 (LMP1) and interferon-γ pathway co-regulated programmed cell death protein 1 ligand (PD-L1). This study further confirmed that LMP1 up-regulated PD-L1 through STAT3, AP-1 and NF-κB pathway. In addition, the up-regulation of interferon-γ on PD-L1 in NPC does not depend on LMP1, but has a synergistic effect with LMP1. Xiang YP et al. [19] found that pY772-EphA2 can further promote the growth of EphA2-dependent nasopharyngeal carcinoma cells in vivo and in vitro by activating Shp2/Erk-1/2 signal pathway, suggesting that it is an important target for the treatment of NPC.

7. Conclusion

Some achievements have been made in the study of LMP1-mediated signal pathways in NPC associated with EBV infection. These signaling pathways are involved in the occurrence and development of NPC and promote the proliferation and metastasis of NPC cells through different pathways, but they are always complex, and the activation pathways of signal pathways and their molecular mechanisms are also intertwined. In different environments and cells, the multiple biological functions of the pathways, and how different pathways coordinate regulation and integration of information, need to be further studied. How to effectively treat diseases caused by EBV at the LMP1 level should also be the focus of future research (not just NPC). At present, a small number of effective components of traditional Chinese medicine have been studied as targeted drugs for malignant tumors. It is hoped that the above signal pathways, combined with existing research techniques and means, the mechanism of the action of more and more traditional Chinese medicines in NPC can be studied. It is further hoped that these studies can provide more choices for the

study of molecular targeted drugs, provide more hope of survival, and eventually provide the cure for patients with LMP1-positive nasopharyngeal carcinoma.

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