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Role of Epstein-Barr virus in the development of nasopharyngeal carcinoma

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Abstract: Southern China experiences larger extent of total cancer pathologies, of which nasopharyngeal carcinoma has the highest incidence under otorhinolaryngeal malignant carcinomas. Risk factor of nasopharyngeal carcinoma varies from hereditary causes to virus infection, among which Epstein-Barr virus (EBV) infection is the mostly investigated. The study into mechanism of EBV in occurrence, development and prognosis of nasopharyngeal carcinoma has been studied for several decades. The pathophysiology in making of EBV into a cancerogen includes proteins as latent membrane protein 1 (LMPs) and nucleic acids as micro-RNAs. In this paper, we reviewed till date studies focusing on relationship between EBV and nasopharyngeal carcinoma.

Keywords: Nasopharyngeal carcinoma; Epstein-Barr virus; LMP1; MicroRNAs

1 Introduction

According to recent research, 80% of nasopharyngeal carcinomas (NPC) in China are found in southern geographical areas. The incidence of NPC in male is two to four times higher than that in female. Radiotherapy is often treatment of choice while the prognosis is not satisfying

ascribed to relapse and cause early metastasis. Causes of NPC are not explicit, while mainstream opinions are that NPC is closely related with latent Epstein-Barr virus (EBV) infection, hereditary factors and environmental factors are also responsible for many kinds of lymphomas and epithelial tumors and hence can modulate mechanisms affecting carcinogenesis, proliferation, apoptosis, death and migration of cells, epigenetically change lymphocyte-specific processes and induce cell immortalization. Mechanisms underlying the carcinogenic effects of EBV involve LMP1, LMP2, microRNA and other molecules that we will introduce in the following sections [1-3].

2 LMP1

EBV-encoded latent membrane protein 1 (LMP1) is a 66-KD integral membrane protein that is closely associated with poor prognosis of NPC. Therefore, LMP1 is considered behind the fact that EBV happens to modulate most of the cell processes including migration, proliferation, metabolism and tumorigenesis through alternation of various kind of target proteins, RNAs and signaling pathways.

Endothelial cell specific molecule (endocan, or called Esm-1), was found in 52% of NPC specimens. Endocan could stimulate migration and invasion of endothelial cells, and indicates a shorter survival in NPC patients. Endocan can be upregulated by LMP1 through the LMP1-activated NF- κ B, MEK-ERK and JNK signaling pathways [4]. The phosphorylation of insulin-like growth factor 1 receptor (IGF1R) can be altered by LMP1, which depends on activation of NF- κ B signaling pathway and could be suppressed by I κ B α and TRAF6. These contributes to the transformation of epithelial cells induced by LMP1 [5]. Through phosphorylation and degradation of I κ B α , LMP1 activates NF κ B signaling pathway [6].

Phosphatase and tension homolog (PTEN) is a major tumor suppressor. LMP1 can induce a DNA methylation of PTEN via DNMT3b transcription up-regulated by LMP1-mediated NF- κ B. Thus tumor suppressor PTEN is silenced at the cellular and molecular level [7]. Expression of tumor necrosis factor α -induced protein 2 (TNFAIP2) is high in

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NPC tissues. This over-expression is transcriptionally induced by LMP1 through C-terminal-activating region (CTAR2) domain of TNFAIP2. NF- κ B participated in this process through a NF- κ B-binding site within the TNFAIP2 promoter. This enhances the expression of TNFAIP2 which further induce cell motility and thus contributes to promoting NPC tumor progression [8]. Glucose transporter-1 (Glut-1) is one of the direct target genes of NF- κ B signaling that is down-stream of activation of mTORC1 by LMP1. LMP1-induced NF- κ B activation leads to upregulation of Glut-1 transcription and growth of NPC cells, which results in aerobic glycolysis, cell proliferation and colony formation [9]. Through Toll-like receptor 3 (TLR3), EBERS induce inflammatory response in which macrophages are recruited in NPC cells. EBERS, LMP1 with NF- κ B form a positive regulatory loop that amplifies the inflammatory signals, which leads to a favorable microenvironment for solid tumor growth [10].

As a signal transducer and a transcriptional activator of many essential genes, the important roles of STAT3 in tumor generation, progression, metastasis and drug-resistance have been thoroughly investigated. LMP1 was found to be able to cause phosphorylation of STAT3 through activation of Janus kinase 3 (JAK3) and extracellular signal-regulated kinase (ERK) and then stimulated STAT3 nuclear accumulation [11]. Bcl-3 induction was mediated by this activation of STAT3. Through its carboxyl-terminal activation domain 1 (CTAR1), LMP1 activates both STAT3 and EGFR [12]. In NPC cells, level and stability of transcription of the HIF-1 α were significantly enhanced by LMP1 via interaction with the ERK1/2 and STAT3 signaling pathways through CTAR1 and CTAR3. ERK1/2/NF- κ B pathway recruited by LMP1 CTAR1 also facilitated HIF-1A promoter activity [13]. Through the JNKs/c-Jun signaling pathway, VEGF expression is increased by LMP1 [14].

Survivin is an inhibitor of apoptosis protein which is specifically expressed in tumor tissues and is related with proliferation of tumor cells. Expression of Survivin could be promoted by LMP1 in G0/G1, S and G2/M phase. LMP1 could also trigger accumulation of Survivin and CDK4 in nuclei and thus keep tumor cells from apoptosis. The function of Survivin is known to be closely connected with tumor suppressor gene P53. P53 protein levels were reduced by LMP1 through the increase in the polyubiquitination of p53 in NPC cells [15, 16].

MicroRNAs (miRNAs) are a collection of endogenous non-coding small RNAs found in eukaryocyte that regulate cell behaviors such as proliferation, apoptosis and tumor progression through binding to target RNAs. LMP1 could suppress miR-1 expression, of which K-ras is found to be a novel direct target. By repressing K-ras expres-

sion, tumour-suppressive effects of miR-1 was suppressed by LMP1 [17]. MicroRNA-21, a biomarker for chemo-resistance, its expression is triggered by LMP1 via the PI3K/Akt/FOXO3a pathway, which lead to the expression of PDCD4 and Fas-L, and at last results in chemo-resistance in NPC cells [18]. Aberrant expression of miR-155, which significantly increased in radio-resistant NPC tissues, can also be induced by LMP. Ubiquitin-1 expression is negatively correlated to MiR-155. The axis of miR-155-UBQLN1 could affect some important genes regulating cell proliferation, cycling, migration and invasion through PI3K/Akt pathway. As well, up-regulation of miR-155 in NPC driven by LMP1 lead to a downregulation of, which results in poor prognosis of NPC patients [19, 20]. Mir-204 inhibited invasion and metastasis of NPC cells partly through targeting cdc42. In NPC cells and tissues, miR-204 is found to be down-regulated, which indicates a more aggressive phenotype of NPC and poor prognostic. By activating Stat-3, LMP1 suppressed miR-204 expression [21]. LMP1 and transcription factor Twist-1 is also associated with miR-10b that was significantly up-regulated in NPC. MiR-10b is related with young age and advanced clinical stage [22].

The repair of DNA double-strand breaks (DSBs) is repressed by LMP1 through inhibiting phosphorylation and activity of DNA-dependent protein kinase (DNA-PK). LMP1 could also reduce the phosphorylation of AMP-activated protein kinase (AMPK), which is associated with glycolysis and resistance to apoptosis mediated by LMP1. The AMPK α (Thr172) reduction is a predictive factor for poorer clinical outcomes of radiation therapy in NPC patients [23]. The LKB1-AMPK pathway has anti-tumor activity via modulation of energy metabolism. LMP1-mediated AMPK inactivation is related to the proliferation and transformation of epithelial cells, which implicates the LMP1-driven pathogenesis of NPC [24].

LMP1 increases glucose and glutamine uptake in NPC cell, stimulates LDHA activity and production of lactate, while reduces pyruvate kinase activity and pyruvate concentrations. PKM2, LDHA and FGFR1 phosphorylation, as well as PDHK1, FGFR1, c-Myc and HIF-1 α expression, are also increased by LMP1 [25].

Rho GTPases, such as Cdc42 and Cdc2, are associated with actin cytoskeleton reorganization, which modulates cell morphology and motility and tumorigenesis. LMP1 can enhance FGD4 activity toward Cdc42, resulting in actin cytoskeleton rearrangement and motility increase of NPC cells [26]. LMP1 can also regulate Op18/stathmin signaling by cdc2 mediation and affect tumor phenotype and metastasis [27].

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which is involved in guideline of

cell proliferation, differentiation and cell cycle. Many aberrant expression of proteins in mTOR signaling pathway are important for tumor occurrence. Through phosphorylation of AKT/mTOR/P70S6K/4EBP1, LMP1 can upregulate the mTOR signaling pathway in NPC cell. Expression of genes in the mTOR pathway such as p-P70S6K, p-4EBP1 are significantly correlated with overall survival of NPC patients [28].

Transcription coactivator TAZ is a member of Hippo-related pathways, which can restrict cell proliferation and induce cell apoptosis. In a recent study, for LMP1-mediated cell proliferation, cancer stem cell-like properties and EMT, TAZ, frequently expressed in LMP1-positive NPC, plays an important role, which provide new insights into oncogenic mechanism of LMP1 [29].

ATOH8 is a transcript factor among the basic helix-loop-helix (bHLH) gene family. LMP1 can impair the occupancy of activated H3K4me3 and enhance the repressive occupancy of H3K27me3 on ATOH8 promoter, which leads to ATOH8 expression inhibition that promote malignant phenotype of NPC [30].

Ezrin, a membrane cross-linker protein, takes parts in signal transduction and phagocytosis of tumor cells and thus implicated in tumor cell metastasis through interaction with cell adhesion molecules. Recent data showed that LMP1-stimulated cell motility and invasion of NPC require the phosphorylation and recruitment of ezrin [31].

Mitogen- and Stress-Activated Kinase 1 (MSK1) is a nuclear kinase that is important for cell proliferation. High level of phosphorylated MSK1 is observed in poorly differentiated NPC tissues. LMP1-promoted cell proliferation is associated with increased MSK1 activity, which may be correlated with Fra-1 and c-Jun induction by it through phosphorylation of histone H3 [32].

Fibronectin is a high molecular weight glycoprotein. As an extracellular matrix protein, it is important in an adhesive growth of cells and is closely related with occurrence, development and prognosis of tumors. Induction of activin A and TGF β 1 and JNK/SAPK signaling are required for LMP1-mediated expression of fibronectin. The expression and activation of the major fibronectin receptor, α 5 β 1 integrin, is also induced by LMP1. Thus, these proteins contribute in the pathogenesis of LMP1-positive NPC by increase the metastatic potential of epithelial cells [33]. Aside of fibronectin, LMP-1 could induce cell surface interactions involving integrin- α 5 and N-cadherin as well and promote EMT of NPC [34].

Several stemness-related gene can be up-regulated by LMP1 and lead to increase of the cell number of side population (SP), enhanced self-renewal properties and tumor initiation ability in vivo. Cancer stem cell (CSC) marker

CD44 and radio-resistance are also regulated by LMP1, which might be the results of inactivation of DNA damage response (DDR) proteins including ATM, Chk1, Chk2 and p53 in EBV-positive NPC cells.[35] Phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway also plays an important role in the CSC properties induction and maintenance in NPC. LMP1, PI3K/AKT, miR-21 and PTEN could constitute a positive feedback loop that regulates LMP1-induced CSCs in NPC cell.[36]Some sequence variations of LMP1 may lead to a potential escape from host cell immune recognition, protecting latent EBV infection and causing an increase in tumorigenicity [37].

Learning from above, LMP1 is so important to EBV-associated NPC that anti-LMP1 HELA/CAR-T cells, LMP1-targeted DNA enzyme and human antibody Fab against LMP1 conjugated with mitomycin C (MMC) can all control NPC development in vitro and in vivo [38-40].

3 MicroRNAs

MiRNAs are small non-coding RNAs which through negatively regulating gene expression post-transcriptionally, mediate cell proliferation, apoptosis, and carcinogenesis. In NPC infected by EBV, few viral proteins are expressed but high levels of BamHI-A rightward transcripts (BARTs) are found to be anticipating in cell functions. EBV-encoded BART-miRNAs, including long noncoding RNAs (lncRNAs) and BART microRNAs (miRNAs) are closely related to EBV pathogenesis in NPC [41, 42].

MiR-BART3, miR-BART7 and miR-BART13 microRNAs are detected to be at abundant levels and regularly secreted extracellular of NPC cells which may make them new biomarkers for diagnosis and clinical predictors of NPC, and circulating examination shows that miR-BART17-5p can be a potential biomarker of a poor prognosis in post-treatment detections [43, 44].

EBV-miR-BART1 is highly expressed in NPC. Reduction of PTEN directly mediated by EBV-miR-BART1 activates PTEN-dependent pathways, PI3K-Akt, FAK-p130(Cas) and Shc-MAPK/ERK1/2 signaling included. As a result, migration, invasion and metastasis of NPC cells increases, and thus drive EMT [45]. EBV-miR-BART1 could also up- and down-modulate a number of metabolism-associated genes, such as PSAT1 and PHGDH [46].

BART promoters can be activated and the expression of BARTs can be modulated by NF- κ B in EBV-infected NPC cells. NF- κ B activity is correlated with expression of BART miRNAs and lnc RNAs in EBV-infected epithelial cells [47].

EBV-miR-BART7-3p is highly expressed in NPC cells. Through targeting PTEN and modulating PI3K/Akt/GSK-3 β signaling, EBV-miR-BART7-3p enhances cell migration/invasion, metastasis and EMT, leading to gain of mesenchymal features and loss of epithelial markers in NPC cells. That makes it correlated positively with node metastasis and clinical stage of NPC [48].

Over-expression of EBV-miR-BART10-3p is found in clinical samples which is correlated with poor prognosis. EBV-miR-BART10-3p directly modulates BTRC gene that encodes beta-transducin repeat containing E3 ubiquitin protein ligase (β TrCP). Through targeting BTRC and regulating the expression of the β -catenin and Snail downstream substrates, EBV-miR-BART10-3p promote the invasion and migration of NPC cells and lead to EMT [49].

Forkhead box P1 (FOXP1) plays a key role in monocyte to macrophage differentiation. EBV-miR-BART11 promotes inflammation-induced NPC carcinogenesis by directly targeting FOXP1 gene and inhibiting TAM differentiation mediated by FOXP1, and induce inflammatory cytokines secretion into the tumor microenvironment [50].

4 Other molecules

EBV-encoded Latent Membrane Protein 2A (LMP2A) is regularly expressed in NPC. Recently studies indicate that LMP2A expression interferes with Syk tyrosine kinase and integrin $\alpha 6\beta 4$ interaction by competitive binding to Syk, which is associated with migration and invasive property of LMP2A-expressing NPC [51].

Epstein-Barr virus-encoded latent membrane protein 2A (LMP2A) is an oncoprotein of EB virus and a well-known NPC activator which could increase tumor invasion through promotion of the epithelial-mesenchymal transition (EMT) of NPC. Overexpression of metastatic tumor antigen 1 (MTA1) significantly correlated with tumor metastasis via the Wnt1 pathway and β -catenin activation. A molecular connection between LMP2 and MTA1 has been established. LMP2A reinforces EMT by induce the expression of MTA1 via activation of the mTOR pathway and 4EBP1-eIF4E axis in NPC [52].

EBNA1 protein is a nuclear protein encoded by EB virus that is highly expressed in NPC tissues, and its expression was associated with transcription of EB virus and NPC lymph node metastasis. Expression of microRNA 200a (miR-200a) and miR-200b can be inhibited by over-expressed EBNA1 that is mediated by transforming growth factor- $\beta 1$. Expression of target genes of these microRNAs, zinc finger E-box binding homeobox 1 (ZEB1) and ZEB2,

are up-regulated, which in turn affect NPC cell morphology and the expression of EMT markers [53].

Peptidyl-prolyl-cis-trans isomerase NIMA-interacting 1 (PIN1) is found to be consistently expressed in almost all EBV-associated NPC cells. It is a vital regulator in cell survival and apoptosis through isomerizing specific phosphorylated amino acid residues. Suppression of PIN1 can lead to inhibition of cyclin D1 expression and activation of caspase-3 in NPC cells and restrain tumor growth. At the same time, PIN1 can regulate proliferation, colony formation and anchorage-independent growth of NPC cell [54].

In NPC cells, the EBV immediate-early protein BZLF1 plays a key role in transformation of EBV infection from latent to lytic forms, and the later form is implicated in human carcinogenesis. BZLF1 functions to bind with several DNA damage response (DDR) proteins and thus impair DNA damage repair and abrogate G2/M checkpoint, which induced genomic instability. In summary, BZLF1 contributes to the carcinogenesis of EBV-associated epithelial malignancies by induction of mis-localization of important DDR proteins, and BZLF1 may be the connection of lytic EBV infection with impaired DNA damage repair [55].

5 Conclusions

EBV plays an essential role in the development of NPC and targeting EBV may a great help in treatment of NPC. And that claims deep insight of underlying mechanisms of NPC derived from EBV infection. Associations of LMP1, EBNA and microRNAs with NPC cell behaviors and important signaling pathways have been elucidated. But targeted therapy has not been exploited, which makes further investigation still in need.

Conflict of interests: No authors report any conflict of interest.

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