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The role of Epstein-Barr virus in the pathogenesis of NasoPharyngeal Carcinoma

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Abstract
Nasopharyngeal Carcinoma (NPC) is a cancer of the head and neck originating from the epithelial cells of the nasopharynx. It is particularly prevalent in South and Southeast Asia as well as in North Africa, where its aggressive nature and high mortality index underscore the imperative for developing efficient diagnostic and therapeutic strategies. This paper examines the impact of Epstein-Barr Virus (EBV) on NPC development by considering its latent genes—such as LMP1, LMP2, EBNA1, and EBNA2—and the oncogenic pathways they initiate, including NF-κB, JAK/STAT, MAPK, PI3K, and Wnt, which collectively drive cell transformation, proliferation, and immune evasion. In addition, we address the role of EBV in promoting genetic instability through both genetic and epigenetic alterations and highlight emerging evidence on EBV genetic heterogeneity as a factor in disease aggressiveness. We further discuss existing diagnostic approaches based on EBV-specific biomarkers such as VCA-IgA, EBNA1-IgA, and viral DNA detection by PCR, and highlight emerging non-invasive methods that incorporate machine learning and smartphone-based imaging, such as the "Nose-Keeper", for early detection and personalized risk stratification. Such AI-powered solutions can potentially lower resource barriers and improve early detection outcomes. Additionally, this paper presents a machine learning–based screening model that integrates clinical, demographic, and serological data to minimize false positives and improve early detection, particularly in resource-limited or non-endemic settings. The paper reviews recent advances in targeted therapies concerning EBV—including immunotherapy, cytolytic virus activation therapy, and gene-targeting strategies—that seek to exploit EBV's unique role in NPC. Thus, the present study emphasizes the need for further large-scale epidemiological and molecular investigations to refine EBV-based diagnostic and therapeutic approaches, with the ultimate goal of improving patient management and survival in this complex disease.

Keywords
Nasopharyngeal carcinoma, Epstein-Barr virus, Early detection, Machine learning, Smartphone-based imaging, Diagnostic algorithms, Immunotherapy, Head and Neck cancer, Cytolytic virus activation therapy, False positive

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Introduction

Nasopharyngeal Carcinoma (NPC) is a type of head and neck cancer that originates in the epithelial cells lining the internal and external surfaces of the nasopharynx. It is located in the nasopharynx, the tissue that connects the nose and mouth to allow air to travel from the nose to the throat. There are different histological types of NPC, including undifferentiated non-keratinizing carcinoma, differentiated non-keratinizing carcinoma, keratinizing squamous cell carcinoma, and basaloid squamous cell carcinoma (1). Although NPC is rare in most parts of the world, it is greatly prevalent in South and Southeast Asia and North Africa, where it ranks as one of the top ten cancers by incidence and mortality. In these high-risk regions, NPC accounts for up to 20% of all cancers, with a 5-year survival rate for advanced stages of < 50% (2). Additionally, 120,434 cases of NPC globally have been recorded, along with 73,482 cases of mortality (3). These figures underscore the urgent need for heightened awareness and research, as NPC's aggressive nature and significant regional disparities highlight gaps in our understanding and treatment of this formidable disease.

NPC is a metastasis-prone malignancy closely associated with Epstein-Barr Virus (EBV). Despite over 90% of the global adult population being infected with EBV asymptomatically, NPC shows a striking geographical and ethnic distribution. In endemic regions, EBV is associated with 95% of NPC cases and 100% of NPC-related mortalities. Although EBV infects more than 90% of the global population for life, only a small fraction of infections eventually result in cancer, demonstrating the significance of additional environmental and genetic risk factors in the development of EBV-related malignancies, including NPC. (4). The interplay between EBV infection, host genetic predisposition (including polymorphisms in the HLA complex and genes such as TLR8), and environmental factors such as tobacco smoking, consumption of dietary nitrosamines, and occupational exposure is critical in NPC pathogenesis (5, 6).

EBV is one of the most common human viruses, also known as a ubiquitous gamma herpes virus, and was first discovered in Burkitt lymphoma cells during the 1960s (7). Since its discovery, EBV has been closely linked to many malignancies, including gastric carcinoma, Hodgkin's lymphoma, and NPC. It is common in both Southern and Northern Asia, where the NPC rates are also higher. An early association between EBV and NPC was discovered through the detection of EBV DNA in NPC-infected cells (6). In addition, EBV is prevalent in 100% of all undifferentiated nonkeratinizing squamous cell carcinoma, indicating its role in certain NPC subtypes (8).

There is strong evidence connecting Epstein-Barr Virus (EBV) and NPC, particularly in endemic regions. This has prompted further investigations into EBV-mediated molecular events to identify new biomarkers for early diagnosis and novel therapeutic targets. It is thus imperative to expand our understanding of the contribution of EBV to NPC to find new biomarkers for early diagnosis and improved treatment strategies. EBV infects cell types such as B cells and epithelial cells, and it is also found in tumor cells derived from NK/T cells and leiomyosarcoma (9). Moreover, EBV contributes to NPC through the expression of its latent genes, which are significant in the carcinogenic process. Some of these genes are LMP1, LMP2, EBNA1, and EBNA2 (10). LMP1 and LMP2 are crucial for cell transformation and proliferation, while EBNA1 and EBNA2 are necessary for the maintenance of the viral genome and the regulation of viral and cellular gene expression. Overall, these latent genes promote cell survival,

proliferation, immune evasion, and the development and progression of NPC (10).

EBV can be used in the early detection and therapy of NPC using specific biomarkers and therapeutic targets. For instance, EBV DNA in the blood can serve as a biomarker to monitor disease progression (11). Additionally, antibodies against EBV proteins—such as VCA and EA—are elevated in NPC patients, indicating that these antibodies can serve as biomarkers for early detection (12, 13). In terms of treatment, immune-based therapies that target EBV-specific cytotoxic T lymphocytes (CTLs) and viral proteins can be employed to recognize and destroy EBV-infected NPC cells (14).

This information highlights the necessity and urgency of further investigating EBV and its role in NPC carcinogenesis, as it could lead to new approaches for both diagnosis and treatment. This review will discuss the role of EBV in NPC and how this knowledge may be utilized for early detection and therapeutic targeting.

Discussion

EBV - induced NPC carcinogenesis

Oncogenesis is another term for the process of turning normal cells into cancerous ones, otherwise referred to as tumorigenesis or, more traditionally, carcinogenesis. This process is thought to be characterized by a progressive sequence of genetic alterations affecting cellular identity and growth, leading to cancer. There are three stages to carcinogenesis: initiation, promotion, and progression. Genetic mutations resulting from environmental factors such as chemicals, radiation, or pathogens, enable oncogenic transformation where the cells grow and proliferate to form a malignancy (15). Although EBV infection alone can initiate oncogenic changes, most EBV carriers never develop NPC, suggesting that EBV's full carcinogenic potential requires additional genetic susceptibilities and environmental stimuli (5).

EBV primarily infects B lymphocytes and epithelial cells through attachment to specific receptors on the cell membrane surface, including CD21, HLA, and EphA2. The fusion of EBV and the host cell membrane can establish either a lytic or latent cycle. In the lytic cycle, the initiation of viral DNA replication and production of viral particles occur, leading to the active spread of infection (16). In the case of NPC, EBV predominantly enters a latent cycle, which is when EBV stays in a host cell without producing viral particles, ensuring that the infection will evade the immune system and establish itself long term in the host. This process of infection and gene expression by EBV – in association with other opportune host cell susceptibilities - contributes to the disruption of normal cellular mechanisms, ultimately leading to the oncogenic transformation of infected cells and the development of NPC.

Once EBV establishes a latent infection in NPC cells, a type II latency pattern is typically observed, characterized by the expression of viral proteins such as EBNA1, LMP1, and LMP2, as well as non-coding RNAs (17). These viral gene products interfere with normal cell-cycle regulation, inhibit apoptosis, and promote genomic instability and are essential for viral genome maintenance, tumor progression, and immune evasion (18). LMP1, a key oncoprotein, acts as a continuously signaling active receptor, activating multiple oncogenic pathways—including NF-κB, JAK/STAT, MAPK, PI3K, and Wnt—thus enhancing cell proliferation, survival, immune evasion, and metastatic behavior. LMP2 contributes to cellular transformation and proliferation, enabling EBV-infected NPC cells to survive and expand despite tumor suppressor mechanisms. EBNA1, on the other hand, plays

a crucial role in viral genome replication and segregation during cell division, ensuring the virus persists in host cells. Additionally, EBV-encoded microRNAs (e.g., those from the BART family) modulate the expression of cellular genes involved in DNA repair and apoptosis, thereby compounding the malignant transformation process (6).

Recent studies have also highlighted the importance of EBV genetic heterogeneity. For example, polymorphisms in the LMP1 gene (including the 30-bp deletion) and variations in EBNA1 have been associated with more aggressive tumor phenotypes and increased NPC risk. Detailed viral genomic analyses of strains isolated from NPC patients (e.g., GD1, GD2, C666-1, M81) provide insights into the role of EBV sequence variation in disease progression and treatment response. Hence, a joint analysis of host and viral genomic variations may yield novel biomarkers for risk stratification and therapeutic targeting (6).

EBV also plays a key role in genetic instability in NPC by disrupting DNA repair mechanisms and cell-cycle checkpoints. For instance, LMP1 represses nucleotide excision repair via the PI3K/Akt/FOXO3a pathway and compromises the G2 checkpoint by impairing Chk1 activation, leading to the accumulation of chromosomal aberrations. Moreover, EBV latent proteins can induce epigenetic alterations such as promoter hypermethylation of tumor suppressor genes (e.g., PTEN) and modifications in histone marks, which together silence critical tumor suppressor genes and further drive oncogenesis (6)

Several oncogenic signaling pathways are hijacked by EBV proteins, contributing to NPC progression. Luo et al. showed that the protein LMP1 activates the NF-κB pathway, which functions as a transcription factor that becomes activated upon phosphorylation by kinases (19). This process enables the NF-κB to translocate into the nucleus, thus driving the transcription of genes involved in inflammation, immunity, and stress response. In NPC-infected cells, there are somatic mutations of genes for regulators of the NF-κB pathway. It has been noted that the majority of these mutations involved downregulation in the NFKBIA inhibitor, CYLD, and in tumor necrosis factor receptor-associated factor 3. CYLD downregulation inhibits the suppression in growth and promotes proliferation, metastasis, and migration of NPC cells. In general, it is clear that unregulated NF-κB pathways or LMP1 lead to an advanced incidence of NPC.

Another pathway implicated in the differentiation of NPCs is the JAK/STAT (13). JAK is a family of intracellular, non-receptor tyrosine kinases that relay signal transduction initiated by cytokines. On being phosphorylated, STAT relocates to the cell nucleus, where it initiates gene expression. STAT3 is generally a signal transducer and activator of transcription that conveys signals from cytokine receptors all the way through to the DNA in the nucleus, promoting genes involved in cell growth and proliferation, survival, differentiation, and immune responses. This pathway is activated through the expression of proteins LMP1 and LMP2; which in turn, originate from EBV infection. After the activation of this pathway, survival, proliferation, and immune evasion influence the impact on NPC cells. The mitogen-activated protein kinase pathway is the pathway through which MAPK transmits signals from cell surface receptors by phosphorylation. Some of the EBV proteins that mediate this pathway are LMP1 and LMP2A. Other implications include cell proliferation and differentiation, cell motility, and hypoxia. Generally, LMP1 and LMP2A act on cell signaling pathways to aid in the growth, progression, and survival of NPC cells and tumors (13).

Other critical pathways influenced by EBV include the PI3K and Wnt signaling pathways. The PI3K, otherwise known as the phosphoinositide 3-kinase pathway, is a major intracellular signaling pathway. It controls certain cellular functions such as growth, proliferation, differentiation, motility, and survival, among others. Latent proteins such as LMP1 and LMP2A from the Epstein-Barr virus take advantage of the PI3K pathway. Activation of the PI3K pathway has implications for cell survival, oncogenesis, resistance to apoptosis, and metabolic changes (13) The Wnt signaling pathway, which controls embryonic development and tissue homeostasis, is activated by EBNA1, LMP1, and EBNA3C, promoting tumor stem-like characteristics, unchecked proliferation, and immune evasion. This pathway is important for NPC tumor progression, enabling cells to avoid maturing and to continue to spread aggressively (13).

EBV in NPC diagnosis

The diagnosis of nasopharyngeal carcinoma typically involves clinical examinations, imaging techniques, and biopsy. However, these methods are often imprecise and unreliable due to the tumor's obscure anatomical location and the overlap of its symptoms with common benign conditions. NPC can present with mild symptoms such as nasal congestion and recurrent infections, which can be mistaken for conditions like allergies or sinusitis. Because of these non-specific clinical manifestations, misdiagnosis rates can be as high as 43% (20, 21). These factors explain why NPC is often diagnosed at an advanced stage, increasing the likelihood of cancer spreading to nearby structures before the patient can seek medical attention. Although imaging modalities like MRI and CT scans are used, they may not always reliably distinguish NPC from other head and neck tumors.

Given the strong association between EBV and NPC (5), several EBV-based biomarkers have been developed to improve early detection and prognosis. Conventional serological markers, such as viral capsid antigen (VCA-IgA), early antigen (EA-IgA), and EBNA1-IgA have been widely used in endemic regions. These antibodies are produced in response to EBV infection, and their elevated levels can signal the presence of NPC before clinical symptoms appear (22). Specifically, EBV-VCA IgA has been identified as an early marker for NPC because its levels increase and persist before the disease becomes clinically apparent (23). For example, plasma EBNA1-IgA has been shown to distinguish early-stage NPC with a specificity of 98.7% and a sensitivity of 91.5%. Combined detection of VCA-IgA and EA-IgA by immunoenzymatic assays has further improved early diagnosis rates, ranging from 55% to 87% in endemic areas (24, 25). ELISA techniques have also enhanced sensitivity and specificity for EBV antigen detection, and quantitative PCR (qPCR) assays targeting EBV genomic regions (such as BamHI-W and EBNA1) have successfully detected cell-free EBV DNA in over 95% of NPC patient plasma samples (26). Importantly, a seminal study found that EBV DNA was present in the sera of 13 out of 42 NPC patients, and it was absent in 82 healthy controls, confirming that circulating EBV DNA likely originates from tumor cells and may serve as a specific diagnostic marker (27)**.** Some studies suggest that EBV DNA obtained from nasopharyngeal swabs may yield even higher accuracy with fewer false positives compared to plasma-based assays (6).

In NPC patients, the concentration of plasma EBV DNA is related to the disease stage, clinical outcome, and overall survival; thus, it can be a potentially important prognostic marker for staging and monitoring therapeutic effects. Despite its potential as a biomarker, variability in EBV DNA assay methods and a

lack of standardization have been major issues; therefore, uniform protocols are required to be reproducible and reliable (11). Standardization of anti-PD-1 drugs to chemotherapy as a first-line treatment for NPC has shown significant improvement in progression-free survival (27).

Recent research has also identified EBV-encoded microRNAs as potential non-invasive biomarkers for NPC. Studies have highlighted the role of the BART family miRNAs (e.g., BART2-5p, BART6-3p, BART7-3p, BART7-5p, BART9-5p, BART11-3p, BART17-5p, and BART19-5p) in the early detection and monitoring of disease (28). These EBV-derived miRNAs, which are elevated in the plasma of NPC patients, may serve as promising molecular markers for diagnosing NPC and assessing treatment response (29, 30). However, further large-scale studies and standardized protocols are needed to validate the efficacy of these EBV-encoded miRNAs as biomarkers in NPC, as existing research has not fully accounted for geographical, genetic, and environmental factors.

Despite their effectiveness in diagnosing EBV infection in suspected cases, serological testing and EBV-based biomarkers alone are still unreliable for mass screening of the general population. While EBNA1-IgA and qPCR-based EBV DNA detection are useful in distinguishing NPC patients from healthy individuals, their positive predictive value (PPV) drops significantly in mass screening settings. In low-prevalence populations, for example, anti-EBV IgA serological tests, such as VCA-IgA and EBNA1-IgA, have shown PPVs as low as 4%, leading to false-positive rates exceeding 95%. Even in high-prevalence (endemic) regions, widespread latent EBV infection further complicates NPC screening, as many individuals test positive for EBV despite not having cancer. This results in a high false-positive rate even in endemic settings, reducing screening efficiency and compliance. Additionally, while EBV DNA detection in plasma has been proposed as a screening tool, its sensitivity for detecting early-stage NPC remains low, further limiting its effectiveness (31).

Several risk assessment models have been developed to improve early detection strategies. Ruan et al. created an environmental risk model incorporating dietary habits, smoking history, and family history of NPC, achieving modest predictive power (AUROC = 0.68–0.74) (32). Zhou et al. expanded this approach by integrating genetic susceptibility factors and EBV serological markers, increasing predictive accuracy to AUROC = 0.77 (33). He et al. further validated a polygenic risk score for NPC, demonstrating that combining genetic risk factors with EBV serology improved PPV from 4.84% to 11.91% (34). However, despite these advancements, overall accuracy, cost, accessibility, and generalizability remain challenges for widespread implementation.

Given these limitations, recent studies have explored the use of machine learning (ML) and artificial intelligence (AI) to enhance NPC detection. Chen et al. (2024) developed and validated an ML-based NPC prediction model using a population-based medical claims database in an Asian population. Their model leveraged demographic data, symptom-related diagnoses, procedures, treatments, and reimbursement information to stratify NPC risk and shorten the prediagnostic period. By analyzing 14 clinically relevant variables, the algorithm achieved high predictive accuracy and demonstrated potential for large-scale implementation. Additionally, ML-driven models incorporating electronic medical records (EMRs) have further improved NPC risk stratification by integrating laboratory results and real-world clinical data, allowing for more efficient identification of high-risk

individuals and reducing diagnostic delays (31).

Beyond traditional clinical markers, AI-powered imaging techniques have also contributed to improving NPC screening and diagnosis. For example, Li et al. used 28,966 white-light nasoharyngoscopic images to develop a convolutional neural network (CNN) that distinguished between normal nasopharynx, NPC, and other nasopharyngeal malignancies with an overall accuracy of 88.7 % (35). Similarly, Xu et al. proposed a deep learning model trained on 4,783 nasopharyngoscopy images to differentiate between NPC and non-malignant nasopharyngeal lesions, achieving satisfactory accuracy and efficiency (36). Furthermore, He et al. introduced a real-time detection framework based on the You Only Look Once (YOLO) algorithm using nasal endoscopy video frames. Their model, however, yielded a sensitivity of 74.3%, indicating room for improvement in early-stage detection (37).

Yue et al. developed a smartphone application, "Nose-Keeper," which uses nasal endoscopic white-light images to detect NPC and five other common nasal diseases (38). This system attained an overall accuracy of 92.27% (95% CI: 90.66%–93.61%) and a sensitivity and specificity for NPC detection of 96.39% and 99.91%, respectively. By integrating deep learning into a user-friendly, mobile-cloud application, “Nose-Keeper” addresses critical diagnostic challenges in resource-limited settings. Users can connect a low-cost nasal endoscope to a smartphone, capture images, and obtain near real-time diagnostic feedback on possible NPC or common benign nasal conditions (38). "Nose-Keeper" is a pioneering tool for primary healthcare providers, enabling efficient and non-invasive screening in resource-limited settings, and it underscores the potential of integrating AI into healthcare diagnostics.

Despite these promising outcomes, several limitations in the “Nose-Keeper” study need to be considered. First, because no prospective validation has been conducted, the model’s performance and reliability in real-world settings remain to be confirmed. Second, “Nose-Keeper” currently lacks an image quality control system, leading to the possibility of inaccurate results if low-quality, blurred, or improperly lit images are submitted. Third, the training and evaluation datasets were derived from high-resolution, clinically acquired images, which may limit the model’s generalizability to non-clinical environments. Fourth, “Nose-Keeper” requires internet connectivity to link to the cloud-based AI model, limiting its use in regions with limited network infrastructure. Collectively, these shortcomings highlight the broader challenges facing AI-based approaches to NPC screening, as described further below (38).

Similarly, integrating ML with nomograms offers a promising avenue to enhance early detection and risk stratification in NPC. These nomograms could incorporate a comprehensive set of variables, including demographic data, lifestyle factors, EBV DNA level, and advanced imaging features. By training ML algorithms on large, multicenter datasets, this model could identify subtle patterns and interactions among variables that traditional statistical methods might overlook. For instance, integrating radiomic features from imaging studies with serological markers could improve the sensitivity and specificity of early detection. The nomograms would assign individualized risk scores, facilitating personalized screening protocols and timely interventions. Prospective studies testing this ML-nomogram approach in diverse populations would be essential to confirm its clinical usefulness and ensure its generalizability (6, 31, 39, 40, 41).

EBV based biomarkers have significantly advanced the diagnosis of nasopharyngeal carcinoma, but persistent challenges, particularly low positive predictive values in large-scale screening, still limit their widespread adoption. Recent developments, including EBV-encoded miRNAs, polygenic risk scores, and AI-powered tools such as "Nose-Keeper," show promise for enhancing early detection. However, the challenges of cost, accessibility, and validation across diverse populations remain. Future efforts should focus on large-scale, prospective studies to confirm these novel approaches' clinical utility and facilitate their integration into routine screening for improved patient outcomes.

Perspectives

Innovative strategies for early detection

To address the challenges of early NPC detection, we propose the development of a machine learning (ML) model that integrates clinical, demographic, and serological data to distinguish EBV-positive individuals with NPC from EBV-positive individuals those without NPC. This model would focus on identifying clinical parameters that are easily diagnosable at the primary care level, without the need for invasive procedures or costly instrumentation.

Table 1. Key features for the proposed ML model

Clinical Symptoms	Demographic and Lifestyle Factors	Serological and Molecular Data
Headaches (location, frequency, severity)	Geographic origin (China, SE Asia, North Africa, Inuit populations)	EBV DNA levels in plasma
Nasal congestion (frequency, medication responsiveness, resistance to OTC decongestants)	Dietary habits (salted fish or preserved vegetables during childhood) (39, 42)	VCA-IgA and EBNA1-IgA titers
Nasal bleeding (frequency, duration)	Occupational exposure (volatile chemicals, nitrosamines, fumes, dust)	Other EBV-related biomarkers (e.g., BART miRNAs)
Neck lumps (size, mobility, tenderness)	Smoking and alcohol consumption	
Hearing loss or tinnitus (unilateral or bilateral)	Family history of NPC	
Recurrent ear infections		

The ML model would be trained on retrospective and prospective datasets to identify patterns predictive of NPC and capable of distinguishing EBV+ yet NPC- individuals from those that are EBV+ and NPC+. By incorporating these features, the algorithm could improve the sensitivity and specificity of NPC screening, in endemic as well as in non-endemic regions where EBV based serological tests have high false-positive rates.

The potential impact of such an integrated ML approach includes a reduction in false positives by combining clinical symptoms with demographic and serological data, thereby minimizing unnecessary clinical examinations and improving screening efficiency. Furthermore, the algorithm could be adapted for use in non-endemic regions where NPC is less prevalent, and it could serve as a decision-support tool for primary care physicians to enable routine, non-invasive screening during regular health visits.

EBV in NPC treatment

The standard treatment modalities for nasopharyngeal carcinoma include radiotherapy and chemotherapy, which are typically used in combination (43). Radiotherapy is the standard of treatment since tumors are very sensitive to radiation. Often, chemotherapy is concomitantly administered in an attempt to enhance the results and efficacy of radiotherapy and to treat advanced-stage cancer (44). Even though such methods are applied in NPC treatment, problems such as toxicity, resistance, and recurrence remain significant clinical issues (45). Many short- and long-term side effects have been associated with radiotherapy and chemotherapy, including fibrosis, chronic sinusitis, and hypothyroidism (45). Some studies also reported secondary cancers (45, 46).

The strong association between EBV and NPC has prompted the development of EBV-targeted therapies that exploit the presence of viral antigens expressed in tumor cells to improve treatment efficacy. Immunotherapy is a type of cancer treatment that helps the immune system fight cancer (47). Approaches include passive and adoptive immunotherapy, which could be used against EBV in NPC. Passive immunotherapy uses monoclonal antibodies against specific antigens on EBV-infected cells, recognizing and binding with the viral proteins to signal the immune system to engage the tumor cells (48). Adoptive immunotherapy techniques use T-cells that specifically target EBV antigens, typically by generating EBV-specific cytotoxic T-lymphocytes (CTLs) from polyclonal T-cell lines. These T-cells are expanded using EBV-transformed lymphoblastoid cell lines (LCL) and are then capable of recognizing and destroying EBV-infected cells (49).

Another EBV-targeted therapy under study is antiviral therapy. The drugs are targeted to restrict the growth and proliferation of EBV-associated tumors. Targeted gene therapies against latent genes of EBV, such as LMP1 and EBNA1, inhibit the proliferation of EBV-infected cells, growth, and survival of the tumor (50). Recent clinical trials have demonstrated that adoptive immunotherapy using EBV-specific CTLs, as well as genetically engineered T-cell receptor (TCR), or chimeric antigen receptor (CAR)–modified T cells directed against EBV antigens (such as LMP1 and LMP2), can significantly reduce tumor burden and prolong overall survival in NPC patients. Moreover, immune checkpoint inhibitors targeting PD-1/PD-L1 and CTLA-4 have been incorporated into treatment regimens to enhance the anti-tumor immune response, particularly in EBV-positive NPC cases (6).

Cytolytic virus activation therapy, which aims to reactivate the latent EBV lytic cycle in tumor cells, is another promising therapeutic approach. Under lytic conditions, the cells express viral kinases that make them susceptible to antiviral drugs like ganciclovir. Agents such as histone deacetylase inhibitors (e.g., SAHA, romidepsin), proteasome inhibitors (e.g., bortezomib), and protein kinase C modulators have been used to induce this latent-to-lytic switch. Recent studies indicate that combining chemotherapeutic agents (e.g., gemcitabine) with lytic inducers and antiviral drugs produces synergistic effects, leading to significant tumor cell death via viral kinase activation and subsequent prodrug conversion (6).

Active immunotherapy approaches also include vaccines targeting EBV antigens (such as LMP1, LMP2, and EBNA1) and gene-targeting methods (such as RNA-cleaving deoxyribozymes and CRISPR/Cas systems) that disrupt viral gene expression and promote tumor cell death. Furthermore, novel peptide-based theragnostic agents and nanoparticle-based drug delivery systems have shown promise in preclinical models for both imaging

and therapy (6). Active immunotherapy enhances tumor antigenicity through the activation of autologous dendritic cells and cytokine-induced killer cells, thereby amplifying the immune response. It leverages the immune system to target and destroy EBV-infected cells associated with the development and progression of NPC (51). Vaccines against EBV antigens (LMP1, LMP2, EBNA1) induce strong immune responses against EBV-infected cells (52). This mechanism can also be achieved through monoclonal antibodies against EBV antigens or tumor-related antigens (52). These antibodies work through mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) or complement activation. During ADCC, antibodies bind to specific antigens, leaving the Fc region exposed. Immune effector cells, mainly NK (natural killer) cells with Fc receptors, recognize and bind this Fc region, then release cytotoxic substances like perforin and granzymes that induce apoptosis in the target cell.

Additionally, immune checkpoint inhibitors (ICIs) substantially contribute to cancer immunotherapy by targeting immune checkpoints such as PD-1 and CTLA-4, which play crucial roles in regulating the immune response (52). By blocking these pathways, T-cells can more effectively destroy cancer cells. High PD-1 expression, often linked to LMP1, has been associated with poor prognosis, recurrence, and metastasis in NPC, particularly in EBV-positive patients. Moreover, using genetically modified viruses to selectively infect and kill cancer cells, sometimes referred to as oncolytic virotherapy, can also reactivate the lytic cycle in latent EBV. This induces the expression of immunogenic proteins and viral kinases that sensitize cells to antiviral immune responses or therapies.

Molecular target therapy is another innovative approach for treating NPC. It utilizes detailed information about the molecular pathways and key molecules involved in tumorigenesis (53). Compared with traditional treatments, molecular targeted therapy often produces fewer side effects, potentially improving patient prognosis and treatment tolerance (53). Several clinical trials have been conducted to evaluate these targeted and immune therapies in various cancers, including NPC. A phase I clinical study of EBV-specific adoptive T-cell therapy, for instance, showed encouraging preliminary results, with some patients experiencing marked tumor regression and prolonged survival (54, 55). Another clinical study examining the combination of PD-1 inhibitors and chemotherapy for NPC demonstrated significant improvement in progression-free survival and overall response rates, making immune checkpoint blockade one of the most promising areas in oncology (56). Oncolytic virotherapy, which involves modifying viruses that selectively lyse tumor cells and enhance the immune response, has also shown promise; however, further research is necessary to establish its full clinical impact.

These sophisticated therapies, now entering clinical practice, offer renewed hope to patients with NPC, especially those with advanced or treatment-resistant disease. By leveraging the unique relationship between EBV and NPC, these innovative treatments aim to optimize therapeutic outcomes, reduce toxicity, and improve the quality of life for patients, ultimately increasing their chances of long-term survival.

Conclusion

Epstein-Barr Virus (EBV) infection, environmental exposure, and genetic predisposition interact intricately to cause nasopharyngeal carcinoma (NPC). The involvement of EBV in NPC development primarily occurs through the expression of latent genes, including LMP1, LMP2, EBNA1, and EBNA2. These genes activate oncogenic

pathways such as NF-κB, JAK/STAT, MAPK, PI3K, and Wnt, resulting in metastasis, immunological evasion, and uncontrolled proliferation. EBV induces genetic and epigenetic changes that make tumors more aggressive and underscore the complex nature of NPC development.

Even with the availability of serological and molecular tests (e.g., VCA-IgA, EBNA1-IgA, BV DNA) that have improved diagnostic accuracy in suspected cases, mass-screening initiatives continue to face significant challenges as low positive predictive values and a high rate of false positives were observed, particularly in low-prevalence settings. Recent innovations, including EBV-encoded miRNA detection, polygenic risk scores, machine learning algorithms, and AI-driven imaging tools such as the "Nose-Keeper" smartphone application, offer promise for enhancing early detection and personalizing screening strategies according to individual risk. However, cost, accessibility, and validation across diverse populations remain major barriers.

In terms of treatment, EBV-targeted approaches that leverage the virus's distinct biology are being incorporated into traditional therapies like radiotherapy and chemotherapy. Immunotherapy, including passive antibody administration, adoptive T-cell transfer, and immune checkpoint inhibitors, are used to precisely target EBV-infected tumor cells. In addition, new treatment options, such as cytolytic viral activation therapies, oncolytic virotherapies, and molecular-targeted interventions, aim to improve treatment efficacy and reduce adverse side effects. Clinical trials have demonstrated the potential of these approaches; however, large-scale prospective studies with standardized protocols are necessary to confirm their effectiveness and ensure widespread clinical utility.

Taken together, a deeper understanding of EBV's contributions to NPC pathogenesis, along with continued refinements in EBV-based diagnostic tools and personalized treatments, will be crucial in reducing the world-wide impact of NPC. As researchers advance machine learning-enabled diagnostics and targeted therapies, integrating these innovations into clinical practice stands to significantly improve early detection, therapeutic outcomes, and overall prognosis and survival in NPC patients.

Abbreviations

NPC: Nasopharyngeal carcinoma, EBV: Epstein-Barr virus, LMP1: Latent membrane protein 1, LMP2: Latent membrane protein 2, EBNA1: Epstein-Barr nuclear antigen 1, EBNA2: Epstein-Barr nuclear antigen 2, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells, JAK/STAT: Janus kinase/Signal transducer and activator of transcription, MAPK: Mitogen-activated protein kinase, PI3K: Phosphoinositide 3-kinase, Akt: Protein kinase B, FOXO3a: Forkhead box O3a, PI3K/Akt/FOXO3a: Signaling pathway involving PI3K, Akt, and FOXO3a, Wnt: Wingless-related integration site, VCA-IgA: Viral capsid antigen immunoglobulin A, EA-IgA: Early antigen immunoglobulin A, PCR: Polymerase chain reaction, qPCR: Quantitative polymerase chain reaction, ELISA: Enzyme-linked immunosorbent assay, AI: Artificial intelligence, ML: Machine learning, OTC: Over-the-counter, DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, miRNA: MicroRNA, BART miRNAs: BamHI-A rightward transcript microRNAs, HLA: Human leukocyte antigen, TLR8: Toll-like receptor 8, CD21: Cluster of differentiation 21, EphA2: Ephrin type-A receptor 2, CTL: Cytotoxic T lymphocyte, NK cells: Natural killer cells, ADCC: Antibody-dependent cellular cytotoxicity, PD-1: Programmed cell death protein 1, PD-L1: Programmed death-ligand 1, CTLA-4: Cytotoxic T lymphocyte-associated protein 4, TCR:

T-cell receptor, CAR: Chimeric antigen receptor, ICI: Immune checkpoint inhibitor, MRI: Magnetic resonance imaging, CT: Computed tomography, EMR: Electronic medical records, CNN: Convolutional neural network, YOLO: You Only Look Once (algorithm), AUROC: Area under the receiver operating characteristic curve, PPV: Positive predictive value, SAHA: Suberoylanilide hydroxamic acid, LCL: Lymphoblastoid cell lines, Chk1: Checkpoint kinase 1

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