Nasopharyngeal carcinoma: A review of current updates

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Abstract. Nasopharyngeal carcinoma (NPC) is a rare malignancy worldwide, but it is endemic in a few areas including Southern China, Southeast Asia, North Africa and the Arctic. The underlying mechanisms behind this remarkable geographic distribution remain unclear. Although Epstein-Barr virus (EBV) infection has been suggested as a necessary cause of undifferentiated NPC, EBV itself is not sufficient to cause this malignancy. Other co-factors, such as environmental risk factors, and/or genetic susceptibility, may interact with EBV to play a role in the carcinogenesis of NPC. Survival rates differ significantly between NPC patients in early stages and late stages. Due to the close associations between EBV infection and NPC risk, EBV-related biomarkers have been used for early detection and screening for NPC in a few high-incidence areas. In the present review article the latest updates are discussed.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is one of the Epstein-Barr virus (EBV)-associated malignancies and has a characterized geographical distribution (1). In southern China, it is one of the major causes of morbidity and mortality. Despite the heavy public-health burden of NPC in southern China and other endemic areas, relatively little is known about the etiology and prevention of NPC. Although certain environmental exposures, including high consumption of salt-preserved fish, tobacco smoking and lack of fresh fruit and vegetable intake, are generally well accepted as NPC risk factors. To date there has been no rigorous population-based case-control study of NPC in southern China. Evidence accumulated so far indicated a probable causal role of EBV in the pathogenesis of undifferentiated NPC (the most common histological subtype of NPC) (1,2). However, despite establishing lifelong latency in the majority of humans, only a small proportion of individuals infected with EBV develop cancer. This indicates that EBV alone is not a sufficient cause for this malignancy. Environmental exposures and/or genetic risk factors likely also play a role in the pathogenesis of this tumor (3).

Despite the unknown etiology, using antibodies against EBV for early diagnosis and screening for NPC has been conducted in a few high-incidence areas in Southern China since the 1970s. Recent studies demonstrated that a combination of IgA antibodies against the Epstein-Barr nuclear antigen 1 (EBNA1/IgA) and VCA/IgA measured by enzyme-linked immunosorbent assay (ELISA) has higher sensitivity, specificity, and positive predictive value compared with the traditional method. Individuals identified as being at high risk of NPC based on EBV serological markers can be offered fiberoptic endoscopy/biopsy and close medical surveillance to enable early diagnosis of NPC and, ideally, reduced mortality. However, the cost-effectiveness of this labour-intensive strategy has yet not been proven, and new biomarkers are needed to more specifically identify the high-risk population, in order to provide screening for NPC in the general population.

2. Clinical features

The symptoms and signs at presentation of NPC include neck masses, epistaxis, nasal obstruction and discharge, headache, and other nonspecific indicators. Furthermore, because the cancer is located in a silent anatomic site, and NPC exhibits

a higher metastatic rate (4), NPC tends to present at advanced stages (clinical stages III and IV) when diagnosed. It has been shown that >70% of patients were at advanced stage when diagnosed in clinics (5). A 10-year survival rate for NPC patients can reach 98% for stage I and 60% for stage II (6). In contrast, median survival is 3 years for patients at advanced stages (7), highlighting that improvements in diagnosis rate could help to reduce NPC mortality. Especially in high-incidence area, patients with symptoms should be clinically assessed for physical signs of the disease. The examination of the nasopharynx is firstly made by an indirect nasopharyngoscope, followed by a direct nasopharyngoscope (fiberoptic endoscope). A biopsy should be performed if a suspicious growth in the nasopharynx is detected. If the suspected tumor is not visible upon endoscopic examination then advanced imaging procedures like CT scan or MRI are preferred choices.

3. Risk factors

Since the malignancy was firstly reported in 1901 (8), the etiology of NPC has remained a puzzle for more than a century. Migrant studies show that when southern Chinese settle in other countries, their incidence of NPC is 10-30 times higher than that of other races, a rare pattern among malignancies suggesting a strong genetic component of NPC risk (9). A higher incidence of NPC is also observed among North African immigrants in Israel and Sweden, when compared to the native Israelis and native Swedes (10). Incidence of NPC among Chinese born in Western countries is still higher than that among Caucasians, although it is about half that of those living in China or migrating within Southeast Asia (10). In addition, compared with those born in southern France, men of French origin born in North Africa also had a higher incidence of NPC (11). The latter findings indicate that in addition to genetics, environmental factors also play an important role in NPC.

To date, established risk factors for type III NPC include Cantonese ethnicity (12), male sex (13), EBV infection, a family history of NPC, high consumption of salt-preserved fish, low intake of fresh vegetables and fruits intake, smoking, and some human leukocyte antigen (HLA) class I alleles (14-17). On the other hand, other HLA genotypes and a history of infectious mononucleosis (IM) may be associated with a decreased risk (18). Further potential risk factors include high consumption of other preserved foods (19), a history of chronic respiratory tract conditions, and genetic polymorphisms in cytochrome P450 2E1 (*CYP2E1*), *CYP2A6*, glutathione S-transferase M1 (*GSTM1*) and *GSTT1* (20,21). Less established risk factors include consumption of herbal medicine, occupational exposures to dust and formaldehyde, and nickel exposure (22).

4. Epstein-Barr virus

EBV, a γ-herpesvirus that infects lymphocytes and epithelial cells, establishes lifelong latency in >90% of adults globally (23). Primary infection with EBV usually occurs early in life and transmission is mainly through saliva. In high NPC incidence areas, such as Hong Kong, Taiwan and mainland China, ~60% of children have been infected by age 2, ~80% by age 6, and almost 100% by age 10 (24). In contrast, age at primary infection is relatively late in children from Western countries, such as US, Denmark and Sweden (24). Although primary infection with EBV is usually asymptomatic, it is associated with certain diseases, including IM and Burkitt's lymphoma (BL), ~30% of HL, certain subtypes of non-Hodgkin lymphoma, type III NPC, and a subset (~10%) of gastric carcinoma (25). It is estimated that ~143,000 deaths worldwide in 2010 could be attributed to EBV-associated malignancies (26).

The link between EBV and NPC was first proposed in 1966 when NPC patients were reported to have higher antibody response against an antigen that was later demonstrated as a product of EBV (27). Since then, extensive evidence suggests that EBV is a potential cause of NPC, especially type III. First, monoclonal EBV genome and viral gene products are detected in virtually all tumors in NPC-endemic areas (28), indicating that the tumors result from clonal proliferations of a single cell that is initially infected with EBV. Second, elevated IgA antibodies against EBV antigens are highly specific markers for subsequent NPC in high-incidence areas (29), while elevated EBV-neutralizing antibodies blocking B-cell infection and anti-gp350 antibodies are inversely associated with NPC risk (29). Third, the expression of viral proteins, such as latent membrane protein 1 (LMP1), LMP2, EBNA1 and EBNA2, has been demonstrated to drive tumor progression in invasive epithelial cancers. Nevertheless, EBV has never been detected in non-cancerous epithelial cells of the nasopharynx (30), and epithelial infection is much less efficient *in vitro* than B-lymphocyte infection (31). The viral target, complement receptor type 2 (CR2), which is presented on B cells and attaches to EBV envelop, gp 350/220, is expressed at low levels on epithelial cells (32). Therefore, other mechanisms of viral entry into epithelial cells have been postulated (33), including attachment to two additional glycoproteins, gHgL and gB (34), cell-to-cell contact, or IgA/secretory component (SC) protein mediation (35).

5. Early-life risk factors

Factors that could potentially alter the oncogenicity of EBV include the age and immune response at the time of primary EBV infection (36). For example, when primary infection is delayed until adolescence, the EBV-related immune response is robust and can lead to symptoms of IM, which is linked to risk of EBV-related HL in adulthood (37). In NPC- endemic areas, IM and HL are not prevalent, leading us to hypothesize that timing at infection may play a role in the development of NPC. Accumulated evidence shows that childhood exposures to certain environmental factors confer a higher risk of NPC (38). Nevertheless, the associations of factors influencing the timing of common childhood infections with the risk of NPC have not been studied. There are a few studies showing that a history of IM is associated with a lower risk of NPC, although commonly based on small number of cases. In high-risk populations, perhaps due to the fact that late infection is rare, it is difficult to estimate the association between a history of IM and NPC risk. In NPC non-endemic areas, however, the rarity of NPC makes the evaluation of this hypothesis a big challenge.

The household environment during childhood, when primary EBV infection is most probable, including number of siblings and population density of the household, could be

important predictors for the immunological control of EBV and eventual EBV-related disease risk (39). Childhood family structure may serve as an indirect indicator of early infection with common childhood pathogens. For example, birth order has been linked to risk of other EBV-related malignancies, such as HL (40), and also to hepatocellular carcinoma (41). Hence, we hypothesized that very early exposure to EBV and other carcinogens may play a role in NPC pathogenesis. Studies on early childhood family structure may lend insights into whether timing of primary infection with EBV is associated with a subsequent risk of NPC.

6. Oral hygiene

Poor oral health, as a modifiable risk factor that is common among the elderly (42,43) has been linked to cancers of the pancreas, esophagus, stomach, and head and neck (44). In the case of NPC, periodontitis may increase inflammation and thus may increase the risk for NPC, given that inflammatory response could be one pathway of carcinogenesis promotion (45). In addition, bacterial load increases with a greater number of teeth lost, and some of the bacteria have been implicated in the production of nitrosamines, which are known carcinogens for NPC development (46). Poor oral health could also increase the risk of NPC by stimulating EBV replication, as indicated by higher viral load among individuals with periodontal disease than those without (47).

Few epidemiological studies have tried to address this research question of whether poor oral hygiene is related to NPC risk. One hospital-based case-control study in Turkey showed that infrequent tooth brushing and an increasing number of decayed teeth were associated with a higher NPC risk (48). Compared with those who brushed teeth daily, those who teeth brushed rarely had an odds ratio (OR) of 6.17 (95% CI, 3.60-10.55). However, when examining poor oral health as a risk factor for cancer in general, any positive associations could be due to residual confounding by smoking, low socioeconomic status, diet, and/or medical history. Detailed risk factor information in a large, population-based case-control study could help to facilitate the rigorous evaluation of oral health as a risk factor for NPC.

7. Familial aggregation

Familial clustering has been consistently reported in NPC high-incidence (49), intermediate-incidence (50), and low-incidence (51) areas. In Southern China, where NPC is endemic, >5% of incident cases reported a positive family history of NPC among the first-degree relatives (52,53). Previous case-control studies in different populations showed that ORs ranged from 2 to 20 in individuals who reported a first-degree family history of NPC compared with those with no such history (54,55). This magnitude of association is among the highest of any malignancy, suggesting that environmental factors themselves cannot fully explain the observed association. Genes and environmental exposures likely play a combined role in the etiology of NPC. An inheritance pattern that cannot be explained by activation of a single major susceptibility gene is supported by results from a complex segregation analysis of familial NPC showing that the etiology of NPC involves interaction of multiple genetic and environmental factors (56).

Whether familial NPC cases differ substantially from sporadic cases in terms of clinical features (i.e. histology, stage and prognosis), ethnicity, sex, age at diagnosis, environment risk factors, EBV serology, and/or genetic risk factors is still controversial (57). A study in the recent past showed that familial cases did not have characteristics notably distinct from sporadic cases (58). On the other hand, others found that familial NPC cases tend to be younger, and have better survival than sporadic cases (59,60). Further, significant modification is reported of the association with family history of NPC by smoking, wood fuel use, and salt-preserved fish consumption, whereas a prospective study did not find an interaction between smoking and family history of NPC. The small number of controls with a positive first-degree family history of NPC and the low power of the statistical test of heterogeneity make it difficult to draw firm conclusions about the joint effects of family history and environmental risk factors. Pooled studies with larger numbers of subjects will enable more powerful tests of such interactions.

To date, previous epidemiologic studies of NPC have been limited in number, size, scope and rigorousness of study design. Few studies have investigated the relative-specific risk among families with affected members. Because most studies have not ascertained all first-degree relatives and are not population-based, absolute NPC risks in the general population with and without a family history in NPC-endemic geographic regions, where the great majority of NPC cases occur worldwide, are largely unknown. The lack of evidence precludes cost-effectiveness modeling of screening for NPC among families with a positive history of NPC.

8. Genetic susceptibility

Only one genome-wide association study (GWAS) (61) utilized samples from >1,000 cases and controls; other GWAS were limited to a few hundred cases and controls. Many investigators have focused on the possible pathogenetic role of HLA molecules, which are required for the presentation of foreign antigens, including viral peptides, to the immune system for targeted lysis. In Chinese and other high-risk Asian populations, *HLA-A2-B46*, and B17 are associated with a 2- to 3-fold increase in NPC risk, whereas an increased risk is associated with *HLA-B5* in Caucasians. One-third to one-half lower risk is found in association with *HLA-A11* across all races, B13 in Chinese and Tunisians, and A2 in non-Chinese. Several other HLA associations have been reported, but must be interpreted with caution due to multiple-testing considerations. Genetic polymorphisms other than *HLA* are also reported. However, most genetic association studies are based on small sample sizes, and the lack of replication precludes a full understanding of genetic influences on NPC development.

9. Screening in high-incidence areas

Several lines of evidence support the notion that testing for antibodies against EBV could a useful screening tool to facilitate the early detection of NPC. First, EBV infection is an early event during tumor progression (62), and the EBV genome and

gene products can be detected in virtually all tumors of type III NPC. Second, VCA/IgA neutralizing antibodies against EBV DNAse), and EA/IgA could be detected in serum even years prior to clinical evidence of the cancer, making them the basis for successful NPC screening tests in high-incidence areas. A few pilot efforts have been made to conduct NPC mass screening in high-incidence counties in southern China since the 1970s (29), using the two biomarkers of VCA/IgA and EA/IgA measured by immunofluorescence assays. More recently, studies in southern China demonstrated that a combination of EBNA1/IgA and VCA/IgA measured by ELISA had a higher diagnostic accuracy [i.e. high sensitivity, specificity, and positive predictive value (PPV)] in both the general population and families with at least two affected relatives.

Although the value of using antibodies against EBV to facilitate NPC diagnosis is generally accepted, there are a few barriers to the implementation of screening for NPC by testing these antibodies in high-incidence populations. First, only a fraction of the ~2% of individuals with elevated titers of VCA/IgA in high-risk areas develop NPC. Second, serologic evidence of EBV reactivation from latency, as indicated by elevated antibody titers against viral lytic antigens, can also be detected in normal individuals, particularly during periods of psychological or physical stress (63), thereby decreasing their specificity. Third, previous efforts were not carefully controlled (i.e. no randomized controlled trial has yet been conducted) and do not permit accurate quantification of the impact of EBV-based screening on detection rates of early-stage NPC and on NPC mortality. These results are required to support evidence-based decisions regarding the efficacy and cost-effectiveness of such screening strategies. Other than biomarkers related to EBV, biomarkers related to the human proteome may also exhibit great potential for early diagnosis of NPC. A handful of studies have used proteomics to investigate potential biomarkers for early diagnosis of NPC (64,65). Although a number of biomarkers have been identified, few have been replicated in other independent studies. Most of them may have biological implications rather than diagnostic capacity. Limited sample size, inappropriate study design, heterogeneity of NPC, and different technologies used across different studies may contribute to the inconsistency of findings.

10. Conclusion

To date, the technology most commonly used for human protein biomarker discovery is mass spectrometry (MS), which is limited to the analysis of a relatively small number of samples in parallel. Recently, plasma antibody profiling technologies, such as antibody suspension bead array assays have been developed for multiplex screening of a large number of proteins in patient cohorts. This advance may bring hope to identify and validate biomarkers for early diagnosis of NPC.

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Authors' contributions

LW was responsible for the conception and design of the study. LW and CL collected the files and revised the manuscript for important intellectual content. CL and LP analyzed and interpreted the files, and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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