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The Role of Plasma Epstein – Barr Virus DNA in Nasopharyngeal Carcinoma

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Abstract

Nasopharyngeal carcinoma (NPC) is a unique tumour which is endemic in Southern China including Hong Kong. While the treatment results for early disease are encouraging, patients with advanced cancer are uniformly associated with poor prognosis. Epstein-Barr virus (EBV), the first virus related to the development of human malignancy, plays an important role in the carcinogenesis of the disease. Over the past decades, researchers have been trying to identify EBV associated biomarkers which allows early diagnosis as well as accurate monitoring of treatment response. With the development of real time quantitative polymerase chain reaction, plasma EBV DNA has been studied with much enthusiasms for its potential role in the management of patients with NPC. Since then, numerous reports have been published regarding the applications of plasma EBV-DNA for early diagnosis, monitoring of treatment response after radiotherapy or surgery, and as a prognosticator predicting oncological results after curative therapy for primary disease or palliative treatments for patients with recurrent/metastatic cancer. On-going studies are performed to investigate its potential use in the screening of at-risk populations in the endemic geographic regions. In the future, it may allow pre-treatment risk stratification for individual patients, so that personalization of treatment protocols can be achieved with potentially better oncological outcome.

Keywords: Nasopharyngeal carcinoma; Epstein-barr virus; Plasma EBV-DNA; Prognosticator; Survival

Introduction

Since the first report in 1910, nasopharyngeal carcinoma (NPC) has been recognized for over 100 years. Compared to other head and neck malignancies, it is unique with respect to its epidemiology, pathology and treatment outcome [1,2]. The incidence of NPC is <1 per 100,000 person years in most countries, making it the 23rd most common cancer in the world [3]. On the other hand, in endemic areas including southern China and Hong Kong, up to 50 per 100,000 populations are affected per year [4]. According to the Hong Kong Cancer Registry, NPC ranked 7th in the most common new malignancy for both gender. In these areas, majority of the tumors are undifferentiated carcinoma (World Health Organization [WHO] type III). Because of the tumor's radio sensitivity, the primary treatment is radiotherapy or concurrent chemoradiation depending on the stage of disease on presentation [5-8], while surgery is reserved for persistent or recurrent tumours after the initial therapy [9,10].

NPC is strongly linked to Epstein-Barr virus (EBV) infection, which is a virus of the herpes family. It is named after Michael Anthony Epstein and Yvonne Barr, who discovered and documented the virus [11]. Apart from causing infectious mononucleosis, it is also found to be associated with certain human malignancies, including Burkitt's lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma. Majority of the adults become infected with the virus and have gained adaptive immunity. Once the initial lytic infection is controlled, the latent phase continues, when the virus persists in the B cells for the rest of the life.

The EBV viral particle has a diameter of 120 – 180 nm. It is a DNA virus consisting of a double stranded DNA enclosed in a protein capsid. The viral capsid is surrounded by a protein tegument and a lipid envelope [12]. The EBV genome is a linear, double-stranded, ~ 172-kb DNA molecule that encodes more than 85 genes. The nomenclature for EBV open-reading frames (ORFs) is based on BamHI-restriction fragment in which they are found. These EBV PRFs are divided into latent and lytic genes, the majority of which are translated into proteins of various functions. EBV also encodes viral-encoded micro-RNAs, which are small non-protein coding RNAs that regulate mRNA at the post-transcriptional level. There are two major clusters, the BamHI fragment H rightward open reading frame 1 (BHRF1) and BamHI-A rightward transcript (BART), coding for 25 precursors and 44 mature viral-encoded microRNAs.

EBV infection was shown to be an early event in NPC, and studies have demonstrated the presence of EBV deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins in the majority of the NPC cancer cells [13-15], origination of tumour cells from the EBV-infected cell [16], as well as the presence of high levels of EBV antibodies in patient with NPC [17-19] and in healthy individuals who subsequently developed NPC [20,21]. Furthermore, EBV was found in dysplastic lesions and carcinoma in situ of the nasopharynx [22,23]. Since then, EBV has received much attention and it has been investigated as a molecular marker for NPC.

For the past decades, researchers have studied EBV serology to be used for screening, diagnosis, follow-up and prognostication of NPC. An ideal tumour marker should have high sensitivity and specificity. Moreover, it should also allow the prediction of prognosis. The immunoglobulin A (IgA) antibody against viral capsid antigen (VCA-IgA), which is either detected by indirect immune fluorescence [24] or enzyme-linked immunosorbent assays (ELISA) [25], is one of the

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most extensively studied serological markers for diagnosis [24,26] and for screening [20,21]. Studies have investigated the clinical usefulness of various anti-EBV antibodies. Pre-treatment assays for antibodies against VCA and early antigen (EA) failed to reflect prognosis [27]. On long-term follow up, post-treatment serial measurements also failed to predict the outcome [28]. A multicenter trial on 319 patients with NPC showed that the initial serology against VCA, EA or EBV nuclear antigen had no prognostic value [29], although increasing level of antibody against EA at 1 year after the initial radiotherapy was significantly associated with disease relapse irrespective of the initial titers. These studies showed the limitations in the clinical use of anti-EBV antibodies in patients with NPC. Possible reason is that the EBV serology represents the host response to viral tumour antigens that can be affected by numerous factors. Clearly, better markers are needed to achieve the goal of early diagnosis and prognostication.

Plasma Epstein-Barr virus DNA

Mutirangura et al. [30] has shown a correlation between tumour apoptosis and the presence of plasma EBV DNA in patients with NPC. Lin et al. [31] showed that sizes of the PCR products for LMP-1 and EBNA-3C were identical in paired samples of the primary tumour and plasma, and direct sequencing of the PCR products demonstrated complete homology of the EBNA-3C in all patients and of the LMP-1 in 63.6% of the patients. As laboratory techniques advances, measurement of specific DNA sequences becomes possible using the real-time quantitative polymerase chain reaction (PCR) technique.

Chang et al. [13] applied the technique to detect Epstein-Barr virus DNA (EBV-DNA) sequences in 50 tumour tissues from patients with NPC. They found that EBV-DNA sequences were detected in 100% of the undifferentiated NPC specimens, in 50% of the moderately differentiated NPC specimens, and in 60% of the keratinized NPC specimens. They also showed that the undifferentiated NPC cells contained higher copy numbers of EBV than the cells from the other two types of NPC. They concluded that it is useful for the detection of EBV-DNA sequences in NPC tumour cells. With the postulation of EBV-DNA being directly released from the tumour cells, Lo et al. [32] have used PCR to detect plasma EBV DNA, and they found that EBV was detected in 55 (96%) of 57 patients with newly diagnosed NPC, whereas only 3 (7%) out of 43 healthy controls has detectable EBV-DNA By studying the patients with NPC in Hong Kong, which is an endemic area, they also have evidence to show that the plasma EBV concentration reflects the tumour burden. Patients with advanced staged disease had significantly higher viral load than those with localized disease (median values 47,047 vs. 5,918 copies/ml, respectively) [33].

EBV Markers for Screening

Historically, NPC has been diagnosed at very late stages because of the nonspecific nature of its symptoms. The oncological outcome of treatment, however, is strongly influenced by the cancer stage on presentation. The cure rate for early stage disease can be as high as 85%, but the corresponding rate for advanced disease is usually less than 30% [34]. Although modern treatment techniques have improved the results of treatment for advanced disease [35], post-treatment complications of these radical treatments have significant effects on the subsequent quality of life [36]. Because the tumour is in the vicinity of various vital structures, such as the brain stem, cervical spine, parotid glands and the eye, radiotherapy related complications are frequent and tend to be significantly more severe in patients with advanced disease. It is advantageous to achieve early cancer detection in order to achieve superior treatment results as well as to minimize the potential complications after treatment.

However, the major obstacle to early diagnosis of NPC is the difficult access due to the deeply seated location of the nasopharynx, coupled with the vague presenting symptoms. In endemic regions such as the southern China including Hong Kong, one way to achieve early diagnosis is by screening asymptomatic population at risk of NPC. This includes people who have a family history of NPC [37-40]. The 1st degree relatives of NPC patients have an increased risk of 4 – 8 folds over the general population. Chan et al. [41] recently demonstrated that plasma EBV DNA analysis is useful for early detection of NPC before it is clinically apparent. He studied 1318 healthy volunteers and identified 3 asymptomatic individuals with NPC at enrolment. Among the subjects with initially positive plasma EBV DNA but did not have NPC, 66% of them had normalized plasma EBV DNA after a median of 2 week. Wong et al. [42] studied 1475 blood samples from patients without history of EBV-related malignancies, and he found that plasma EBV DNA was detected in 33 (2.24%) patients. Among them, 1 patient had elevated plasma EBV DNA as well as positive VCA and EA IgA, and he was subsequently found to have NPC. O et al. [43] investigated the EBV IgA and DNA assays as a screening tool for NPC in a nonendemic United States population. He demonstrated that series testing provided sensitivity, specificity, positive and negative predictive values of 90.6%, 93.5%, 78.4% and 97.5%, respectively. Parallel testing, on the other hand, increased the sensitivity to 100%. However, information on the role of plasma EBV-DNA in the screening of high-risked population is still limited. Baizig et al. [44,45] showed that the level of EBV-DNA load varied according to the condition of the tumour, and is not reliable to use as a screening tool. Ongoing studies are being performed to better investigate the potential role of EBV DNA as a screening tool.

EBV DNA for Monitoring of Treatment Response

Lo et al. [46] studied the plasma of NPC patients during radiotherapy, daily during the first week of treatment followed by weekly afterwards until the end of the therapy, and described the changes in plasma EBV-DNA concentration during radiation therapy. They found that an initial surge in plasma EBV-DNA level was observed in all patients during the first treatment week. This is likely to be explained by the liberation of EBV-DNA after cancer cell death induced by the ionizing radiation. Afterwards, the plasma EBV-DNA concentration dropped rapidly with a median half-life of 3.8 days. These findings showed that the clearance rate of the EBV-DNA may reflect the radio sensitivity of the tumour, which in turn may have treatment implications in the future. The same phenomenon of rapid clearance of plasma EBV-DNA was observed after salvage nasopharyngectomy for recurrent nasopharyngeal carcinoma [46,47].

Chan et al. [48] explored the application of serial measurements of plasma EBV-DNA as a non-invasive way to monitor the treatment response in NPC. He studied 31 patients with advanced stage undifferentiated NPC who received neoadjuvant chemotherapy followed by concurrent chemoradiation. The high mean pre-treatment EBV DNA level (28, 938 copies/ml) reflected the tumour load of the cohort. A decreasing plasma EBV-DNA level was observed in the majority (96.8%) of patients during neoadjuvant chemotherapy, and in all patients who completed the concurrent chemoradiation. With a median follow-up duration of 33.7 months, 6 distant and 3 locoregional failures were detected. Plasma EBV DNA was significantly elevated in 88.9% of patients with treatment failure, while remained undetectable for those in remission. Studies from non-endemic regions also concurred the use of plasma EBV-DNA to detect tumour recurrence during follow-up. Ferrari et al. [49] studied 36 consecutive patients after induction chemotherapy followed by chemoradiation for NPC

in a non-endemic area. They showed that plasma EBV DNA useful to predict recurrence, as 5 of the 7 patients who relapsed had plasma EBV-DNA copy numbers that was significantly higher than those in remission. None of the patients who did not experience a recurrence had raised EBV-DNA concentration, giving its overall accuracy of 94.4% in detecting tumour recurrence.

EBV DNA as Prognosticator for Recurrent/Metastatic Tumour

Apart from being used as a tool for screening and post-therapy monitoring, pre-treatment plasma EBV-DNA has been extensively studied to predict loco-regional failure as well as distant metastasis. Studies have shown that elevated plasma EBV-DNA levels measured before and at 6 weeks after radiotherapy were significantly associated with disease recurrence and inferior survival in patients with locoregionally advanced NPC [49-51]. Hou et al. [52] showed that plasma EBV-DNA concentration before- and after-treatment have important clinical significance. Pre-treatment plasma EBV-DNA concentration reflects tumour burden, whereas the rate of circulating plasma EBV-DNA clearance after treatment predicts the risk of distant metastasis and overall survival. Leung et al. [53] studied 376 patients with NPC of various stages treated with curative intent radiation therapy and found that the pre-treatment plasma EBV-DNA level is an independent prognostic factor for subsequent survival. The current NPC treatment is dichotomized between late stage (stage III and IV) disease, which is treated with chemoradiotherapy, and early stage disease, which is treated by radiotherapy alone. Conclusions from multiple randomized trials and meta-analyses have led to the adoption of concurrent chemoradiation for patients with advanced-stage disease. While those with stage I tumours are adequately dealt with by radiotherapy alone, the optimal treatment for patients with stage II disease is less well defined [53,54]. The authors proposed that patients with stage II disease could be stratified by the pre-treatment EBV-DNA levels into a poor-risk subgroup, which are treated with concurrent chemoradiation, and a good-risk subgroup, which are adequately treated by radiotherapy alone.

In the setting of recurrent or metastatic disease, plasma EBV-DNA level may also bear prognostic implications. Wang et al. [55] studied the circulating EBV-DNA of 30 patients with tumour relapse and 4 patients with previously untreated metastatic NPC. The patients were treated with systemic chemotherapy followed by local radiation and oral maintenance chemotherapy. At 12 weeks, 14 patients demonstrated complete response (41.2%), 12 patients had partial responses (35.3%), 7 patients had stable diseases (20.6%), and 1 patient suffered from disease progression (2.9%). They found that the circulating EBV-DNA concentration have no significant effect on the oncological outcome. However, patients with rapid clearance of their plasma EBV-DNA have significantly higher rate of complete response and better overall survival. Multivariate analysis revealed a significant effect of the plasma EBV-DNA clearance on survival. By studying 127 patients with metastatic or recurrent NPC, An et al. [56] demonstrated a significantly better survival in patients with low pre-treatment plasma EBV-DNA level. Patients with undetectable post-treatment plasma EBV-DNA have better survival and tumour response. Significantly better survival was observed in patients with a rapid clearance of plasma EBV-DNA after 1 cycle of chemotherapy. They concluded that plasma EBV-DNA level before treatment and their clearance allows early discrimination between good and poor responders to palliative therapy.

Esptein-Barr virus DNA in Surgery for Recurrent Nasopharyngel Carcinoma

Despite the apparent improved outcome using intensity-modulated radiotherapy [56] and concurrent chemoradiation [57,58] for NPC, the treatment for persistent or recurrent disease remains challenging. Surgical salvage offers better local tumour control and survival [59-61] as well as less post-treatment morbidities than reirradiation.

Information on the role of circulating EBV-DNA in the prediction of surgical outcome for recurrent NPC, however, is limited. Previous studies showed that after nasopharyngectomy for locally recurrent NPC, the circulating EBV-DNA dropped rapidly, with a median half-life of 139 minutes only [46]. After a median follow-up of 6.7 days, EBV-DNA was undetectable in 8 out 11 patients. One of the 8 patients with undetectable EBV-DNA and all the patients with elevated EBV-DNA after surgery subsequently developed clinical relapse, showing that failure of elimination of plasma EBV-DNA predicts disease recurrence [62]. Chan et al. [63] studied 64 patients with recurrent NPC after previous radiotherapy. Among them, 42 patients had local tumour recurrence, and 83.3% of them had clear resection margins during maxillary swing nasopharyngectomy. Their plasma EBV DNA concentration before surgery was significantly lower than those with positive resection margins. The receiver operating characteristic (ROC) curveshowed that the cutoff plasma EBV-DNA concentration that predicted the status of resection margins was 425 copies. For the remaining 22 patients who had regional failure treated by neck dissection, 42.3% of the metastatic lymph nodes demonstrated histological evidence of extra-capsular tumour invasion. The pre-operative plasma EBV-DNA, however, failed to identify these patients from those without extra-capsular spread. It is important in this study that, 10 patients in the subjects had no detectable plasma EBV-DNA before surgery despite the presence of histologically proven recurrent undifferentiated NPC, which were all EBV encoded RNA (EBER) positive. Therefore, plasma EBV-DNA concentration should be checked as part of the routine investigations prior to operation, as it provides a baseline reference as to whether it is useful as a follow-up tool after surgery. Obviously, patients with negative plasma EBV-DNA before surgery cannot rely on the tumour marker for follow-up in the future.

Conclusions

Nasopharyngeal carcinoma is a unique tumour in the head and neck region that is strongly associated with EBV infection. Measurement of circulating EBV-DNA by real time quantitative PCR allows early diagnosis of NPC. Evidence shows that it is also useful in the monitoring of treatment response after radiotherapy and surgery, and as a prognosticator for recurrent and metastatic disease. These may have potential implications in the individualization of treatment protocol to maximize the oncological outcome of treatment.

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