

Original Article

Association between interferon gamma 13-CA-repeats polymorphism and metastasis of nasopharyngeal carcinoma in a population of Northern China

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Abstract: Interferon Gamma gamma (IFN- γ) 13-CA-repeats polymorphism is associated with a variety of diseases; here we report its association with nasopharyngeal carcinoma (NPC) metastasis in a retrospective analysis of a cohort of 220 NPC patients in the northern China. The results showed that the distributions of CA13-/CA13-genotypes were significantly higher in the patients with lymph node metastasis ($P<0.05$) and distant metastasis ($P<0.001$); there was a significant difference between NPC patients with stage I+II and those with stage III+IV regarding CA13+/CA13- ($P<0.001$) and CA13-/CA13- genotypes ($P<0.001$); further analysis showed a more pronounced difference between NPC patients with stage I+II+III and those with stage IV for CA13-/CA13-genotype ($P<0.001$), whereas no difference was found for CA13+/CA13- genotype ($P=0.790$). Thus, we identify that IFN- γ 13-CA-repeat polymorphism is significantly associated with the metastasis of NPC, which may provide insights into its prognosis and individualized treatment.

Keywords: Polymorphism, interferon gamma, nasopharyngeal carcinoma, metastasis

Introduction

Nasopharyngeal carcinoma (NPC) as a common cancer originating from the mucosal epithelium of nasopharynx shows an extremely high morbidity in southern China [1]. Now even in northern China, its prevalence is increasing yearly with the severe pollution. Although the etiology of NPC is obscure, recent understandings tend to believe that this complication is most likely to be caused by interactions of several complex mechanisms in which viral factors, environmental factors as well as genetic factors are implicated. The comprehensive understandings of cancer development in previous decades have made the treatment strategies more systematic [2, 3]; however, the genetic basis that can be utilized to predict the prognosis and to develop individualized treatment strategy are still lacking and to be explored.

A large body of evidence has highlighted the critical roles of immunological surveillance in

the development and progression of cancer [4, 5], and some of the ideas have already been translated to clinical application and become a standard treatment for several cancers [6]. Interferon gamma (IFN- γ), a cytokine secreted by activated T cells and natural killer (NK) cell, exhibits dramatic antiviral, antitumor and immunomodulation effects by enhancing the activity of immune cells, up-regulating antigen presentation and increasing the sensitivity of tumor cells to apoptotic signals [7]. Previous studies have demonstrated that endogenous IFN- γ protected against tumors by accelerating host defense response towards cancer cells [8-10]. Particularly, given Epstein-Barr virus (EBV) is the only surest etiological factor of NPC, the endogenous antiviral capacity may be crucial for its clinical prognosis. Regardless of the recommendation in which IFN- β been used as the adjuvant therapy for NPC in a previous review [3], the role of IFN- γ on this cancer has scarcely been determined.

Table 1. Clinical features of study populations

| Variables | NPC | Controls |
|-----------------------|-----------------|-----------------|
| Sample size | 220 | 220 |
| Gender (male/female) | 156/64 | 157/63 |
| Age (year) | 50.7 \pm 15.9 | 50.4 \pm 15.1 |
| Tumor stages | | |
| I | 41 | |
| II | 57 | |
| III | 97 | |
| IV | 25 | |
| Lymph node metastasis | | |
| NO | 85 | |
| N+ | 135 | |
| Distant Metastasis | | |
| MO | 195 | |
| M+ | 25 | |

The studies demonstrating the associations of gene polymorphisms and cancers have broadened the understanding of genetic susceptibility of tumors in populations. To date, two functional polymorphisms including the microsatellite polymorphism of CA repeats in IFN- γ gene have been reported. The CA repeats polymorphism, which locates in the first intron of gene encoding IFN- γ , has been shown associated with its gene transcription. To present studies, although it has been shown that 12-CA-repeats is best for IFN- γ production, whereas 13-CA-repeats polymorphism greatly impaired the generation of IFN- γ [11], conflicting result indicating the opposite effect of 12-CA-repeats on IFN- γ production as well as tumor development and progression was also presented [12]. Several studies have demonstrated the association between 13-CA-repeats polymorphism and a variety of diseases [11, 13, 14]; however, its association with NPC has never been evaluated. In this case-control study, we reviewed a cohort of 220 NPC patients with diverse tumor stages. The present study revealed that the 13-CA-repeats polymorphism is significantly associated with the metastatic process of NPC.

Materials and methods

Study subjects

A total of 220 patients who suffered from NPC and admitted in three hospitals in Tianjin, China (Tianjin Third Central Hospital, Tianjin Medical University Cancer Institute and Hospital and the Affiliated Hospital of Logistics University of Chinese People's Armed Police Forces) from

August 2010 to July 2014 were recruited in this study, the diagnosis of all the subjects were pathologically proved. The staging for NPC patients was based on the criteria as described in the AJCC Cancer Staging Manual (AJCC Cancer Staging Manual, 7th Ed. New York, NY: Springer, 2010, pp 41-56). For healthy control group, 220 volunteers who underwent physical examination and proved to be normal in the outpatient department were included. All the subjects were excluded from cardiovascular, cerebral, hepatic and nephritic diseases, and all of the study subjects belong to a Han population of northern China. 5 mL EDTA-anticoagulant peripheral blood sample of each individual was collected and stored at -20°C for the following experiments. The study was approved by the ethic committee of Tianjin medical University, and informed consent from each individual was obtained.

Genomic DNA isolation

The genomic DNA from the peripheral blood sample was isolated using a TIANamp Blood DNA Kit from Tiangen Biotechnology Co., Ltd (Beijing, China) according to the manufacturer's instructions. The DNA samples were stored at -20°C.

Genotyping CA-repeats polymorphisms by standard PCR

The CA-repeats microsatellite polymorphisms were genotyped by PCR based method. We synthesized the primers from Invitrogen (Shanghai, China) according to a previously published literature [15] for standard PCR reaction. The primer sequence were 5'-GCTGTCATAATAATATT-CAGAC-3' for forward primer and 5'-CGAGCTTT-AAAAGATAGTTCC-3' for reverse primer. After the PCR reactions were completed, the DNA products were separated by non-denaturing polyacrylamide gel electrophoresis. The gels were analyzed and photographed in a Bio-Rad GelDoc XR+system (CA, USA). A 15% blind, random sample of study subjects was genotyped twice by different persons and the reproducibility was 100%. Further confirmation of each genotype was carried out by gene sequencing (Takara, Dalian, China).

Statistical analysis

All the data were processed and analyzed by SPSS 19.0 software. The comparisons of the rates and indicated clinical parameters

Table 2. Comparison of CA13 between NPC patients and healthy controls

| | controls | Patients | P | NPC | | | | | |
|-------------|----------|----------|-------|------------|------------|-------|------------|------------|--------|
| | n | n | | LN (+) | LN(-) | P | DM (-) | DM (+) | P |
| CA13+/CA13+ | 96 | 101 | 1 | 41 (30.4%) | 39 (45.9%) | 1.00 | 94 (48.2%) | 7 (28.0%) | 1.000 |
| CA13+/CA13- | 97 | 94 | 0.761 | 53 (39.3%) | 32 (37.6%) | 0.160 | 86 (44.1%) | 8 (32.0%) | 0.790 |
| CA13-/CA13- | 27 | 25 | 0.756 | 41 (30.4%) | 14 (16.5%) | 0.007 | 15 (7.7%) | 10 (40.0%) | <0.001 |

LN, Lymph node metastasis; P value was calculated by chi-square test and a Fisher's exact test. DM, distant metastasis.

Table 3. The distribution of genotypes for TNM stages among NPC patients

| Genotypes | The TNMs of NPC | | | | P |
|-------------|-----------------|----|-----|----|--------|
| | I | II | III | IV | |
| CA13+/CA13+ | 30 | 30 | 35 | 7 | <0.001 |
| CA13+/CA13- | 9 | 24 | 53 | 8 | |
| CA13-/CA13- | 2 | 3 | 9 | 10 | |

P value refers to significance value among all the groups on CA13 polymorphism, and was calculated by person χ^2 -square test.

between groups were performed by χ^2 test, a P value less than 0.05 was considered to be statistically significant.

Results

General characteristics of the study subjects

We firstly inspected the clinical features in both NPC and healthy control group, as presented in **Table 1**, no significant difference regarding sex and age were observed (sex (male/female): 156/64 vs. 157/63, $P>0.05$; age: 50.7 \pm 15.9 vs. 50.4 \pm 15.1, $P>0.05$). Detailed clinical features with regard to the tumor stages, tumor size, lymph node metastasis as well as distant metastasis were also shown. The numbers of NPC patients in stage I to stage IV are 41, 57, 97 and 25, respectively. 85 patients did not show lymph node metastasis, whereas 135 patients have lymph node involvement; most of the patients (195 cases) did not have distant metastasis, whereas only 25 patients showed metastatic complications.

The 13-CA-repeats polymorphism distribution in NPC patients and healthy controls

As shown in **Table 2**, there was no significant difference in the distribution of 13-CA-repeats genotypes between healthy control and NPC patients group ($P=1,0.761$ and 0.756 for

CA13+/CA13+, CA13+/CA13- and CA13-/CA13-, respectively). Particularly, in NPC group, the CA13-/CA13-homozygous patients are more likely to have lymph node metastasis, with a rate of 16.5% in lymph node negative patients versus 30.4% in lymph node positive patients ($\chi^2=7.417$, $P=0.007$). Similar result was observed in terms of distant metastasis, the number of patients who have CA13-/CA13-homozygous genotype consists of 40.0% of the distant metastatic cases ($\chi^2=18.78$, $P<0.0001$).

The association between 13-CA-repeats polymorphism and NPC metastasis

To dissect the role of the 13-CA-repeats microsatellite polymorphism in NPC metastasis, we summarized all the NPC cases regarding their TNM stages. The general information of distributions of 13-CA-repeats genotypes for TNM stages was presented in **Table 3**. We further observed significant difference between patients with stages I+II and stages III+IV for both CA13+/CA13- heterozygous ($\chi^2=11.04$, $P<0.001$) and CA13-/CA13- homozygous genotypes ($\chi^2=11.23$, $P<0.001$) (**Table 4**). By contrast, we found a more pronounced difference in patients with stage IV compared with those with stages I+II+III but only for the CA13-/CA13-homozygous genotype, with a rate of 40.0 % in the former versus 7.2% in the latter ($\chi^2=20.16$, $P<0.001$) (**Table 4**).

Discussion

In the current case-control study, we report that the 13-CA-repeat microsatellite polymorphism is associated with the metastasis of NPC. Our conclusion is evidenced by the significant difference in the rate of CA13-/CA13-genotype between the metastasis positive and negative patients, further analysis of distribution of each genotype for TNM stages strengthened their association. To the best of our knowledge, it is the first study demonstrating the associations

Table 4. The genotype distribution of CA13 by NPC TNM stage

| Genotypes | The TNM stages of NPC | | | | | |
|-------------|-----------------------|------------|--------|------------|------------|--------|
| | I+II | III+IV | P | I+II+III | IV | P |
| CA13+/CA13+ | 60 (61.2%) | 42 (34.4%) | 1.000 | 95 (48.7%) | 7 (28.0%) | 1.000 |
| CA13+/CA13- | 33 (33.7%) | 61 (50.0%) | <0.001 | 86 (44.1%) | 8 (32.0%) | 0.790 |
| CA13-/CA13- | 5 (5.6%) | 19 (15.6%) | <0.001 | 14 (7.2%) | 10 (40.0%) | <0.001 |

between the 13-CA-repeat microsatellite polymorphism and the progression of NPC.

The rapid advancement of the genetics and genomics has shed some new light on the role of polymorphisms in the development and progression of cancer. Several gene polymorphisms have been shown to be associated with nasopharyngeal carcinoma; for instance, the roles of the polymorphisms of several oxidative stress-related genes and microRNAs have been recently addressed [16, 17]. As with other types of cancers, the immunological factors such as cytokines and transcription factors also show a great impact on NPC susceptibilities and carcinogenesis [18-22]. IFN- γ , which is previously depicted to play an anti-tumor role, has been studied both experimentally and clinically. It has been shown that there exist two functional polymorphisms in the encoding gene of IFN- γ ; and particularly, one of them, named +874 A/T was found to have no association with NPC risk in a Tunisian population [23]. The role of the other polymorphism, which is referred to as CA-repeat microsatellite polymorphism and locates in the first intron of IFN- γ encoding gene, has been reported. Experimental studies showed that it is closely related to its gene expression [24]. Several studies demonstrating the association between IFN- γ and complications such as graft rejection response and type I diabetes mellitus [25, 26] have provided implications for the possible association between 13-CA-repeats polymorphism and NPC susceptibility or progression. Given that the cytokine driving immune responses are disrupted in NPC, our study thus hypothesized that the 13-CA-repeats polymorphism might be a strong predictor or risk factor of NPC. After analyzing the distributions of each genotype in NPC patients for TNM stages, we could draw a conclusion that 13-CA-repeats microsatellite polymorphism is associated with the metastasis of NPC.

Accumulating evidences have shown that IFN- γ therapy exerts protective actions to reduce the

tumor recurrence rate and promote the survival rate [24, 27]. The endogenous IFN- γ protecting against tumors by accelerating host defense response towards cancer cells has also been demonstrated [8-10], indicating the pivotal role that IFN- γ plays in cancer development. Our study, which suggests that the CA13-/CA13-genotype is associated with increased risk of metastasis, is reminiscent of the increased production of IFN- γ in these patients compared with those with CA13+/CA13+ genotype. Our study reinforced the role of cytokine in the progression of cancer. Previous study declared that IFN- γ and C-reactive protein levels were not correlated; rather, IL-1, IL-6 and TNF- α administered rodent model exhibited cachexia phenotype which is identical to the end stage of nasopharyngeal carcinoma [28, 29]. However, we propose a contrary assumption that IFN- γ might exert a detrimental effect on NPC metastasis especially in those who have CA13-alleles. However, whether the 13-CA-repeats polymorphism is associated with decreased IFN- γ in the scenario of NPC is still to be investigated as the conflicting results are still presented in other systems. NPC is a multifactorial complex disease, although it's not clear how the hazard factors are interacted to promote the carcinogenesis process, the most common subtype, which is characterized by non-keratinizing undifferentiated cancer cell type, is strongly associated with EBV. A previous study has already demonstrated that the risk of EBV reactivation in CA13+/CA13+ homozygous was significantly increased after allogeneic haematopoietic stem cell transplantation [30]. This report seems to be conflict to our study, but the heterogeneity between cancer biology and normal cell biology may provide some instructive information on this discrepancy, and help to explain why CA13-/CA13- homozygous genotype has higher risk of lymph node and distant metastasis.

Despite the strong association between 13-CA-repeats polymorphism of IFN- γ and NPC metastasis revealed by our study, there are still

some limitations need to be mentioned. As nasopharynx is constantly exposed to environmental factors, the time and the level of exposure at diagnose may vary in each patient; what is more, as a retrospective study, we did not follow these patients for survival analysis, as reported previously that 12-CA-repeats is significantly associated with increased survival time in pancreatic cancer [15], it is conceivable that 13-CA-repeats may have an influence on survival duration in the context of NPC; finally, the number of cases in our study is relatively small and these cases were confined to a small region and ethnic origins, which limits the power of our interpretation. Although our study is a single-centered, chart-based and retrospective study, our data highlighted the significant association between IFN- γ polymorphism and NPC metastasis, which suggested that this polymorphism within IFN- γ gene has prominent prognostic value and may be incorporated into the emerging concept of tailoring individualized treatment for NPC patients.

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Disclosure of conflict of interest

None.

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