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Oncogene mutational profile in nasopharyngeal carcinoma

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Zi-Chen Zhang[1,*]
Sha Fu[1,*]
Fang Wang[1]
Hai-Yun Wang[1]
Yi-Xin Zeng[2]
Jian-Yong Shao[1]

[1]Department of Molecular Diagnostics, [2]Department of Experimental Research, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center of Cancer Medicine, Guangzhou, People's Republic of China

*These authors contributed equally to this work

Abstract: Nasopharyngeal carcinoma (NPC) is a common tumor in Southern China, but the oncogene mutational status of NPC patients has not been clarified. Using time-of-flight mass spectrometry, 238 mutation hotspots in 19 oncogenes were examined in 123 NPC patients. The relationships between mutational status and clinical data were assessed with a χ^2 or Fisher's exact test. Survival analysis was performed using the Kaplan–Meier method with the log-rank test. In 123 patients, 21 (17.1%) NPC tumors were positive for mutations in eight oncogenes: six patients had *PIK3CA* mutations (4.9%), five *NRAS* mutations (4.1%), four *KIT* mutations (3.3%), two *PDGFRA* mutations (1.6%), two *ABL* mutations (1.6%), and one with simultaneous mutations in *HRAS*, *EGFR*, and *BRAF* (1%). Patients with mutations were more likely to relapse or develop metastasis than those with wild-type alleles (P=0.019). No differences or correlations were found in other clinical characteristics or in patient survival. No mutations were detected in oncogenes *AKT1*, *AKT2*, *CDK*, *ERBB2*, *FGFR1*, *FGFR3*, *FLT3*, *JAK2*, *KRAS*, *MET*, and *RET*. These results demonstrate an association between NPC and mutations in *NRAS, KIT, PIK3CA, PDGFRA*, and *ABL*, which are associated with patient relapse and metastasis.

Keywords: NPC, oncogene, mutation

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor that originates in the upper lining epithelium of the human retronasal cavity.[1] It is generally rare, but is more common in certain geographic regions, such as Southeast Asia, North Africa, and especially Southern China.[2] The annual incidence of NPC is up to 30 per 100,000 in Guangdong (a province in Southern China), which is 50-fold higher than that in the Western world.[3] There is a clear relationship between NPC and this specific region, with convincing evidence of a correlation with Epstein–Barr virus (EBV) infection.[4] Although this tumor is sensitive to radiotherapy, therapy can fail in patients with advanced stage disease, as the disease is highly invasive and metastatic in nature.[5,6]

Because of the significant correlation between NPC and EBV, most studies of NPC have focused on EBV-related proteins and genes, such as EBER (EBV-encoded RNA) and LMP (EBV-associated membrane antigen).[7,8] Gene linkage studies have also been conducted.[9] Moreover, several chromosome regions, such as 3p21.3-1-21.2, and the human leukocyte antigen (HLA) haplotypes have been linked to the development of NPC.[10–12] Previous studies have also investigated the expression of other genes, such as *TP53* in NPC.[13–16] *C-KIT* and *PIK3CA* mutations have been detected in NPC cell lines and NPC specimens.[17,18] However, few studies have examined the genomic mutations of NPC.

Correspondence: Jian-Yong Shao
Department of Molecular Diagnostics, Sun Yat-sen University Cancer Center, 21 Qing Cai Gang Road, Guangzhou, 510060, People's Republic of China
Tel +86 020 8734 5599
Fax +86 020 8734 5599
Email shaojy@sysucc.org.cn

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) can detect multiple gene mutations with high sensitivity and accuracy. Using this technology, Patrick et al showed that *RAS* mutations are more frequent in cutaneous squamous cell tumor patients treated with *RAF* inhibitors than in those not so treated.[19] Kang et al also detected *EGFR* T790M mutations in patients with non-small-cell lung cancer using MALDI-TOF MS, which detected and quantified the mutations highly sensitively.[20] In this study, a panel of 19 oncogenes including *EGFR*, *RAS* family genes, *KIT*, and *PIK3CA*, were analyzed for 238 possible mutations in tumor tissues from 123 NPC patients. The purpose of this study was to analyze the mutational status of multiple genes in NPC samples and clarify the possible relationships between these mutations and the characteristics of NPC patients.

Materials and methods

Clinical samples

Formalin-fixed paraffin-embedded samples were obtained from 123 patients with pathologically diagnosed NPC between October 1991 and July 2002 at Sun Yat-sen University Cancer Center (SYSUCC) (Guangzhou, People's Republic of China). Informed consent and clinicopathological information were obtained from all patients. Disease stage was classified or reclassified according to the People's Republic of China 1992 NPC TNM staging system.[21] The clinicopathological characteristics of the 123 NPC patients are summarized in Table 1. Institute Research Medical Ethics Committee of SYSUCC granted approval for this study.

Table 1 Clinical characteristics of 123 NPC patients

Characteristic	Number of patients
Sex	
Male	95 (77.2%)
Female	28 (22.8%)
Age (years)	
Median	46
≤46	66
>46	57
Overall survival (months)	
Median	57
Range	6–120
Progression-free survival (months)	
Median	52
Range	5–118
Clinical stage	
I+II	32 (26.0%)
III+IV	91 (74.0%)
Relapse/metastasis	
No	86 (69.9%)
Yes	37 (30.1%)
Therapeutic modality	
No treatment	17 (13.8%)
Radiotherapy alone	83 (67.5%)
Chemotherapy alone	1 (0.8%)
Radiochemotherapy	22 (17.9%)
WHO histological classification	
NKUC	101 (82.1%)
NKDC	22 (17.9%)
KSCC	0 (0%)

Abbreviations: NPC, nasopharyngeal carcinoma; WHO, World Health Organization; NKUC, non-keratinizing undifferentiated carcinoma; NKDC, non-keratinizing differentiated carcinoma; KSCC, keratinizing squamous cell carcinoma.

DNA extraction

We chose paraffin blocks containing more than 60% tumor cells from hematoxylin and eosin stained sections of each tumor. Sections (4–6 μm) were cut and transferred to 1.5 mL Eppendorf tubes for DNA extraction. DNA was extracted using the QIAamp DNA Formalin-fixed Paraffin-embedded Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. The quantity and quality of the isolated DNA were tested using a Nanodrop ND-2000 Spectrophotometer (Thermo Scientific, Niederelbert, Germany). The final DNA samples were diluted to 10 ng/μL for analysis.

OncoCarta assay

A total of 238 possible mutations in 19 oncogenes were investigated in 123 NPC samples using the OncoCarta Panel (v 1.0; Sequenom Inc., San Diego, CA, USA). This panel is a set of predesigned and prevalidated assays for sensitive and efficient mutation screening by the parallel analysis of 238 possible mutations across the following 19 common oncogenes: *ABL1*, *AKT1*, *AKT2*, *BRAF*, *CDK*, *EGFR*, *ERBB2*, *FGFR1*, *FGFR3*, *FLT3*, *HRAS*, *JAK2*, *KIT*, *KRAS*, *MET*, *NRAS*, *PDGFRA*, *PIK3CA*, and *RET*. The mutation types of each gene are list in Table S1.

In brief, 20 ng of DNA was amplified using 24 sets of OncoCarta PCR primers. An extension reaction based on the OncoCarta extension primers was then performed. After a cation exchange resin was used to remove salts, the products were spotted onto a 384-well SpectroChipII using the MassARRAY Nanodispenser RS1000 (Sequenom Inc.) and analyzed on a MALDI-TOF mass spectrometer (Sequenom Inc.). We chose high performance liquid chromatography purified water as the blank control and normal human somatic cells as the negative control in each experiment.

Data analysis

Mutation data was analyzed using the software MassARRAY Typer (v4.0; Sequenom Inc.), using a cutoff mutation

frequency of 1%. A successful experiment should show that the sample figure was typical and the blank control had no peak (Figure 1).

Statistical analysis

The statistical analysis was performed using SPSS software (v 16.0; SPSS Inc., Chicago, IL, USA). The relationships between the patients' mutational status and clinical data were assessed with a χ^2 or Fisher's exact test. Kaplan–Meier analysis was used to compare differences in the survival rate of the groups. A *P*-value of less than 0.05 was considered statistically significant. A multivariate analysis was used to check the possible relationship between factors. Overall survival (OS) and progression-free survival (PFS) were calculated for the 123 NPC patients from the date of surgery until death or the last follow-up. The clinical data follow-up date was updated to October 2012.

Results

Mutation profiles of NPC tumors

Mutations were detected in 17.1% (21/123) of NPC tumors, with one NPC patient having two simultaneous mutations.

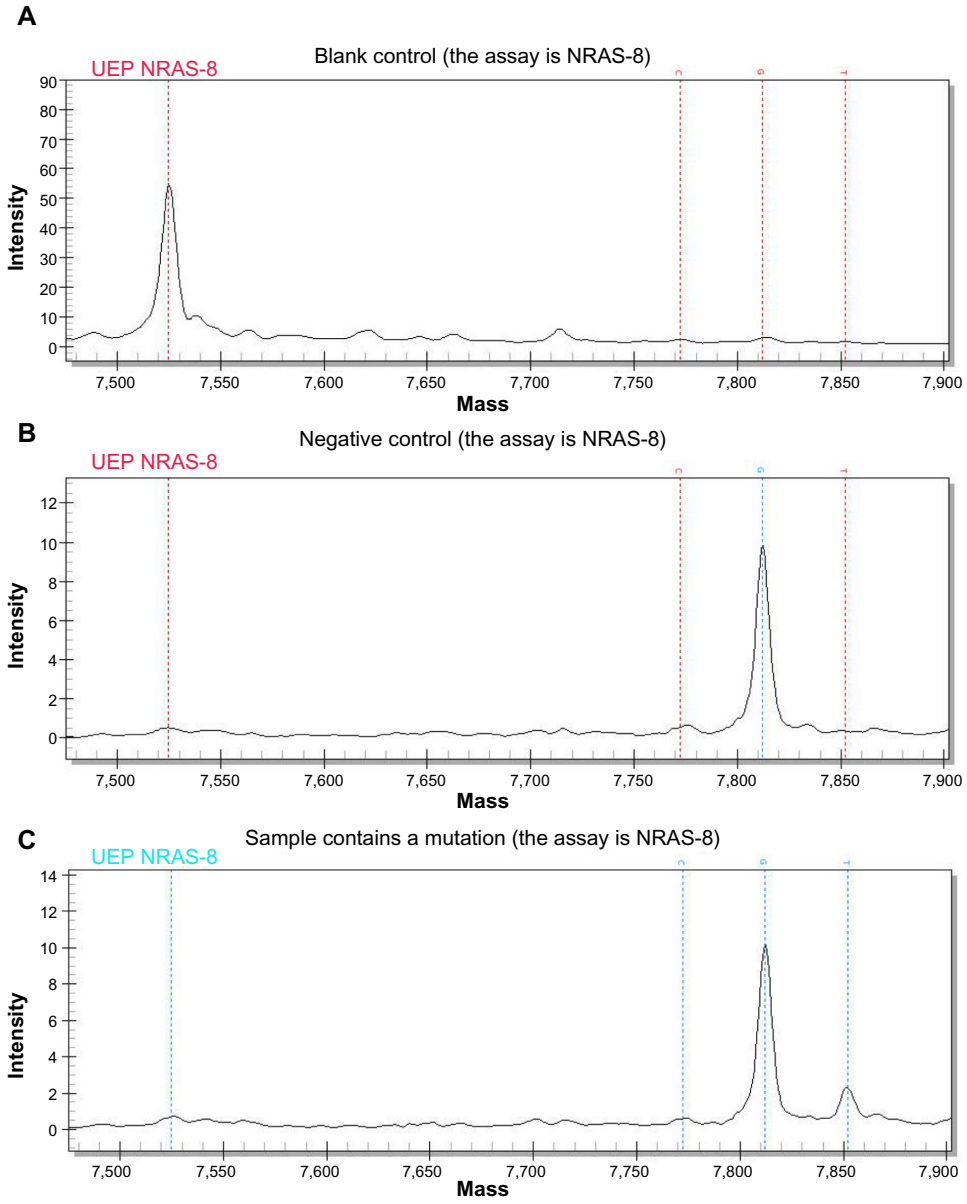

Figure 1 Representative graphs showing the mutations detected by time-of-flight mass spectrometry using *NRAS*-8. (**A**) Blank control. Only a peak representing the uncombined probe is apparent, with no sample peaks. (**B**) Negative control. A peak for the negative sample is shown, with no mutation peak or close chemical noise peak. However, there is a clear standard peak for the wild-type sample. (**C**) A typical mutation peak. The wild-type peak and a mutation peak are apparent, with no abnormality noted in the blank control (**A**) or negative control (**B**).

Therefore, a total of 21 patients presented with 22 mutations. The mutations affected eight oncogenes, as follows: six in *PIK3CA* (three E542K, two H1047Y, and one R38H), five in *NRAS* (one G12D, two G13D, and two Q61K), four in *KIT* (two V559I, one V559A, and one D52N), two in *PDGFRA* (two T647I), and two in *ABL* (two E255K); *HRAS* (G13S) and *EGFR* (E709A) mutations were found in one NPC tumor each; one NPC tumor had a *BRAF* mutation (G464E) together with a *PIK3CA* mutation (E545K). No mutation was detected in the remaining 102 (82.9%) NPC tissues (with no mutations in *AKT1*, *AKT2*, *CDK*, *ERBB2*, *FGFR1*, *FGFR3*, *FLT3*, *JAK2*, *KRAS*, *MET*, or *RET*). The mutational profiles and distributions of the NPC tumors are shown in Table 2.

Correlations between oncogene mutations and patient clinicopathological characteristics

We divided the patients into groups according to their clinical characteristics, and calculated the mutation rate in each group. We then assessed the relationships between the mutational status and clinical data using the χ^2 or Fisher's exact test. The results are presented in Table 3. There was an association between oncogene mutations and relapse/metastasis of NPC (P=0.019, Table 3). We also tested the intersubject effects with a univariate analysis and found no effect between all factors (Table S2). No significant correlation between the presence of an oncogenic mutation and other clinicopathological parameters, such as age, sex, clinical stage, or WHO histological grade was found (P>0.05, Table 3).

Table 2 Mutation status of the 21 positive cases

Sample ID	Gene	Mutation	Ratio	
			MT	WT
NPC053	*PIK3CA*	R38H	0.07	0.93
NPC125	*PIK3CA*	H1047Y	0.07	0.93
NPC064	*PIK3CA*	H1047Y	0.08	0.92
NPC041	*PIK3CA*	E542K	0.07	0.93
NPC113	*PIK3CA*	E542K	0.21	0.79
NPC029	*PIK3CA*	E545K	0.05	0.95
	BRAF	G464E	0.18	0.82
NPC056	*PDGFRA*	T674I	0.10	0.90
NPC087	*PDGFRA*	T674I	0.10	0.90
NPC093	*NRAS*	G12D	0.12	0.88
NPC042	*NRAS*	G13D	0.10	0.90
NPC050	*NRAS*	G13D	0.11	0.89
NPC022	*NRAS*	Q61K	0.14	0.86
NPC044	*NRAS*	Q61K	0.21	0.79
NPC021	*KIT*	D52N	0.10	0.90
NPC120	*KIT*	V559A	0.29	0.71
NPC099	*KIT*	V559I	0.16	0.84
NPC121	*KIT*	V559I	0.22	0.78
NPC030	*ABL*	E255K	0.14	0.86
NPC038	*ABL*	E255K	0.13	0.87
NPC019	*EGFR*	E709A	0.14	0.86
NPC031	*HRAS*	G13S	0.08	0.92

Abbreviations: WT, wild-type; MT, mutation.

Table 3 Correlations between oncogene mutation and clinicopathological characteristics of NPC patients

Characteristics	Mutation n=21 (17.1%)	Wild-type n=102 (82.9%)	*P*-value
Age (years)			0.359
<46	12 (20.3%)	47 (79.7%)	
≥46	9 (14.1%)	55 (85.9%)	
Sex			0.656
Male	17 (17.9%)	78 (82.1%)	
Female	4 (14.3%)	24 (85.7%)	
Clinical stage			0.800
I+II	5 (15.6%)	27 (84.4%)	
III+IV	16 (17.6%)	75 (82.4%)	
Relapse/metastasis			0.019
No	10 (11.8%)	75 (88.2%)	
Yes	11 (28.9%)	27 (71.1%)	
WHO histological classification			0.437
NKUC	16 (15.8%)	85 (84.2%)	
NKDC	5 (22.7%)	17 (77.3%)	
KSCC	0 (0%)	0 (0%)	

Abbreviations: NPC, nasopharyngeal carcinoma; WHO, World Health Organization; NKUC, non-keratinizing undifferentiated carcinoma; NKDC, non-keratinizing differentiated carcinoma; KSCC, keratinizing squamous cell carcinoma.

Correlation between oncogene mutation and patient survival

We estimated the survival of NPC patients by comparing the OS and PFS of the mutation and wild-type subgroups (Figure 2). The mean survival time (MST) in the mutation subgroup did not differ significantly from that of the wild-type subgroup (OS: 86.5 vs 90.0, P>0.05, Figure 2A; PFS: 83.6 vs 88.6, P>0.05, Figure 2B).

Because the *PIK3CA*, *KIT*, and *NRAS* oncogenes had higher mutation frequencies of the oncogenes analyzed, we also assessed the correlation between the clinicopathological characteristics of the patients and the presence of these mutations, but found no significant correlation (Table S3).

Discussion

Various aspects of NPC have been widely investigated because it is an important cancer of the head and neck. However, few studies have examined the role of mutations in NPC, and the results of such studies are controversial. In this study, our data show that *PIK3CA*, *KIT*, and *RAS* are the oncogenes most susceptible to mutations in NPC, whereas mutations of *BRAF*, *PDGFRA*, *ABL1*, and *EGFR* occur less frequently. Many of the mutations described here have never been previously reported in NPC samples. A summary of

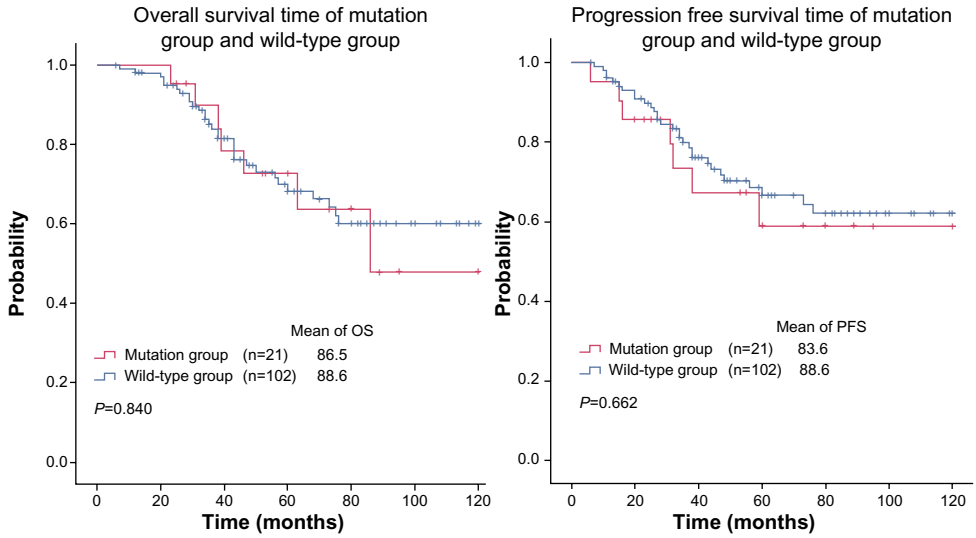

Figure 2 Kaplan–Meier survival curves for NPC patients. (**A**) Overall survival (OS) of the NPC patients with oncogene mutations vs that of wild-type patients. (**B**) Progression-free survival (PFS) of NPC patients with oncogene mutations vs that of wild-type patients.

oncogenes' mutations in NPC in our study and other literature is shown in Table 4.[17,18,22–33]

In a comparison of the mutation frequencies in the subgroups of patients with and without relapse or metastasis, we found that NPC patients who relapsed or developed metastases had higher mutation frequencies (28.9% vs 11.8%, respectively, *P*=0.019).

The *PIK3CA* gene encodes the p110α catalytic subunit of PI3K and plays an important role in many tumors. Mutations of this gene are reportedly located in exons 9 and 20, with

Table 4 Summary of oncogene mutations in NPC

Gene	Mutation status in this study	Mutation status in other NPC studies	More NPC literature
ABL1	1.6% (2 of 123)	No report	No report
AKT1	0%	No report	No report
AKT2	0%	No report	No report
BRAF	0.8% (1 of 123)	0% (0 of 65) (sequencing)[33]	No report
CDK4	0%	No report	Expression of mRNA and protein (RT-PCR and IHC)[22]
EGFR	0.8% (1 of 123)	0% (0 of 60) (sequencing)[23]; 0% (0 of 102) (sequencing)[24]	Expression rate 65.6% (IHC)[25]; expression rate 70.9% (IHC)[26]
ERBB2	0%	No report	Expression rate 37.5% (IHC)[25]; amplification rate 43.3% (C-PCR)[27]
FGFR1	0%	No report	No report
FGFR3	0%	No report	No report
FLT3	0%	No report	No report
HRAS	0.8% (1 of 123)	No report	No report
KRAS	0%	0% (0 of 45) (sequencing)[33]; no mutation in 4 NPC cell lines (sequencing)[28]	No report
NRAS	4.1% (5 of 123)	No report	Amplification (CGH)[25]
JAK2	0%	No report	No report
MET	0%	No report	No expression (IHC)[29]; expression rate 91.1% (IHC)[30]
KIT	3.3% (4 of 123)	5 cell lines reported intron mutation (sequencing)[18]	No report
PDGFRA	1.6% (2 of 123)	No report	No report
PIK3CA	4.9% (6 of 123)	4.3% (2 of 46) (clone sequencing)[17]; 0% (0 of 27) (sequencing)[32]; 9.6% (7 of 73) (sequencing)[33]; 1.13% (1 of 88) (sequencing)[31]	21.6% amplification (RT-PCR)[31]
RET	0%	No report	No report

Abbreviations: CGH, comparative genomic hybridization; IHC, immunohistochemistry; NPC, nasopharyngeal carcinoma; RT-PCR, real-time polymerase chain reaction; C-PCR, competitive polymerase chain reaction; mRNA, messenger RNA.

hotspots at E542K, E545K, and H1047Y.[34] In our study, 83.3% (5/6) of all the *PIK3CA* mutations identified occurred at these hotspots. We found that NPC patient survival did not correlate significantly with the presence of *PIK3CA* mutations, which is consistent with a previous study (Figure S1).[33] In NPC cell lines, the inhibitor NVP-BEZ235 was found to selectively inhibit the proliferation of NPC cells carrying *PIK3CA* mutations.[35] Currently, mTOR inhibitors are used as therapies for cancers in which the PI3K/AKT/mTOR pathway is activated. Although mutation rate is not so high, PIK3CA is also worthy as a research object of targeted therapy in NPC.

It is well established that the RAS/RAF/ERK pathway plays an important role in tumor development. *KRAS*, *HRAS*, and *NRAS* mutations occur in at least one-third of all human cancers, with *KRAS* mutations being the most common.[28,36,37] In the present study, we detected mutations of *NRAS* and *HRAS*, but not in *KRAS*. *KRAS* mutation rate in all tumors is estimated to be 25%–30%.[38] But here, in NPC, *KRAS* mutation is particularly scarce. We detected *NRAS* mutations at Q61K, G13D, and G12D and a *HRAS* mutation at G13S, all of which are acknowledged hotspots. Consistent with our results, previous studies have detected no mutations in codons 12, 13, or 61 of *KRAS* in NPC specimens or NPC cell lines.[28,33] These data suggest that *RAS* mutations exist in NPC, but that *KRAS* mutations are rare.

KIT is a type III receptor tyrosine kinase that initiates multiple downstream signaling pathways, such as the PI3K/AKT and JAK/STAT pathways. *KIT* gene mutations are mainly found in melanomas, and imatinib is an effective inhibitor of this oncogene. Here, we observed mutations V559I and V559A, which are the most common *KIT* mutation types. V559I is considered to confer resistance to imatinib, whereas V559A reportedly confers sensitivity to imatinib.[39,40] PDGFRA belongs to the type III tyrosine kinase family. In fact, there appears to be a close relationship between *KIT* and *PDGFRA*, and the correlation between them has been widely investigated.[41,42] *PDGFRA* mutation T674I confers imatinib resistance. In the present study, both mutations of *KIT* (3.3%) and *PDGFR* (1.6%) were detected in NPC tissues; this result is consistent with other reports.[43,44] Further clinical trials are required to evaluate the correlation between NPC patients with *KIT* and *PDGFR* mutations and their response to the drug imatinib.

EGFR is a cell-surface protein that binds to EGF, and mutations in *EGFR* are associated with a wide variety of tumors. *EGFR* mutations are very frequent in non-small-cell lung cancer, with exons 18, 19, 20, and 21 being the predominantly mutated regions.[45] In our study, only one NPC patient was positive for an *EGFR* mutation, resulting in a mutation rate of less than 1%. This mutation was E709A, which is encoded in exon 20 and usually reported in lung cancer.[46,47] This result may suggest that the treatment of NPC patients with tyrosine kinase inhibitors may not be an effective strategy.

Detection of multiple mutations status in NPC was also one of our aims. It can provide more information about treatment and prognosis than single mutation detection. Herein, one NPC sample was detected to have two simultaneous mutations (*PIK3CA* and *BRAF*). Studies have suggested that the concurrent presence of *PIK3CA* and *BRAF* mutations predict resistance to everolimus.[48,49]

We should not ignore the negative results of this study. This report describes the analysis of 238 potential mutations in 19 oncogenes in 123 NPC samples to gain a preliminary understanding of mutational status of these 19 oncogenes. The negative results of this study indicate that mutations are rare in *NPC*, *AKT1*, *AKT2*, *CDK*, *ERBB2*, *FGFR1*, *FGFR3*, *FLT3*, *JAK2*, *KRAS*, *MET*, and *RET*, suggesting that drugs targeting these genes may be ineffective.

This study had several limitations. Compared with deep sequencing, MALDI-TOF MS offers high-throughput and is less expensive. However, the comprehensiveness of detection is inadequate. Many deserted or concealed mutations cannot be detected using this method, so we may miss some important genes in NPC.

Conclusion

In summary, a small number of mutations in *NRAS, KIT, PIK3CA, PDGFRA,* and *ABL* are present in NPC, whereas mutations in other genes, including *AKT1, AKT2, CDK, ERBB2, FGFR1, FGFR3, FLT3, JAK2, KRAS, MET*, and *RET*, are extremely rare. The presence of oncogene mutations in NPC patients is associated with relapse and metastasis.

Acknowledgments

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Author contributions

JYS and YXZ conceived and designed the experiments; ZCZ and SF performed the experiments; ZCZ and FW analyzed

the data; JYS contributed the reagents/materials; ZCZ and JYS wrote the paper; HYW acquired clinical samples and follow-up clinical information. All authors read and approved the final manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Mutation subtypes detected by the OncoCarta Panel (v 1.0; Sequenom Inc., San Diego, CA, USA) MassARRAY

Gene	Mutation subtypes
ABL1	G250E; F317L; M351T; E355G; F359V; H396R; Q252H; Y253H; Y253F; E255K; E255V; D276G; F311L; T315I
AKT1	rs11555435; rs11555431; rs11555432; rs12881616; rs11555433; rs11555436; rs34409589
AKT2	S302G; R371H
BRAF	G464R; F595L; G596R; L597S; L597R; L597Q; L597V; T599I; V600E; V600K; V600R; V600L; K601N; K601E; G464V
CDK	R24C; R24H
EGFR	R108K; S768I; V769_D770insASV; V769_D770insCV; D770_N771>AGG; V769_D770insASV; V769_D770insASV; D770_N771insG; N771_P772>SVDNR; P772_H773insV; H773>NPY; H773_V774insNPH; H773_V774insPH; H773_V774insH; V774_C775insHV; T263P; T790M; L858R; L861Q; A289V; G598V; E709K; E709H; E709A; E709G; E709V; G719S; G719C; G719A; M766_A767insAI; E746_T751del; E746_A750del; E746_A750del; E746_T751del; E746_A750del; E746_T751del; S752D; L747_E749del; L747_T750del; L747_S752del; L747_T751del; L747_S752del; P753S; L747_T751del; A750P; T751A; T751P; T751I; S752I/F; L747_Qins; E746_T751del; Iins; E746_A750del; T751A; E746_T751del; Vins; E746_A750del; Vins; L747_E749del; A750P; L747_T750del; Pins; L747_S752del; Qins; T751; S752_I759del
ERBB2	L755P; G776S; G776LC; G776VC; A775_G776insYVMA; P780_Y781insGSP; P780_Y781insGSP; S779_P780insVGS
FGFR1	S125L; P252T
FGFR3	G370C; Y373C; A391E; K650Q; K650E; K650T; K650M
FLT3	I836del; D835H; D835Y
HRAS	G12V; G12D; G13C; G13R; G13S; Q61H; Q61H; Q61L; Q61R; Q61P; Q61K
JAK2	V617F
KIT	D52N; V559del; V559_V560del; V560del; P551_V555del; Y553_Q556del; Y570_L576del; E561K; L576P; P585P; D579del; Y503_F504insAY; K642E; D816V; D816H; D816Y; V825A; E839K; M552L; Y568D; F584S; W557R; W557R; W557G; V559D; V559A; V559G; V559I; V560D; V560G; K550_K558del; K558_V560del; K558_E562del
KRAS	G12V; G12A; G12D; G12C; G12S; G12R; G12F; G13V; G13D; A59T; Q61E; Q61K; Q61L; Q61R; Q61P; Q61H; Q61H
MET	R970C; T992I; Y1230C; Y1235D; M1250
NRAS	G12V; G12A; G12D; G12C; G12R; G12S; G13V; G13A; G13D; G13C; G13R; G13S; A18T; Q61L; Q61R; Q61P; Q61H; Q61E; Q61K
PDGFRA	V561D; I843_S847>T; D842V; T674I; F808L; D846Y; N870S; D1071N; D842_H845del; I843_D846del; S566_E571>K
PIK3CA	R88Q; H1047Y; R38H; C901F; M1043I; M1043I; N345K; C420R; P539R; E542K; E545K; Q546K; H701P; H1047R; H1047L
RET	C634R; C634W; C634Y; E632_L633del; M918T; A664D

Table S2 Tests of between-subjects effects

Source	Type III sum of squares	*df*	Mean square	*F*	*P*-value
Corrected model	6.784[a]	20	0.339	1.777	0.033
	6.318	1	6.318	33.087	0.000
Sex	0.024	1	0.024	0.128	0.721
Mutation	1.250	1	1.250	6.544	0.012
Age	0.012	1	0.012	0.065	0.799
WHO	0.258	1	0.258	1.351	0.248
Clinical staging	0.003	1	0.003	0.018	0.893
Sex * mutation	3.536×10^{-5}	1	3.536×10^{-5}	0.000	0.989
Sex * age	0.284	1	0.284	1.486	0.226
Sex * WHO	0.031	1	0.031	0.165	0.686
Sex * clinical staging	0.053	1	0.053	0.276	0.600
Mutation * age	0.251	1	0.251	1.316	0.254
Mutation * WHO	0.012	1	0.012	0.065	0.800
Mutation * clinical staging	0.473	1	0.473	2.475	0.119
Age * WHO	0.414	1	0.414	2.168	0.144
Age * clinical staging	0.027	1	0.027	0.141	0.708
WHO * clinical staging	0.307	1	0.307	1.607	0.208
Sex * mutation * age	0.000	0	–	–	–
Sex * mutation * WHO	0.000	0	–	–	–
Sex * mutation * clinical staging	0.000	0	–	–	–
Sex * age * WHO	0.462	1	0.462	2.419	0.123
Sex * age * clinical staging	2.665×10^{-5}	1	2.665×10^{-5}	0.000	0.991
Sex * WHO * clinical staging	0.037	1	0.037	0.195	0.660
Mutation * age * WHO	0.000	0	–	–	–
Mutation * age * clinical staging	0.000	0	–	–	–
Mutation * WHO * clinical staging	0.000	0	–	–	–
Age * WHO * clinical staging	0.000	0	–	–	–
Sex * mutation * age * WHO	0.000	0	–	–	–
Sex * mutation * age * Clinical staging	0.000	0	–	–	–
Sex * mutation * WHO * Clinical staging	0.000	0	–	–	–
Sex * age * WHO * Clinical staging	0.000	0	–	–	–
Mutation * age * WHO * Clinical staging	0.000	0	–	–	–
Sex * mutation * age * WHO * clinical staging	0.000	0	–	–	–
Error	19.476	102	0.191		
Total	38.000	123			
Corrected total	26.260	122			

Note: Dependent variable: relapse/metastasis.
Abbreviation: WHO, World Health Organization.

Table S3 Correlations between PIK3CA, NRAS, KIT mutations and clinicopathological characteristics of NPC patients

Characteristics	PIK3CA			NRAS			KIT		
	Yes	No	*P*-value	Yes	No	*P*-value	Yes	No	*P*-value
Age (years)			1.000			1.000			1.000
<46	3	47		2	4		2	47	
≥46	3	55		3	5		2	55	
Sex			0.334			1.000			1.000
Male	6	78		4	7		3	78	
Female	0	24		1	2		1	24	
Clinical stage			0.333			0.611			1.000
I+II	0	27		2	2		1	27	
III+IV	6	75		3	7		3	75	
Relapse/metastasis			0.333			0.611			0.068
No	6	75		3	7		1	75	
Yes	0	27		2	2		3	27	
WHO histological classification			0.587			1.000			0.147
NKUC	6	85		4	8		2	85	
NKDC	0	17		1	1		2	17	
KSCC	0	0		0	0		0	0	

Abbreviations: NPC, nasopharyngeal carcinoma; WHO, World Health Organization; NKUC, non-keratinizing undifferentiated carcinoma; NKDC, non-keratinizing differentiated carcinoma; KSCC, keratinizing squamous cell carcinoma.

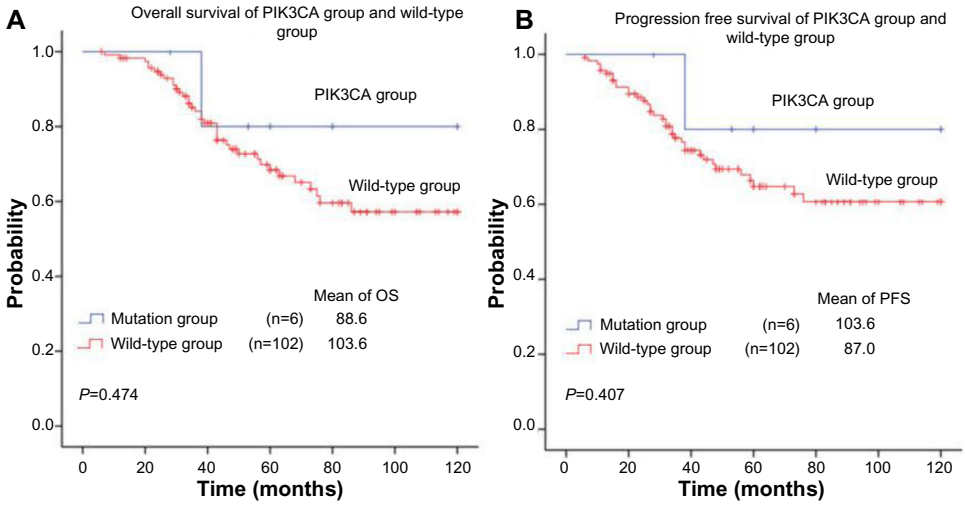

Figure S1 Kaplan–Meier survival curves for NPC patients classified as either with or without PIK3CA mutations. (**A**) Overall survival (OS) curve of NPC patients with PIK3CA mutations have no difference in wild-type NPC patients; (**B**) progression-free survival (PFS) curve of NPC patients with PIK3CA mutations also have no difference in wild-type NPC patients.
Abbreviation: NPC, nasopharyngeal carcinoma.