Hotspot mutations in common oncogenes are infrequent in nasopharyngeal carcinoma

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Abstract. Oncogene mutations contribute to carcinogenesis and can provide potential therapeutic targets for clinical anticancer management. However, oncogene mutation patterns in nasopharyngeal carcinoma (NPC) have yet to be fully elucidated. To gain insight into mutation patterns in NPC, a high-throughput OncoCarta panel assay was used to determine 238 hotspot mutations across 19 common oncogenes in 8 NPC cell lines and 160 NPC patient samples from southern China. Statistical analyses were further conducted to identify associations between oncogene mutations and selected clinicopathological characteristics. In total, we identified 24 mutations across 11 oncogenes in 17 (10.6%) NPC patients. Four patients exhibited mutations in at least one oncogene. We also identified a *PIK3CA* H1047R mutant in 7 NPC cell lines. In addition, oncogene mutations showed no correlation with either risk habits (smoking and drinking) or other clinical characteristics except for TNM stage. *KIT* mutations were associated with poorer overall and relapse-free survival. Furthermore, *KIT* mutations together with age and N stage were independent prognostic factors in NPC. Taken together, the present study is the first report on mutations in multiple oncogenes in NPC. We found that hotspot oncogene mutations are infrequent in NPC patients from southern China. The lack of hotspot mutations requires a comprehensive characterization of gene mutations in NPC for developing new therapeutic targets in the future.

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Introduction

Nasopharyngeal carcinoma (NPC) is an endemic disease in southern China and Southeast Asia, with a global incidence of 84,400 new cases annually and a mortality of 51,600 in 2008 (1). After primary treatment with radiotherapy or chemoradiotherapy, a significant proportion of endemic NPC patients, particularly those with stage III or IV, relapsed locoregionally and/or systemically (2,3). The median overall survival after recurrence is generally poor and ranges from 7.2 to 22 months (4-6). Therefore, new therapeutic strategies are required.

Cancer derives from the progressive accumulation of abnormalities in cellular DNA which provides growth advantages to cancer cells (7). Somatic mutations become useful targets and biomarkers in selecting personalized therapy for many solid tumors. For example, molecular driven therapeutic targets such as epidermal growth factor receptor (*EGFR*) and abnormal fusion of echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase (*EML4-ALK*) genes have resulted in a paradigm shift in the treatment of lung adenocarcinoma (8,9). Furthermore, *KRAS (*Kirsten rat sarcoma viral oncogene homolog) and *BRAF* (v-raf murine sarcoma viral oncogene homolog B) oncogene mutations are positively associated with resistance to anti-EGFR drugs (10,11). Hence, current anticancer therapy depends more on the knowledge of genetic alterations in specific tumors.

Lifestyle exposure such as salted fish, EBV infection, smoking and drinking has consistently been linked to NPC risk (12), predicate on the hypothesis that genomic alternations as a result of lifestyle exposure may be a reason for NPC oncogenesis. Despite the fact that chromosomal abnormalities (13) together with amplification of certain oncogenes have been identified in NPC (14,15), information regarding oncogene mutations in NPC is limited (16). Several studies demonstrated that oncogene phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) mutation was an uncommon event in NPC patients (17-19). No *EGFR* kinase domain mutation was found in 60 Moroccan NPC patients (20). *BRAF* and

Table I. Clinicopathological characteristics of NPC patients (n=160).

Characteristics	No. of patients (n=160)	Mutation		P-value[a]
		Absent n (%)	Present n (%)	
Age (years)[b]	160	47.4±12.1	47.9±11.5	0.86[c]
Gender				
Female	36	32 (88.9)	4 (11.1)	1.00
Male	124	111 (89.5)	13 (10.5)	
Histology				
Differentiated	6	5 (83.3)	1 (16.7)	1.00
Undifferentiated	154	138 (89.6)	16 (10.4)	
Smokers				
Yes	81	73 (90.1)	8 (9.9)	0.58
No	71	62 (87.3)	9 (12.7)	
Alcohol consumption				
Yes	29	28 (96.6)	1 (3.4)	0.25
No	123	107 (87.0)	16 (13.0)	
VCA-IgA				
<1:80	18	15 (83.3)	3 (16.7)	0.64
≥1:80	141	127 (90.1)	14 (9.9)	
EA-IgA				
<1:10	23	19 (82.6)	4 (17.4)	0.45
≥1:10	136	123 (90.4)	13 (9.6)	
T stage				
T1-2	37	30 (81.1)	7 (18.9)	0.12
T3-4	123	113 (91.9)	10 (8.1)	
N stage				
N0-1	71	62 (87.3)	9 (12.7)	0.62
N2-3	89	81 (91.0)	8 (9.0)	
TNM stage				
I-II	15	10 (66.7)	5 (33.3)	0.01
III-IV	145	133 (91.7)	12 (8.3)	

[a]Fisher's exact test. [b]Mean ± SD. [c]Student's t-test. TNM, tumor-node-metastasis.

RAS mutants were observed to be absent in 65 NPC samples (17). *KIT (*v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) intron mutation was reported in NPC cell lines (21). Therefore, hotspot mutations in a group of actionable oncogenes remain to be investigated in larger studies.

In the present study, a high throughput OncoCarta™ ver. 1.0 mutation profiling panel was used to determine the prevalence of 238 hotspot mutations across 19 oncogenes in 8 NPC cell lines and 160 NPC patients. This panel interrogates with oncogenes with known targeted drugs or genes that interact with oncogenic pathways (22-24). Furthermore, the association between oncogene mutations and clinicopathological factors of NPC patients was also investigated in our study.

Materials and methods

Patient samples. The present study included 160 formalin-fixed paraffin-embedded (FFPE) tumor samples and matched peripheral blood cell samples from adult patients with newly diagnosed NPC. All samples were obtained from the Sun Yat-sen University Cancer Center between January 2006 and December 2009 and were collected before patients underwent treatment (radiotherapy or chemotherapy). All tissue slides were pathologically diagnosed by at least two independent pathologists according to the World Health Organization (WHO) classification (J.Z. and J.Y.).

The clinicopathological characteristics of all patients, including age, gender and clinical staging were collected and summarized in Table I. Clinical staging was classified according to the criteria of the 7th edition of the AJCC Cancer Staging Manual. The follow-up duration was calculated from the first day of treatment to either the day of death or the day of last examination. The median follow-up time was 40.0 months (range, 1.87-67.33). This study was approved by the Institutional Ethics Review Boards of Sun Yat-sen University Cancer Center and written informed consent was obtained from all patients.

Table II. Mutations detected with the OncoCarta™ ver. 1.0 kit.

No.	Genes	Targeted mutations
1	ABL1	D276G, E255K, E255V, F311L, F317L, F359V, G250E, H396R, M351T, Q252H, T315I, Y253F, Y253H
2	AKT1	E17del, E319G, L357P, P388T, Q43X, V167A, V167A, V461L
3	AKT2	R371H, S302G
4	BRAF	D594V, D594G, F468C, F595L, G464R, G464V, G464E, G466R, G469S, G469E, G469A, G469V, G469R, G596R, K601E, K601N, L597Q, L597V, L597S, L597R, T599I, V600E, V600K
5	CDK4	R24C, R24H
6	EGFR	A289V, D770_N771>AGG, D770_N771insG, E709A, E709G, E709V, E709K, E709H, E746_A750del, E746_A750del, V ins, E746_A750del, T751A, E746_T751del, I ins, E746_T751del, S752D, E746_T751del, V ins, G598V, G719A, G719S, G719C, H773_V774insH, H773_V774insNPH, H773>NPY, L747_E749del, A750P, L747_S752del, P753S, L747_S752del, Q ins, L747_T750del, P ins, L747_T751del, L858R, L861Q, M766_A767insAI, N771_P772>SVDNR, P772_H773insV, R108K, S752_I759del, S768I, SNP C2255T, T263P, T751A, T790M, V769_D770insASV, V769_D770insCV, V774_C775insHV
7	ERBB2	A775_G776 insYVMA, G776S, G776LC, G776VC, L755P, P780_Y781 insGSP, S779_P780 insVGS
8	FGFR1	P252T, S125L
9	FGFR3	A391E, G370C, K650Q, K650E, K650T, K650M, Y373C
10	FLT3	D835H, D835Y, I836del
11	HRAS	G12V, G12D, G13C, G13R, G13S, Q61H, Q61H, Q61K, Q61L,Q61R, Q61P
12	JAK2	V617F
13	KIT	D52N, D579del, D816H, D816Y, D816V, E561K, E839K, F584S, K550_K558del, K558_E562del, K558_V560del, K642E, L576P, M552L, P551_V555del, P585P, V559_V560del, V559D, V559A, V559G, V559del, V559I, V560D, V560G, V560del, V825A, W557R, W557R, W557G, Y503_F504insAY, Y553_Q556del, Y568D, Y570_L576del
14	KRAS	A59T, G12A, G12C, G12D, G12F, G12R, G12S, G12V, G13V, G13D, Q61E, Q61K, Q61H, Q61H, Q61L, Q61R, Q61P
15	MET	M1250T, R970C, T992I, Y1230C, Y1235D
16	NRAS	A18T, G12C, G12R, G12S, G12V, G12A, G12D, G13C, G13R, G13S, G13V, G13A, G13D, Q61E, Q61K, Q61H, Q61L, Q61R, Q61P
17	PDGFRA	D1071N, D842_H845del, D842V, D846Y, F808L, I843_D846del, I843_S847>T, N870S, S566_E571>K, T674I, V561D
18	PIK3CA	C420R, C901F, E542K, E545K, H1047R, H1047L, H701P, M1043I, M1043I, N345K, P539R, Q546K, R38H, R88Q
19	RET	A664D, C634R, C634W, C634Y, E632_L633del, M918T

Cell lines. The NPC cell lines SUNE-1, CNE-1, C666-1, CNE-2, HONE-1, HNE-1, 5-8F and 6-10B were obtained and maintained in RPMI-1640 (Invitrogen, Beijing, China) supplemented with 10% fetal bovine serum (Gibco, Montevideo, Uruguay) as previously described (25). The immortalized nasopharyngeal epithelial cell line (NP69) was cultured in keratinocyte serum-free medium (Invitrogen, NY, USA) supplemented with bovine pituitary extract (BD Biosciences, San Jose, CA, USA) (25). All cell lines were passaged for less than ten generations and incubated at 37˚C in a 5% CO_2 incubator.

DNA extraction. For all FFPE tumor samples, hematoxylin and eosin (H&E) stained slides were reviewed by two pathologists (Z.J. and J.Y.) to ensure a percentage of tumor cells >70% as previously described (26,27). Eight 10 μm unstained FFPE tissue sections of each sample were deparaffinized by xylene wash (20 min) followed by two 100% ethanol washes. DNA was extracted from the pellets, cell lines and matched peripheral blood cell samples using the Qiagen DNA extraction Kits (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The quality and quantity of DNA was determined using the NanoDrop ND1000 Spectrophotometer and gel agarose electrophoresis.

Oncogene mutation detection and analysis. DNA samples were amplified using the OncoCarta™ v1.0 Kit (Sequenom, San Diego, CA, USA) containing 24 pools of PCR primers and extension primers that allow the detection of 238 pathogenic mutations in 19 oncogenes (*ABL1, AKT1, AKT2, BRAF, CDK4, EGFR, ERBB2, FGFR1, FGFR3, FLT3, JAK2, KIT, MET, HRAS, KRAS, NRAS, PDGFA, PIK3CA* and *RET*) (Table II) (23,28). The extension products were analyzed based on the matrix-assisted laser desorption ionization-time of flight mass

Table III. Summary of specific mutations detected in NPC samples using MassArray.

Gene	Mutation	Allele	Allele frequency		Sample
			WT	Mut	
AKT1	E17K	G	0.6	0.4	56
BRAF	L597S	G	0.77	0.23	17
	G469R	T	0.911	0.089	104
CDK4	R24H	T	0.73	0.27	17
	R24C	A	0.57	0.43	26
	R24C	A	0.868	0.132	116
EGFR	N771_P772>SVDNR	GCGT	0.91	0.09	48
	N771_P772>SVDNR	GCGT	0.83	0.17	106
	T790M	T	0.924	0.076	100
	H773_V774insNPH	AA..AC	0.909	0.091	104
	R108K	A	0.91	0.09	149
FGFR3	Y373C	G	0.85	0.15	26
KIT	V559I	A	0.724	0.276	12
	E839K	A	0.901	0.099	104
	K558_V560del	DEL	0.899	0.101	157
KRAS	A59T	T	0.89	0.11	13
	G12D	T	0.639	0.287	105
MET	R970C	T	0.92	0.08	17
	R970C	T	0.895	0.105	129
NRAS	A18T	T	0.89	0.12	13
PDGFRA	T674I	T	0.85	0.15	14
	T674I	T	0.69	0.31	17
	T674I	T	0.94	0.06	96
PIK3CA	E545K	A	0.894	0.106	153

Mutations are represented as amino acidic changes found for each gene and the proportions between the wild type (WT) and mutated (Mut) alleles frequency for each alteration.

spectrometry (MALDI-TOF) technology on the Sequenom MassArray platform (28). The experiments were conducted according to the manufacturer's instructions, as previously described (28,29). The spectra were analyzed by MassArray Typer Analyzer® ver. 4.0.4.20 Software (Sequenom) which automates the identification of mutants by comparing ratios of wild-type (WT) peaks to all suspected mutants and adjusting these peaks when adducts are detected in the spectrum. All mutations detected were manually reviewed by three different persons (N.J., N.L. and F.Y.). Mutation peaks that appeared in both tumor and matched blood cell DNA were not considered as somatic mutations and were excluded from further analysis.

Statistical analysis. Statistical analyses were conducted using SPSS ver. 20 Software. The χ^2 test and Fisher's exact test were used to assess differences in the distribution of clinical variables and oncogene mutation status. Kaplan-Meier analysis was used to determine survival; the differences between genotypes were compared using the log-rank test. HR values were calculated using univariate Cox regression analysis. Multivariate Cox regression analysis was used to test the independent significance, in which *KIT* mutation, age, gender, T and N stages were used as covariates. All tests were two-tailed, and P-values <0.05 were considered to indicate a statistically significant difference.

Results

Oncogene mutations detected in NPC. Of the 160 NPC patients, 17 (10.6%) had at least one oncogene mutation and 4 (23.5%) of them had two or more mutations. In total, we identified 24 mutations located in 11 genes by Sequenom OncoCarta kit (Table III). *EGFR* variants were detected in 5 tumors, followed by *CDK4*, *KIT* and *PDGFRA* mutations in 3 patients and *KRAS*, *BRAF* and *MET* (*MET* proto-oncogene) mutations in 2 patients. *FGFR3* (fibroblast growth factor receptor 3), *AKT1* (v-akt murine thymoma viral oncogene homolog 1), *PIK3CA* and *NRAS* mutations were only detected in one sample (Table III). We did not detect any mutations in the remaining eight oncogenes. Patients with *BRAF* mutations were WT in *KRAS*, which was consistent with previous findings in colon cancer (30). Direct sequencing was adopted to validate the MassArray findings. Representative figures of detected mutations are shown in Fig. 1A and B.

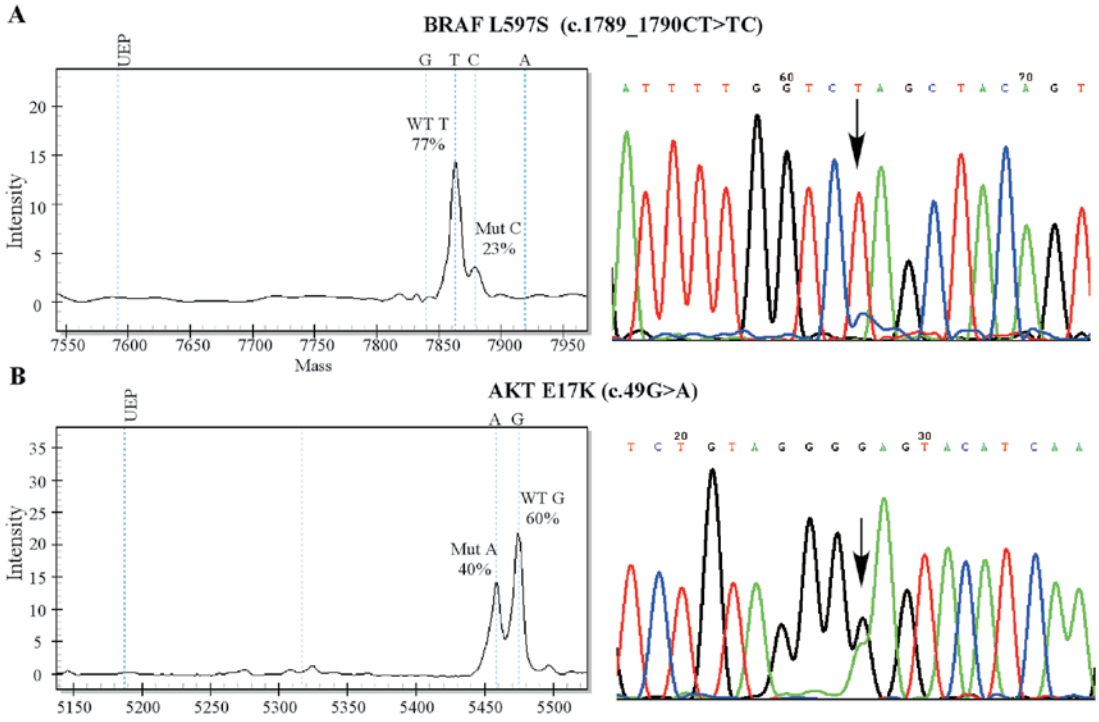

Figure 1. Representative examples of mutations detected by MassArray and DNA direct sequencing. (A and B) The expected positions for the unextended primer (UEP), and the extension products (mutant and WT) from assays A*KT*1_7 and *BRAF*_12-13 by MassArray indicated in the left panel. The proportion of peak areas and the specific base are also shown. *BRAF* L597S (c.1789_1790CT>TC) and *AKT1* E17K (c.49G>A) mutations were confirmed by direct sequencing (black arrows indicate the mutation site in the right panel of A and B).

We next determined the 238 hotspot mutations in 8 NPC cell lines: 5-8F, 6-10B, SUNE-1, CNE-1, C666-1, CNE-2, HONE-1 and HNE-1. *PIK3CA* c.3140A>G (H1047R) was the only mutation observed in 7 NPC cell lines, but not in C666-1 (Fig. 2). The results were confirmed by direct sequencing (Fig. 3). Notably, the *PIK3CA* c.3140A>G mutation was also found absent in an immortalized nasopharyngeal epithelial cell line NP69 by direct sequencing (Fig. 3).

Taken together, hotspot oncogene mutations which are common in other solid tumors are infrequent events in NPC.

Correlation between mutational profile and clinicopathological characteristics. The relationship between clinicopathological factors and mutation patterns were assessed. As shown in Table I, oncogene mutations were significantly associated with the TNM stage of NPC patients (P=0.01). However, there was no association between oncogene mutations and clinical characteristics such as age, gender, histopathological grade, EBV-related antigen levels, tumor (T) and lymph node (N) stage of NPC patients (P>0.05, Table I). We also did not find any difference in risk habits (smoking or alcohol consumption) in patients with or without oncogene mutations (P>0.05, Table I).

Furthermore, Kaplan-Meier analysis did not show significant differences in overall survival (OS) and relapse-free survival (RFS) between patients with and without oncogene mutations (P>0.05) (Fig. 4A). Patients with *KIT* mutation were associated with poorer OS (HR, 8.34; 95% CI, 2.47-28.21; P<0.001) and RFS (HR, 8.20; 95% CI, 1.03-64.92; P=0.02) (Fig. 4B). Furthermore, we performed multivariate analyses with *KIT* mutation, age, gender, T and N stages as covariates (P<0.05). We found that the *KIT* mutation (HR, 5.94; 95% CI, 1.73-20.42; P<0.01), age (HR, 1.69; 95% CI, 1.11-2.58; P=0.01) and N stage (HR, 1.56; 95% CI, 1.02-2.41; P=0.04) were independent prognostic factors associated with OS in NPC patients (Table IV).

Table IV. Summary of the multivariable analysis of prognostic factors for overall survival and risk score in NPC.

Variables	P-value	HR	95% CI
Age group (years) (≤47 vs. >47)	0.01	1.69	1.11-2.58
Gender (Male vs. female)	0.06	0.58	0.32-1.02
T stage (T3-T4 vs. T1-T2)	0.18	1.42	0.85-2.37
N stage (N2-N3 vs. N1-N0)	0.04	1.56	1.02-2.41
KIT mutation	<0.01	5.94	1.73-20.42

Discussion

In recent years, identification of somatic mutations as key perturbations that promote tumorigenesis has become an essential component in determining the management of certain malignancies. For example, oncogenic mutations in *EGFR*, *KRAS* have been clinically used as target and sensi-

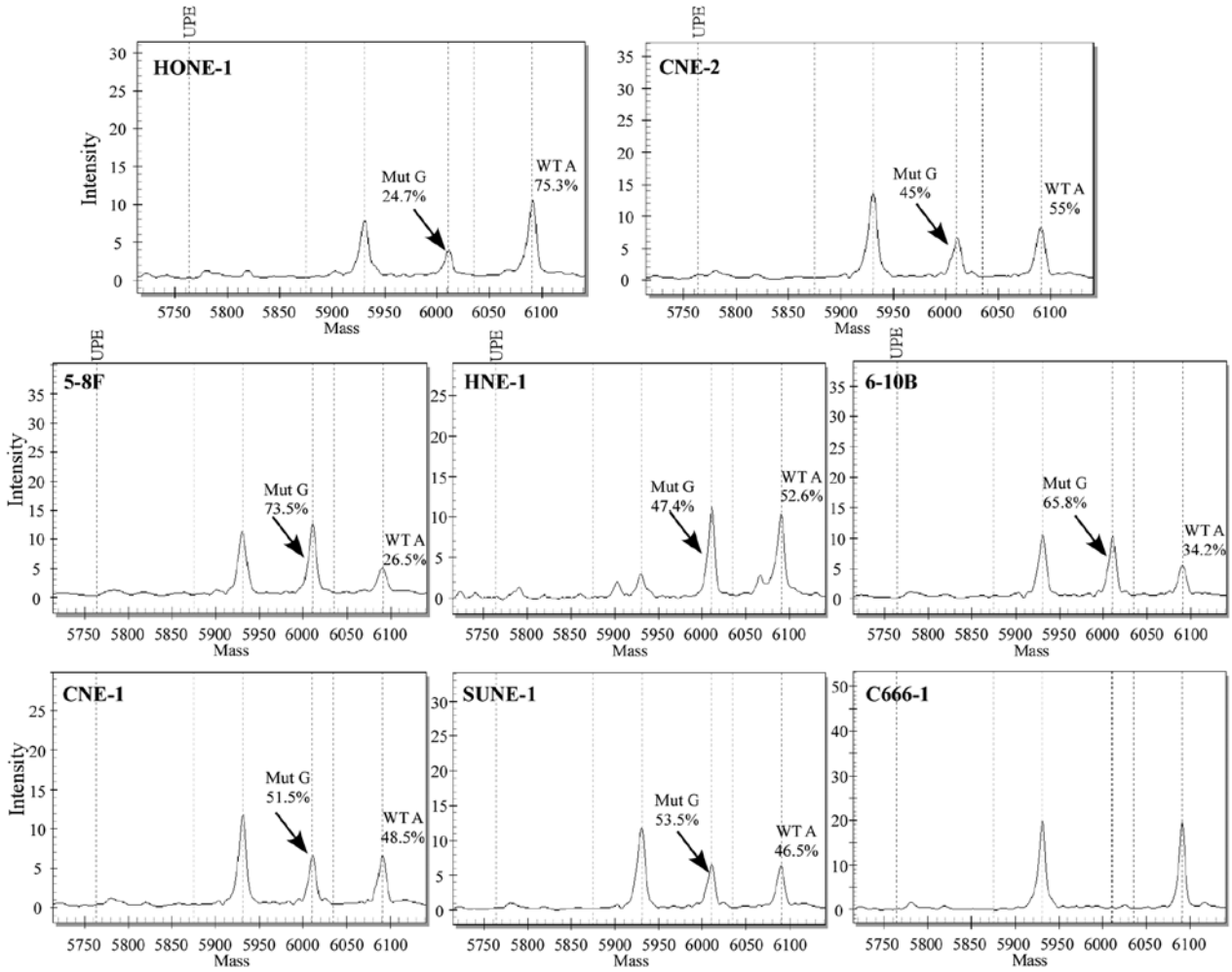

Figure 2. Seven NPC cell lines showing *PIK3CA* H1047R mutation. The expected peak positions for the UEP and the extension products (Mutant G and WT A) using the *PIK3CA*_9 assay in HONE-1, CNE-2, 5-8F, HNE-1, 6-10B, CNE1, SUNE1 and C666-1 cell lines are shown. The arrows indicate the mutation peaks. Other peaks included in these spectra that were not marked are results of multiplexing but not part of the designated assays.

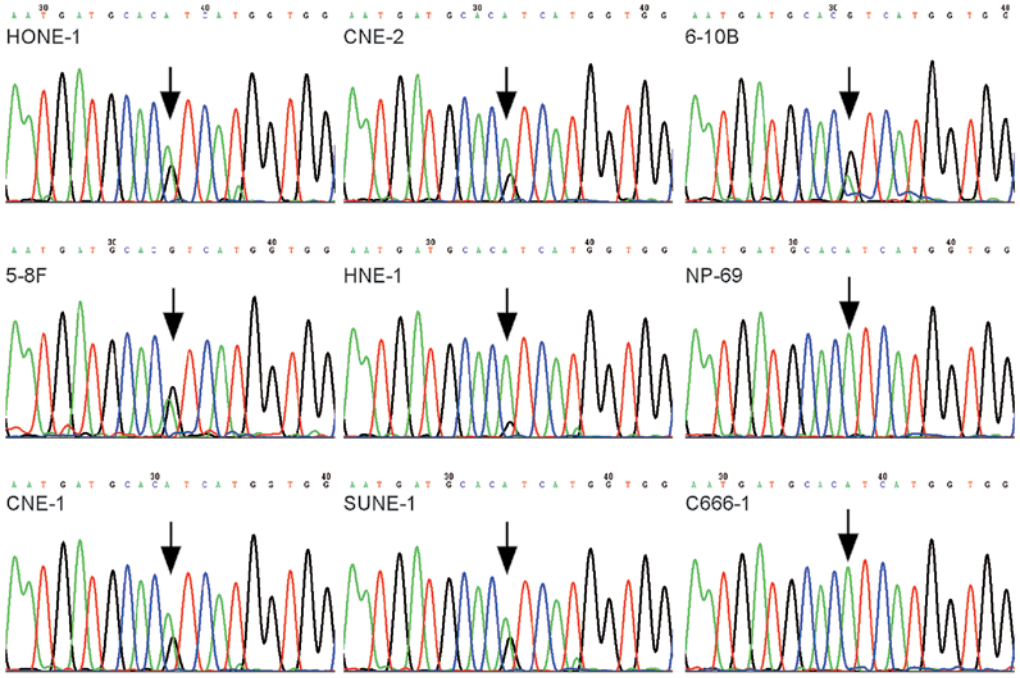

Figure 3. *PIK3CA* H1047R mutation confirmed in NPC cell lines by direct sequencing. *PIK3CA* H1047R mutation was confirmed by direct sequencing in 8 NPC cell lines and a normal nasopharyngeal epithelial cell line NP69 (black arrows indicate the mutation site).

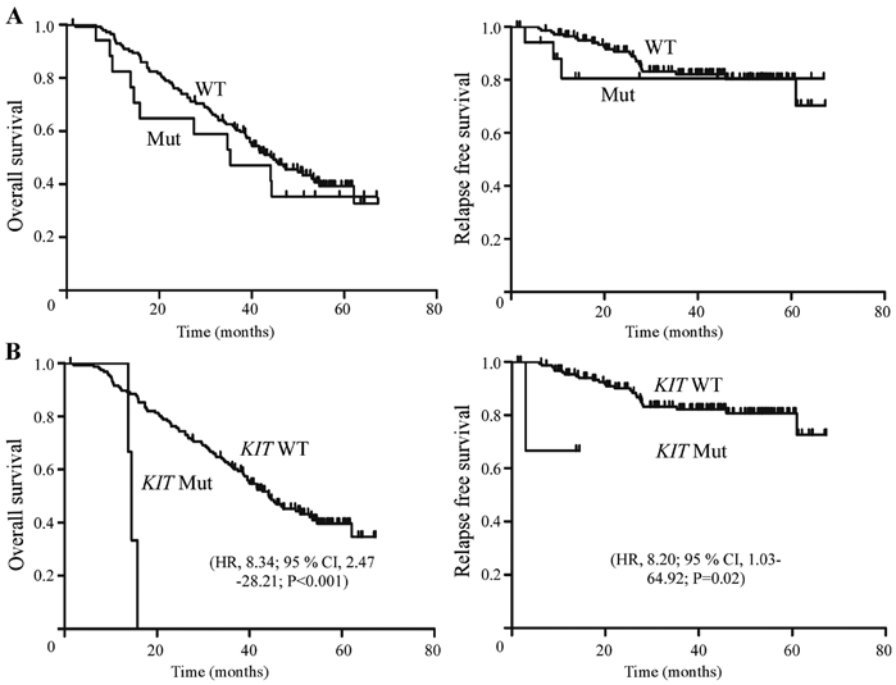

Figure 4. *KIT* mutations correlate with poorer survival in NPC patients. Kaplan-Meier curves of overall survival (OS) and relapse-free survival (RFS) in NPC patients stratified by mutation levels with respect to over time. Hazard ratio (HR) values and confidence interval (CI) were calculated by the univariate Cox regression analysis. (A) There was no significant difference in OS and RFS between NPC patients with or without mutations. (B) Patients with *KIT* mutations exhibited poorer OS and RFS in comparison with *KIT* WT patients.

tivity biomarkers in the treatment of non-small cell lung carcinoma (31). However, knowledge regarding oncogene mutational patterns in NPC remains limited, especially in patients from NPC prevalent southern China. Thus, a high-throughput OncoCarta panel was adopted in the current study to determine 238 hotspot mutations across 19 oncogenes in NPC. This panel provides a cost effective and efficient technology for detecting known hotspot mutations in FFPE samples (23,32). Moreover, information concerning 10 of the 19 oncogenes investigated by the OncoCarta™ ver. 1.0 assay is either limited or absent in COSMIC database in NPC (http://www.sanger.ac.uk/cosmic).

The present study demonstrated that oncogene mutations were uncommon in NPC with 10.6% of patients carrying one or more mutations across 11 oncogenes. There was no difference between patients with or without oncogene mutations in risk habits (tobacco and alcohol consumption) and EBV infection status, both of which are historically associated with NPC. Consistent with previous reports, known *PIK3CA* mutations were detected in both NPC cell lines and tissues with similar mutation frequency compared to COSMIC database (Table V) (17,33). Furthermore, we found a correlation between *KIT* mutation and poorer survival in NPC patients. *KIT* mutation together with age and N stage were independent prognostic factors for NPC. The identification of mutated oncogenes in NPC is encouraging as it may provide insight into the etiology of NPC and influence future clinical management.

PIK3CA, BRAF, EGFR, KIT, KRAS, HRAS, NRAS, PDGFRA and *MET* (34) oncogenes have been previously reported to be absent in NPC according to the COSMIC database, whereas mutations in gene *AKT1, CDK4, FGFR3* and *ABL1* have not yet been studied in NPC (Table V) (35). In the present study, mutations in these oncogenes were reported in NPC for the first time. This could be explained by the use of a more sensitive MALDI-TOF technology which is as sensitive as next generation sequencing, and more sensitive than Sanger sequencing, as Su *et al* demonstrated (36). Secondly, according to the COSMIC database, most of the previous studies were based on cohorts <100 samples (Table V). Furthermore, this difference may also be due to different pathological characteristics or population background.

Table V. Mutations detected in the present study compared with the COSMIC database.

Genes	COSMIC database Mut/total cases (%)[a]	Present study Mut cases (%; n=168)[a]
AKT1	N	1 (0.6)
BRAF	0/65 (0)	2 (1.2)
CDK4	N	3 (1.8)
EGFR	0/78 (0)	5 (3.0)
FGFR3	N	1 (0.6)
KIT	0/3 (0)	3 (1.8)
KRAS	0/74 (0)	2 (1.2)
MET	0/5 (0)	2 (1.2)
NRAS	0/18 (0)	1 (0.6)
PDGFRA	0/3 (0)	3 (1.8)
PIK3CA	8/105 (7.6)	9 (5.4)

[a]Including data of NPC cell lines. N, not determined.

The *PIK3CA* gene encodes the 110 kDa catalytic subunit of PI3K. Upon activation, PIK3CA generates an activating signaling cascade involved in cell growth, survival, proliferation, motility and morphology (37,38). A study by Or *et al* previously reported a one base substitution of c.3140A>G (H1047R) in the *PIK3CA* gene in CNE-2 and HONE-1, but not in C666-1 cells line (33). We confirmed the same mutant in 7 NPC cell lines, including CNE-2 and HONE-1. However, EBV positive C666-1 and an immortalized nasopharyngeal epithelial cell line NP69 showed absence of this mutation. Since the c.3140A>G mutant acts as a gain-of-function mutation in *PIK3CA* kinase domain (39) and appears only in EBV negative NPC cell lines, we speculate that this mutation may play an important role in the transformation of EBV-negative NPC cell lines, which deserves further investigation. We did not find this mutant in 160 patient samples among which 98.1% of patients showed detectable EBV-related antigens. Instead, a *PIK3CA* c.1633G>A (E545K) mutation was detected in one NPC patient. This mutant was previously reported by Chou *et al* in 4 NPC patients (17). Moreover, in comparison with the mutation rate of 7.6% (8/105) reported by COSMIC database (including cell line data), this study found a similar mutation frequency of 5.6% (8/168) in *PIK3CA* oncogene (Table V) (18,33,40).

The proto-oncogene *c-KIT* encodes a transmembrane tyrosine kinase receptor which plays important roles in the hematogenous system, placenta, heart, lung, and midgestational kidney (41,42). Gain-of-function mutations of the *c-KIT* gene promote constitutive phosphorylation of *KIT* and, consequently, activation of downstream PI3K/AKT, Src family kinases and MAPK pathways (42). In our study, we found *KIT* K558_V560del, E839K and V559I mutations in three NPC patients. K558_V560del and V559I are juxtamembrane mutations located in exon 11 of *KIT* oncogene which have been observed in GISTs (43,44) and aggressive systemic mastocytosis (ASM), respectively (44). These two mutants showed spontaneous KIT phosphorylation (45) and could transform IL-3-dependent Ba/F3 cells into IL-3-independent growth in the absence of KIT ligand stem cell factor (SCF) (46). In contrast, KIT^{E839K} was not spontaneously phosphorylated in response to exogenous SCF and thus lacked cell transforming ability (45). KIT phosphorylated mutants could be inhibited by KIT inhibitors such as imatinib and dasatinib (47). These data together with our findings that *KIT* mutation correlated with poor survival in NPC, suggest that targeting KIT could be a potential therapeutic strategy in the treatment of NPC. We did not find any *KIT* mutation in NPC cell lines. This is consistent with a study by Huang *et al* (21) in which no *KIT* exon 9-21 hotspot mutation was found in 5 NPC cell lines. However, the fact that *KIT* mutation was only detected in 3/160 NPC patients requires broader studies in the future. *KIT* DNA amplification, protein overexpression and their clinical relevance also warrants further investigation in NPC.

Mutations in exon 18-21 of *EGFR* tyrosine kinase domain are present in lung cancer, and some of them are related to response to anti-EGFR agents such as gefitinib or erlotinib (8,24). EGFR tyrosine kinase domain mutations have been previously found absent in 60 Moroccan patients (20) and four NPC cell lines (48). In our series, we detected five NPC cases carrying four EGFR mutants (Table III). EGFR T790M, H773_V774insNPH and N771_P772>SVDNR mutations located in EGFR exon 20 could change the crystal structures of EGFR which lead to resistance to EGFR inhibitors (49). EGFR R108K is a gain-of-function mutation in EGFR exon 3 which has been reported in glioma. It would be of interest to determine whether these mutants affect the response to EGFR-TKIs in NPC patients in the future.

In the present study, we first reported three *CDK4* mutations in NPC. Cyclin-dependent kinase 4 (CDK4) is the chief catalytic subunit of the regulatory cyclin D that governs G1-to-S phase cell cycle progression (50). Dominant activating mutations affecting codon 24 of the *CDK4* gene (R24H or R24C) render CDK4 insensitive to p16INK4 inhibition and are responsible for multiple neoplasia developing (50,51). Collectively, these data indicated that *CDK4* mutation may play a role in oncogenesis in a subset of NPC patients. Moreover, mutations affect the RAS/RAF pathway and growth factor receptors such as MET, PDGFRA, FGFR3 were also found in NPC in our study. Since all these molecules are popular targets in anticancer treatment, NPC patients with these mutations may benefit from therapies targeting these oncogenes

In summary, this study provided evidence for understanding oncogenic mutational patterns in NPC. Our data showed lower oncogene mutation frequencies in NPC compared to other solid tumors (52-55). The presence of mutations in a few key oncogenes may ultimately be important in clinical management of NPC and requires future verification.

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