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PIK3CA amplification is predictive of poor prognosis in Tunisian patients with nasopharyngeal carcinoma

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PI3Ks (phosphatidylinositol 3-kinases) are lipid kinases that regulate signalling pathways involved in cell proliferation, motility, and adhesion. Somatic mutations and amplification of the *PIK3CA* gene have been reported in various types of human cancers. However, little is known about the frequency and prognosis role of *PIK3CA* activation in nasopharyngeal carcinoma (NPC). This study was conducted with the aim to screen for *PIK3CA* mutations in the two hot spot regions (exons 9 and 20) and to investigate for the *PIK3CA* gene amplification combined with the expression analysis of the phosphorylated Akt (pAkt). We showed that among 88 specimens, none had mutation in the helical domain (exon 9) and only one (1.13%) had mutation in the kinase domain (exon 20). On the other hand, *PIK3CA* gene amplification was found in 21.6% of cases and was strongly associated with distant metastasis ($P = 0.002$), lymph node involvement ($P = 0.032$), and advanced tumor stage ($P < 0.001$). Moreover, patients with *PIK3CA* copy number gain have a significant reduced overall survival time (P log rank = 0.02). We concluded that *PIK3CA* gene amplification is frequent in NPC and occurs in the advanced stage of NPC. Moreover, our finding emphasizes the association of *PIK3CA* gene amplification with worse prognosis in nasopharyngeal carcinoma. (*Cancer Sci* 2009; 100: 2034–2039)

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway plays an important role in cell growth and proliferation initiated by activation of receptor tyrosine kinases and in tumor genesis and progression.[1,2] Activation of cell-surface receptors recruits PI3K, which phosphorylates the phosphatidylinositol 4,5-bisphosphate substrate to generate phosphatidylinositol 3,4,5-trisphosphate, recognized by the protein kinase Akt and its regulator PDK1. In the cell membrane, Akt is activated through phosphorylation at serine and threonine residues and thus modulates the expression of several genes involved in suppression of apoptosis and cell cycle progression.[3] *PIK3CA*, the gene encoding the 110-kDa subunit of PI3K, was mapped to 3q26, an area amplified in various human cancers including ovarian, head and neck, breast, urinary tract, and cervical cancers.[3–5] The *PIK3CA* copy number gains are associated with increased *PIK3CA* transcription, P110-alpha protein expression, and PI3K activity in ovarian cancer.[6] Beside gene amplification, high frequency of *PIK3CA* mutations have been reported in colorectal, ovarian, lung and breast cancers.[7,8] A majority of somatic mutations in *PIK3CA* are missense mutations clustering in exons 9 and 20, which encode a part of the helical and kinase domains, respectively.[7–9] In *vitro* study has demonstrated that one of the most frequently observed *PIK3CA* mutations in human cancers, the H1047R (exon 20), is associated with increased kinase activity, indicating that the *PIK3CA* mutations actually activate the PI3K pathway.[10,11]

In nasopharyngeal carcinoma only few reports that studied *PIK3CA* have been available. In one small previous study of 40 patients with primary NPC, no mutations were detected in exons 9 and 20 of the *PIK3CA* gene, whereas copy number gains were found in 75% of these tumors without *PIK3CA* mutations.[12] In a second recent study, *PIK3CA* mutations were detected in 9.6% of primary NPC tumors, and no association was found with clinico-pathological features and prognosis.[13] Thus, the relation of *PIK3CA* amplification to major clinico-pathological parameters and patient outcome still remains unknown in NPC.

In Tunisia, NPC represents the most frequent head and neck cancer with a bimodal pattern of age distribution.[14,15] Indeed, in the endemic regions of the South-East Asia, there is only one major peak of incidence at about the age of 50 while in North Africa including Tunisia, an additional minor peak of incidence occurs between the ages of 10–20 years, and represents about 25% of all NPC patients.[15–17]

In this study, we aimed to investigate the frequency of *PIK3CA* mutations and amplification in Tunisian patients with NPC and their effect on patient survival and major clinical parameters.

Materials and Methods

Tumor specimens and DNA extraction. Primary NPC biopsies were collected from 88 patients who underwent resection before any treatment at the Sfax University Hospital in the south of Tunisia. The median age is 40 years (range, 10 to 80 years) at the time of diagnosis. The clinical stage was determined according to the tumor, node, and metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC).[18] Histological type was determined on tissues sections according to the World Health Organization criteria. Our patients were grouped as followed: 41cases (47%) of undifferentiated type (UCNT) and 47 cases (53%) of non-keratinizing carcinoma. The majority of patients (83%) had palpable neck lymph nodes (N+). In addition, 10 histological normal hyperplasia tissues were collected from patients with clinical symptoms indicative of NPC but nasopharyngeal biopsies did not show tumoral cells. These specimens were used as controls.

Genomic DNA was extracted from frozen sections using the Wizard SV Genomic DNA purification system according to the manufacturer's protocol (Promega, France).

Informed consent for use of all human specimens in this study was obtained under a protocol approved by CHU Habib Bourguiba (Sfax-Tunisia) institutional review board.

PCR-SSCP. PCR amplification targeting the *PIK3CA* gene areas of interest were performed using two primer pairs based on the *PIK3CA* sequence obtained from Gene Bank NM_006218.

To optimize the size of the PCR fragments for polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) analysis and avoid co-amplification of the pseudogene,

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primer pairs were chosen to individually amplify exon 9 of *PIK3CA* using two-step PCR according to the protocol described previously[19] Primary PCR was performed using the following primers: forward 5′-AACTT CAGCAGTTACTATTCTGT-GAC-3′ and reverse: 5′-GATTTTCCACA AATATCA ATT TA CAA-3′ generating 575 bp. PCR amplification was performed in 25 μL containing 2 nM of dNTPs, 1.5 mM MgCl2, 50 mM KCl, 0.2 mM of each primer, and 1 U of Taq DNA polymerase (Fermentas, France). PCRs were done with the touchdown conditions: an initial 3 min denaturation at 95°C followed by 25 cycles of 1 min each at 94°C, 61°C, and 72°C, and a single final extension step for 5 min at 72°C. The nested PCR was done using the following primers: forward 5′-CTGTGAATCCA-GAGGGGAAA-3′ and reverse: 5′-ACAG AGAATCTCC-ATTTTAGCA-3′ to generate 217 bp products. PCR were done with an initial 3 min denaturation at 95°C followed by 25 cycles of 1 min each at 94°C, 60°C, 72°C, and a final extension step for 5 min at 72°C. After amplification, PCR products were diluted 5-fold in loading buffer containing 20 mM/L EDTA, 94% formamide and 0.05% of bromophenol blue and xylene cyanol. After denaturation at 94°C for 10 min, each sample was immediately chilled on ice for 5 min and applied on 6% non denatured polyacrylamide gel containing 10% glycerol. Electrophoresis was performed at 6°C with constant voltage (100 V) in 1X TBE buffer for 20 h. DNA was visualized using the DNA Silver Staining Kit according to the manufacture's recommendations (Amersham-Biosciences, Athens, Greece).

PCR-DHPLC analysis. *PIK3CA* exon 20 was screened for mutations by denaturating high performance liquid chromatography (DHPLC) (Wave Nucleic Acid Fragment Analysis System; Transgenomic, Omaha, USA). Briefly, PCR was performed using primers: forward 5′-TTT GCTCCAAACTGACCAAAC-3′ and reverse: 5′-CAGTGCAGTGTGGAATCCAG-3′ giving a 360 bp DNA fragment. The cycling conditions consisted of an initial 5 min denaturation at 95°C followed by 35 cycles of 45 s each at 94°C, 60°C, 72°C, and a single final extension for 5 min at 72°C. PCR products displaying different profiles compared to the wild type were sequenced using the Big Dye Cycle Sequencing Kit with the ABI Prism 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

***PIK3CA* copy number quantification.** To determine the level of *PIK3CA* gene amplification, copy number quantification was carried out using quantitative real-time PCR (iCycler iQ System; Bio-Rad, France) with the *GAPDH* gene (glyceraldehyde-3-phosphate dehydrogenase) as the internal control. The PCR reaction was carried out with the QTM Syber Green Supermix (Bio-Rad) using 50ng of DNA in a 25 μL final reaction volume. The thermal cycling conditions were as follows: 5 min at 95°C and 50 cycles of 15 s at 95°C, 15 s at 58°C, and 15 s at 72°C. The GAPDH primers were: 5′-ACCCACTCCTCCACCTTTG-3′ (forward) and 5′-ACCCACTCCTCCACCTTTG-3′ (reverse) to generate a 188 bp fragment. The *PIK3CA* primers were: 5′AAATGAAAGCTCACTCTGGATT-3′ (forward) and 5′-TGT GCAATTCCTATGCAATCG-3′ (reverse) to generate a fragment of 81 bp. Each sample was run in duplicate for both target and endogenous genes. Each case was repeated twice and the mean value was calculated.

The amount of PIK3CA-specific template was estimated in each carcinoma with respect to the control gene. The relative representation of the PIK3CA copy number (PIK3CA gain) in each tumor with respect to non-tumor tissue is given by the formula $2^{-\Delta\Delta Ct}$, which is a simplified calculation tool derived from the ΔΔCt method for gene expression analysis.[20]

The $\Delta\Delta C_t = (C_{t\ PIK3CA} - C_{t\ GAPDH})_{samples} - (C_{t\ PIK3CA} - C_{t\ GAPDH})_{calibrator}$. Normal nasopharyngeal mucosa (10 cases) were used to normalize the data. The differences between the cycle threshold value (C_t: the cycle at which the fluorescence rises appreciably above the background fluorescence) of each tumor and the mean cycle threshold value of the normal nasopharyngeal mucosa were designated as ΔC_t. PIK3CA copy number gain or amplification ≥3 were considered significant. For statistical evaluation, the cases were sub-divided into three groups with respect to the PIK3CA gene copies number (0–3 no amplification, 3–4 moderate amplification, >4 high amplification).

PAkt protein expression. To evaluate Akt phosphorylation status, we performed western blot analysis on available NPC samples that allowed us to perform protein extraction. Frozen tumors were collected in sodium dodecyl sulfate lysis buffer and proteins were extracted by Trizol Reagent (Invitrogen, France). Approximately 50 μg of total proteins was denatured in loading buffer for 10 min, electrophoresed on 10% SDS-PAGE, and electroblotted to Hybond-P (Amersham Biosciences). The membrane was incubated overnight with primary antibody anti-Akt Thr308 (rabbit polyclonal IgG, 1:200; Santa Cruz Biotechnology, Hildelberg, Germany), then with anti-β-actin (antimouse monoclonal antibody, 1:500; Santa Cruz Biotechnology) at 4°C. The membrane was washed three times in PBS with 0.1% Tween 20 at room temperature and incubated with horseradish peroxidase–labeled secondary antibody (goat antirabbit IgG or goat antimouse IgG; Sigma-Aldrich, France) for 1 h at room temperature. Signal detection was performed by horseradish peroxidase chemiluminescent reaction (ECL plus; Amersham Biosciences).

Statistical analysis. Statistical analyses were performed using SPSS (SPSS, Inc., Chicago, IL, USA) 13.0 statistical software for Windows. The χ^2 test was used to determine associations between the amplification of *PIK3CA* gene and various clinicopathological features of NPC. Analyses of disease free survival (end-point = loco-regional recurrence or distant metastases) and cancer-specific survival (end-point = death from NPC) were performed using Kaplan–Meier survival plots, and the significance was tested using the log-rank test. *P*-values of < 0.05 were considered significant.

Results

Mutations analysis. Because more than 80% of mutations in the *PIK3CA* gene occur in helical (exon 9) and kinase (exon 20) domains, we screened these exons in 88 primary NPC tumors using PCR-SSCP (for exon 9) and PCR-DHPLC (for exon 20). For SSCP analysis, we used nested PCR with a primer design that avoids confounding *PIK3CA* pseudogene sequences because *PIK3CA* shows a high homology (98%) with a genomic fragment in the Cat Eye Syndrome region. As positive control, we used two DNA samples with G1633A (E545K) or G1624A (E542K) mutations. A representative example of PCR-SSCP is shown in Figure 1. No aberrant shifted bands were found in the exon 9 of the 88 NPC specimens tested compared with DNA from non-tumoral nasopharyngeal mucosa used as negative control (Fig. 1).

For exon 20 (360 bp) we used the PCR-DHPLC as prescreening approach for primary NPC specimens using as a control one DNA with a wild-type sequence. Only one synonymous point mutation (C3231T) leading to the replacement of the codon ''cct'' with ''tct'' (both encoding leucin 2710) was found in exon 20 in one NPC case (Fig. 2A,B). No other mutations were detected in exons 9 and 20.

***PIK3CA* gene amplification in NPC.** To determine the *PIK3CA* gene copy number, we performed quantitative PCR on 88 primary tumors, and 10 normal nasopharyngeal mucosa were used as calibrator to normalize the data. Standard curves for *PIK3CA* and GAPDH amplification were generated and showed linearity over the range used. Most samples showed no difference in GAPDH amplification between tumor and normal samples. A representative example of GAPDH and *PIK3CA* amplification in tumor and normal samples is shown in Figure 3.

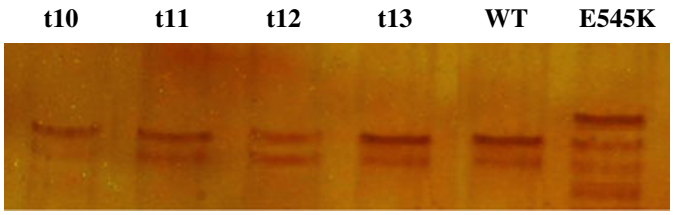

Fig. 1. Single strand conformation performance liquid (SSCP) analysis of PIK3CA exon 9 (wild type [WT]). WT DNA sample from non-tumoral tissues. t10, t11, t12, and t13, DNA from nasopharyngeal carcinoma (NPC) tumors. E545K is a DNA with mutation E>K in position 545 of PIK3CA exon 9.

Among the 88 cases, 19 (21.6%) showed a gain of gene copy number; among them, 16 cases (84%) showed more than four copies and three (16%) had a moderate amplification with three to four copies (Table 1).

Correlation between *PIK3CA* amplification and clinico-pathological parameters. The relationship of *PIK3CA* amplification with clinico-pathologic variables is summarized in Table 2. Overall, tumors with *PIK3CA* amplification show more aggressive behavior. In univariate analysis, *PIK3CA* amplification is significantly higher in tumors with advanced tumor stage (T4, 45% *vs* T1-3, 4%; $P = 0.001$), with lymph node (N+,100% *vs* N0, 0%; $P = 0.032$), and with distant metastasis (M+, 60% *vs* M0, 19.6%; $P = 0.009$). Multivariate analysis using the logistic regression model showed a strong association with tumor stage ($P = 0.003$, odds ratio [OR] = 0.084, 95% confidence interval [CI] = 0.017–0.42) and distant metastasis ($P = 0.009$, OR = 0.147, 95% CI = 0.035–0.617) whereas the association with lymph node involvement was lost.

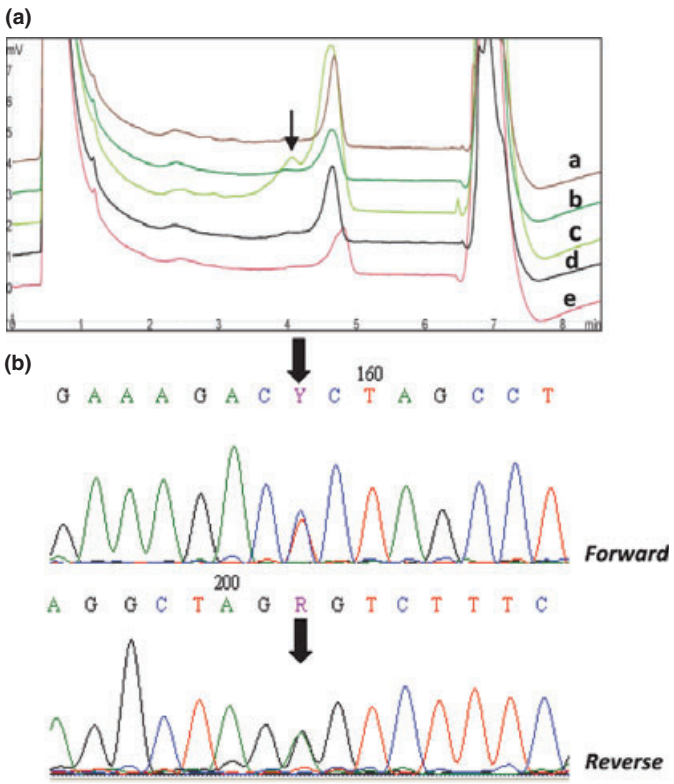

Fig. 2. (a) Denaturating high performance liquid chromatography (DHPLC) profiles of PIK3CA exon 20 (a) wild type DNA sample (parts b, c, d, and e) correspond to DNA from NPC biopsies: note the small peak (shown by the black arrows) at 4.3 min that was not observed in the control sample which indicates the existence of heterozygous change. (b) Sequence analysis of exon 20 (NPC126) showing an additional peak on the DHPLC profile. The arrows indicate the heterozygous mutation Y 3231R.

Relationship of *PIK3CA* amplification and patient survival. Survival data was available for only 53 patients, among them 27 patients died of their disease. The Kaplan–Meier method for overall survival revealed significant association in the survival times of patients with respect to *PIK3CA* amplification (log-rank test, $P = 0.028$, Fig. 4a). Moreover in the group of patients with advanced NPC (TNM stage IV), *PIK3CA* amplification was associated with a poor survival (Fig. 4b) suggesting once again that activation of *PIK3CA* via copy number gain is associated with worse prognosis in NPC. No significant association between *PIK3CA* amplification and disease free survival was noted in our series.

pAkt-protein expression in NPC tissues. To further evaluate the functional significance of the *PIK3CA* gene amplification, we studied the expression of active Akt (pAkt) on 19 available NPC samples, 10 of them without *PIK3CA* amplification and nine with high amplification level (Table 1). We found a perfect correlation between pAkt expression and *PIK3CA* amplification since all tested specimens with more than four copies of the *PIK3CA* gene showed pAkt expression, whereas none of the 10 cases without *PIK3CA* amplification exhibited the activated Akt (Fig. 5).

Furthermore, and as shown in Table 1, all patients with tumors exhibiting *PIK3CA* gene amplification and pAkt expression had lymph nodes and advanced T-stage, and 11 out of 19 patients developed distant metastasis. Our result suggests that *PIK3CA* gene amplification and pAkt expression occur in advanced NPC stage.

Discussion

The PI3K/Akt pathway plays a central role in many physiological processes and is implicated in malignant transformation by promoting cell cycle progression, and invasion.[21] Many studies have already reported *PIK3CA* somatic mutation in a wide variety of human cancers, such as lung, colorectal, ovarian, gastric, and breast cancer.[22–25] Functional significance of these missense mutations was suggested because they are clustered in the helical (exon 9) and kinase domains (exon 20); also the mutant protein showed increased kinase activity[26] and oncogenic potential.[22]

In NPC, there are only a few published reports on *PIK3CA* mutations and whether it affects the clinical behavior and patient prognosis; however, to the best of our knowledge, the effect of *PIK3CA* amplification on patient survival is not documented in NPC. In this study, we confirmed that *PIK3CA* mutations are rare in NPC (1.13%) compared to other cancers such as colon[27] and breast cancer.[28] Our results are concordant with those of two independent studies conducted on Asian patients, showing the absence or the low frequency (9%) of *PIK3CA* mutations in the two hotspot regions.[12,13]

Beside mutation, *PIK3CA* amplification has been reported in many human cancers. In this study, we investigated by quantitative PCR (qPCR) the status of *PIK3CA* in 88 NPC. Nineteen out of 88 cases (21.6%) harbored amplification. A previous study employing array-based comparative genomic hybridization and FISH analysis showed that *PIK3CA* amplification was observed in 24 out of 32 cases (75%). Discrepancies between qPCR and FISH or CGH methods were previously observed in a study assessing the *PIK3CA* gene in ovarian carcinoma.[26] This could be due to the fact that FISH or CGH methods are not able to detect changes restricted only to a small genomic region and the qPCR approach seems to be more appropriate to assess genetic aberrations limited to a single gene.

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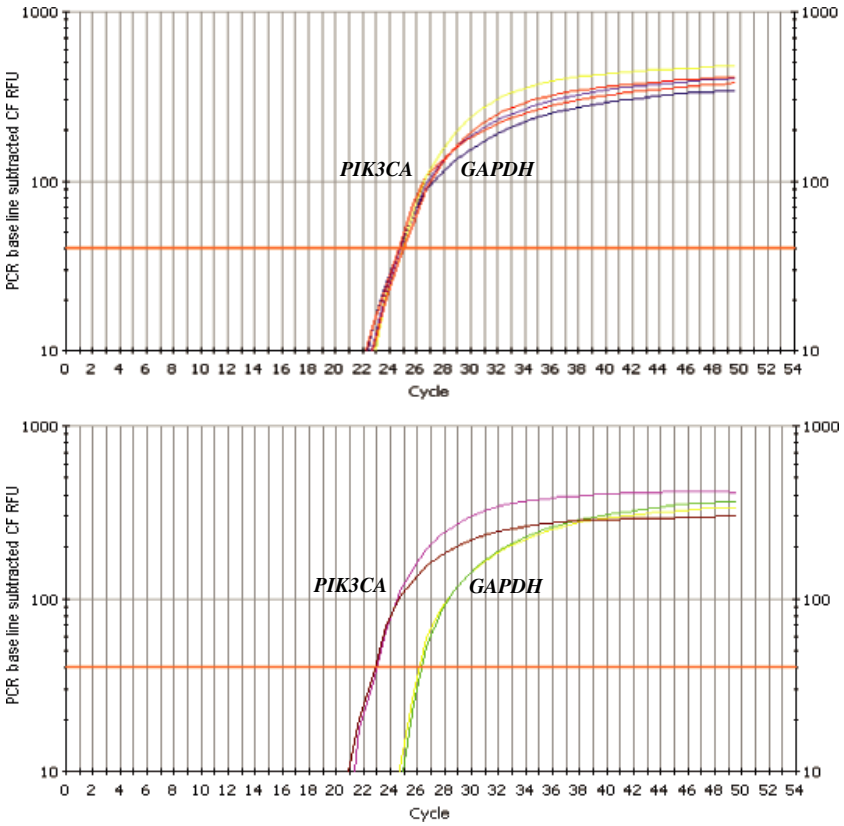

Fig. 3. Determination of *PIK3CA* gene copy number by quantitative PCR. Relative fluorescence of *PIK3CA* and GAPDH reactions are plotted against cycle number. The horizontal line marks the threshold used for assessment of C_t value. Upper, normal nasopharyngeal epithelia used as a negative control. Bottom, example of nasopharyngeal carcinoma (NPC) case showing genomic amplification of the *PIK3CA* gene.

Table 1. Clinical features of nasopharyngeal carcinoma (NPC) patients with *PIK3CA* gene amplification and pAkt expression

Cases	Age (year)	Histology (WHO type)‡	Tumor stage	Lymphe node	Distant metastasis	pAKT expression	PIK3CA copy number	
							3–4	>4
T1†	64	Non-keratinizing carcinoma	T3	N+	M1	+	ND	+
T2	17	Non-keratinizing carcinoma	T4	N+	M1	ND	+	ND
T3	41	Undifferentiated (UCNT)	T4	N+	M0	ND	ND	+
T4	46	Undifferentiated (UCNT)	T4	N+	M1	ND	ND	+
T5	28	Undifferentiated (UCNT)	T4	N+	M1	ND	ND	+
T6	20	Non-keratinizing carcinoma	T4	N+	M1	ND	+	ND
T7†	13	Non-keratinizing carcinoma	T4	N+	M1	+	ND	+
T8†	64	Non-keratinizing carcinoma	T4	N+	M0	+	ND	+
T9	77	Undifferentiated (UCNT)	T4	N+	M1	ND	ND	+
T10†	74	Undifferentiated (UCNT)	T4	N+	M0	+	ND	+
T11	64	Non-keratinizing carcinoma	T4	N+	M0	ND	+	ND
T12†	66	Undifferentiated (UCNT)	T4	N+	M0	+	ND	+
T13†	40	Non-keratinizing carcinoma	T4	N+	M1	+	ND	+
T14†	54	Non-keratinizing carcinoma	T4	N+	M0	+	ND	+
T15†	40	Non-keratinizing carcinoma	T4	N+	M0	+	ND	+
T16	48	Undifferentiated (UCNT)	T4	N+	M0	ND	ND	+
T17†	43	Non-keratinizing carcinoma	T3	N+	M1	+	ND	+
T18	55	Non-keratinizing carcinoma	T4	N+	M1	ND	ND	+
T19	42	Non-keratinizing carcinoma	T4	N+	M1	ND	ND	+

†The nine cases tested for pAkt expression. ‡American Joint Committee on Cancer (AJCC, 5th edition), N+, lymph node involvement. Metastatic status defined as M0 in the absence of clinical or radiological evidence of distant metastasis at the initial workup, M1 in the other cases (synchronous metastasis).

In line with our result, recent studies based on qPCR detected *PIK3CA* amplification in 24.6% and 22% of ovarian cancers.[26,29] Moreover, it was reported in thyroid tumors that *PIK3CA* gene mutation is not a common mechanism in the activation of *PIK3CA*, whereas gene amplification occurs with a relatively high frequency. This suggests that the genetic alteration may play an important role in the tumorigenesis of thyroid cancer.[30]

Table 2. Correlation between *PIK3CA* amplification and clinical features

	Number	PIK3CA amplification (%)
Cases	88	19 (21.6)
Age (years)		
≤30	23	4 (21)
>30	65	15 (79)
Histology (WHO type)†		
Undifferentiated (UCNT)	41	7 (37)
Non-keratinizing carcinoma	47	12 (63)
Tumor stage		
T1–3	48	2 (11)
T4	40	17 (89)
P-value (χ^2 test)		0.001
Lymph node		
N0	15	0 (0)
N+	73	19 (100)
P-value (χ^2 test)		0.032
Metastasis		
M0	72	8 (42)
M1	16	11 (58)
P-value (χ^2 test)		0.002
Clinical stage		
Stage I–III	18	0 (0)
Stage IV	70	19 (100)
P-value (χ^2 test)		0.013

†American Joint Committee on Cancer (AJCC, 5th edition), N+, lymph node involvement. Metastatic status defined as M0 in the absence of clinical or radiological evidence of distant metastasis at the initial workup, M1 in the other cases (synchronous metastasis). WHO, World Health Organization.

In the current study, the univariate and multivariate analysis revealed a significant association between *PIK3CA* amplification and tumors with aggressive behavior, i.e. advanced tumor stage and presence of distant metastasis. All cases with stage IV showed *PIK3CA* amplification compared to specimens in stage I–III. Recently, Chou *et al.* reported that *PIK3CA* mutation was slightly influenced by sex but had no significant relationship to other clinicopathological characteristics in NPC.[13]

On the other hand, little has been known regarding the impact of *PIK3CA* mutation in NPC on patient survival. One recent study has shown that disease-specific survival was not significantly affected by *PIK3CA* mutations.[13] However, in colon cancer, *PIK3CA* mutations were associated with a significant increase in colon cancer–specific mortality.[27,31]

To the best of our knowledge, this is the first study demonstrating the prognosis value of *PIK3CA* amplification in NPC. We showed that *PIK3CA* amplification is associated with a poor overall survival (P log rank = 0.02). In addition, patients with TNM stage IV have a worse survival in a background where *PIK3CA* is amplified than those in the same stage without *PIK3CA* amplification. This suggests once again that activation of *PIK3CA* is a marker of poor prognosis in NPC. Further, to investigate the functional implication of *PIK3CA* amplification, we evaluated the expression of active Akt (pAkt) on 19 available NPC samples. The pAkt was observed in all tumors carrying *PIK3CA* amplification (nine out of nine) while pAkt was not detected in tumors without *PIK3CA* amplification. This result is in line with previous reports and confirms the importance of the protein kinase Akt as a downstream effector of *PIK3CA* in NPC. In lung cancer, it was shown that *PIK3CA* mutations and gains were strongly correlated with expression of activated pAkt.[32]

In summary, our study shows that *PIK3CA* amplification is associated with aggressive tumor behavior and poor survival in patients with nasopharyngeal carcinoma. We also confirm that

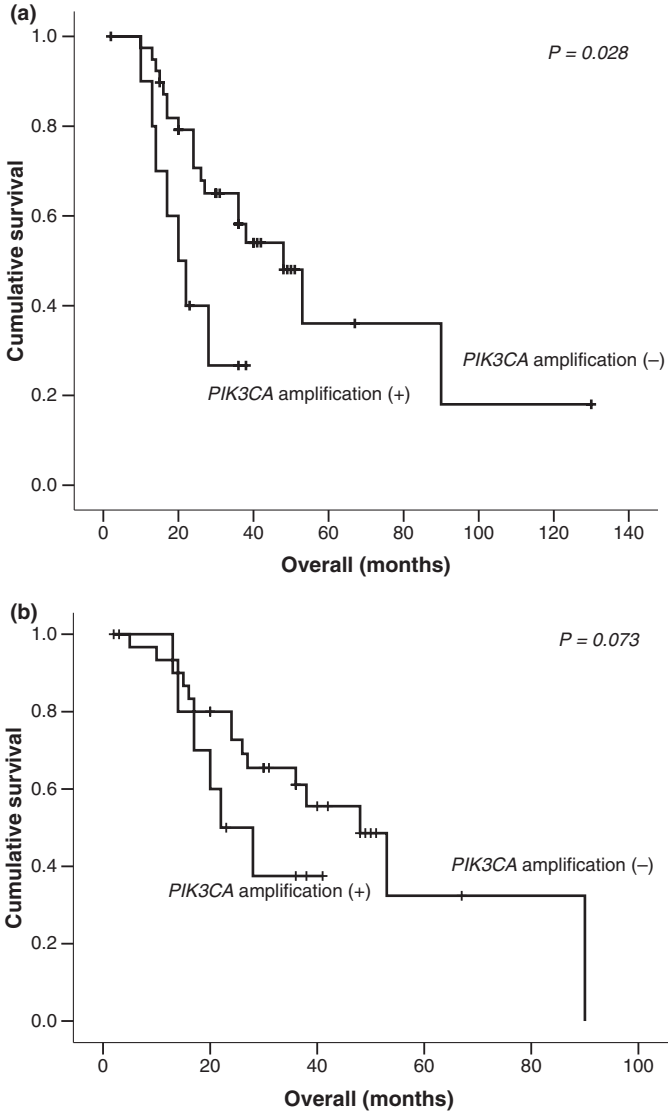

Fig. 4. Kaplan–Meier curves for (a) overall survival and (b) overall survival in patients with stage IV nasopharyngeal carcinoma (NPC) according to *PIK3CA* amplification status.

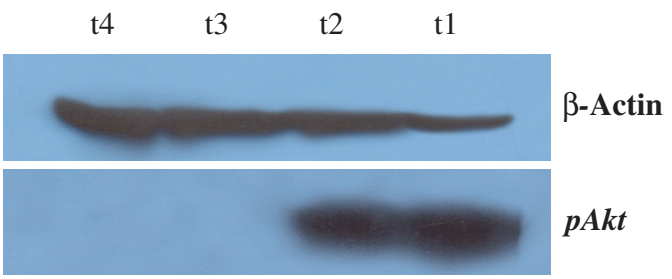

Fig. 5. Western blot analysis of Akt phosphorylation in nasopharyngeal carcinoma (NPC) samples. Anti-pAkt was used as a primary antibody to visualize pAkt (52 kDa), and β-actin levels were examined to ensure the integrity of the protein preparations. Note that t1 and t2 samples harbor more than four copies of the *PIK3CA* gene, while t3 and t4 do not carry *PIK3CA* amplification as determined by quantitative PCR.

mutations in exons 9 and 20 of the *PIK3CA* gene are not a common event in NPC as in other epithelial cancers. Our findings suggest the implication of *PIK3CA* as an oncogene with a

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prognostic relevance in NPC. Specific inhibitors of the PI3K/Akt pathway could be a promising target for the treatment of patients with NPC.

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Disclosure Statement

The authors guarantee that there are no financial or personal relationships with other people or organizations that might pose a conflict of interest in connection with this work.

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