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PIP5K3 upregulation correlates with unfavorable prognosis in patients with nasopharyngeal carcinoma

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Summary. Background. Nasopharyngeal carcinoma (NPC) is prevalent in East and Southeast Asia, with metastasis and recurrence leading to poor prognosis. Identifying prognostic biomarkers is essential.

Methods. We analyzed differentially expressed genes involved in the phosphatidylinositol metabolic process (GO: 0046488) and tumorigenesis (GSE12452) in NPC. Associations between PIP5K3 expression and clinicopathological features were assessed using chi-square tests and Cox proportional hazards models.

Results. PIP5K3 was significantly upregulated in NPC tissues, correlating with advanced stage (p=0.001) and nodal metastasis (p<0.001). High PIP5K3 expression was associated with worse disease-specific survival (DSS), distant metastasis-free survival (DMeFS), and local recurrence-free survival (LRFS). Multivariate analysis confirmed its association with poor prognosis (DSS: HR=4.321, DMeFS: HR=2.883, LRFS: HR=2.249; all p<0.001).

Conclusions. PIP5K3 upregulation is associated with unfavorable clinical outcomes in NPC and may serve as a novel prognostic biomarker and potential therapeutic target.

Key words: Nasopharyngeal carcinoma, PIP5K3, Phosphatidylinositol metabolism-related genes

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Introduction

NPC arises from the epithelial cells of the nasopharynx and represents the most prevalent malignancy in this anatomical region (Shah and Nagalli, 2024). It is distinct from other head and neck cancers due to its unique geographic and epidemiologic distribution, with particularly high incidence rates in East and Southeast Asia, southern China, and North Africa (Chen et al., 2019). This distribution pattern suggests a multifactorial etiology involving genetic susceptibility, dietary exposure to nitrosamine-rich foods, and Epstein-Barr virus (EBV) infection (Stan et al., 2022). The World Health Organization (WHO) classifies NPC into three histological subtypes based on cellular differentiation: type 1 (keratinizing squamous cell carcinoma), type 2 (differentiated nonkeratinizing carcinoma, and type 3 (undifferentiated nonkeratinizing carcinoma), with type 3 being the most common (Chua et al., 2016; Sinha et al., 2024). The disease stage guides treatment strategies and may involve radiotherapy, chemotherapy, targeted therapy, immunotherapy, or a combination of these therapies. Due to the anatomical location and radiosensitive nature of NPC, radiotherapy is the standard treatment for early-stage disease, whereas advanced stages typically require a combined modality approach (Feng et al., 2021; Wong et al., 2021; Liu et al., 2024). Early-stage NPC is associated with a favorable prognosis, with five-year overall survival rates approaching 95%. In contrast, outcomes for patients with locally advanced or metastatic NPC remain suboptimal. Metastasis and recurrence, either during or after treatment, are significant challenges that adversely impact the long-term prognosis (Perri et al., 2019; Wong

et al., 2021). Consequently, effective local and regional tumor control is crucial, as recurrence often precedes distant metastatic spread (Kieser and Sterz, 2015; Stan et al., 2022). In this context, identifying novel biomarkers is crucial for enhancing diagnostic precision and informing therapeutic decision-making for recurrent or metastatic NPC.

Cancer cell migration and invasion are fundamental biological processes underlying metastasis (Wu et al., 2021). These processes are mediated by a coordinated interplay of molecular components, including adhesion molecules, receptor tyrosine kinases, cytoskeletal proteins, and intracellular signaling proteins (Bozzuto et al., 2010). Among the various signaling pathways, the phosphatidylinositol metabolic pathway has been implicated in cancer progression by enhancing cell motility and invasion. In humans, the phosphatidylinositol 3-phosphate/phosphatidylinositol 5-kinase, type III (PIP5K3) gene encodes phosphatidylinositol 3-phosphate 5-kinase, type III, a protein involved in calcium-mediated signaling, intracellular signaling cascades, phosphatidylinositol metabolic processes, and protein folding (Shisheva, 2008; gen6.genecards.org). Accumulating evidence has linked PIP5Ks to the development and progression of several cancers, including breast, ovarian, and prostate cancers (Semenas et al., 2014; Chen et al., 2015; Cao et al., 2017; Tran et al., 2018; Wang et al., 2022). Additionally, PIP5K3 has been proposed as a potential therapeutic target in several tumor types, including B-cell non-Hodgkin lymphoma, multiple myeloma, prostate cancer, and non-small cell lung cancer, due to its involvement in modulating tumor invasiveness (Baird et al., 2013; Gayle et al., 2017; de Campos et al., 2020; Qiao et al., 2021).

Despite these advances, the role of PIP5K3 in NPC remains largely unexplored. To date, there is limited evidence regarding its biological relevance in this malignancy. Addressing this gap, the present study investigates the prognostic value of PIP5K3 expression in NPC. Specifically, the study evaluates the association between PIP5K3 expression levels and clinical outcomes, aiming to establish its potential as a prognostic biomarker and to further elucidate its role in NPC pathogenesis.

Materials and methods

Analysis of expression patterns from a publicly accessible NPC transcriptome dataset

The transcriptome dataset GSE12452 was obtained and analyzed, focusing on mRNA expression data from the Gene Expression Omnibus database (National Center for Biotechnology Information, Bethesda, MD). This dataset comprised 31 NPC tissue samples and 10 non-neoplastic nasopharyngeal mucosal epithelial tissue samples. Gene expression levels were determined using the original files on the Affymetrix Human Genome U133 Plus 2.0 Array platform, analyzed via Nexus Expression 3 software (BioDiscovery, USA), without prior selection or filtering of probe combinations. To identify relevant targets, a comprehensive comparative analysis of gene expression was conducted, with emphasis on genes involved in the phosphatidylinositol metabolic process (GO:0046488). Genes exhibiting significant differential expression ($\log_2$ ratio >2, $p<0.01$) were prioritized for further investigation.

Patients and tumor specimens

For this study, paraffin-embedded tissue blocks were obtained from 520 patients diagnosed with NPC at Chi Mei Medical Center between January 1993 and December 2020. The study protocol was approved by the Institutional Review Board of Chi Mei Medical Center (IRB10302013). Patients with metastatic disease were excluded from the cohort. Two pathologists reassessed the histological subtypes according to the current WHO classification system. Tumor staging was determined according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system. The expression of EBV-encoded small RNA (EBER), which is indicative of EBV infection, was detected by *in situ* hybridization (ISH) as reported in our previous study (Lee et al., 2015).

Immunohistochemical (IHC) staining and assessment of PIP5K3 expression

Tissue samples from NPC cases were initially diagnosed through histopathology and preserved using formalin fixation and paraffin embedding, followed by IHC staining. Sections of 4 μm thickness were cut and placed on adhesive slides (SuperFrost Plus, Thermo Scientific). They were then baked at 60°C for 50 minutes to remove paraffin and subsequently cleaned in xylene. We hydrated the sections by dipping them in 100% ethanol and xylene, followed by a wash in distilled water. A mixture of 95% ethanol and 0.3% hydrogen peroxide was applied for five minutes to block endogenous peroxidase activity, followed by rinsing in distilled water.

Antigen retrieval was performed by boiling the slides in citrate buffer (pH 6.0) at 125°C for four minutes. After immersion in distilled water, sections were treated with Tris-buffered saline (TBS, Dako, Denmark) for three minutes, then washed. Primary antibodies (anti-phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase, type III [PIP5K3]; polyclonal, Novus Biologicals; dilution 1:100) were applied, followed by incubation with a secondary horseradish peroxidase (HRP) polymer reagent at room temperature for 30 minutes. After additional washing, chromogen development was performed using diaminobenzidine at room temperature for 10 minutes. Finally, slides were counterstained with Mayer hematoxylin (Histolab), mounted with Pertex (Histolab), and prepared for analysis and interpretation. Two

pathologists, who were blinded to the clinical data, evaluated the immunostaining. The staining of PIP5K3 was scored using the H-score method. It was calculated with the following equation: H score=$\sum Pi$ (i+1), where i is the intensity of immunostaining (ranging from 0 to 4), and Pi is the percentage of stained tumor cells of intensity, varying from 0 to 100%. High expression of PIP5K3 was defined as an H-score greater than the median value.

Statistical analysis

The chi-square test was used to evaluate the statistical significance of the relationship between clinicopathological characteristics and PIP5K3 expression. Data analysis was performed using SPSS software (version 14), focusing on three primary outcomes: disease-specific survival (DSS), distant metastasis-free survival (DMeFS), and local recurrence-free survival (LRFS). These outcomes were measured from the date of radiotherapy initiation to the occurrence of the respective event. The median follow-up duration was 66.5 months (mean, 72.2 months; range, 3-186 months). For multivariate analysis, the Cox proportional hazards model was applied. All statistical tests were two-sided, and a p-value of less than 0.05 was considered statistically significant.

Ethics statement

All participants provided informed consent before enrolling in the biobank. The study, including the use of de-identified NPC tissues from the biobank, was approved by the Ethics Committee and Institutional Review Board of Chi Mei Medical Center (IRB10302013). It adhered to the ethical guidelines of the Declaration of Helsinki and relevant government regulations.

Results

PIP5K3 shows significant upregulation among phosphatidylinositol metabolism-related genes in NPC

The GSE12452 dataset from the Gene Expression Omnibus database was analyzed to identify differentially expressed genes involved in the phosphatidylinositol metabolic process. This analysis aimed to reveal potential metabolic genes associated with tumor development in NPC. Transcriptome analysis showed significant differential expression of several phosphatidylinositol metabolism-related genes in NPC tissues (n=31) compared with non-NPC tissues (n=10) (Fig. 1). Among these, PIP5K3 exhibited the highest fold change between tumor and non-tumor tissues (log2 ratio=1.1922; p<0.001; Table 1). Based on these bioinformatic results, we further examined the relationship between PIP5K3 expression, clinicopathological parameters, and prognosis in NPC.

Association between PIP5K3 expression and clinicopathological parameters in NPC

A total of 520 patients with histologically confirmed NPC were included in this study to evaluate the relationship between PIP5K3 expression and clinicopathological features. The cohort consisted of 111 patients aged 60 years or older and 409 patients younger than 60 years, including 385 males and 135 females. Immunohistochemical staining revealed minimal PIP5K3 expression in non-neoplastic nasopharyngeal epithelium (Fig. 2A). In contrast, tumors with low PIP5K3 expression (Fig. 2B) were generally associated with earlier clinical stages, while those with high PIP5K3 expression (Fig. 2C) tended to present at more advanced stages. High PIP5K3 expression was significantly associated with higher nodal (N) status (p<0.001) and advanced AJCC stage (p=0.001). However, no significant association was observed between PIP5K3 expression and patient sex, age, primary tumor (T) stage, histological grade, or EBER expression (Table 2).

High PIP5K3 expression is associated with poor prognosis in NPC

Survival analysis demonstrated a significant association between PIP5K3 expression and clinical outcomes. In univariate analysis (Table 3), improved DSS was associated with early T stage (p<0.001), lower

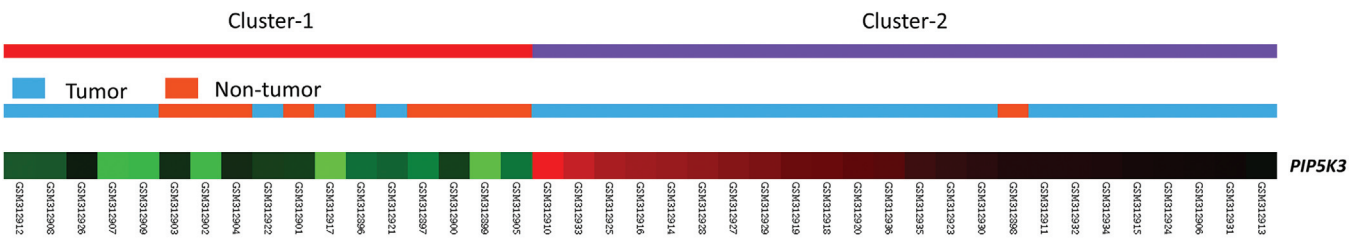

Fig. 1. Heatmap illustrating differentially expressed genes between NPC patients and non-NPC tissues derived from the GSE12452 dataset. The analysis focuses on genes related to phosphatidylinositol metabolism (GO: 0046488). The green coloration indicates genes with reduced expression levels, whereas red signifies genes with elevated expression. NPC: nasopharyngeal carcinoma. GO: Gene Ontology.

nodal stage (p<0.001), earlier AJCC stage (p<0.001), lower histological grade (p=0.017), negative EBER status (p=0.044), and lower PIP5K3 expression (p<0.001). Similarly, better DMeFS correlated with early T stage (p<0.001), lower nodal stage (p=0.004), earlier AJCC stage (p<0.001), and lower PIP5K3 expression (p<0.001). Improved LRFS was also significantly associated with T stage (p<0.001), earlier AJCC stage (p<0.001), and reduced PIP5K3 expression (p<0.001). In multivariate survival analysis (Table 4), advanced AJCC stage (III-IV) was identified as a strong independent predictor of poor outcomes across all survival metrics: DSS (hazard ratio [HR]=2.219, 95% confidence interval [CI]=1.360-3.621, p=0.001), DMeFS (HR=2.607, 95% CI=1.538-4.418, p<0.001), and LRFS (HR=2.314, 95% CI=1.309-4.089, p=0.004). Notably, high PIP5K3

Table 1. Summary of gene expression alterations related to phosphatidylinositol metabolic process (GO: 0046488) and associated with tumorigenesis in the transcriptome of nasopharyngeal carcinoma (GSE12452).

Probe	Tumor to non-tumor comparison		Gene Symbol	Gene Name	Biological Process
	Comparison log ratio	Comparison *p*-value			
213111_at	1.1922	<0.001	*PIP5K3*	Phosphatidylinositol-3-phosphate/ phosphatidylinositol 5-kinase; type III	Calcium-mediated signaling, cellular protein metabolic process, intracellular signaling cascade, phosphatidylinositol metabolic process, protein folding
1553047_at	-0.1715	0.157	*PIP4K2B*	Phosphatidylinositol-5-phosphate 4-kinase; type II; beta	Cell surface receptor-linked signal transduction, phosphatidylinositol metabolic process
1553048_a_at	-0.06	0.624	*PIP4K2B*	Phosphatidylinositol-5-phosphate 4-kinase; type II; beta	Cell surface receptor-linked signal transduction, phosphatidylinositol metabolic process
1553917_at	0.0046	0.962	*PIP5K3*	Phosphatidylinositol-3-phosphate/ phosphatidylinositol 5-kinase; type III	Calcium-mediated signaling, cellular protein metabolic process, intracellular signaling cascade, phosphatidylinositol metabolic process, protein folding
1557719_at	0.0031	0.976	*PIP5K3*	Phosphatidylinositol-3-phosphate/ phosphatidylinositol 5-kinase; type III	Calcium-mediated signaling, cellular protein metabolic process, intracellular signaling cascade, phosphatidylinositol metabolic process, protein folding
201080_at	0.1314	0.734	*PIP4K2B*	Phosphatidylinositol-5-phosphate 4-kinase; type II; beta	Cell surface receptor-linked signal transduction, phosphatidylinositol metabolic process
201081_s_at	0.0081	0.905	*PIP4K2B*	Phosphatidylinositol-5-phosphate 4-kinase; type II; beta	Cell surface receptor-linked signal transduction, phosphatidylinositol metabolic process
205570_at	0.0128	0.902	*PIP4K2A*	Phosphatidylinositol-5-phosphate 4-kinase; type II; alpha	Glycerophospholipid metabolic process, phosphatidylinositol metabolic process, phosphorylation
205632_s_at	-0.3003	0.385	*PIP5K1B*	Phosphatidylinositol-4-phosphate 5-kinase; type I; beta	Phosphatidylinositol metabolic process, phosphorylation
207391_s_at	-0.0444	0.893	*PIP5K1A*	Phosphatidylinositol-4-phosphate 5-kinase; type I; alpha	Glycerophospholipid metabolic process, phosphatidylinositol metabolic process, phosphorylation, signal transduction
210256_s_at	0.0937	0.228	*PIP5K1A*	Phosphatidylinositol-4-phosphate 5-kinase; type I; alpha	Glycerophospholipid metabolic process, phosphatidylinositol metabolic process, phosphorylation, signal transduction
211205_x_at	0.0584	0.469	*PIP5K1A*	Phosphatidylinositol-4-phosphate 5-kinase; type I; alpha	Glycerophospholipid metabolic process, phosphatidylinositol metabolic process, phosphorylation, signal transduction
212518_at	0.0997	0.498	*PIP5K1C*	Phosphatidylinositol-4-phosphate 5-kinase; type I; gamma	Axonogenesis, phosphatidylinositol metabolic process
212829_at	-0.4204	0.067	*PIP4K2A*	Phosphatidylinositol-5-phosphate 4-kinase; type II; alpha	Glycerophospholipid metabolic process, phosphatidylinositol metabolic process, phosphorylation
217477_at	0.0337	0.822	*PIP5K1B*	Phosphatidylinositol-4-phosphate 5-kinase; type I; beta	Phosphatidylinositol metabolic process, phosphorylation
218942_at	-0.2443	0.394	*PIP4K2C*	Phosphatidylinositol-5-phosphate 4-kinase; type II; gamma	Phosphatidylinositol metabolic process
231179_at	-0.079	0.519	*IHPK3*	Inositol hexaphosphate kinase 3	DNA repair, apoptosis, calcium-mediated signaling, phosphatidylinositol metabolic process, positive regulation of DNA recombination, protein amino acid phosphorylation, vesicle-mediated transport
243116_at	-0.0603	0.470	*PIP5KL1*	Phosphatidylinositol-4-phosphate 5-kinase-like 1	Phosphatidylinositol metabolic process

expression emerged as a significant predictor of unfavorable outcomes, including poorer DSS (HR=4.321, 95% CI=2.829-6.599, $p<0.001$, Fig. 3A), reduced DMeFS (HR=2.883, 95% CI=1.943-4.278, $p<0.001$, Fig. 3B), and shortened LRFS (HR=2.249, 95% CI=1.473-3.436, $p<0.001$, Fig. 3C). These findings

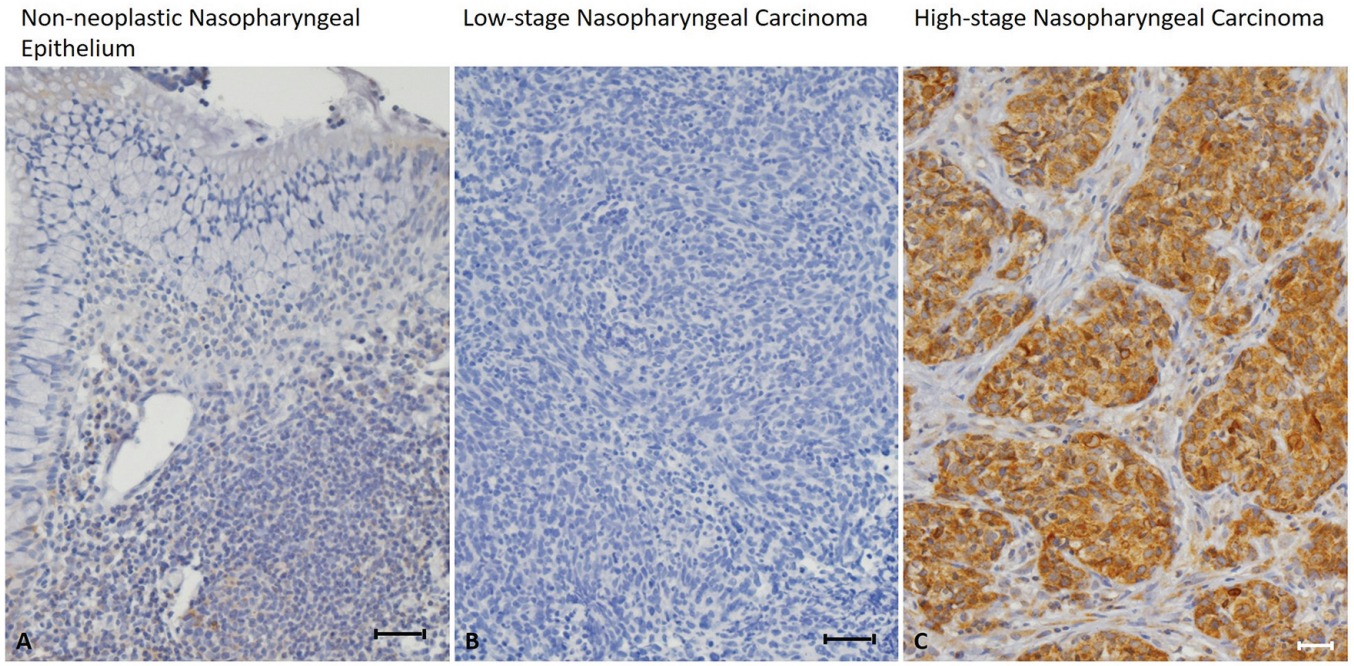

Fig. 2. Immunohistochemical staining of PIP5K3: minimal PIP5K3 expression **(A)**, low PIP5K3 expression **(B)**, and high PIP5K3 expression **(C)**. PIP5K3=phosphatidylinositol 3-phosphate/phosphatidylinositol 5-kinase, type III. Scale bars: 500 μm.

Table 2. Associations between PIP5K3 expression and other important clinicopathological variables.

Parameters	Category/case number (%)		PIP5K3 expression		*p*-value
			Low (%)	High (%)	
Gender	Male	385 (74)	191 (50)	194 (50)	0.764
	Female	135 (26)	69 (51)	66 (49)	
Age (years)	<60 years	409 (79)	203 (50)	206 (50)	0.748
	≥60 years	111 (21)	57 (51)	54 (49)	
Primary tumor (T)	T1	156 (30)	78 (50)	78 (50)	0.996
	T2	145 (28)	72 (50)	73 (50)	
	T3	76 (15)	39 (51)	37 (49)	
	T4	143 (28)	71 (50)	72 (50)	
Nodal status (N)	N0	80 (15)	58 (72)	22 (28)	**<0.001***
	N1	174 (33)	118 (68)	56 (32)	
	N2	223 (43)	73 (33)	150 (67)	
	N3	43 (8)	11 (26)	32 (74)	
Stage	I	34 (7)	16 (47)	18 (53)	**0.001***
	II	106 (20)	71 (67)	35 (33)	
	III	183 (35)	85 (46)	98 (54)	
	IV	197 (38)	88 (45)	109 (55)	
Histological grade	Keratinizing	9 (2)	4 (44)	5 (56)	0.935
	Non-keratinizing	65 (12)	32 (49)	33 (51)	
	Undifferentiated	446 (86)	224 (50)	222 (50)	
EBER	Negative	4 (1)	2 (50)	2 (50)	>0.950
	Positive	516 (99)	258 (50)	258 (50)	

*Statistically significant.

suggest that PIP5K3 expression levels may serve as a valuable biomarker for risk stratification and prognostic assessment in NPC.

Discussion

This study is the first to demonstrate that high PIP5K3 expression is significantly associated with adverse clinical outcomes in NPC. Utilizing transcriptomic analysis and immunohistochemical validation within a substantial clinical cohort, our findings indicate that PIP5K3 is markedly upregulated in NPC tissues relative to non-tumorous controls. Furthermore, its overexpression is associated with advanced nodal stage,

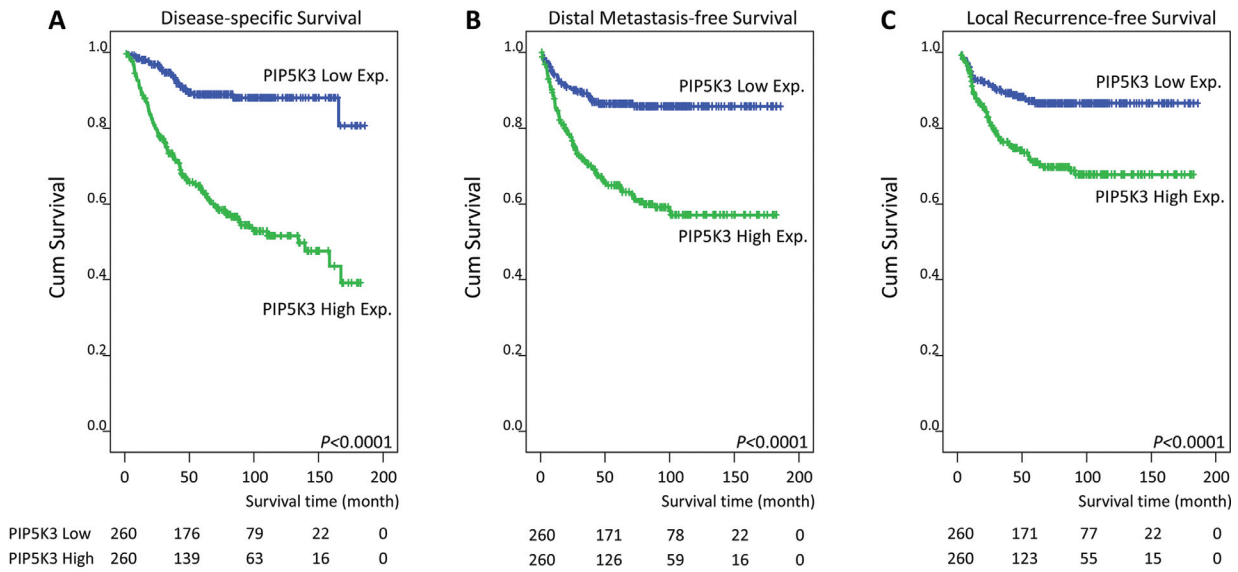

Fig. 3. The Kaplan–Meier Plotter analysis illustrates the association between PIP5K3 expression levels and survival outcomes in patients with NPC. High expressions of PIP5K3 are significantly associated with poorer survival rates, including disease-specific survival (DSS) **(A)** (p<0.001), distal metastasis-free survival (DMeFS) **(B)** (p<0.001), and local recurrence-free survival (LRFS) **(C)** (p<0.001). NPC=nasopharyngeal carcinoma. PIP5K3=phosphatidylinositol 3-phosphate/phosphatidylinositol 5-kinase, type III.

Table 3. Univariate log-rank analysis.

Parameters	Category	No. of case	DSS		DMeFS		LRFS	
			No. of events (%)	*p*-value	No. of events (%)	*p*-value	No. of events (%)	*p*-value
Gender	Male	385	111 (29)	0.387	97 (25)	0.876	74 (19)	0.722
	Female	135	27 (20)		31 (23)		26 (19)	
Age (years)	<60 years	409	114 (28)	0.1664	107 (26)	0.126	81 (20)	0.374
	≥60 years	111	24 (22)		21 (19)		19 (17)	
Primary tumor (T)	T1-T2	301	60 (20)	**<0.001***	49 (16)	**<0.001***	44 (15)	**<0.001***
	T3-T4	219	78 (36)		79 (36)		56 (26)	
Nodal status (N)	N0-N1	254	49 (19)	**<0.001***	50 (20)	**0.004***	42 (17)	**0.076**
	N2-N3	266	89 (33)		78 (29)		58 (22)	
Stage	I-II	140	19 (14)	**<0.001***	16 (11)	**<0.001***	14 (10)	**<0.001***
	III-IV	380	119 (31)		112 (29)		86 (23)	
Histological grade	Keratinizing/non-keratinizing	74	32 (43)	**0.017***	19 (26)	0.776	18 (24)	0.466
	Undifferentiated	445	106 (24)		109 (24)		81 (18)	
EBER	Negative	4	3 (75)	**0.044***	1 (25)	0.967	4 (100)	0.388
	Positive	516	135 (26)		127 (25)		416 (81)	
PIP5K3 expression	Low (H-score<median)	260	27 (10)	**<0.001***	34 (13)	**<0.001***	32 (12)	**<0.001***
	High (H-score>=median)	260	111 (43)		94 (36)		68 (26)	

*Statistically significant; DSS, disease-specific survival; DMeFS, distal metastasis-free survival; LRFS, local recurrence-free survival.

higher AJCC stage, and diminished survival outcomes, including DSS, DMeFS, and LRFS. These results identify PIP5K3 as a potential independent prognostic biomarker in NPC. Importantly, multivariate Cox regression analysis within our cohort verified that high PIP5K3 expression independently predicts poor prognosis across all primary outcome measures, regardless of tumor stage. This underscores its clinical significance and potential application in risk stratification beyond conventional staging parameters.

Phosphoinositides, although constituting a minor component of membrane lipids, have garnered increasing attention due to their crucial role in a wide array of biological processes and the pathogenesis of various diseases (Jin and Xue, 2023). Phosphoinositide kinases are emerging as potential therapeutic targets for a wide range of human diseases, including cancer, diabetes mellitus, viral infections, neurodegenerative diseases, and inflammatory disorders (Burke et al., 2023). These kinases are generally classified into three primary families: the PIP kinase superfamily (including PIP5K3, PIP4Kα/β/γ, and PIP5Kα/β/γ), the PI3K superfamily (encompassing all classes of PI3Ks), and type II PI4Ks (PI4K2A/B) (Burke et al., 2023). Among these, PIP5K3, also known as PIKfyve, was first described in 1999 and belongs to an evolutionarily conserved enzyme family that generates phosphatidyl-inositol 3,5-bisphosphate (PtdIns(3,5)P2) (Sbrissa et al., 1999; Shisheva, 2008). Functioning as both a lipid and protein kinase, PIP5K3 regulates multiple cellular activities, including nuclear transport, cytoskeletal dynamics, stress-induced signaling pathways, membrane trafficking, gene transcription, and cell cycle regulation (Shisheva, 2008). In cancer biology, PIP5K3 has been implicated in tumorigenesis and metastasis; its knockdown leads to a substantial reduction in cancer cell migration (Ikonomov et al., 2013; Oppelt et al., 2013, 2014). Our subsequent immunohistochemical analysis of patient samples further corroborated this finding, demonstrating a significant association between elevated PIP5K3 expression and advanced nodal status, as well as later AJCC stages. These clinical associations strongly support our initial gene expression data and reinforce the potential oncogenic role of PIP5K3 in the progression of NPC.

The observed association between high PIP5K3 expression and unfavorable survival outcomes in NPC aligns with its reported involvement in the progression of various malignancies, including breast, ovarian, prostate cancers, and head and neck squamous cell carcinoma (Stransky et al., 2011; Semenas et al., 2014; Chen et al., 2015; Burke, 2018; Tran et al., 2018; Wang et al., 2022, Jin and Xue, 2023; Tortorella et al., 2023). Mechanistically, PIP5K3 contributes to the synthesis of phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2), a pivotal element of the PI3K/AKT signaling pathway, which is frequently dysregulated in oncogenesis and is recognized for its role in promoting cellular survival and proliferation (Duewell et al., 2024). Moreover, PIP5K3 has been implicated in autophagy (Vicinanza et al., 2015), a process with a complex role in cancer, capable of both inhibiting tumor growth and enhancing cancer cell survival under stress conditions (Yang et al., 2011). Modulating PIP5K3 activity may influence autophagy, thus impacting cancer cell viability and resistance to therapy (Vicinanza et al., 2015; Sharma et al., 2019). Significantly, our multivariate analysis identified high PIP5K3 expression as an independent prognostic indicator of DSS, DMeFS, and LRFS, irrespective of the AJCC stage, thereby emphasizing its substantial prognostic relevance in NPC patients.

The identification of PIP5K3 as a critical factor in NPC prognosis also suggests its potential as a therapeutic target. For instance, research has identified PIP5K3 as a potential therapeutic target in B-cell non-Hodgkin lymphoma (B-NHL). Apilimod, which selectively targets PIP5K3, induces cytotoxicity in B-NHL by disrupting lysosomal function and demonstrates broad anticancer activity both *in vitro* and *in vivo* across all B-NHL subtypes (Gayle et al., 2017). More recently, preclinical studies in pancreatic ductal adenocarcinoma have shown that PIP5K3 inhibition via apilimod can

Table 4. Multivariate survival analysis.

Parameter	Category	DSS			DMeFS			LRFS		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Stage	I-II	1		0.001*	1	-	<0.001*	1		**0.004***
	III-IV	2.219	1.360-3.621		2.607	1.538-4.418		2.314	1.309-4.089	
PIP5K3 expression	Low Exp.	1	-	**<0.001***	1	-	<0.001*	1	-	**<0.001***
	High Exp.	4.321	2.829-6.599		2.883	1.942-4.278		2.249	1.473-3.436	
Histological grade	Keratinizing/non-keratinizing	1	-	0.019*	-	-	-	-	-	-
	Undifferentiated	0.610	0.404 -0.922		-	-	-	-	-	-
EBER	Negative	1	-	0.768	-	-	-	-	-	-
	Positive	0.835	0.252-2.766		-	-	-	-	-	-

*Statistically significant; DSS, disease-specific survival; DMeFS, distal metastasis-free survival; LRFS, local recurrence-free survival; HR, hazard ratio; CI, confidence interval.

robustly suppress autophagic flux, impair lysosomal degradation, and inhibit tumor growth with greater potency than traditional autophagy inhibitors. These findings, based primarily on *in vitro* assays and animal models, suggest that PIP5K3 inhibition may represent a promising strategy for tumors with high autophagic dependence (Cheng et al., 2025; DeLiberty et al., 2025). Although further clinical validation is needed, this preclinical evidence provides a rationale for exploring PIP5K3 inhibition as a therapeutic strategy in NPC, particularly in advanced cases prone to metastasis and recurrence.

However, certain limitations must be acknowledged in our study. Firstly, this was a retrospective, single-center cohort study. Therefore, validation of these findings in larger, multi-center cohorts is essential for future research. Secondly, although our study indicates a significant association, the exact regulatory mechanisms of PIP5K3 and the specific downstream molecules involved in PIP5K3-mediated adverse prognosis in NPC patients remain incompletely understood. Future research should focus on exploring the molecular pathways regulated by PIP5K3 that contribute to the proliferation and progression of NPC tumors.

Conclusion

In conclusion, this is the first study to report that PIP5K3 may serve as a relevant prognostic biomarker in NPC. Our results demonstrated a significant association between PIP5K3 expression and clinicopathological features. In NPC patients, elevated PIP5K3 expression was explicitly associated with poorer DSS, DMeFS, and LRFS. These findings suggest that PIP5K3 could be a novel diagnostic and prognostic marker for NPC patients.

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Data availability statement. The datasets generated and/or analyzed during the course of this study are available from the corresponding author upon reasonable request.

References

Baird A.M., Dockry E., Daly A., Stack E., Doherty D.G., O'Byrne K.J. and Gray S.G. (2013). IL-23R is epigenetically regulated and modulated by chemotherapy in non-small cell lung cancer. Front. Oncol. 3, 162.

Bozzuto G., Ruggieri P. and Molinari A. (2010). Molecular aspects of tumor cell migration and invasion. Ann. Ist. Super. Sanita 46, 66-80.

Burke J.E. (2018). Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. Mol. Cell 71, 653-673.

Burke J.E., Triscott J., Emerling B.M. and Hammond G.R.V. (2023). Beyond pi3ks: 298 Targeting phosphoinositide kinases in disease. Nat. Rev. Drug. Discov. 22, 357-299.

Cao S., Chen C., Xue J., Huang Y., Yang X. and Ling K. (2017). Silencing of type Iγ phosphatidylinositol phosphate kinase suppresses ovarian cancer cell proliferation, migration and invasion. Oncol. Rep. 38, 253-262.

Chen C., Wang X., Xiong X., Liu Q., Huang Y., Xu Q., Hu J., Ge G. and Ling K. (2015). Targeting type Iγ phosphatidylinositol phosphate kinase inhibits breast cancer metastasis. Oncogene 34, 4635-4646.

Chen Y.P., Chan A.T.C., Le Q.T., Blanchard P., Sun Y. and Ma J. (2019). Nasopharyngeal carcinoma. Lancet 394, 64-80.

Cheng C., Hu J., Mannan R., He T., Bhattacharyya R., Magnuson B., Wisniewski J.P., Peters S., Karim S.A., MacLean D.J., Karabürk H., Zhang L., Rossiter N.J., Zheng Y., Xiao L., Li C., Awad D., Mahapatra S., Bao Y., Zhang Y., Cao X., Wang Z., Mehra R., Morlacchi P., Sahai V., Pasca di Magliano M., Shah Y.M., Weisman L.S., Morton J.P., Ding K., Qiao Y., Lyssiotis C.A. and Chinnaiyan A.M. (2025). Targeting pikfyve-driven lipid metabolism in pancreatic cancer. Nature 642, 776-784.

Chua M.L.K., Wee J.T.S., Hui E.P. and Chan A.T.C. (2016). Nasopharyngeal carcinoma. Lancet 387, 1012-1024.

de Campos C.B., Zhu Y.X., Sepetov N., Romanov S., Bruins L.A., Shi C.X., Stein C.K., Petit J.L., Polito A.N., Sharik M.E., Meermeier E.W., Ahmann G.J., Armenta I.D.L., Kruse J., Bergsagel P.L., Chesi M., Meurice N., Braggio E. and Stewart A.K. (2020). Identification of PIKfyve kinase as a target in multiple myeloma. Haematologica 105, 1641-1649.

DeLiberty J.M., Roach M.K., Stalnecker C.A., Robb R., Schechter E.G., Pieper N.L., Taylor K.E., Pita L.M., Yang R., Bang S., Drizyte-Miller K., Ackermann S.E., Nicewarner Peña S.R., Baldelli E., Min S.M., Drewry D.H., Petricoin E.F., 3rd, Morris J.P.t., Der C.J., Cox A.D. and Bryant K.L. (2025). Concurrent inhibition of the ras-mapk pathway and pikfyve is a therapeutic strategy for pancreatic cancer. Cancer Res. 85, 1479-1495.

Duewell B.R., Faris K.A. and Hansen S.D. (2024). Molecular basis of product recognition during pip5k-mediated production of pi(4,5)p(2) with positive feedback. J. Biol. Chem. 300, 107631.

Feng Y., Dai Z., Yan R., Li F., Zhong X., Ye H., Chen C., Fan S., Qing C., Pan Y. and Sun H. (2021). Outcomes of recurrent nasopharyngeal carcinoma patients treated with salvage surgery: A meta-analysis. Front. Oncol. 11, 720418.

Gayle S., Landrette S., Beeharry N., Conrad C., Hernandez M., Beckett P., Ferguson S.M., Mandelkern T., Zheng M., Xu T., Rothberg J. and Lichenstein H. (2017). Identification of apilimod as a first-in-class PIKfyve kinase inhibitor for treatment of B-cell non-hodgkin lymphoma. Blood 129, 1768-1778.

Ikonomov O.C., Filios C., Sbrissa D., Chen X. and Shisheva A. (2013). The pikfyve-arpikfyve-sac3 triad in human breast cancer: Functional link between elevated sac3 phosphatase and enhanced proliferation of triple negative cell lines. Biochem. Biophys. Res. Commun. 440, 342-347.

Jin Y. and Xue J. (2023). Lipid kinases pip5ks and pip4ks: Potential drug targets for breast cancer. Front. Oncol .13, 1323897.

Kieser A. and Sterz K.R. (2015). The latent membrane protein 1 (LMP1). Curr. Top. Microbiol. Immunol. 391, 119-149.

Lee Y.E., He H.L., Chen T.J., Lee S.W., Chang I.W., Hsing C.H. and Li C.F. (2015). The prognostic impact of RAP2A expression in patients with early and locoregionally advanced nasopharyngeal carcinoma

in an endemic area. Am. J. Transl. Res. 7, 912-921.

Liu H., Tang L., Li Y., Xie W., Zhang L., Tang H., Xiao T., Yang H., Gu W., Wang H. and Chen P. (2024). Nasopharyngeal carcinoma: Current views on the tumor microenvironment's impact on drug resistance and clinical outcomes. Mol. Cancer 23, 20.

Oppelt A., Lobert V.H., Haglund K., Mackey A.M., Rameh L.E., Liestøl K., Schink K.O., Pedersen N.M., Wenzel E.M., Haugsten E.M., Brech A., Rusten T.E., Stenmark H. and Wesche J. (2013). Production of phosphatidylinositol 5-phosphate via pikfyve and mtmr3 regulates cell migration. EMBO Rep. 14, 57-64.

Perri F., Della Vittoria Scarpati G., Caponigro F., Ionna F., Longo F., Buonopane S., Muto P., Di Marzo M., Pisconti S. and Solla R. (2019). Management of recurrent nasopharyngeal carcinoma: Current perspectives. Onco Targets Ther. 12, 1583-1591.

Qiao Y., Choi J.E., Tien J.C., Simko S.A., Rajendiran T., Vo J.N., Delekta A.D., Wang L., Xiao L., Hodge N.B., Desai P., Mendoza S., Juckette K., Xu A., Soni T., Su F., Wang R., Cao X., Yu J., Kryczek I., Wang X.M., Wang X., Siddiqui J., Wang Z., Bernard A., Fernandez-Salas E., Navone N.M., Ellison S.J., Ding K., Eskelinen E.L., Heath E.I., Klionsky D.J., Zou W. and Chinnaiyan A.M. (2021). Autophagy inhibition by targeting PIKfyve potentiates response to immune checkpoint blockade in prostate cancer. Nat. Cancer 2, 978-993.

Sbrissa D., Ikonomov O.C. and Shisheva A. (1999). Pikfyve, a mammalian ortholog of yeast fab1p lipid kinase, synthesizes 5-phosphoinositides. Effect of insulin. J. Biol. Chem. 274, 21589-21597.

Semenas J., Hedblom A., Miftakhova R.R., Sarwar M., Larsson R., Shcherbina L., Johansson M.E., Härkönen P., Sterner O. and Persson J.L. (2014). The role of PI3K/AKT-related PIP5K1α and the discovery of its selective inhibitor for treatment of advanced prostate cancer. Proc. Natl. Acad. Sci. USA 111, E3689-3698.

Shah A.B. and Nagalli S. (2024). Nasopharyngeal carcinoma. In: Statpearls. StatPearls Publishing LLC. Treasure Island (FL).

Sharma G., Guardia C.M., Roy A., Vassilev A., Saric A., Griner L.N., Marugan J., Ferrer M., Bonifacino J.S. and DePamphilis M.L. (2019). A family of pikfyve inhibitors with therapeutic potential against autophagy-dependent cancer cells disrupt multiple events in lysosome homeostasis. Autophagy 15, 1694-1718.

Shisheva A. (2008). PIKfyve: Partners, significance, debates and paradoxes. Cell Biol. Int. 32, 591-604.

Sinha S., Winters R. and Gajra A. (2024). Nasopharyngeal cancer. In: Statpearls. StatPearls Publishing LLC. Treasure Island (FL).

Stan D.J., Niculet E., Lungu M., Onisor C., Rebegea L., Vesa D., Bezman L., Bujoreanu F.C., Sarbu M.I., Mihailov R., Fotea S. and Tatu A.L. (2022). Nasopharyngeal carcinoma: A new synthesis of literature data (review). Exp. Ther. Med. 23, 136.

Stransky N., Egloff A.M., Tward A.D., Kostic A.D., Cibulskis K., Sivachenko A., Kryukov G.V., Lawrence M.S., Sougnez C., McKenna A., Shefler E., Ramos A.H., Stojanov P., Carter S.L., Voet D., Cortés M.L., Auclair D., Berger M.F., Saksena G., Guiducci C., Onofrio R.C., Parkin M., Romkes M., Weissfeld J.L., Seethala R.R., Wang L., Rangel-Escareño C., Fernandez-Lopez J.C., Hidalgo-Miranda A., Melendez-Zajgla J., Winckler W., Ardlie K., Gabriel S.B., Meyerson M., Lander E.S., Getz G., Golub T.R., Garraway L.A. and Grandis J.R. (2011). The mutational landscape of head and neck squamous cell carcinoma. Science 333, 1157-1160.

Tortorella E., Giantulli S., Sciarra A. and Silvestri I. (2023). Ar and pi3k/akt in prostate cancer: A tale of two interconnected pathways. Int. J. Mol. Sci. 24.

Tran M.H., Seo E., Min S., Nguyen Q.T., Choi J., Lee U.J., Hong S.S., Kang H., Mansukhani A., Jou I. and Lee S.Y. (2018). NEDD4-induced degradative ubiquitination of phosphatidylinositol 4-phosphate 5-kinase α and its implication in breast cancer cell proliferation. J. Cell. Mol. Med. 22, 4117-4129.

Vicinanza M., Korolchuk V.I., Ashkenazi A., Puri C., Menzies F.M., Clarke J.H. and Rubinsztein D.C. (2015). Pi(5)p regulates autophagosome biogenesis. Mol. Cell 57, 219-234.

Wang T., Sarwar M., Whitchurch J.B., Collins H.M., Green T., Semenas J., Ali A., Roberts C.J., Morris R.D., Hubert M., Chen S., El-Schich Z., Wingren A.G., Grundström T., Lundmark R., Mongan N.P., Gunhaga L., Heery D.M. and Persson J.L. (2022). PIP5K1α is required for promoting tumor progression in castration-resistant prostate cancer. Front. Cell Dev. Biol. 10, 798590.

Wong K.C.W., Hui E.P., Lo K.W., Lam W.K.J., Johnson D., Li L., Tao Q., Chan K.C.A., To K.F., King A.D., Ma B.B.Y. and Chan A.T.C. (2021). Nasopharyngeal carcinoma: An evolving paradigm. Nat. Rev. Clin. Oncol. 18, 679-695.

Wu J.S., Jiang J., Chen B.J., Wang K., Tang Y.L. and Liang X.H. (2021). Plasticity of cancer cell invasion: Patterns and mechanisms. Transl. Oncol. 14, 100899.

Yang Z.J., Chee C.E., Huang S. and Sinicrope F.A. (2011). The role of autophagy in cancer: Therapeutic implications. Mol. Cancer Ther. 10, 1533-1541.

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