

Frequency of *PIK3CA* mutations in different subsites of head and neck squamous cell carcinoma in southern Thailand

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Background: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations have been reported in many cancers, including head and neck squamous cell carcinoma (HNSCC). The frequency of these mutations varies among tumor locations and might be relevant to treatment outcomes among HNSCC. In this study, we examined the frequency of *PIK3CA* mutations in the different subsites of HNSCC. **Methods:** Ninety-six fresh biopsy specimens were investigated for mutations in *PIK3CA* exons 4, 9, and 20 using allele-specific real-time polymerase chain reaction. Patient characteristics and survival were analyzed and compared between specimens with or without *PIK3CA* mutations. **Results:** The study included primary tumors originating from the oral cavity (n=63), hypopharynx (n=23), and oropharynx (n=10). We identified mutations in 10.4% of patients (10 of 96 specimens). The overall mutational frequency was 17.4% (4/23) and 9.5% (6/63) in the hypopharynx and oral cavity, respectively. No patients with oropharyngeal carcinoma had mutations. Among the 10 mutant specimens, five were missense mutations (exon 9 [E545K] in two samples and exon 20 [H1047R] in three samples) and five were silent mutations in exon 20 (T1025T). Mutations were not found in exon 4. Among 84 patients with available clinical data, we found no significant differences in clinical characteristics and survival based on the presence or absence of *PIK3CA* mutations. **Conclusions:** The results indicate that *PIK3CA* mutations are involved in HNSCC carcinogenesis, and the hypopharynx should be considered a primary site of interest for future studies, particularly in Southeast Asian populations.

Key Words: *PIK3CA* mutations; Head and neck squamous cell carcinoma; Oral cavity carcinoma; Hypopharyngeal carcinoma; Oropharyngeal carcinoma

Received: October 27, 2021 **Revised:** December 23, 2021 **Accepted:** January 4, 2022

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Head and neck cancer includes all tumors that arise from various anatomical subsites, including the oral cavity, oropharynx, nasopharynx, hypopharynx, larynx, paranasal sinuses, and salivary glands. Although histologic subtypes are diverse, malignancies of the epithelial lining, namely squamous cell carcinoma, are the most common [1].

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, with approximately 890,000 new cases diagnosed and 450,000 deaths reported in 2008. Among all HNSCC, the oral cavity is the most common subsite of a primary tumor [2]. The burden of HNSCC varies across regions of the world. In Thailand, HNSCC is one of

the most common cancers, with approximately 10,000 new cases diagnosed annually. The age-standardized incidence rates of HNSCC in males and females are 15.7 and 10.7, respectively [3]. The dominant tumor locations of HNSCC in males are the oral cavity, nasopharynx, and larynx; and the most common location in females is the oral cavity [3].

At the time of diagnosis, most patients with HNSCC have locally advanced, unresectable tumors. Despite advanced multidisciplinary treatments, including surgery with either or both radiotherapy and chemotherapy, only modest improvements in outcomes have been achieved over the past 20 years. HNSCC molecular profiling and biomarker studies have provided infor-

mation regarding rapid diagnosis, prognosis, and surveillance for recurrence and metastasis. However, biomarkers that serve as potential targets of novel therapies to improve survival outcomes are important. Among the signaling pathways associated with tumor proliferation and survival, the phosphoinositide 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway is the most frequently altered oncogenic pathway in HNSCC [4,5].

The phosphatidylinositol 3-kinase gene (*PI3K*) encodes PI3K enzymes (PI3Ks), which ultimately trigger various downstream signaling pathways that result in cell survival, apoptosis, transformation, metastasis, and cell migration. PI3Ks are classified into three major subclasses (class I, II, and III) that are determined based on PI3K structure and substrate specificity. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) is a 34-kb gene located on chromosome 3q26.3 that consists of 20 exons coding for 1,068 amino acids, yielding a protein with a molecular weight of 124 kDa (p110 α catalytic subunit protein, class I) [6]. Several studies previously reported alterations in *PIK3CA* in many cancers. The presence of *PIK3CA* mutations is associated with prognosis and might predict response to PI3K inhibitors [6,7]. In HNSCC, the frequency of *PIK3CA* mutations varies among primary tumor locations [6,8-15]; hence, the frequency of these mutations from different tumor subsites might be relevant to determine treatments and outcomes among HNSCCs. Furthermore, the mutation rate in *PIK3CA* might be diverse among ethnicities [15,16]; the mutation rate is unknown in Southeast Asian populations, including the Thai population. Therefore, in the present study, we evaluated the frequency of *PIK3CA* mutations in patients with squamous cell carcinoma of the oral cavity, oropharynx, and hypopharynx in southern Thailand. Furthermore, the clinical characteristics and survival between patients with or without *PIK3CA* mutations were analyzed and compared.

MATERIALS AND METHODS

Patients and specimens

During 2002–2003, 96 fresh tissues (63 from the oral cavity, 10 from the oropharynx, and 23 from the hypopharynx) were obtained from patients registered at the head and neck clinic of Songklanagarind Hospital. The tissue samples were obtained from a biopsy or sampling of surgical resection specimen, after which they were snap frozen and stored at -80°C until DNA extraction. All collected tissues were subsequently reviewed to ensure cancer site and pathological diagnosis of cancer by a pathologist (P.T).

All cases were primary tumors that had not been treated with radiation or chemotherapy.

Clinicopathological parameters of the patients were retrieved from medical records. The patient information extracted from the database was date of birth, sex, vital status, history of smoking and alcohol consumption, date of diagnosis, stage, and treatment. Pathological information was obtained from pathological reports. Date of death was obtained from the national civil registration system.

Mutation detection using conventional polymerase chain reaction and DNA sequencing

Genomic DNA was extracted from fresh tissue using an E.Z.N.A Tissue DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA), and the procedures were performed according to the manufacturer's instructions. *PIK3CA* gene analysis was performed in exons 4, 9, and 20; 100 ng of DNA was amplified using polymerase chain reaction (PCR) with the following primer sets: *PIK3CA*-exon 4 forward (5'-CATCTTATTCCAGACGCATTTC-3'), *PIK3CA*-exon 4 reverse (5'-AGATTACTGTATAGTGCAAGA AAA-3'), *PIK3CA*-exon 9 forward (5'-CCAGAGGGGAAAAA TATGACA-3'), *PIK3CA*-exon 9 reverse (5'-CATTTTAGCACT TACCTGTGAC-3'), *PIK3CA*-exon 20 forward (5'-CATTTGC TCCAACTGACCA-3'), and *PIK3CA*-exon 20 reverse (5'-TG AGCTTTCATTTTCTCAGTTATCTTTTC-3'). The PCR conditions for *PIK3CA* exons 4 and 9 were denaturation at 95°C for 3 minutes; amplification for 45 cycles at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds; and extension at 72°C for 7 minutes. The PCR conditions for *PIK3CA* exon 20 were denaturation at 95°C for 3 minutes; amplification for 45 cycles at 95°C for 45 seconds, 58°C for 45 seconds, and 72°C for 1 minute; and extension at 72°C for 7 minutes. All gene sequencing was performed with an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequences were analyzed to ascertain point mutations in *PIK3CA* exons 4, 9, and 20. The conventional PCR technique was performed in certain samples to determine the reference point mutations. Then, allele-specific real-time PCR was performed for the remaining tissue samples.

Allele-specific real-time PCR

Reference point mutations were used to design allele-specific probes (dual-labeled fluorogenic probes) to develop a real-time PCR technique. The allele-specific probes were composed of short DNA sequences covering the mutational areas. The probes were labeled with two fluorescent dyes: HEX for the *PIK3CA*

Table 1. Allele-specific probes

Probe name	Type	Sequence
<i>PIK3CA</i> exon 9 G>A	Wild-type probe	5'-(HEX) CTCTCTGAAATCACTGAGCAGGAGAA (BHQ-1)-3'
<i>PIK3CA</i> exon 9 G>A	Mutated probe	5'-(FAM) CTCTCTGAAATCACTAAGCAGGAGAA (BHQ-1)-3'
<i>PIK3CA</i> exon 20 A>G	Wild-type probe	Anti-sense 5' (HEX) AGCCACCATGATGTGCATCATT (BHQ-1)-3'
<i>PIK3CA</i> exon 20 A>G	Mutated probe	Anti-sense 5' (FAM) AGCCACCATGACGTGCATCATT (BHQ-1)-3'
<i>PIK3CA</i> exon 20 C>T	Wild-type probe	5'-(HEX) ACATTCGAAAGACCCTAGCCTTAGAT (BHQ-1)-3'
<i>PIK3CA</i> exon 20 C>T	Mutated probe	5'-(FAM) ACATTCGAAAGACTCTAGCCTTAGAT (BHQ-1)-3'

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

wild-type allele and FAM for the mutated allele (Sigma-Aldrich, Singapore). BHQ-1 was used as a quencher. For all samples, 20 ng of DNA was obtained and subjected to real-time PCR using previously described primer sets and allele-specific probe sets (Table 1). PCR mixtures contained 10 μ L of 2 \times FastStart Essential DNA Probes Master buffer (Roche Diagnostics, Mannheim, Germany), 0.5 μ L forward primer, 0.5 μ L reverse primer, 0.25 μ L wild-type probe, 0.25 μ L mutated probe, 6.5 μ L molecular grade water, and 20 ng DNA in a final volume of 20 μ L. The real-time PCR conditions for *PIK3CA* exon 9 were 95°C, 3 minutes and (95°C, 10 seconds; 58°C, 30 seconds) \times 45 cycles. The real-time PCR conditions for *PIK3CA* exon 20 were 95°C, 3 minutes and (95°C, 10 seconds; 62°C, 30 seconds; 72°C, 30 seconds) \times 45 cycles. Template controls were not included in each real-time PCR run. All samples were subsequently subjected to real-time PCR using the probes. Five mutant specimens served as positive controls.

Statistical analysis

Descriptive statistics were used to compare the data between the two groups of patients. The chi-square test or Fisher exact test was used to compare proportional data, and Student's t test or the Mann-Whitney U test was used to assess the differences in continuous data. Descriptive results are presented as the median (continuous variable) and percentage (categorical variable).

Duration of follow-up and overall survival (OS) time were calculated from the date of diagnosis until the date of last follow-up or date of death from any cause, respectively. The data of patients who were alive at the last date of follow-up, December 20, 2021, were censored. The survival curve was calculated using the Kaplan-Meier method. Comparisons of survival curve among groups were performed using the log-rank test.

Stata/MP ver. 17.0 (StataCorp, College Station, TX, USA) was used to perform all analyses. p-values < .05 were considered statistically significant.

Table 2. Patient demographics and baseline characteristics

Variable	Total (n=84)	Wild-type <i>PIK3CA</i> (n=74)	Mutant <i>PIK3CA</i> (n=10)	p-value
Age (yr)				.100
Median \pm SD	68 \pm 12.6	68 \pm 12.8	70 \pm 9.7	
Sex				.307
Male	49 (58.3)	45 (60.8)	4 (40.0)	
Female	35 (41.7)	29 (39.2)	6 (60.0)	
Smoking				.324
Yes	47 (56.0)	43 (58.1)	4 (40.0)	
No	37 (44.0)	31 (41.9)	6 (60.0)	
Alcohol drinking				.182
Yes	44 (52.4)	41 (55.4)	3 (30.0)	
No	40 (47.6)	33 (44.6)	7 (70.0)	
Tumor location				.380
Oral cavity	57 (67.9)	51 (68.9)	6 (60.0)	
Tongue	14 (16.7)	13 (17.6)	1 (10.0)	
Gums	13 (15.4)	12 (16.2)	1 (10.0)	
Buccal mucosa	11 (13.1)	10 (13.5)	1 (10.0)	
Floor of mouth	6 (7.1)	5 (6.7)	1 (10.0)	
Lip	5 (6.0)	3 (4.1)	2 (20.0)	
Hard palate	4 (4.8)	4 (5.4)	0	
Retromolar area	2 (2.4)	2 (2.7)	0	
Oral cavity (unspecified)	2 (2.4)	2 (2.7)	0	
Oropharynx	6 (7.1)	6 (8.1)	0	
Base of tongue	6 (7.1)	6 (8.1)	0	
Hypopharynx	21 (25.0)	17 (23.0)	4 (40.0)	
Pyramidal sinus	21 (25.0)	17 (23.0)	4 (40.0)	
Clinical stage				.628
I	16 (19.0)	14 (18.9)	2 (20.0)	
II	17 (20.3)	13 (17.6)	4 (40.0)	
III	19 (22.6)	18 (24.3)	1 (10.0)	
IVa	29 (34.5)	26 (35.1)	3 (30.0)	
IVb	1 (1.2)	1 (1.4)	0	
IVc	2 (2.4)	2 (2.7)	0	
Treatment				.337
Surgery	23 (27.4)	18 (24.3)	5 (50.0)	
Surgery followed by RT	42 (50.0)	38 (51.4)	4 (40.0)	
RT	13 (15.5)	12 (16.2)	1 (10.0)	
Best supportive care	6 (7.1)	6 (8.1)	0	

Values are presented as number (%) unless otherwise indicated.

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; SD, standard deviation; RT, radiotherapy.

RESULTS

The samples used in this study comprised 96 HNSCC samples diagnosed from 2002–2003. The study included primary tumors originating from the oral cavity (n = 63), hypopharynx (n = 23), and oropharynx (n = 10). The patient demographics and their baseline clinical characteristics were available for 84 cases and are shown in Table 2; 49 cases were male, and the median age at diagnosis was 68 years.

We initially identified three *PIK3CA* point mutations using conventional PCR (Fig. 1A–C): one in exon 9 and two in exon 20. Mutations were not detected in exon 4. We then applied these known mutations as a reference to design allele-specific probes (dual-labeled fluorogenic probes) as shown in Table 1.

We found *PIK3CA* mutations in 10 of 96 samples (10.4%) with all heterozygous mutations. When the patients were classified based on mutational events in the primary tumor location, 17.4% (4/23) with hypopharyngeal squamous cell carcinoma (HPSCC) and 9.5% (6/63) with oral cavity squamous cell carcinoma (OSCC) had *PIK3CA* mutations. No patient (0/10) with oropharyngeal squamous cell carcinoma (OPSCC) had *PIK3CA* mutations (Table 3). Clinical characteristics between patients with or without *PIK3CA* mutations were not statistically significantly different (Table 2). The patient characteristics and details of *PIK3CA* mutations are shown in Table 4. Two patients had exon 9 mutations (Fig. 2A) and eight had exon 20 mutations (Fig. 2B, C). The 10 mutations were categorized as five missense and five silent mutations, which corresponded to E545K in exon 9 and T1025T and H1047R in exon 20. E545K (GAG1633A-AG) and H1047R (CAT3140CGT) are missense mutations, and T1025T (ACC3075ACT) is a silent mutation.

The majority of patients with wild-type *PIK3CA* (51.4%) underwent surgery followed by adjuvant radiotherapy; however, patients with *PIK3CA* mutations underwent only surgery (50.0%), as shown in Table 2. The median duration of follow-up was 2.11 years (95% confidence interval [CI], 1.21 to 3.26). The 5-year OS rates in the wild-type *PIK3CA* and mutant *PIK3CA* were 35%

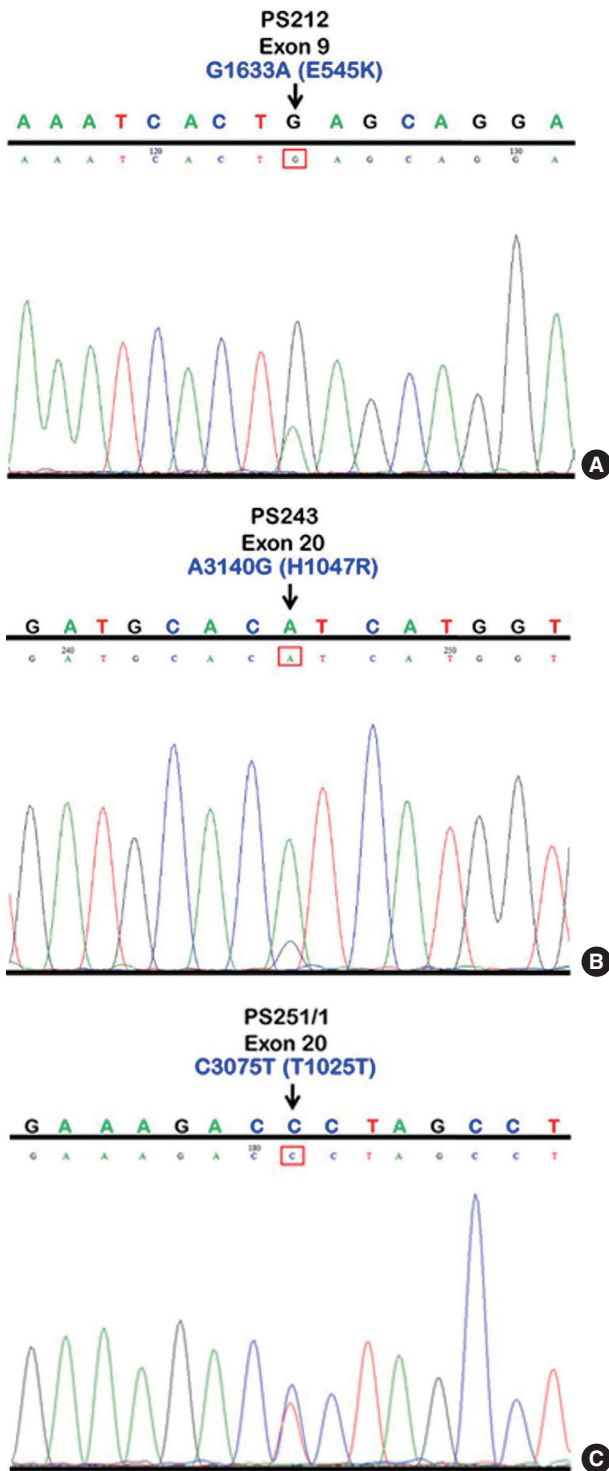


Fig. 1. *PIK3CA* mutations in exons 9 and 20 using conventional PCR as illustrated in sequencing chromatograms showing a missense mutation in sample No. PS212 at position 1633, G>A (E545K) in exon 9 (A), a missense mutation in sample No. PS243 at position 3140, A>G (H1047R) in exon 20 (B), and a silent mutation in sample No. PS251/1, C>T (T1025T) in exon 20 (C). *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PCR, polymerase chain reaction.

Table 3. Frequency of *PIK3CA* mutations in HNSCC by subsite

Subsites of HNSCC	<i>PIK3CA</i> mutation		Overall <i>PIK3CA</i> mutations
	Exon 9	Exon 20	
Oral cavity	0/63 (0)	6/63 (9.5)	6/63 (9.5)
Oropharynx	0/10 (0)	0/10 (0)	0/10 (0)
Hypopharynx	2/23 (8.7)	2/23 (8.7)	4/23 (17.4)

Values are presented as number (%).

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; HNSCC, head and neck squamous cell carcinoma.

Table 4. Mutational status of *PIK3CA* in HNSCC

Sample No.	Age at diagnosis (yr)	Sex	Tumor location	Exon	Nucleotide	Codon change	Amino acid change	Type of mutation
PS212	76	Male	Pyriiform sinus	9	G1633A	GAG to AAG	E545K	Missense
PS220	62	Female	Pyriiform sinus	9	G1633A	GAG to AAG	E545K	Missense
PS243	72	Male	Pyriiform sinus	20	A3140G	CAT to CGT	H1047R	Missense
PS251/1	63	Male	Pyriiform sinus	20	C3075T	ACC to ACT	T1025T	Silent
OR183	58	Female	Buccal mucosa	20	C3075T	ACC to ACT	T1025T	Silent
OR214	90	Female	Tongue	20	C3075T	ACC to ACT	T1025T	Silent
OR378	79	Female	Gums	20	C3075T	ACC to ACT	T1025T	Silent
OR391	69	Male	Floor of mouth	20	C3075T	ACC to ACT	T1025T	Silent
OR205	79	Female	Lip	20	A3140G	CAT to CGT	H1047R	Missense
OR209	66	Female	Lip	20	A3140G	CAT to CGT	H1047R	Missense

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; HNSCC, head and neck squamous cell carcinoma.

(95% CI, 24 to 46) and 58% (95% CI, 23 to 82), respectively (Fig. 3). There was no statistically significant difference in OS rate between the two groups ($p = .625$).

DISCUSSION

In HNSCC, the PI3K/AKT/mTOR pathway is the most frequently altered oncogenic pathway associated with tumor proliferation and survival [4,5]. Reportedly, *PIK3CA* mutations are associated with advanced stage of HNSCC, particularly in subjects with oral squamous cell carcinoma, indicating that *PIK3CA* mutations play an important role in the carcinogenesis of HNSCC [9,15]. In the present study, we evaluated the frequency of *PIK3CA* mutations in the different subsites of HNSCC in the Thai population.

When divided by primary tumor location, we detected a higher mutation rate in HNSCC than in OCSCC (17.4% vs. 9.5%). We also found a slightly higher *PIK3CA* mutation rate in HNSCC than reported in a study in the Chinese population (13.6%) [16]. The frequency of *PIK3CA* mutations in OCSCC in the present study (9.5%) was slightly higher than the 7.4% frequency reported in the Japanese population [9]. Notably, Kommineni et al. [15] reported a substantially high *PIK3CA* mutation rate of 52% in OCSCC tumor samples from Indian patients. Taken together, these findings might highlight the effect of diverse mutation rates even among Asian populations.

Human papillomavirus (HPV) infection is a well-known risk factor associated with OPSCC. Nichols et al. [17] reported a higher frequency of *PIK3CA* mutations in HPV-related OPSCC than HPV-negative tumors (28% vs. 10%). In the present study, we found no *PIK3CA* mutation in any patient with OPSCC. This result was discordant from those of a study by Beaty et al. [18] showing a *PIK3CA* mutation rate of 21% in patients with HPV-associated OPSCC. The reason for the discordance might be the

small sample cohort in the OPSCC subgroup in the present study. Furthermore, information regarding HPV status in these patients was unknown due to the lack of sufficient specimens. However, in previous studies, the proportion of HPV-related OPSCC in Thai patients was remarkably low (15%–20%) [19,20] compared with 40%–60% reported in Western patients [21]. Thus, the correlation between *PIK3CA* mutations and HPV status should be further explored in Asian populations.

In the present study, *PIK3CA* mutations in exon 4 were not detected. In contrast, a previous study reported mutations in exon 4, with a rate of 2.6% in an oral cavity carcinoma cell line (primary tumor originated in the tongue) [8]. In the present study, the overall 10.4% mutation rate of *PIK3CA*, which included 2.1% in exon 9 (E545K) and 8.3% in exon 20 (H1047R and T1025T), was not significantly different from previous studies (9%–13% in non-nasopharyngeal carcinoma), as shown in Table 5. However, a higher mutation rate of 21% in pharyngeal carcinoma and 21% in oropharyngeal carcinoma were reported in two studies [11,18]. In those studies, a more advantageous technique, a novel mutant-enriched sequencing method, was used for analysis, which could explain the difference in results.

We found two of the three types of *PIK3CA* mutations, E545K and H1047R, which have been previously reported as hotspot mutations [22–24]. E545K is located on exon 9 in the helical domain of *PIK3CA*, and H1047R and T1025T are encoded by exon 20 within the kinase domain [25]. The E545K mutation disrupts an inhibitory charge–charge interaction between p110 α and the N-terminal SH2 domain of the p85 regulatory subunit, and the H1047R mutation increases the binding affinity between p110 α and the negatively charged phosphatidylinositol substrate [26–28]. The functional effect of these two hotspot mutations has been well established in many studies, showing the gain-of-function PI3K activity and downstream signaling *via* the AKT/mTOR pathway [22,23]. Furthermore, the benefit of *PIK3CA* mutations

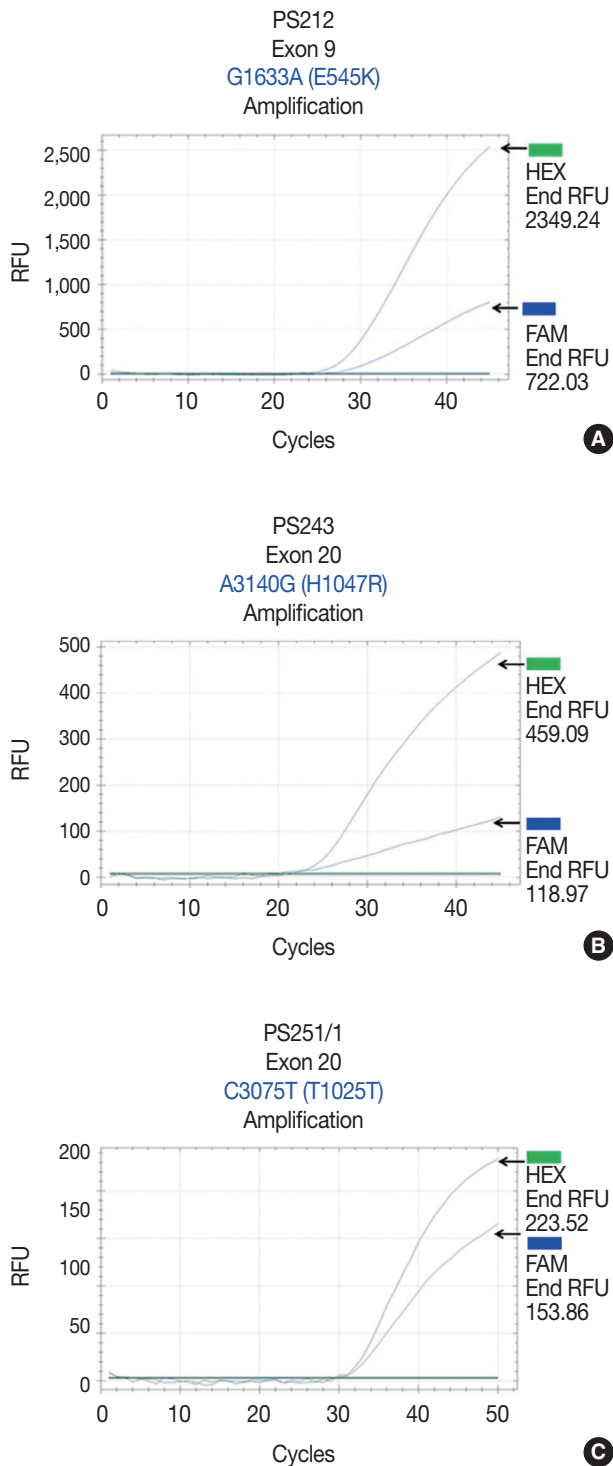


Fig. 2. Amplification plot of allele-specific real-time PCR showing *PIK3CA* mutations in exon 9 and exon 20 and the HEX (green, wild-type *PIK3CA* allele) and FAM (blue, mutated *PIK3CA* allele) signals. (A) A missense mutation in sample No. PS212 at position 1633, G>A (E545K) in exon 9. (B) A missense mutation in sample No. PS243 at position 3140, A>G (H1047R) in exon 20. (C) A silent mutation in sample No. PS251/1, C>T (T1025T) in exon 20. PCR, polymerase chain reaction.

as a predictive marker for novel targeted therapies has been demonstrated. Alpelisib, an oral selective PI3K inhibitor, has been implemented in current practice for patients with advanced breast cancer harboring *PIK3CA* mutations [29,30]. The efficacy and safety of PI3K or mTOR inhibitors are being evaluated in several clinical trials for patients with advanced HNSCC [31]. In contrast to the previously mentioned two hotspot mutations, T1025T is categorized as a silent mutation. Notably, we detected the T1025T mutation, which was significantly high in OCSCC, in 4 of 5 mutant cases in the present study. This was concordant with a previous study in which the T1025T mutation was reportedly prominent in OCSCC [15]. However, whether the T1025T silent mutation is the oncogenic driver in HNSCC remains controversial. Kommineni et al. [15] reported a significantly higher frequency of T1025T silent mutation in patients with HNSCC (41.0%) than in healthy controls (16.7%, $p < .001$). In contrast, Dogruluk et al. [28] identified T1025T as a genetic variant in the human germline.

In the present study, clinical significance was not observed based on the presence or absence of *PIK3CA* mutations. This finding was similar to a previous study in which significant difference was not observed in clinical characteristics between patients with wild-type *PIK3CA* or mutant *PIK3CA* OPSCC [18]. However, the presence of a *PIK3CA* mutation in that study was associated with poorer disease-free survival among patients receiving de-intensified definitive chemoradiotherapy for HPV-related OPSCC [18]; survival did not differ between the two groups in the present study. This contradictory finding might be explained by the small sample cohort in the present study. A larger study in which *PIK3CA* mutations are investigated as a potentially prognostic

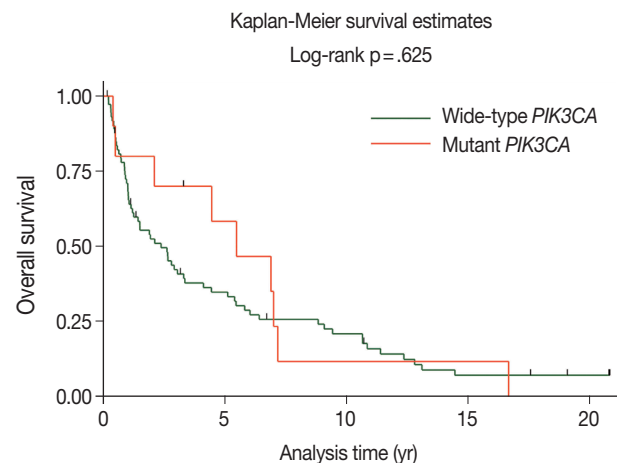


Fig. 3. Kaplan-Meier survival curves of overall survival for patients with wild-type *PIK3CA* or mutant *PIK3CA*. *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

Table 5. Previous studies of *PIK3CA* mutations in HNSCC

Authors (year)	Location of HNSCC	<i>PIK3CA</i> mutations (%)	Type of mutation	Exons
Qiu et al. (2006) [8]	HNSCC	11 (4/38)	Point mutation	4, 9, and 20
Kozaki et al. (2006) [9]	Oral cavity	9 (11/122)	Point mutation	9 and 20
Qiu et al. (2008) [11]	Pharynx	21 (5/24)	Point mutation	9 and 20
Murugan et al. (2008) [12]	HNSCC	13 (7/54)	Point mutations, frameshift	9 and 20
Kommineni et al. (2015) [15]	HNSCC	60.46	Point mutation	9 and 20
Wu et al. (2017) [16]	Hypopharynx	13.6	Point mutation	9 and 20
Beaty et al. (2020) [18]	Oropharynx	21 (16/77)	Point mutation	9 and 13
Lui et al. (2013) [32]	HNSCC	12.6 (19/151)	Point mutation	9, 20, and novel mutations

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; HNSCC, head and neck squamous cell carcinoma.

factor in patients with OPSCC and non-OPSCC is warranted.

To the best of our knowledge, this is the first study to report the frequency of *PIK3CA* mutations in HNSCC in Thailand. However, the limitations of the study included small sample size, particularly in the OPSCC subgroup, and lack of available data regarding HPV status. The absence of *PIK3CA* mutations in OPSCC might be due to the smaller number of samples tested than those at other subsites. Furthermore, testing for *PIK3CA* mutations in normal tissue from these patients was not performed; hence, conclusions cannot be drawn regarding involvement of the T1025T silent mutation in the pathogenesis of HNSCC or its involvement as a genetic variant in the human germline.

In conclusion, our study showed a higher frequency of *PIK3CA* mutations in HPSCC than in OCSCC. The results suggest that these mutations are involved in carcinogenesis and could serve as biomarkers for novel targeted therapies as well as immunomodulators in the era of immune checkpoint inhibitors. Furthermore, the hypopharynx is suggested as a primary site of interest for further studies, particularly in Southeast Asian populations.

Ethics Statement

This study was approved by the Institutional Review Board of Faculty of Medicine, Prince of Songkla University (REC 56-498-14-1) with a waiver of informed consent.

Availability of Data and Material

All data generated or analyzed during the study are included in this published article (and its supplementary information files).

Code Availability

Not applicable.

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Conceptualization: AD. Data curation: PT (Phatcharaporn Thongwatchara), TD. Formal analysis: AD, PT (Phatcharaporn Thongwatchara), PT (Paramee Thongsuksai), TD, SLG. Funding acquisition: AD. Investigation: PT (Phatcharaporn Thongwatchara). Methodology: PT (Phatcharaporn Thongwatchara), PT (Paramee Thongsuksai). Project administration: AD. Resources: PT (Phatcharaporn Thongwatchara), PT (Paramee Thongsuksai). Supervision: AD, PT (Paramee Thongsuksai). Validation: PT (Phatcharaporn Thongwatchara), PT (Paramee Thongsuksai). Visualization: AD, PT (Paramee Thongsuksai). Writing—original draft: AD, PT (Phatcharaporn Thongwatchara). Writing—review & editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

Funding Statement

This study was funded by the Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand.

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