

Aim of the study: Important signalling pathways play fundamental roles in the pathogenesis of thyroid carcinoma (TC). PTEN, mTOR, PI3K-p85 and K-Ras are the principal factors involved in these signalling pathways. To immunohistochemically examine the expressions of PI3K, mTOR and PTEN in patients suffering from follicular TC, papillary TC or variants thereof, as well as to investigate KRAS mutations via PCR to determine their clinical and prognostic relevance to differentiated thyroid cancer.

Material and methods: The expression of PTEN, PI3K-p85 and mTOR was immunohistochemically examined, and the mutation of K-Ras was examined via PCR. The results obtained were compared to the clinico-pathologic characteristics of the patients.

Results: A significant correlation was found between p85 expression and lymphovascular invasions and between PTEN expression and multifocality ($p = 0.048$ and $p = 0.04$, respectively), and a correlation between p85 and capsular invasion was found, with a borderline statistical significance ($p = 0.056$). No expression of PTEN, p85 or Mtor was detected in normal tissue. K-Ras mutation was examined in 66 of the 101 patients (57.4%), and the percentage of patients exhibiting a K-Ras mutation was 17.4%. All of the patients exhibiting a K-Ras mutation were women ($p = 0.047$). The disease-free survival was 44.6 months (95% CI: 37.9–51.3) and was statistically significantly higher in the group that displayed level 1 or lower expression of p85 ($p = 0.043$).

Conclusions: The expression levels of the aforementioned markers were significantly higher in TC cells than in normal tissue. A significant correlation was detected between K-Ras mutation and gender. This study demonstrates that p85 and PTEN are markers that should be evaluated in further studies of TC.

Key words: thyroid cancer, PTEN, mTOR, PI3K, K-Ras.

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Evaluation of PTEN, PI3K, MTOR, and KRAS expression and their clinical and prognostic relevance to differentiated thyroid carcinoma

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Introduction

In thyroid carcinoma (TC), the phosphoinositide-3(OH) kinase (PI3K)/Akt and mitogen-activated protein (MAP) kinase pathways are significantly involved in tumourigenesis. After the activation of RTKs by growth factors, RET/PTC and RAS play a role in both pathways. PI3K, the mammalian target of rapamycin (mTOR), and Kirsten rat sarcoma (KRAS) signalling are responsible for cell growth, proliferation and survival. Phosphatase and tensin homolog (PTEN) is a tumour suppressor [1].

PI3Ks contain several subunits. Class I PI3Ks are well defined and consist of a p85 regulatory and a p110 catalytic subunit [2–5]. The PI3K/Akt pathway is activated as a result of the binding of the p85 subunit of PI3K to the subunits of activated tyrosine residues present on an activated growth factor receptor or via interaction with a RAS protein [6]. PTEN plays many roles in several subcellular locations, including that of a negative regulatory lipid protein phosphatase involved in the PI3K-associated signalling pathway. The primary function of PTEN is the negative regulation of the PI3K pathway, which it performs by removing the phosphate from position 3 of phosphoinositides via its phosphatase activity [7]. PTEN deactivation was detected in different types of carcinoma [8–10]. mTOR is a regulatory protein, involved in the PI3K/Akt/mTOR pathway, which increases the uptake of iodine from thyroid cells to promote cell proliferation and survival. Genetic alterations occurring in this pathway play a role in the progression of TC. mTOR is a highly attractive target via several therapeutic strategies [11]. RAS is an oncogene family that regulates two important signalling pathways in thyroid cancer: the MAP kinase/extracellular signal-regulated kinase (Ras/Raf/MEK/ERK) and PI3K/Akt signalling pathways. N-, H- and K-Ras are the three essential members of this family. The activation of oncogenic RAS causes point mutations that affect the GTP-binding domain (codon 12 or 13) in exon 1 or the GTPase domain (codon 61) in exon 2. These mutations fix the RAS proteins in an activated state, thereby causing chronic stimulation, genomic instability, additional mutations and malignant transformation of these genes. RAS mutations were detected in follicular adenomas and in the follicular variant of papillary thyroid carcinoma (PTC) [12, 13]. In TC, the prevalence of RAS mutations is approximately 20–40%. The role of RAS in the progression of thyroid tumours is unclear [1].

The important role that these genetic alterations play in TC is an important guide for the development of new gene-based diagnostic, prognostic and therapeutic strategies. Although the standard therapeutic strategy for the treatment of thyroid cancer still includes surgery, radioactive iodine (RAI) treatment and thyroid suppression therapy, an understanding of the specific factors that constitute the signalling pathways involved in TC has provided

opportunities for targeted therapies [1, 14, 15]. As a result of the establishment of these targets of thyroid cancer, several targeted therapies, such as sorafenib, gefitinib, axitinib, motesanib, sunitinib, imatinib, and pazopanib, were generated. Clinical studies are ongoing to determine the effectiveness of these drugs in patients suffering from locally advanced and metastatic disease, who have exhibited resistance to previous therapeutic strategies (i.e. surgery and RAI) [14].

In light of these results, the aim of our study was to immunohistochemically examine the expressions of PI3K, mTOR and PTEN in patients suffering from follicular (FTC), papillary TC (PTC) or variants thereof, as well as to investigate KRAS mutations via PCR to determine their clinical and prognostic relevance to differentiated thyroid cancer.

Material and methods

Patient selection

This study enrolled a cohort of 101 patients suffering from differentiated thyroid carcinoma, who were diagnosed and followed between 2005 and 2010 by the Cukurova University Faculty of Medicine in Adana, Turkey. The patient database of the hospital was screened. The patients were staged based on the TNM staging system. The patients' demographic data, such as age, sex, city of residence, telephone number, date of diagnosis, concomitant systemic diseases, the presence of secondary cancer, family history of cancer, personal history of radiotherapy, tumour diameter, status of the lymph nodes, the presence of distant metastasis, the surgical method performed, therapies administered and characteristics including lymph vascular invasion (LVI), capsular invasion (CI) and multifocality of the existing pathological specimens, were recorded. Paraffin blocks of the tumour were retrieved from the pathology archives. All patients with inadequate follow-up data and those with inadequate pathology specimens were excluded from the study. A total of 160 patients with histologically confirmed differentiated thyroid cancer were selected for analysis in this study. Of these, paraffin embedded tissue material/tissue blocks were available from 133 patients, of which 106 were suitable for analysis. There was not adequate material from five patients, who were therefore excluded from the study. We evaluated a total of 101 cases of differentiated thyroid carcinoma. The mean age of the patients was 46.3 ± 12.1 years. Tissue specimens from a total of 101 patients suffering from DTC, normal thyroid tissue near the tumour specimens and 23 pathology specimens from patients suffering from Hashimoto thyroiditis (HT) were analysed. The paraffin blocks were stained for immunohistochemical analysis, and DNA samples were isolated for PCR analysis. This study was approved by the Cukurova University Faculty of Medicine Ethics Committee (approval date 02.12.2011, approval number 17).

Performance of PCR of K-RAS

First, DNA was isolated using a QIAmp DNA FFPE kit. The quantity of DNA was measured using a Nan photometer (Version: 7122VI.6.1, Serial number: 1562), and based

on this result, real-time PCR of K-Ras was performed. For this purpose, an Entrogen kit specific for genomic mutations in K-Ras codon 12, 13 or 61 (Instrument Type: Applied Biosystems 7500 Real-Time PCR System) was used.

Immunohistochemical staining for PTEN, PI3K and mTOR

Formalin-fixed, paraffin-embedded (FFPE) blocks of thyroid tumour specimens were retrieved and reviewed to confirm the presence of normal TC and normal thyroid tissue near the cancerous area or HT. Thyroidectomy material was used. The samples and controls were sectioned (5 micrometres) and stained using the Dako Autostainer Plus Staining System (Dako USA, Carpinteria, California (CA), USA). The primary antibodies used were: a PI3K-p85 monoclonal antibody (Clone SP62, M3624, Spring Bioscience, Pleasanton, CA, USA), a PTEN mouse monoclonal antibody (Clone 28H6, NCL-PTEN, Novocastra Laboratories, United Kingdom) and an mTOR rabbit anti-human polyclonal antibody (Clone N/A, Spring Bioscience, Pleasanton, CA, USA).

Finally, after rinsing with deionised water, the slides were counterstained using haematoxylin, dehydrated, mounted using a toluene-based mounting medium (Thermo Scientific Richard-Allan) and coverslipped. The samples were evaluated by two independent pathologists from the Cukurova University Department of Pathology. All of the samples were evaluated by the same investigators. The samples stained with PTEN, mTOR or p85 antibody were examined using a light microscope at a magnification of 400 \times . Positive staining was considered as nuclear staining for PTEN, cytoplasmic and membranous staining for mTOR and cytoplasmic staining for p85. The expressions of PTEN, mTOR and p85 in the tumour cells were graded between 0 and 3. No staining in the tumour cells was considered as 0, while 1–10%, 10–50% and > 50% staining were defined as 1, 2 and 3, respectively (Figs. 1–3).

Statistical analysis

Statistical analysis of the data was performed using the SPSS 18.0 software package. The results were evaluated by an investigator from the Department of Biostatistics. The

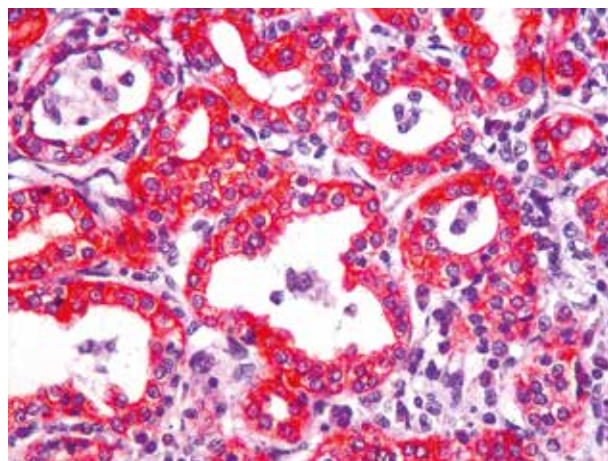


Fig. 1. mTOR +3 positive staining 400 \times high field power

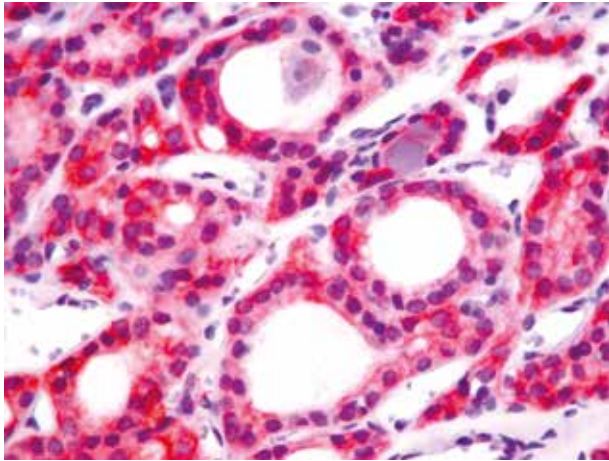


Fig. 2. p85 +3 positive staining 400× high field power

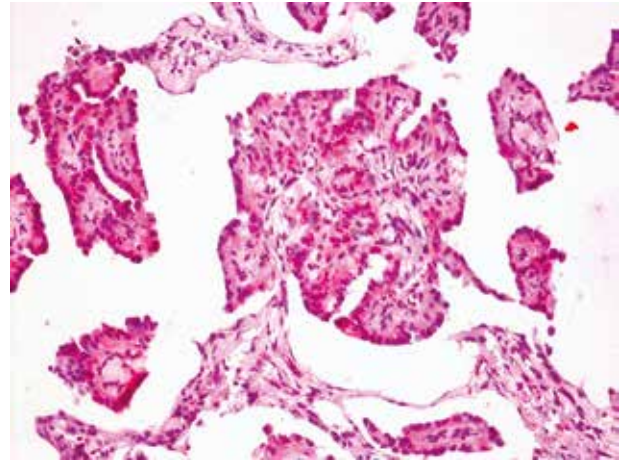


Fig. 3. PTEN +1 positive staining 400× high field power
NOTE: The preparations stained with PTEN, mTOR and p85 antibodies were examined using a light microscope at a magnification of 400×. Positive staining was considered as nuclear staining for PTEN, cytoplasmic and membranous staining for mTOR and cytoplasmic staining for p85. PTEN, mTOR and p85 expressions of the tumour cells were graded between 0 and 3. No staining in tumour cells was considered as 0, whereas 1–10%, 10–50% and > 50% stainings were evaluated as negative, +1, +2 and +3, respectively

Table 1. Demographic and pathological characteristics of the patients

	Papillary carcinoma 81.2% (n = 82)	Follicular carcinoma 18.8% (n = 19)
Gender	Male: 72.7% (n = 16) Female: 83.5% (n = 66)	Male: 27.3% (n = 6) Female: 16.5% (n = 13)
Mean age (age ± SD)	45.32 ±12.7	45.74 ±12.04
LVI	13 (68.4%)	6 (31.6%)
Capsule invasion	12 (54.5%)	10 (45.5%)
Multifocality	24 (88.9%)	3 (11.1%)
Hashimoto Thyroiditis	20 (87%)	3 (13%)
Stage	I: 56 (68.3%) II: 19 (23.2%) III: 3 (4.9%) IV: 2 (3.7%)	I: 11 (55%) II: 6 (30%) III: 1 (5%) IV: 3 (10%)

Table 2. Expression rates of PTEN, m TOR and p85

Expression rate	PTEN; n (%)	mTOR; n (%)	P85; n (%)
0/1+	30 (29.7)	3 (3)	26 (25.7)
2+	25 (24.8)	22 (21.8)	30 (29.7)
3+	46 (45.5)	76 (75.2)	45 (44.6)

categorical measurements were expressed as the number and the percentage, while the numerical measurements were expressed as the mean and the standard deviation. Categorical measurements were compared between the groups using the χ^2 test. Disease-free survival and PTEN, mTOR and PI3K-p85 expression were examined using the Log-Rank test with Kaplan-Meier analysis.

Results

This study included 101 patients. Of these patients, 81.2% (n = 82) suffered from PTC and 18.8% (n = 19) suffered from FTC. The mean age of the patients (mean age ± SD) was 46.3 ±12.1 years. Of these 101 patients, 78.2% (n = 79) were women and 21.8% (n = 22) were men. The demographic and pathologic characteristics of the

patients are presented in Table 1. Of the patients, 89.1% (n = 90) had undergone total thyroidectomy and 10.9% (n = 11) had undergone partial thyroidectomy. Neck dissection was performed on 10.9% (n = 8) of the patients. In 5% of the patients (n = 5), lymph node involvement was detected. RAI treatment was administered to all patients except one, and only one patient received sorafenib treatment.

The normal thyroid tissue (areas near the tumour tissue lacking cancer cells) of all 101 patients was assessed for all of the biomarkers, and no expression was detected in the normal tissue. Twenty-three patients who suffered from HT were also analysed, and no staining for PTEN, PI3K-p85 or mTOR was detected. The rates of expression of PTEN, mTOR and p85 are presented in Table 2.

The tumour size (mean diameter (cm) ± SD) and the expression of PTEN, mTOR and p85 were compared. No statistically significant association was detected between these groups ($p = 0.439$, $p = 0.818$ and $p = 0.584$, respectively).

There was no significant correlation between LVI or CI and PTEN expression, whereas a significant correlation was found between multimodality and PTEN expression. Level 3 PTEN expression was more frequent in patients suffering from thyroid cancer with multifocality ($p = 0.04$) (Table 3). No statistically significant correlation was found between mTOR expression and LVI, CI or multifocality ($p = 0.392$, $p = 0.65$ and $p = 0.156$, respectively). Although there was no statistically significant correlation between multifocality and p85 expression, a statistically significant correlation was found between LVI and p85. The patients that expressed high levels of p85 exhibited less LVI ($p = 0.048$), i.e. there was an inverse correlation. Of the 22 patients exhibiting CI, 31.8% (n = 7) had a score of 1 or lower, 45.5% (n = 10) had a score of 2, and 22.7% (n = 5) had a score of 3,

Table 3. Comparison of PTEN, mTOR and p85 by pathological characteristics

Expression rate	LVI, n (%)		p	Multifocality, n (%)		p	CI, n (%)		p	
	+	-		+	-		+	-		
PTEN	0/1+	5 (26.3)	25 (30.5)	0.39	9 (33.3)	21 (28.4)	0.04	6 (27.3)	24 (30.4)	0.778
	2+	7 (36.8)	18 (22)		2 (7.4)	23 (31.1)		9 (40.9)	16 (20.3)	
	3+	7 (36.8)	39 (46.7)		16 (59.3)	30 (45.5)		7 (31.8)	39 (49.4)	
mTOR	0/1+	0	3 (3.7)	0.39	0	3 (4.7)	0.15	0	3 (3.8)	0.65
	2+	6 (31.6)	16 (19.5)		9 (33.3)	13 (17.5)		5 (22.7)	17 (21.5)	
	3+	13 (68.4)	63 (76.8)		18 (66.7)	58 (78.4)		17 (77.3)	59 (77.3)	
P85	0/1+	4 (21.1)	22 (26.8)	0.04	10 (38.5)	16 (21.6)	0.29	7 (31.8)	19 (24.1)	0.056
	2+	10 (52.6)	20 (24.4)		17 (22.7)	23 (31.1)		10 (45.5)	20 (25.3)	
	3+	5 (26.3)	40 (48.4)		17 (22.7)	35 (47.3)		5 (22.7)	40 (50.6)	

for p85. Of the 79 patients lacking CI, 50.6% (n = 40) had a score of 3 for p85 expression (p = 0.056) (Table 3).

During follow-up, recurrence was detected in only three patients. Of these three patients experiencing recurrence, two (66.7%) exhibited local recurrence and one (33.3%) exhibited distant metastasis. The expressions of PTEN, mTOR and p85 and the rate of disease-free survival (DFS) were evaluated, but because all of the patients remained alive, the overall survival data could not be provided. In these three patients who experienced recurrence, no statistically significant correlation was observed for PTEN, mTOR or p85 staining (p = 0.889, p = 0.758 and p = 0.76, respectively) (Figs. 4–6). Recurrence was not found in any of the patients in whom K-ras mutation was analysed. DFS analysis was performed based on the expression levels of PTEN, mTOR and p85.

K-ras mutation: In 34.7% (n = 35) of the 101 patients, K-ras mutation was impossible to analyse due to technical issues. Although 57.4% of the patients (n = 58) lacked any mutation, 7% (n = 8) exhibited a mutation. Among the

patients in whom a mutation was detected, six patients (5.9%) exhibited a mutation in the 12th codon of exon 1, one patient (1%) exhibited a mutation in the 13th codon of exon 1 and one patient (1%) exhibited a mutation in the 61st codon of exon 2. Of the patients who exhibited a K-ras mutation in the 12th codon of exon 1, one patient (1%) displayed a substitution from glycine to alanine, three patients (3%) displayed a substitution from glycine to valine, one patient (1%) displayed a substitution from glycine to arginine and one patient (1%) displayed a substitution from glycine to cysteine. When KRAS mutation was compared by sex, none of the male patients (n = 20) exhibited a mutation, whereas 17.4% of the female patients (n = 8) exhibited a mutation. There was a statistically significant difference between these groups (p = 0.047). When evaluated based on histological subtypes, 84.9% of the patients suffering from PTC lacked any mutation (n = 45), whereas 15.1% (n = 8) exhibited a mutation. All of the patients suffering from FTC (n = 13) lacked any mutation. No statistically significant difference was found between these groups (p = 0.154).

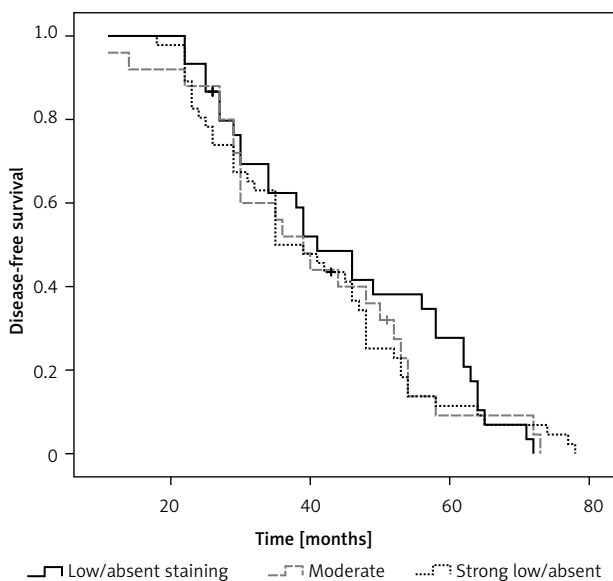


Fig. 4. PTEN expression and disease-free survival
Disease-free survival was found to be 44.8 months (95% CI: 38.9–50.7) in patients with PTEN expression 1(+)/(-), 40.6 months (95% CI: 34.1–47.1) in patients with PTEN expression 2(+) and 40 months (95% CI: 36.08–45.2) in patients with PTEN expression 3(+) (p = 0.779)

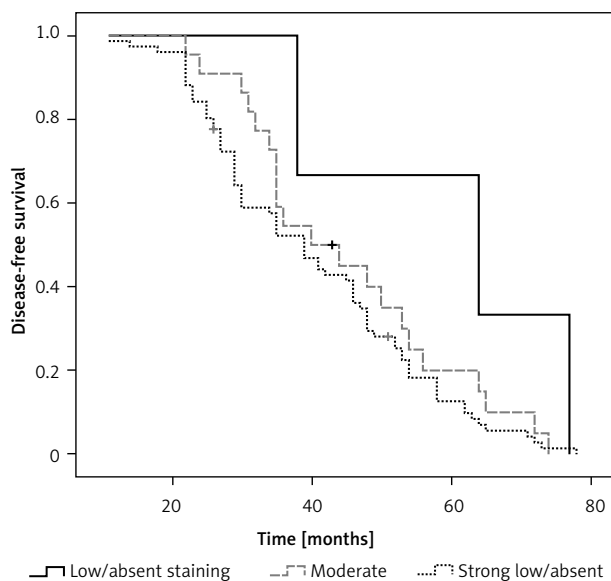


Fig. 5. mTOR expression and disease-free survival
Disease-free survival was found to be 59 months (95% CI: 37.1–82.1) in patients with mTOR expression 1(+)/(-), 45 months (95% CI: 38.5–51.6) in patients with mTOR expression 2(+) and 40 months (95% CI: 36.7–43.9) in patients with mTOR expression 3(+) (p = 0.155)

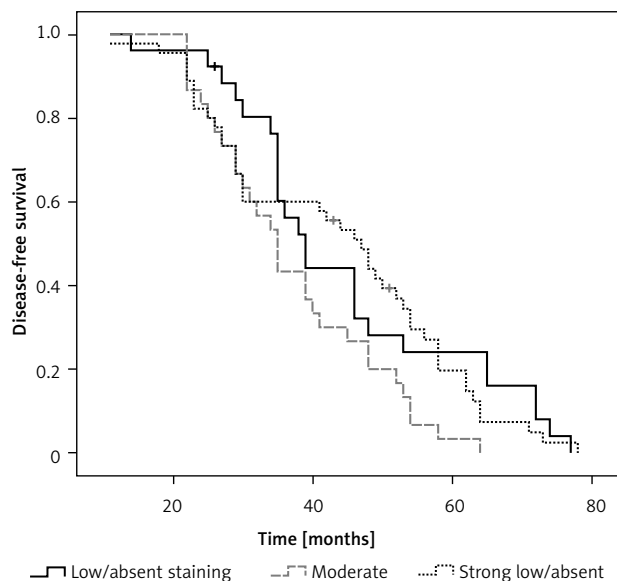


Fig. 6. p85 expression and disease-free survival
Median disease-free survival was found to be 44.6 months (95% CI: 37.9–51.3) in patients with p85 expression 1(+)/(–), 37.1 months (95% CI: 32.8–41.5) in patients with p85 expression 2(+) and 43.5 months (95% CI: 38.4–48.6) in patients with p85 expression 3(+) ($p = 0.043$)

KRAS mutations were evaluated based on tumour size, LVI, CI and multifocality. No statistically significant difference was found between these groups ($p = 0.707$, $p = 0.520$, $p = 0.317$ and $p = 0.418$, respectively) (Table 4).

Discussion

The understanding of specific factors in the signalling pathways involved in TC provide opportunities for targeted therapies [1, 14–17]. Previously, these signalling pathways involved in TC have frequently been immunohistochemically investigated in rat and cancer cell culture models. In a study conducted by Kemmochi *et al.* [18], phosphorylated inactivated PTEN and active Akt isoforms were immunohistochemically evaluated using capsulated invasive follicular cell-derived carcinomas in rats. The expression of inactive PTEN and activated Akt isoforms, elements of the PI3K pathway, was found to be higher in capsular carcinomas compared to parenchymal carcinomas [18]. In a study conducted by Sozopoulos *et al.*, the mutational status and the expression of mediators of the PI3K/Akt pathway were examined in PTC both genetically and immunohistochemically. Although these genetic alterations primarily manifest as phosphorylation and activation of Akt, increased

active phosphorylated (p)-Akt was not detected using immunohistochemical methods [19].

Cowden syndrome is important because of the demonstrated role of the PI3K/Akt signalling pathway in the development of FTC. In TCs, especially FTC and ATC, somatic PTEN mutations may occur [16, 20, 21]. In TC, loss of heterozygosity or gene deletion were frequently detected in the PTEN gene [22]. Bruni *et al.* [23] demonstrated that PTEN inactivation played a role in thyroid carcinogenesis and that PTEN suppressor activity was mediated by the cyclin-dependent kinase inhibitor p27^{kip1}. In our study, when we examined PTEN expression immunohistochemically for loss of PTEN from the tissue, such a result might be caused by various mutations likely to occur in this gene. We did not detect a significant difference in PTEN expression between patients suffering from PTC or FTC ($p = 0.159$).

PI3K-p85, mTOR and PTEN, constituents of the PI3K pathway, are highly expressed in HT and do not exhibit any expression in normal thyroid cells. Larson *et al.* [24] reported the expression of components of the PI3K pathway after immunohistochemical evaluation of HT and PTC thyroid tissue and normal thyroid tissue. While p-Akt, Akt1 and Akt2, components of the PI3K pathway, were highly expressed in HT and TC cells, they did not detect any expression in normal follicular cells [24, 25]. No expression in normal thyroid tissue or HT tissue was detected in this study.

These factors act in concert in the pathway of carcinogenesis, and a loss of PTEN causes Akt activation. It was demonstrated that in mice, the thyroid gland proliferation that developed due to the PTEN deficiency was associated with mTOR activity [4]. mTOR, which is activated by PI3K and causes the proliferation of various cells, is an important effector of this pathway [25]. In another study performed by Yeager *et al.* [26], although the loss of PTEN was found to cause continuous autonomous and homogeneous growth of the hyperplastic gland and the follicular adenoma in thyroid follicular cells in mice, it was also revealed that chronic PI3K/Akt activation led to mTOR/S6K1 activation [11, 26].

Loss of PTEN and activation of the PI3K/Akt pathway are also associated with tumour growth, aggressiveness and poor prognosis [27]. In our study, we examined the clinical and prognostic characteristics as well as the expression of these factors. In previous studies, including animal studies, Akt1 activation was demonstrated to cause tumour invasion in PTC and FTC. In animal studies, PI3K/Akt activation was found to play an important role in the development of FTC-related metastases [28]. It is understood that in TCs, CI and distant metastasis are important

Table 4. Comparison of Kras mutation by pathological characteristics

		K-ras		p
		wild	mutant	
LVI	yes 21.2% (n = 14)	19.6% (n = 13)	1.6% (n = 1)	0.52
	no 78.8% (n = 52)	68.1% (n = 45)	10.7% (n = 7)	
Capsule invasion	yes 27.3% (n = 18)	25.7% (n = 17)	1.6% (n = 1)	0.317
	no 72.7% (n = 48)	61.8% (n = 41)	10.9% (n = 7)	
Multifocality	yes 72.7% (n = 48)	62% (n = 41)	10.7% (n = 7)	0.418
	no 27.3% (n = 18)	24.2% (n = 16)	3.1% (n = 2)	

parameters of unresponsiveness to RAI therapy, disease progression and mortality. Multiple studies are being performed to investigate several factors that affect the function of Akt, particularly Akt1, in tumour invasion and metastasis [28, 29].

A statistically significant association was found between p85 expression and LVI in this study ($p = 0.048$). A borderline correlation was detected between p85 expression and CI ($p = 0.056$). A significant correlation was found between PTEN expression and multifocality ($p = 0.04$).

RAS activates both the MAPK and PI3K/Akt pathways. Ras mutation, which was most commonly detected in TC, includes a N-Ras mutation in codon 61, which has been observed in other studies. H-Ras and N-Ras mutations in codon 61 or codon 12/13 were more rarely detected [13, 16, 20, 21, 30]. However, there are also some studies that demonstrated that K-Ras mutation was the most commonly detected mutation in TC [31, 32]. In the study performed by Ricarte-Filho *et al.* [33], the most commonly detected mutated isoform in poorly differentiated TC (PDTC) was N-ras [33]. In thyroid cancer, its prevalence is approximately 20–40% [6]. In other studies, this rate varied. Based on several studies, the prevalence rate ranges from 0–50% in PTC and from 14–62% in FTC [31, 33]. In the study performed by Ricarte-Filho *et al.*, it was found that in PDTC, RAS mutations were more common compared to BRAF mutations (12% vs. 44%, $p = 0.002$) [33]. In our study, we determined the mutation rate of K-RAS to be 17.4%.

In some studies, Ras mutations were commonly detected in the follicular variant of FTC and PTC, in which PI3K/Akt pathway serves as the main signalling pathway [34–36]. Especially for FTCs, the primary activator of PI3K/Akt pathway is a Ras mutation. In some studies, a good correlation was demonstrated between Ras mutation and Akt phosphorylation in TC [22, 31]. All of our patients who exhibited a K-Ras mutation suffered from PTC. None of the patients suffering from FTC exhibited any K-Ras mutation. These results highlight the role of mutations of K-ras, a member of the Ras subfamily, in the development of PTC.

Some studies demonstrated that RAS mutation was a poor prognostic factor in the course of TC and was associated with a more aggressive course [33, 37]. In the study performed by Rivera *et al.*, TC patients exhibiting a Ras mutation were found to have a better prognosis, and the median survival duration of these patients was 6.6 years [38].

RAS mutation has been correlated with histological characteristics, including loss of differentiation in the tumour and clinico-pathologic parameters that indicate tumour aggressiveness, including tumour size and vascular invasion [33]. Garcia-Rostan *et al.* concluded that RAS mutations correlated with aggressive tumour behaviour and poor prognosis and that they could be used as prognostic determinants in their study [31].

In our study, a correlation was detected only between sex and Kras mutation. All of the mutations were detected in women ($p = 0.047$). Despite the demonstration of this correlation, which had not been previously reported, we believe that the inequality of our patient population with respect to sex, such that females comprised a 69.7% majority of the total patient population, affected this result.

Concomitance of the physiological manifestations of the RAS mutation and PTEN down-regulation has been demonstrated to cause TC in transgenic mouse models [37]. Therefore, in our study, we examined more than one parameter that was involved in the same signalling pathway, but we could not detect any correlation between these various parameters and the subtype, the pathological features or the clinical and prognostic characteristics of the tumour. We did not detect any association between KRAS mutation and tumour size, disease stage, histological subtype, LVI, CI or multifocality ($p = 0.707$, $p = 0.68$, $p = 0.368$, $p = 0.52$, $p = 0.317$ and $p = 0.418$, respectively).

Surgery remains the primary treatment modality for PTC. RAI and thyroid hormone suppression often complement the treatment plan. Although thyroid hormone suppression may decrease the incidence of disease recurrence and RAI may treat metastases, lymph node dissection (LND) is the typical therapeutic strategy for clinically evident cervical lymph node metastases [39, 40]. Specific inhibitors of the PI3K/Akt signalling pathway develops against several target molecules involved in this pathway may be important therapeutics for the treatment of thyroid cancer (inhibition of Akt phosphorylation using perifosine or inhibition of mTOR inhibition using temsirolimus) [41]. A specific therapy for oncogenic Ras mutations in thyroid cancer is still lacking. It was found that, in animal models, combined treatment with MEK and PI3K/AKT/mTOR pathway inhibitors was more efficient than monotherapy [33]. In the clinical studies performed, farnesyl transferase inhibitors targeted mutant RAS, which could, in future, be considered as a therapeutic for the treatment of poor and undifferentiated TCs [31].

In conclusion, these results are important for two reasons. First, these biomarkers have prognostic and perhaps therapeutic importance. In particular, p85 was the most relevant biomarker of those examined in this study for predicting DFS. The other interesting result of this study is that a statistically significant difference in K-ras mutation was detected between men and women.

The authors declare no conflict of interest.

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