Original Article

Mutational Profile of Papillary Thyroid Carcinoma in an Endemic Goiter Region of North India

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Abstract

Introduction: Mitogen activated protein kinase (MAPK) pathway is regularly altered in papillary thyroid carcinomas (PTCs). Serine/threonine-protein kinase B-Raf (BRAF) V600E mutations were observed very frequently in PTC along with less frequent rat sarcoma (RAS) and rearranged during transfection (RET) gene, also known as RET/PTC translocation. The present study aimed to analyze the mutational profile of PTCs from an endemic Goiter area of North India. **Methodology:** Tissues from 109 PTC patients were used to isolate DNA and RNA. BRAF V600E was detected by restriction fragment length polymorphism-polymerase chain reaction (PCR). RAS mutations were screened by using Sanger's sequencing method. RET/PTC rearrangements were analyzed by real-time PCR. **Results:** BRAF V600E mutation was detected in 51.38% (56/109) of PTCs, whereas RAS mutations were less frequent. No RET/PTC rearrangements were observed. BRAF V600E was found to be associated with the aggressive clinicopathological features such as lymph node metastasis, distant metastasis, higher tumor-node-metastasis stages, and high-risk groups. **Conclusion:** The prevalence of BRAF V600E is high in patients from Indian Subcontinent and found to be associated with aggressive features of PTC. Concomitant mutations of BRAF V600E and RAS mutations impart more aggressiveness to PTCs.

Keywords: BRAF V600E, papillary thyroid carcinoma, RAS mutations

Introduction

Thyroid cancer is the most common malignancy of endocrine system and its prevalence and detection continue to rise.[1,2] However, it has a wide range of clinicopathologic behavior ranging from indolent papillary thyroid carcinoma (PTC) to lethal anaplastic thyroid carcinoma. Recent studies have conclusively established that thyroid tumorogenesis, and progression is initiated and driven by specific genetic alterations.[3-5] Among the thyroid carcinomas, most prevalent type worldwide is PTC.[6] Most of the studies at molecular level have revealed that genes in MAP kinase pathway are frequently altered in PTCs,[7,8] especially mutations in RAF (BRAF) and RAS (KRAS) gene family.[9-15] Around 5% of PTCs also harbor RET/PTC translocations.[16-18] Among all genetic alterations detection of a BRAF activating point mutation which has been found to be very frequent.[14] This point mutation results from a thymine to adenine transversion at 1799 position (T1799A) in exon 15 which leads to valine to glutamine substitution at residue 600 (V600E).[19] BRAF V600E subsequently activates the BRAF by continuous phosphorylation. BRAF is the strongest activator of MAP kinase signaling pathway resulting in continuous cell proliferation.[20] However, the prevalence of BRAF V600E mutation in PTC has varied from 25% to 86%.[10,14,21-24] The second uncertainty is about the association of BRAF V600E with the biological behavior of PTC. Although systematic reviews have concluded that BRAF V600E harboring PTC were associated with increased prevalence of aggressive features such as lymph node metastasis, extrathyroidal extension, distant metastasis, recurrence and higher tumor stages.[25,26] Few single institutional studies did not support this argument.[27] The third uncertainty is the correlation of BRAF V600E with the histological subtype of PTCs. The spectrum of PTC includes aggressive subtypes such as tall cell variant (TCV), columnar cell variant, and diffuse sclerosing variants.[28]

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Thus the aim of the study is to establish the overall prevalence of genetic alterations in MAP kinase pathway (BRAF V600E, RAS mutations, and RET/PTC translocations) in an endemic goiter population of North India and to explore the correlation of BRAF V600E mutations with the aggressive features of PTC. Further to access the association of BRAF V600E with histological subtypes of PTC.

Methodology

Sample selection and storage

This study was conducted in a tertiary medical center. All samples were taken from a period of 14 years (2000–2014). A total of 109 patients were selected on availability of tumor tissue from a total of 574 PTCs operated. A total of 12 nontumorous tissues were taken from the excised mass, and 109 tumor tissue samples were collected from the surgically removed thyroid mass in the surgery. All these tissues had been directly frozen using liquid nitrogen and were further stored in deep freezer (−80°C) in vials for RNA isolations. The formalin-fixed paraffin-embedded (FFPE) tissue samples were histologically confirmed as PTCs by the pathologists. FFPE tissue from tumor sections was used for DNA isolation. Informed consent was obtained from all cases before surgery. This study was approved by the Institutional Ethical Committee (IEC code: 2012–172-EMP-66).

Clinicopathological parameters

Hospital information system was used to collect the patient's clinical and laboratory details. Histopathological details were reviewed by pathologist. The tumor was subgrouped on the basis of lymph node metastasis, size of the tumor, extrathyroidal extension, distant metastasis, and recurrence. All samples were further classified by tumor-node-metastasis classification of American Joint Committee on Cancer, 8th edition from stage 1–4.

Screening for BRAF V600E, RAS mutations

The hematoxylin and eosin-stained slides were reviewed for maximum tumor area and marked on the slide. Four sections of 10-micron thickness were cut from the FFPE issue blocks. The tumor area was scraped and collected in a microcentrifuge tube, and then, DNA was extracted using QIAamp FFPE tissue kit (Qiagen, Germany). The quality and quantity of the DNA was measured by the NanoDrop 2000c (Thermo Fisher Scientific, US). BRAF V600E and RAS mutation were also analyzed in 12 cases of nontumor thyroid tissue.

Polymerase chain reaction-restriction fragment length polymorphism and Sequencing for BRAF V600E

BRAF exon 15 was amplified by polymerase chain reaction (PCR) using the following primers: Forward 5'GCTTGCTCTGATAGGAAAATGAG3'; reverse5'GA TACTCAGCAGCATCTCAGG-3'. PCRs conditions were Initial denaturation at 95ºC for 5 min, followed by 40 cycles of denaturation at 94ºC for 20 s, annealing at 56ºC for 20 s, and elongation at 72ºC for 20 s, and a final extension at 72ºC for 10 min.

The denatured PCR products were electrophoresed and digestion of the 237-base pair (bp) PCR fragment with restriction endonuclease TspRI yielded 3 major bands of 117 bp, 87 bp, and 33 bp for the wild-type allele [Figure 1a and b]. The BRAF V600E mutation abolished the restriction sites, resulting in a prominent band of 237 bp from the mutant allele and residual bands from the normal allele.[29] The samples which showed a band of 237 bp indicating BRAF mutation were confirmed by Sanger sequencing using Applied Biosystems 3500 genetic analyzer [Figure 1c].

RAS mutation screening

Point mutations in codons 12/13 of the H-RAS, K-RAS, and codon 61 of N-RAS genes were analyzed by Sanger sequencing method DNA isolated from the FFPE tissue was amplified for KRAS, HRAS, and NRAS using following PCR primers; codon 12/13 KRAS-A (5′-GGCCTGCTGAAAATGACTGA-3′) and D (5′-TAGCTGTATCGTCAAGGCAC-3′), codon 12/13 of HRAS 5-TGA GGA GCG ATG ACG GAA-3 and 5-GCG CTA GGC TCA CCT CTA T-3, codon 61 NRAS; 5′-CCT GTT TGT TGG ACA TAC TG-3 and 5′-CCT GTA GAG GTT AAT ATC CG-3′ were used for the sequencing.[30,31]

RET/papillary thyroid carcinoma rearrangement

Total RNA was isolated from the tissue samples using TRizol reagent (Thermo Fisher Scientific, US). Quantity and quality were measured by NanoDrop 2000c (Thermo Fisher Scientific, US). Further complementary DNA (cDNA) was synthesized using RevertAid First strand cDNA synthesis kit (Thermo Fisher Scientific, US). Primers and Taqman probe for RET/PTC1 were used as described elsewhere.[32]

Statistical analysis

Categorical variables were analyzed using Fisher's exact probability test and Chi-square test (Graph pad prism 6, GraphPad Software Incorporation, California, USA). $P < 0.05$ was considered statistically significant.

Results

Clinicopathological features of papillary thyroid carcinomas

One hundred and nine cases were randomly selected from a total of 574 operated PTC cases from the period of 14 years. This 109 cases of PTC included 75 cases of classical PTCs, 28 cases of follicular variant of PTC (FVPTC), 5 cases of TCV, and one case of oncocytic variant of PTC [Table 1]. All the patients were subcategorized on the basis of clinicopathological features such as age, sex, size of the tumor, lymph node metastasis, distant metastasis, and recurrence of the tumor.

The occurrence of PTC in the age group above 45 was slightly higher (54.13%) than age group below 45 and 60.55% of patients with PTC were female. Out of 109 patients, 39 patients had lymph node metastasis (35.78%). Extrathyroidal extension was found in 17 patients (15.60%), and 20 of the patients were detected with distant metastasis (18.35%). About 45% of patients with distant metastasis were detected

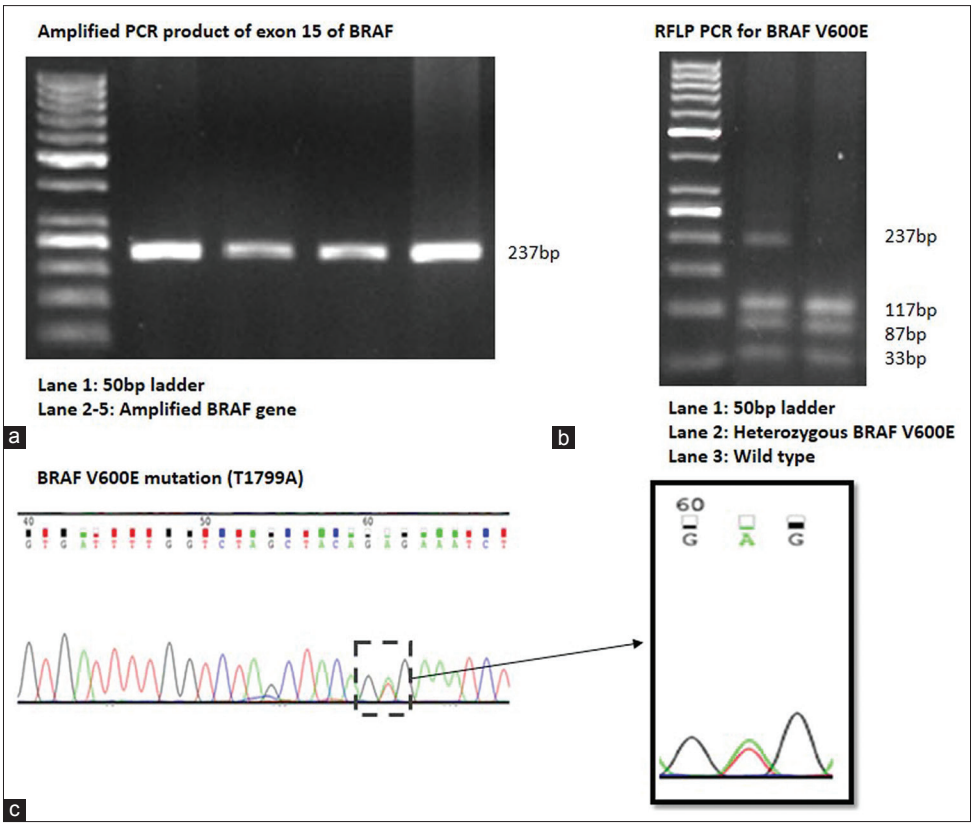

Figure 1: (a) Representative AGE image of polymerase chain reaction product of BRAF gene (b) polymerase chain reaction-restriction fragment length polymorphism of the wild-type and mutant heterozygous BRAF gene. (c) The sequencing result shows a mixture of T and A at the 1799 site

on the initial stages of diagnosis, and 55% were detected later on follow-up. Further these patients were classified on the basis of American Thyroid Association (ATA) staging into Stage 1, 2, 3, and 4. Stage 1 and 2 together accounted for 70/109 patients (64.2%). Stage 3 and 4 together accounted for 39/109 patients (35.8%) who showed aggressive behavior. Further, these patients were stratified into three risk categories based on ATA 2009 guidelines, 42 (38.53%) were included in low risk, whereas 17 (15.60%) patients were in intermediate-risk group. High-risk category contained 50 (45.87%) patients.

Mutational profile of papillary thyroid carcinoma

BRAF mutations

BRAF V600E mutation was detected in 51.38% (56/109) of PTC patients, whereas none of the normal thyroid tissue showed BRAF mutations. Classical PTC and tall-cell variant were detected with higher BRAF V600E mutations (54.7% and 60%, respectively), whereas FVPTC showed 40% of BRAF V600E mutations. The only case of oncocytic variant of PTC did not show any BRAF mutation [Table 1].

RAS mutations

Eight NRAS mutations (7.34%), four each of Q61R and Q61K [Figure 2a and b], were detected in 109 samples. No mutations were found in HRAS and KRAS genes. NRAS mutations were found in four cases each of FVPTC (14.29%; 4/28 cases) and classical PTCs (5.71%; 4/70 cases).

Table 1: Mutational profile of papillary thyroid carcinomas

Number of PTC samples (*n*=109)	BRAF V600E (%)	RAS mutations (%)	RET/PTC
Classical PTC (*n*=75)	41 (54.67)	4 (5.71)	0
Follicular variant of PTC (*n*=28)	12 (42.86)	4 (14.29)	0
Tall-cell variant (*n*=5)	3 (60)	0	0
Oncocytic variant of PTC (*n*=1)	0	0	0
Total (*n*=109)	56 (51.38)	8 (7.34)	0

PTC: Papillary thyroid carcinoma

RET/papillary thyroid carcinoma translocations

No RET/PTC 1 and RET/PTC 3 translocations were detected in any of the PTC cases.

Correlation between mutational profile and clinicopathological features

BRAF V600E was found to be more common than NRAS mutation in PTCs. RAS mutations did not show any correlation with any of the clinicopathological features. Increased incidence of BRAF V600E mutation was found to be associated with age ≥45 years group, lymph node metastasis, distant metastasis, and higher stages (stage 3 and 4). RET/PTC translocations were not detected in any case. BRAF mutation status was correlated with age, gender, lymph node metastasis,

extrathyroidal extension, distant metastasis, clinical stage, and risk groups [Table 2]. BRAF V600E mutations were significantly found higher ($P = 0.0273$) in patients with lymph node metastasis and age group above 45 years ($P = 0.0209$). Although gender and extrathyroidal extension had no significant association with BRAF mutation, patients with distant metastasis were found to be significantly associated with BRAF mutation ($P = 0.0059$).

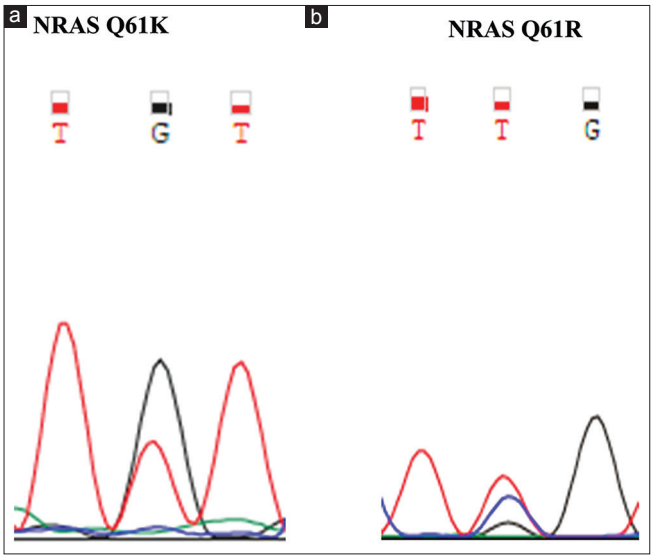

Figure 2: Results of N RAS (a) N RAS Q61K mutation (b) N RAS Q61R mutation

As the disease progressed to stage 3 and 4, more number of patients (74.36%) were detected with BRAF V600E mutations ($P = 0.0006$). There was an increase in the frequency of BRAF V600E mutation in high-risk group of disease than the intermediate- and low-risk groups ($P = 0.036$). Thus, in summary, three factors of clinically aggressive PTCs such as age ≥45 years, lymph node metastasis, and distant metastasis showed significant association with BRAF V600E mutation.

We found that three patients had concomitant BRAF V600E and NRAS mutations. Further when the clinicopathological features of these three cases were analyzed, all the patients had at least one of the aggressive features. All patients were above 45 years age. Lymph node metastasis was observed in all the patients. Extrathyroidal extension was found in two of the patients. Two of the patients were detected with distant metastasis and one among them had extrathyroidal extension. No recurrence occurred in any of the patient. All cases had undergone total thyroidectomy and high-dose radioiodine therapy.

Discussion

Molecular profiling has become an important and effective approach to understand the pathogenesis of thyroid cancers. Most of the previous studies found that MAP kinase pathway is frequently altered in PTCs, especially BRAF, RAS, and RET/PTC gene alterations which activate the MAP kinase pathway. Thus, these genetic alterations lead

Table 2: BRAF V600E and clinicopathological features of papillary thyroid carcinoma

Particulars	(*n*=109), *n* (%)	BRAF positive (%)	BRAF negative (%)	*P*[a]
Age (years)				
<45	59 (54.13)	24 (40.67)	35 (59.33)	0.0209
≥45	50 (45.87)	32 (64)	18 (36)	
Gender				
Male	43 (39.45)	25 (58.14)	18 (41.86)	0.3273
Female	66 (60.55)	31 (46.97)	35 (53.03)	
Lymph node metastasis				
Present	39 (35.78)	26 (66.67)	13 (33.33)	0.0273
Absent	70 (64.22)	30 (42.86)	40 (57.14)	
Extrathyroidal extension				
Present	17 (15.60)	11 (64.70)	6 (35.30)	0.2946
Absent	92 (84.40)	45 (48.91)	47 (51.09)	
Distant metastasis				
Present	20 (18.35)	16 (80)	4 (20)	0.0059
Absent	89 (81.65)	40 (44.94)	49 (55.06)	
Stage				
1 and 2	70 (64.22)	27 (38.57)	43 (61.43)	0.0006
3 and 4	39 (39.45)	29 (74.36)	10 (25.64)	
Recurrence				
Present	24 (22.02)	16 (66.67)	8 (33.33)	0.1085
Absent	85 (77.98)	40 (47.06)	45 (52.94)	
Risk				
Low	42 (38.53)	14 (33.33)	28 (66.67)	0.0076[b]
Intermediate	17 (15.60)	9 (52.94)	8 (47.06)	
High	50 (45.87)	33 (66)	17 (34)	

[a]Two-sided Fisher's exact test was performed. [b]Chi-square test was performed

to uncontrolled cell proliferation and tumor formation.[20] Among the abovementioned three mutations, BRAF V600E mutations are the most frequent mutations in PTC.[23,24] The prevalence of BRAF V600E around the world range from 25% to 87% with comparatively higher rate of occurrence in Asian countries.[10,14,21-24] A recent study by Zhang *et al*. in Chinese population found a high rate of BRAF V600E mutations (86.5%).[24] Another study conducted in 688 patients by Park *et al*. in Korean population detected 69.2% BRAF V600E mutations.[23] Nakayama *et al*. detected 65% BRAF V600E in a series of study in Japanese population.[22] In Indian subcontinent, Khan *et al*. conducted a study in patients from Kashmir and found only 25% of BRAF V600E mutations in 60 patients[21] whereas other two studies by Nair *et al*. and Chakraborty *et al*. detected high frequency of BRAF V600E mutations (around 50%).[27,33] The prevalence of BRAF V600E mutations in our study (51.38%) is in agreement with the studies by Nair *et al*. and Chakraborty *et al*. from Indian subcontinent.

The association between BRAF V600E and clinicopathological factors has been addressed in some multicentric systematic reviews and high-volume single-center studies;[25,26,34] however, there are conflicting reports on the association between BRAF V600E and aggressive clinicopathological features of PTC.[25,26,34,35] Xing *et al*., Li, Carol, *et al*., Kim, Tae Hyuk, *et al*., and Kebebew *et al*. found an association between BRAF V600E and aggressive Trovisco *et al*. found no such association.[28] In agreement with the previous studies,[25,26,34,35] BRAF V600E was found to be significantly correlated with higher age group, lymph node metastasis, distant metastasis, intermediate- and high-risk category, and advanced tumor stage (stage 3 and 4) in our study. There is one study by Chakraborty *et al*. from Indian subcontinent, in which BRAF V600E mutation was found to be associated with lymph node metastasis and extrathyroidal extension.[33] Furthermore, BRAF V600E was found to be associated with PTCs with local and distant spreads ($P = 0.001$) in our study. Thus, BRAF V600E is proven to be a predictor of locoregional and distant metastasis. Our study also supports the argument by Namba *et al*. that PTCs with BRAF V600E mutation are advanced cancers with metastasis.[36]

The prevalence of BRAF V600E mutation varies from one histological subtype from the other. There are few studies on the prevalence of BRAF V600E mutations in histological subtypes of PTC and most of those studies were on FVPTCs.[37] Trovisco *et al*. conducted a study on the prevalence of BRAF V600E mutations in histological subtypes and found 53% of BRAF V600E in cPTCs and 33% in TCVs.[28] The study could not find any BRAF V600E mutations in FVPTC. In contrast, in our study, TCV and FVPTC harbored higher rate of BRAF V600E (60% and 42.86%, respectively) mutations, but the rate of BRAF V600E in cPTC was similar to the previous studies including the abovementioned study.

RAS mutation is an early step in thyroid tumorigenesis.[11] However, mutations in RAS gene are reported less frequently in PTCs.[38] RAS mutations were also found less frequently (7.34%) in our study. To the best of our knowledge, this is the first study on frequency of RAS mutations in Indian subcontinent. Similar to the previous reports[37] around the world, we detected a higher frequency of RAS mutations in FVPTCs than classical PTCs (14.29% vs. 5.71%). However, RAS mutations were not found to be associated with any adverse clinicopathological features in our patient group.

RET/PTC rearrangements are rarely detected in sporadic PTCs (0%–5%).[32] We also did not find any RET/PTC rearrangements in our study.

Another point to be emphasized is that concomitant mutations are likely to impart aggressiveness to PTC.[39] Although we found only three concomitant mutations, but all the three patients presented with advanced disease (two with distant metastasis and one with extrathyroidal extension). Therefore, concomitant RAS and BRAF mutations may require a more aggressive and close follow-up even after total thyroidectomy and radiotherapy.

Conclusion

BRAF V600E is the most common mutation and its prevalence is high in Indian patients with PTC while RAS mutations are less frequent. The present study also provided evidence that BRAF V600E correlates with the aggressive papillary thyroid cancers and concomitant mutations of BRAF V600E and RAS mutations impart more aggressiveness to PTCs.

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Conflicts of interest

Prof. Amit Agarwal received funding for the extramural project from Department of Science and Technology (DST), New Delhi, India (SR/SO/HS-0097/2010 Dt. 19.06.2012).

References

1. Davies L, Welch HG. Increasing incidence of thyroid cancer in the united states, 1973-2002. JAMA 2006;295:2164-7.
2. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: Update on epidemiology and risk factors. J Cancer Epidemiol 2013;2013:965212.
3. Ciampi R, Romei C, Pieruzzi L, Tacito A, Molinaro E, Agate L, *et al.* Classical point mutations of RET, BRAF and RAS oncogenes are not shared in papillary and medullary thyroid cancer occurring simultaneously in the same gland. J Endocrinol Invest 2017;40:55-62.
4. Lee SE, Hwang TS, Choi YL, Kim WY, Han HS, Lim SD, *et al.* Molecular profiling of papillary thyroid carcinoma in Korea with a high prevalence of BRAFV600E mutation. Thyroid 2017;27:802-10.

5. Vuong HG, Altibi AM, Abdelhamid AH, Ngoc PU, Quan VD, Tantawi MY, *et al.* The changing characteristics and molecular profiles of papillary thyroid carcinoma over time: A systematic review. Oncotarget 2017;8:10637-49.
6. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in thyroid cancer incidence and mortality in the united states, 1974-2013. JAMA 2017;317:1338-48.
7. Nikiforov YE. Thyroid carcinoma: Molecular pathways and therapeutic targets. Mod Pathol 2008;21 Suppl 2:S37-43.
8. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, *et al.* Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855-67.
9. Bongarzone I, Pierotti MA, Monzini N, Mondellini P, Manenti G, Donghi R, *et al.* High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma. Oncogene 1989;4:1457-62.
10. Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, *et al.* BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst 2003;95:625-7.
11. Karga H, Lee JK, Vickery AL Jr., Thor A, Gaz RD, Jameson JL, *et al.* Ras oncogene mutations in benign and malignant thyroid neoplasms. J Clin Endocrinol Metab 1991;73:832-6.
12. Namba H, Rubin SA, Fagin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol 1990;4:1474-9.
13. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, *et al.* BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene 2003;22:4578-80.
14. Xu X, Quiros RM, Gattuso P, Ain KB, Prinz RA. High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res 2003;63:4561-7.
15. Ezzat S, Zheng L, Kolenda J, Safarian A, Freeman JL, Asa SL, *et al.* Prevalence of activating ras mutations in morphologically characterized thyroid nodules. Thyroid 1996;6:409-16.
16. Bounacer A, Wicker R, Caillou B, Cailleux AF, Sarasin A, Schlumberger M, *et al.* High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. Oncogene 1997;15:1263-73.
17. Jhiang SM. The RET proto-oncogene in human cancers. Oncogene 2000;19:5590-7.
18. Santoro M, Carlomagno F, Hay ID, Herrmann MA, Grieco M, Melillo R, *et al.* Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. J Clin Invest 1992;89:1517-22.
19. Sapio MR, Posca D, Troncone G, Pettinato G, Palombini L, Rossi G, *et al.* Detection of BRAF mutation in thyroid papillary carcinomas by mutant allele-specific PCR amplification (MASA). Eur J Endocrinol 2006;154:341-8.
20. Mitsutake N, Miyagishi M, Mitsutake S, Akeno N, Mesa C Jr., Knauf JA, *et al.* BRAF mediates RET/PTC-induced mitogen-activated protein kinase activation in thyroid cells: Functional support for requirement of the RET/PTC-RAS-BRAF pathway in papillary thyroid carcinogenesis. Endocrinology 2006;147:1014-9.
21. Khan MS, Pandith AA, Azad N, Hussain MU, Masoodi SR, Wani KA, *et al.* Impact of molecular alterations of BRAF in the pathogenesis of thyroid cancer. Mutagenesis 2014;29:131-7.
22. Nakayama H, Yoshida A, Nakamura Y, Hayashi H, Miyagi Y, Wada N, *et al.* Clinical significance of BRAF (V600E) mutation and ki-67 labeling index in papillary thyroid carcinomas. Anticancer Res 2007;27:3645-9.
23. Park AY, Son EJ, Kim JA, Youk JH, Park YJ, Park CS, *et al.* Associations of the BRAF(V600E) mutation with sonographic features and clinicopathologic characteristics in a large population with conventional papillary thyroid carcinoma. PLoS One 2014;9:e110868.
24. Zhang Q, Liu BJ, Ren WW, He YP, Li XL, Zhao CK, *et al.* Association between BRAF V600E mutation and ultrasound features in papillary thyroid carcinoma patients with and without hashimoto's thyroiditis. Sci Rep 2017;7:4899.
25. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, *et al.* The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: A meta-analysis. Cancer 2012;118:1764-73.
26. Li C, Lee KC, Schneider EB, Zeiger MA. BRAF V600E mutation and its association with clinicopathological features of papillary thyroid cancer: A meta-analysis. J Clin Endocrinol Metab 2012;97:4559-70.
27. Nair CG, Babu M, Biswas L, Jacob P, Menon R, Revathy AK, *et al.* Lack of association of B-type raf kinase V600E mutation with high-risk tumor features and adverse outcome in conventional and follicular variants of papillary thyroid carcinoma. Indian J Endocrinol Metab 2017;21:329-33.
28. Trovisco V, Vieira de Castro I, Soares P, Máximo V, Silva P, Magalhães J, *et al.* BRAF mutations are associated with some histological types of papillary thyroid carcinoma. J Pathol 2004;202:247-51.
29. Park SY, Park YJ, Lee YJ, Lee HS, Choi SH, Choe G, *et al.* Analysis of differential BRAF(V600E) mutational status in multifocal papillary thyroid carcinoma: Evidence of independent clonal origin in distinct tumor foci. Cancer 2006;107:1831-8.
30. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW 2nd, Tallini G, *et al.* RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: Evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab 2003;88:2318-26.
31. Richman SD, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM, *et al.* KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: Results from the MRC FOCUS trial. J Clin Oncol 2009;27:5931-7.
32. Sheils OM, O'Leary JJ, Sweeney EC. Assessment of ret/PTC-1 rearrangements in neoplastic thyroid tissue using taqMan RT-PCR. J Pathol 2000;192:32-6.
33. Chakraborty A, Narkar A, Mukhopadhyaya R, Kane S, D'Cruz A, Rajan MG, *et al.* BRAF V600E mutation in papillary thyroid carcinoma: Significant association with node metastases and extra thyroidal invasion. Endocr Pathol 2012;23:83-93.
34. Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, *et al.* The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann Surg 2007;246:466-70.
35. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, *et al.* Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol 2015;33:42-50.
36. Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, *et al.* Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. J Clin Endocrinol Metab 2003;88:4393-7.
37. Rivera M, Ricarte-Filho J, Knauf J, Shaha A, Tuttle M, Fagin JA, *et al.* Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. Mod Pathol 2010;23:1191-200.
38. Carta C, Moretti S, Passeri L, Barbi F, Avenia N, Cavaliere A, *et al.* Genotyping of an italian papillary thyroid carcinoma cohort revealed high prevalence of BRAF mutations, absence of RAS mutations and allowed the detection of a new mutation of BRAF oncoprotein (BRAF(V599lns)). Clin Endocrinol (Oxf) 2006;64:105-9.
39. Zou M, Baitei EY, Alzahrani AS, BinHumaid FS, Alkhafaji D, Al-Rijjal RA, *et al.* Concomitant RAS, RET/PTC, or BRAF mutations in advanced stage of papillary thyroid carcinoma. Thyroid 2014;24:1256-66.