

## **Papillary Thyroid Cancer: Genetic Alterations And Molecular Biomarker Investigations**

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## **Abstract**

Papillary thyroid cancer (PTC) is the most prevalent form of malignancy among all cancers of the thyroid. It is also one of the few cancers with a rapidly increasing incidence. PTC is usually contained within the thyroid gland and generally biologically indolent. Prognosis of the cancer is excellent, with less than 2% mortality at 5 years. However, more than 25% of patients with PTC developed a recurrence during a long term follow-up. The present article provides an updated condensed overview of PTC, which focuses mainly on the molecular alterations involved and recent biomarker investigations.

Keywords: Papillary thyroid cancer, molecular alteration, genetic signature, biomarker, diagnostics.

## Introduction

Thyroid tumours are now broadly classified as [follicle](#)-derived (thyroid epithelial) neoplasms, other epithelial tumours, non-epithelial tumours and secondary tumours based on pathological, clinical and genetic characteristics [1, 2]. These tumours can be benign, borderline or malignant, depending on their biological behaviour within the body. The follicular adenoma, hyalinising trabecular tumour, encapsulated follicular-patterned thyroid tumours, papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, Hürthle cell tumour, poorly differentiated thyroid carcinoma, anaplastic thyroid carcinoma and squamous cell carcinoma [comprise](#) the major thyroid epithelial neoplasms. Some of the other epithelial tumours found in the thyroid gland include medullary carcinoma, salivary gland-type tumours, mucinous carcinoma and thymic tumours, whilst tumours like paraganglioma, peripheral nerve sheath, vascular, smooth muscle, solitary fibrous and histiocytic tumours, lymphoma and teratoma [fall](#) under the non-epithelial tumours of the thyroid (Figure 1).

The well differentiated thyroid cancer such as follicular and papillary carcinomas [account](#) for 95% of all thyroid cancer cases and are generally associated with a good prognosis and/or survival rate when diagnosed early [3, 4]. On the other hand, the poorly or undifferentiated anaplastic thyroid carcinoma, albeit a rare cancer, is almost always fatal [5]. Among all cancers of the thyroid, papillary thyroid cancer is the most prevalent form of thyroid malignancy. In the present literature review article, we provide an updated condensed overview which focuses mainly on the molecular alterations involved [in tumorigenesis and recent biomarker investigations of PTC](#).

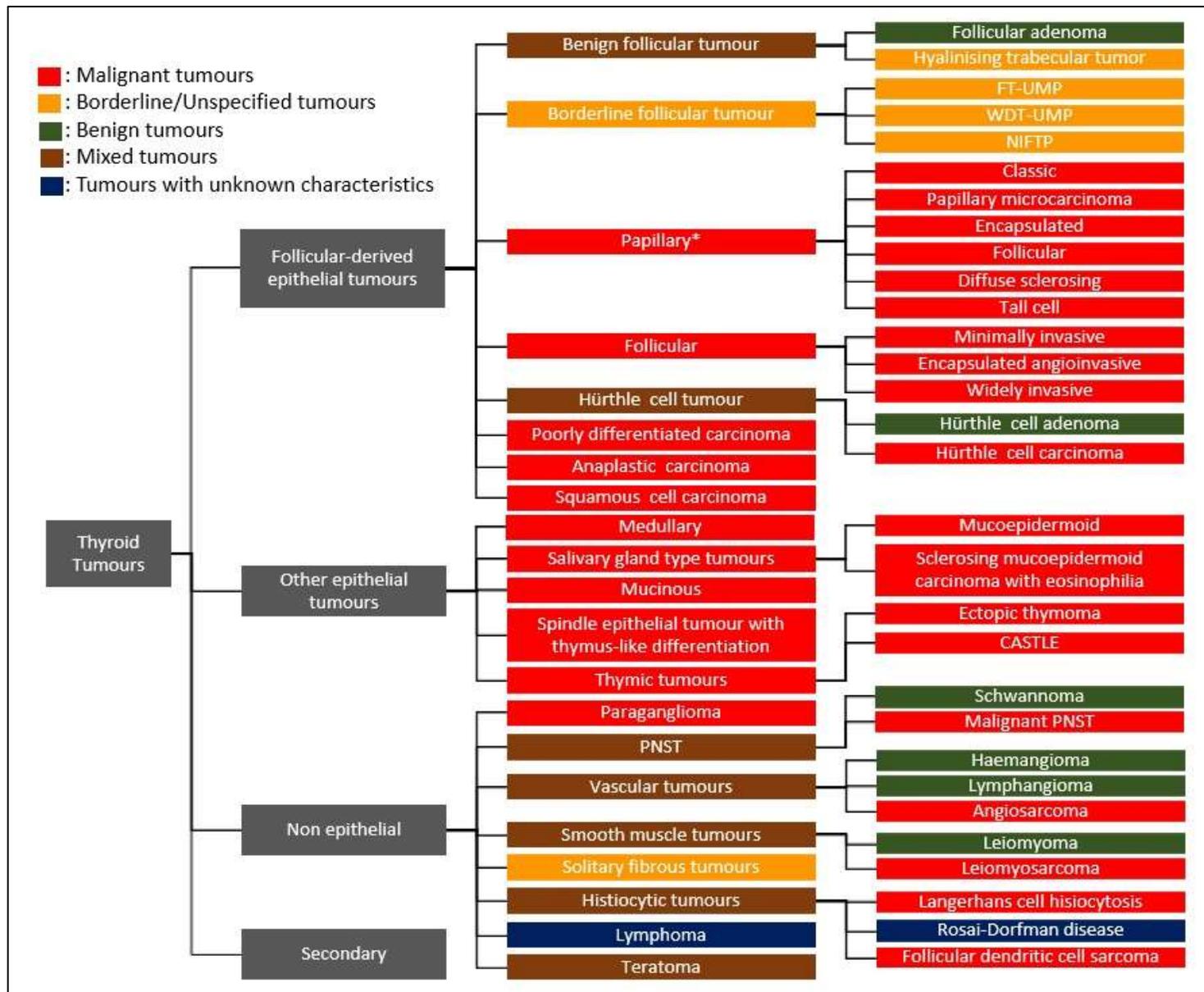


Figure 1 Classification of thyroid tumours. \*There are 15 variants of papillary thyroid carcinoma but only the top 6 listed variants are included in this illustration [1]; FT-UMP, Follicular tumour of uncertain malignant potential; WDT-UMP, Well-differentiated tumour of uncertain malignant potential; NIFTP, Non-invasive follicular thyroid neoplasm with papillary nuclear features; CASTLE, Carcinoma showing thymus-like differentiation/intrathyroid epithelial thymoma; PNSTs, Peripheral nerve sheath tumour.

## **Papillary Thyroid Cancer**

PTC constitutes approximately 80% of all thyroid cancer cases [6]. It is also the most prevalent thyroid cancer subtype in countries having iodine-sufficient or iodine-excess diets [7]. The incidence of PTC is on the rise [8]. The reasons are unclear but may reflect improvements that contribute to earlier detection of the cancer [9]. PTC can occur at any age and has rarely been diagnosed as a congenital tumour [10]. It is usually detected in the third to fifth decades of the patients' life, with the mean age at 40 years. The incidence of PTC increases with age, and women are more frequently affected than men, in ratios of 2:1 to 4:1 [11]. The aetiology of PTC has evoked much interest. The only notable or well-established environmental factor that is related to the progression of PTC is a previous history of radiation exposure [12]. The dropping of the atomic bomb on Hiroshima and Nagasaki at the end of World War II in 1945 and the explosion of the Chernobyl nuclear power station in 1986 confirmed the carcinogenic effect of radiation that led to PTC [13]. Other suggested risk factors include pre-existing benign thyroid disease or having a family history of PTC [14].

### **Risk of PTC among patients with nodular goitre**

The reported risk of PTC malignancy among patients with benign nodular goitre is not consistent. Earlier studies comparing patients with multiple nodular goitre with those having a single thyroid nodule showed no difference in cancer prevalence [15]. Later studies suggested patients with a solitary thyroid nodule carry a higher risk of thyroid cancer than patients with multiple thyroid nodules [16]. However, these views are no longer tenable as numerous subsequent studies have reported a significant risk of PTC in the patients with multiple thyroid nodules [17, 18].

PTC is apparently the most common variety of thyroid malignancy that was incidentally detected in patients with benign thyroid goitre [19, 20]. The risk of PTC in multinodular goitre is reported to vary from as low as 6% to as high as 21.2% [21, 22]. An earlier study by Alevizaki *et al.* [23] has reported that more than 50% of PTC cases were incidentally detected in elderly patients operated for pre-existing multinodular goitre. Variation in the frequencies of thyroid cancer in Graves' disease has also been observed, with 0.5% to 18.7% of the patients having PTC [24].

The demographics of PTC patients are also surrounded by controversies. In an epidemiological survey of 838 patients with multinodular goitre, Luo *et al.* [25] showed younger age, male sex, fewer nodules and smaller nodule size to be predictors of incidental thyroid cancer. Similar findings of higher risk of malignancy among young males with thyroid nodules have also been independently reported [18]. On the contrary, Wang *et al.* [17] reported that the female gender and multiple lesions showed higher risk of malignancy, although the risk for smaller nodule size was similarly high. The data of Bombil *et al.* [21] on 166 cases of PTC patients with concomitant multinodular goitre further adds to the controversy when older age (mean age was 46 years) as well as the female gender were associated with higher risk for malignancy.

## **Diagnosis of PTC**

Fine needle aspiration and cytology (FNAC) is the [method](#) of choice in the diagnosis of PTC [3]. The minimally invasive and rapid procedure involves the use of a narrow gauge needle to obtain a sample of a lesion for microscopic examination. During this procedure, thyroid biopsy specimens are classified by their cytological appearance into benign, suspicious (or indeterminate), or malignant cells [12]. Aspiration smear from PTC may

reveal papillary structure, but preoperative diagnosis is mainly based on the recognition of typical nuclear characteristics, such as ‘Orphan Annie’ nuclei intranuclear pseudoinclusions (due to cytoplasmic invaginations) and nuclear grooves (folds in the nuclear membrane) [11]. The presence of psammoma bodies (calcium salt deposits) in a cervical lymph node [provides further evidence of PTC](#) [26].

The accuracy rate of diagnosis with FNAC is about 90% when correlated with the postoperative diagnosis of surgical specimens [27]. In order to improve the diagnostic yield of FNAC, ultrasonography is usually carried out. The technique is extremely valuable in selecting appropriate nodules to aspirate within a multinodular thyroid or to select a site within a nodule [28]. It can detect presence of nodules that are too small to be palpated, multiple nodules and central or lateral neck lymphadenopathy. Ultrasonography also provides precise dimensions of a nodule for patient’s monitoring [29]. Ultrasound features, such as microcalcifications, hypoechoic appearance, increased vascularity, and irregular borders, are useful to differentiate malignancy in PTC patients with benign goitre [30]. Compared to other forms of thyroid malignancy, distant metastasis, usually to the lungs and bones, is less frequent in PTC [31].

## **Prognosis and Treatment**

Poor prognostic factors of PTC include older age at diagnosis, male gender, large tumour size, and extrathyroidal growth [11]. An aggressive approach in the management and treatment of the disease may render nearly 90% of the patients cancer-free [32]. Surgery is usually the first-line therapy for PTC. The extent of surgery is dependent on the size of the primary tumour and absence or presence of lymph node metastasis [33]. In cases of malignancy with a diameter of more than 1 cm, total thyroidectomy is usually performed

[34]. Oral administered radioiodine is usually recommended subsequent to surgery in all high-risk patients as this will ablate the remaining thyroid cells. A retrospective study performed by Mazzaferri [35] showed lower recurrence rates and improved survival when the remaining thyroid tissue was ablated with radioiodine therapy. For patients whose cancer no longer takes up iodine, tyrosine kinase inhibitors are often prescribed [36].

### **Recurrence of PTC**

PTC was earlier considered to be of low risk for recurrence, with 99% survival at 20 years after surgery [37]. However, in a retrospective study of 269 patients, Grogan *et al.* [38] reported more than 25% of the subjects had been detected with recurrence of PTC during a long term follow-up. In 11% of the cases, recurrences from PTC occurred more than 20 years after treatment, hence, leading to the recommendation for a lifelong follow-up of PTC patients. The risk factors identified for PTC recurrence included follicular variant of PTC, older age, cervical lymph node involvement and stage 4 tumors. More recently, Chien *et al.* [39] identified 676 genes associated with increased risk of PTC recurrence. Upregulation of the genes, which are involved in DNA repair and regulation of the cell cycle, as well as thyroid de-differentiation appeared to have a negative impact on patients' survival.

### **Molecular alterations in PTC**

Substantial molecular genetic alteration studies performed in the last two decades have given better insights in the understanding of the progression of PTC. PTC is often characterized by *RET* chromosomal rearrangement, or point mutation of *RAS* or *BRAF* proto-oncogenes, all of which are able to trigger the activation of mitogen-activated protein kinase (MAPK) cascade (Figure 2). Mutations of the *BRAF*, *RAS* or *RET* genes are found in nearly

70% of PTC cases [40]. Genetic events subsequent to the mutations may further lead to numerous different variants of PTC [41]. These variants may be identified via the different histopathologic features. The most common are the classical, follicular and tall cell variants. Among the variants of PTC, tall cell and columnar cell variants are biologically more aggressive. Table 1 demonstrates the association of *RET*, *BRAF* and *RAS* gene alterations with the three common PTC variants and their characteristic features [42].

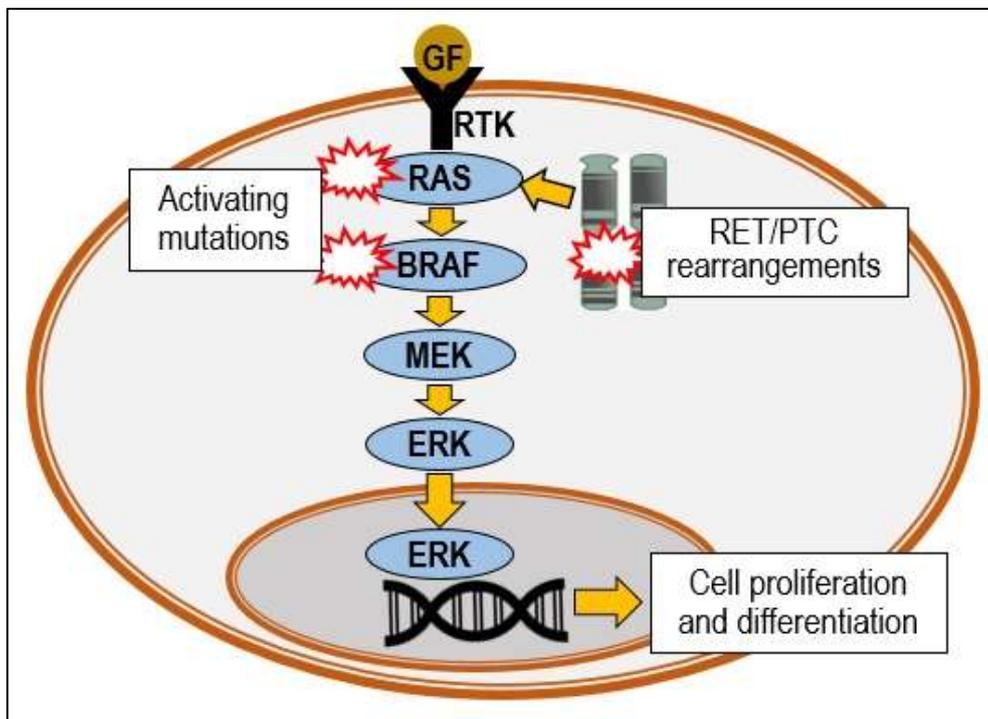


Figure 2 Oncogenic activation of MAPK pathway. The pathway is triggered by binding of growth factor (GF) to a receptor tyrosine kinase (RTK), which activates the RAS, BRAF, MEK and ERK phosphorylation cascade. MEK: MAPK kinase; ERK: extracellular-signal-regulated kinase.

**Table 1 Common PTC variants, their characteristic features and associated gene alterations.**

PTC Variant	Classical	Tall cell	Follicular
Nuclear features	Pronounced	Pronounced	Less pronounced
Psammoma bodies	Common	Common	Rare
Lymph node metastasis	Common	Common	Rare
Extrathyroidal extension	Common	Very common	Rare
Tumour stage at presentation	Early/Advance	Advance	Intermediate
Gene alteration	<i>RET/BRAF</i>	<i>BRAF</i>	<i>RAS</i>

### ***RET* rearrangements and PTC**

The *RET* chromosomal rearrangement was first reported in PTC by Fusco *et al.* [43]. *RET* is a proto-oncogene which encodes a plasma membrane bound RET tyrosine kinase receptor for ligands of the glial-derived neurotrophic factor family (GFL) [44]. The RET protein is expressed in the thyroid parafollicular or C cells, whilst, its expression in the thyroid follicular cells remains disputable.

*RET*/PTC-related carcinogenesis occurs by chromosomal rearrangements, which happens when the C-terminal kinase domain of RET is linked to the promoter and N-terminal domains of unrelated gene(s) [45]. This rearrangement causes *RET* to be placed under the transcriptional control of its fusion partner gene promoters, and allows the aberrant expression of chimeric protein of the receptor in epithelial follicular thyroid cells [46]. The fusion leaves the tyrosine kinases domain of the RET receptor intact, and enables the *RET*/PTC chimeric oncoprotein to bind SHC protein adapter which leads to stimulation of

the RAS-RAF-MAPK signalling cascade [47]. As a consequence of the rearrangement, the MAPK pathway becomes unrestrained and **chronically activated** [48]. In addition, the *RET/PTC* rearrangement has also been suggested to cause deletion of the intracellular juxta membrane domains of the receptor, the extracellular ligand-binding domain and the signal sequence, causing the relocation of the *RET/PTC* protein into the cytosol as well as preventing it from interacting with its negative regulators [49].

To date, there are at least 13 different forms of *RET/PTC* rearrangements that have been detected, and these vary according to the different genetic fusion partners [50]. Indeed, these mutations are almost exclusively found in PTC [48]. Among the rearrangements, *RET/PTC1* and *RET/PTC3* are apparently most common [48, 51], accounting for more than 90% of all the rearrangements [52]. Both *RET/PTC1* and *RET/PTC3* oncogenes are created by a paracentric, intrachromosomal inversion of chromosome 10q, in which *RET* is fused to the activating genes *CCDC6* (also known as *H4*) and *NCOA4* (also known as *ELE1* or *RFG*), respectively [49]. At least ten separate genes located on different chromosomes (intrachromosomal rearrangements) have also been shown to arrange with the *RET* gene, although less frequently [52].

*RET/PTC* rearrangements occur more frequently in patients less than 45 years old [53]. It is particularly common in paediatric PTC cases and those involving radiation exposures, both from the external beam radiation therapy as well as from nuclear accidents [48, 54]. In a study of post-Chernobyl paediatric patients, the *RET/PTC1* rearrangement was found to be associated with the classical variant of PTC, whilst the *RET/PTC3* rearrangement appeared to be more commonly present in solid variant PTC tumours [54]. In addition, *RET/PTC* rearrangements have also been documented in papillary microcarcinomas at a high frequency, suggesting that they are an early event in the carcinogenesis of PTC [55]. In transgenic mice experiments, the introduction of thyroid-targeted *RET/PTC1* oncogene has been shown to

induce a PTC-liked morphological changes, particularly in the nuclear cytologic features and presence of local invasion [56].

### ***BRAF* oncogene and PTC**

BRAF is part of a signalling pathway known as the RAS/MAPK pathway. It is a member of the RAF family of serine-threonine kinases. The activation of BRAF is prompted by binding of RAS to the cell membrane [57]. These kinases are intracellular effectors of the MAPK signalling cascade, which relay the signals downstream and regulate the expression of several genes that are responsible for cell proliferation, differentiation and apoptosis [48].

In thyroid cancer, point mutations, chromosomal rearrangement or small in-frame insertions or deletions can lead to the activation of *BRAF* [57]. The mutations of *BRAF* is mutually exclusive with the *RET/PTC* rearrangement and other common genetic alterations, and is most likely to have an independent oncogenic role as shown in the development of *BRAF* mutation-initiated PTC in a transgenic mouse studies [58]. The BRAF<sup>V600E</sup> is the most commonly reported mutation in patients with PTC [59], while the rarer K601E mutation has been detected in the follicular variant of PTC and benign thyroid adenomas [60].

Earlier studies have associated BRAF<sup>V600E</sup> mutation with poor prognosis [59, 61]. The high kinase activity of this mutant may drive genetic instability in PTC, facilitating secondary genetic alteration of members of the phosphoinositide 3-kinase-Akt serine/threonine kinase (PI3K-AKT) pathway and mediate its progression to a more aggressive cancer [48]. Since then, there have been many reports, including a few meta-analyses that acknowledged the association of this mutation with high risk clinicopathological features such as lymph node metastases, extrathyroidal invasion, recurrence rate and advanced clinical stage [53, 62, 63]. However, the prognostic value of the BRAF<sup>V600E</sup> mutation in PTC was made questionable

when the statistical data from a large multicentre retrospective study was shown to be insignificant after being adjusted for clinical and clinicopathologic risk factors such as patient age, extrathyroidal invasion, lymph node metastasis, and distant metastasis [64]. Nevertheless, the subsequent data of Xing *et al.* [65] that was generated from a study of more than 2000 patients from eight different countries again highlighted the potential prognostic value of BRAF<sup>V600E</sup> mutation in predicting the risk of PTC recurrence. In this case, the association of BRAF<sup>V600E</sup> mutation with PTC recurrence maintained its significance even after the data was adjusted for the same clinicopathologic risk factors.

### **RAS oncogenes and PTC**

RAS, which is upstream of BRAF, is a family of GTP-binding proteins that regulate cell growth via the MAPK and PI3K-AKT pathways. Almost one-third of human tumours are presented with *RAS* mutations [48]. Mutations of *RAS* were initially reported in thyroid cancer in 1988 [66]. They are found in a wide variety of thyroid tumours including follicular adenomas, follicular carcinomas, poorly differentiated carcinomas, undifferentiated carcinomas as well as papillary carcinomas. Three members of the *RAS* gene family (*HRAS*, *NRAS* and *KRAS*) have been shown to be mutated in thyroid cancer. The most common *RAS* mutations were detected in the *NRAS* gene, followed by *HRAS*, and least frequently, *KRAS* [67]. However, later it became clear that the *RAS* mutations are predominantly related to poorly differentiated thyroid carcinomas and anaplastic thyroid cancers than PTC [68, 69], which suggests the role of RAS is more inclined to the progression rather than the initiation of tumours. In a recent report of 199 cases of non-invasive follicular thyroid neoplasm with papillary-like nuclear features, more than half were apparently attributed to *RAS* mutations [70].

Point mutations in the *RAS* genes typically occur in codons 12, 13 and 61. The most frequent mutations lie in codon 61 of *NRAS* and to a lesser extent in *HRAS* [45]. *RAS* mutations have been described to alter GTP-binding affinity or intrinsic GTPase activity [49]. *RAS* mutants demonstrated distinctive roles in activating the PI3K-AKT and MAPK pathways. In human cancer, whilst the *KRAS* mutant was detected to be a preferential activator of the MAPK pathway, the *NRAS* mutant preferentially activates the PI3K-AKT pathway [71]. In PTC, genetic alterations of *RAS* occur in mutual exclusivity with the *BRAF* genes [72], suggesting that the *RAS* mutation is similar to the mutation of *BRAF*, which is capable of affecting PTC independently. The *RAS* proteins that are translated from the mutant genes cause the constitutive activation of downstream effector pathways that eventually resulted in aberrant cell proliferation and differentiation.

### **Genetic PTC biomarkers**

Mutations in *RET*, *BRAF* and *RAS*, when analysed simultaneously using DNA microarrays, have been shown to generate distinctive expression profiles that can be used as a genetic signature for their accurate classification [73]. Aside from these mutations, the overall differences in the expression of more than 200 other genes between PTC and normal thyroid tissues when taken together showed strong potential to be utilized as a molecular signature to discriminate the cancer [74]. However, when the data generated from numerous subsequent gene profiling studies were compiled, an overlap between genes regarded as specific to PTC or follicular thyroid carcinoma was frequently detected and became conspicuous (Table 2). One possibility for the “overlap” is that PTC and follicular thyroid carcinoma may occur via a common oncogenic pathway. Alternatively, this could have also been attributed to the small sample size of most of the studies, disagreement between

pathologists when diagnosing the lesions, and/or the lack of evaluation of other types of thyroid tumours. Nevertheless, some common observation can already be derived from these analyses, such as the upregulated expression of *LGALS3*, *SERPINA1*, *MET*, *KRT19*, *FN1* and *TIMP1*, as well as downregulated expression of *TPO*, *SLC26A4*, *DIO1/2* and *TFF3* in the well differentiated thyroid carcinomas. However, it is generally clear that more analyses are required before any meaningful conclusions can be made.

### **MicroRNA PTC biomarkers**

MicroRNAs (miRNAs) are small endogenous non-coding RNAs of approximately 22 nucleotides in length. They have key roles in post-transcriptional regulation of genes by repressing translation and/or degrading their messenger RNA targets in the cytosol, as well as in the alteration of gene expression in the nucleus [75]. At the translational level, it was estimated that miRNAs modulate up to 60% of the coding genes in the human genome. Because of their ubiquitous role in gene regulation, miRNAs are involved in many intracellular regulatory processes, such as differentiation, proliferation and apoptosis. Hence, dysregulation of miRNAs has been associated with many pathological disorders, including different types of cancer. The altered levels many different miRNAs have also been linked with the metastatic and invasive potential of cancers [76]. Since their early inception studies, there are now more than 2000 human miRNAs that have been annotated in the database and the number is still rapidly increasing [77].

**Table 2: Gene expression profiling of patients with PTC using microarray.**

Upregulated genes*	Downregulated genes*	Sample analysed	Reference
<i>CITED1, CHI3L1, ADORA1, SCEL, ODZ1, <b>MET</b>, EPS8, <b>LGALS3</b>, <b>KRT19</b>, CST6, SDC4, DUSP6, TSSC3, <b>TIMP1</b>, <b>SERPINA1</b>, LAMB3, MUC1, PROS1, SFPTB</i>	<i>MT1G, <b>DIO1</b>, <b>DIO2</b>, <b>TPO</b>, BCL2; DUSP1, FOSB, CRABP1</i>	8 PTCs and matched normal	[74]
<i><b>MET</b>, MMP11, PLAB, <b>FNI</b>, HIF1, HLA-DBP1, AHR, <b>TIMP1</b></i>	<i>GADD153, PKD1, CYR61, HBA1, DPC4/SMAD4, DLG3</i>	18 PTCs and 3 normal	[78]
<i><b>TIMP1</b>, GPR51, <b>LGALS3</b>, <b>SERPINA1</b>, TSSC3, IGFBP5</i>	<i><b>TFF3</b>, CRABP1, FCGBP, <b>TPO</b>, ID1, RB1</i>	8 PTCs, 6 NGs, and matched normal	[79]
<i>TACSTD2, AMM, AHR, MKP2, <b>FNI</b>, <b>KRT19</b>, HGFR, <b>LGALS3</b>, HER3, TIMP, Cathepsin C, TGF alpha, <b>SERPINA1</b>, SYND4, EPS8, NRP2, ALDH4, Dystrophin, COL8A1, HLA-DQ beta</i>		14 PTCs and 21 benign lesions	[80]
<i>CCND1, FGF, VEGFB, PDGFA, BMP5, TGRB, HGFL, FGFR2, IGF1, MPK, CDH1, CHD3</i>	<i><b>TPO</b>, NIS, TSHR</i>	7 PTCs and matched normal	[81]
<i>ZBTB3, GFER, GPC1, TMSB4X, BMP7, Cathepsin H, <b>SERPINA1</b>, CNN3, <b>MET</b>, <b>LGALS3</b>, CEACAM7, TGFB114, HLA-B, ADAM19, KCNAB</i>	<i>PAX8, TG</i>	16 PTCs, 13 FTCs, and 17 normal	[82]
<i><b>MET</b>, <b>FNI</b>, ADORA1, HMG A2, DTX4, QPTC, TACSTD2, AHR, RXRG, SDC4, <b>KRT19</b>, ICAMI, UDP-GALE, RAB27A, SNED1</i>	<i><b>DIO1</b>, BCL2, <b>TPO</b>, MT1G, EMID1, CDH16, ITPR1, HGD, CA4, ID3</i>	57 PTCs, 61 benign lesions, and 62 normal	[83]
<i>CDH3, SYTL5, LRP4, CHI3L1, SCEL, NMU, SOX4, BAX, RUNX1, <b>LGALS3</b>, <b>KRT19</b>, PLAB</i>		9 PTCs and 11 normal	[84]
<i>CST6, CXCL14, BCAN, DHRS3, <b>LGALS3</b>, SLC34A2, NMU, NRGN</i>		28 PTCs, 17 FTCs and 14	[85]

		normal	
<i>SLC34A2, TM7SF4, COMP, KLK7, KCNJ2, <b><u>SERPINA1</u></b></i>	<i>FOXA2, SLC44A, LYVE-1, OTOS, TFPC2L1</i>	35 PTCs and matched normal	[86]
<b><u>SERPINA1</u></b> , <b><u>KRT19</u></b> , <i>CITED1, CDH3, DPP4, <b><u>TIMPI</u></b>, PROS1, LAMB3</i>	<b><u>TFF3</u></b>	PTCs and NG	[87]
<i>CDH3, NGEF, PROS1, <b><u>MET</u></b>, CTXN1, SDC4, HPN, NRCAM, PSD3, GALE, SCEL</i>	<i>LIFR, MAFB, BMP2, ANKRD37</i>	19 PTCs and 7 normal	[88]
	<i>CUX2, <b><u>TFF3</u></b>, FOXA2</i>	7 PTCs and 7 normal	[6]
<b><u>MET</u></b> , <b><u>SERPINA1</u></b> , <b><u>LGALS3</u></b> , <b><u>FNL</u></b> , <i>LAMB3, COL13A1</i>	<i>TNFRSF11B</i>	16 PTCs and matched normal	[89]
	<i>CXCL12</i>	3 PTCs and matched normal	[90]

\*Commonly expressed genes are shown in bold and underline. FTC: follicular thyroid carcinoma; PTC: papillary thyroid carcinoma; NG: nodular goitre.

Compared to the genetic analyses, studies on the applications of miRNAs as biomarkers for PTC are relatively more recent. Currently, there have been several reports confirming that PTC is consistently associated with overexpression of specific miRNAs such as mir-146b, miR-221 and miR-222 compared to normal thyroid tissues (Table 3). The expression of these miRNAs was apparently associated with features indicative of tumour aggressiveness such as extrathyroidal extension, recurrence, lymph node or distant metastasis and BRAF<sup>V600E</sup> mutation [91, 92]. PTC tumours were also reported to have alterations in the process of miRNA biogenesis. Compared with benign thyroid neoplasms and normal thyroid tissues, the transcription of an RNA endonuclease Dicer, which is involved in the biogenesis and targeting of miRNAs in PTC tissues, was demonstrated to be downregulated. This alteration was also correlated with the same tumour aggressiveness features [93].

**Table 3 Dysregulated tissue and circulating miRNA in PTC.**

Sample	miRNA	Oncogenic alteration	Reference
Tissue (PTC vs Normal)	↑ 146b, 221, 222	n.d	[94]
	↑ 181b, 221, 222	n.d.	[95]
	↑ 146b, 221, 222; ↓ 187	BRAF <sup>V600E</sup>	[40]
	↑ 187	RET/PTC, RAS	
	↑ 21, 203	BRAF <sup>V600E</sup>	[92]
	↑ 451	n.d.	[17]
	↓ 137	n.d.	[96]
	↓ 451a	n.d.	[97]
	↑ 146b	BRAF <sup>V600E</sup>	[91]
	↑ 146b-5p, 146b-3p, 221-3p, 222-5p, 222-3p	n.d.	[98]
↓ 1179, 486-5p, 204-5p, 7-2-3p, 144-5p, 140-3p			
Serum/Plasma (PTC vs Normal)	↑ let-7e, 151-5p, 222	n.d.	[99]
	↑ 146b, 222	n.d.	[100]
	↑ 190; ↓ 95	n.d.	[101]
Serum/Plasma (PTC vs BTN)	↑ let-7e, 151-5p, 222	n.d.	[99]
	↑ 25-3p, 451a	n.d.	[102]
	↑ let7b-5p, 10a-5p; ↓ 146a-5p, 199b-3p	n.d.	[103]
	↑ 9-3p, 124-3p	n.d.	[104]
	↓ 151-5p, 221, 222	n.d.	[105]

↑: overexpressed; ↓: underexpressed; n.d.: not determined; BTN: benign thyroid nodules.

miRNAs have also been found in the blood circulation, with their detections in the serum/plasma, erythrocytes, platelets and nucleated blood cells. The serum/plasma miRNAs are remarkably very stable, and because of that, there have been considerable studies performed to explore the potentials of the extracellular circulating miRNAs for utilization as biomarkers for PTC to distinguish it from normal healthy individuals as well as those with benign thyroid masses (Table 3). Some of these miRNAs also showed strong prognostic utility potentials although there appear to be some minor discrepancies in the reported results of different groups of researchers. For more detailed updates of the diagnostic and prognostic values of miRNAs in PTC as well as all other different types of thyroid tumours, readers are encouraged to read a recent review article by Celano *et al.* [106].

### **Proteome-based PTC biomarkers**

Proteomics have been increasingly applied in the search for new diagnostic and prognostic protein biomarkers in cancers [107]. The study usually involves separation of complex mixtures of proteins by two-dimensional electrophoresis (2-DE), differential in-gel electrophoresis (DIGE) or liquid chromatography and identification of resolved proteins by mass spectrometry and database query. Proteomics analyses are usually used in combination with more conventional procedures such as northern and western blotting as well as immunohistochemical staining. For a more quantitative determination of protein amounts, enzyme-linked immunosorbent assay (ELISA) is a method of choice. In PTC, these techniques have been performed to identify proteins that are aberrantly expressed in tissue and serum samples between patients with the cancer and normal healthy individuals or those with benign thyroid goitre (Table 4). In addition, proteomics have also been used to analyse proteins expressed by PTC cell lines, cyst fluid and urine samples.

One of the earliest reported proteomics study of PTC is on the increased expression of prohibitin and ATP synthase D chain in tissue samples of patients relative to the controls [108]. Later, Brown *et al.* [109] using DIGE and peptide mass fingerprinting, analysed pooled protein extracts from PTC patients' tissues and reported differentially expressed heat shock protein 70 (HSP70), peroxiredoxin (PRDX) and an isoform of S100 protein (S100A6 protein), compared with matched normal thyroid tissues. Interestingly, overexpression of all the three proteins in PTC tissues was subsequently corroborated by several other independent studies; HSP70 by Abdullah *et al.* [110], PRDX by Giusti *et al.* [111] and Lee *et al.* [14], and S100A6 by Sofiadis *et al.* [112] and Martínez-Aguilar *et al.* [113]. In addition, overexpression of alpha1-antitrypsin (A1AT) in PTC tissue samples was also reported in three separate studies [14, 110, 111].

Currently, the proteomics analysis of serum samples of patients with PTC is apparently restricted to only two reports. Using SELDI-TOF MS, which analyses only low molecular weight polypeptides, Fan *et al.* [114] reported different altered levels of serum haptoglobin alpha1 chain, and apolipoproteins C1 and C3 in PTC patients, whilst Abdullah *et al.* [110] reported that PTC patients with history of benign thyroid goitre showed altered serum levels of A1AT, alpha 1-beta glycoprotein, apolipoprotein A-IV and alpha 2-HS glycoprotein relative to those without the history. The field of quantitative proteomics has progressed rapidly with the development of new state-of-the-art techniques such as iTRAQ (isobaric tag for relative and absolute quantitation) and SWATH (sequential window acquisition of all theoretical spectra) mass spectrometry. Already these techniques have been used to identify proteins with altered abundance in tissue and urine samples of patients with PTC [113, 115], which hopefully can be used to discriminate the cancer from follicular thyroid cancer and/or non-cancer.

**Table 4 Aberrantly expressed proteins in PTC.**

<b>Comparison</b>	<b>Samples</b>	<b>Aberrant Proteins</b>	<b>Techniques</b>	<b>References</b>
PTC, FTC, benign, and normal tissues	Tissue	↑ CTSB, ATP5H, and PHB	2-DE	[107]
PTC and normal tissues	Tissue	↑ S100A6, PRDX, and HSP70	2-D DIGE, MALDI-TOF MS, and IHC	[109]
PTC and benign tissues	Tissue and FNAC	↑ LGALS1 and LGALS3	2-DE, LC-MS, and IHC	[116]
FV-PTC, TCV-PTC and benign tissues	FNAC	↑ TTR, FLC, A1AT, GAPDH, LDH-B, APOA1, ANXA1, DJ-1 protein, cofilin-1, and PRDX1	2-DE, MALDI-TOF MS, and Western blotting	[111]
PTC and healthy controls	Serum	↑ HAP $\alpha$ 1; ↓ APOC1 and APOC3	SELDI-TOF-MS, LC-MS-MS and ProteinChip immunoassays	[114]
PTC and normal tissues	Tissue	↓ ANXA3	2-DE, MALDI-TOF-MS, Western blotting, Northern blotting and IHC	[117]
PTC, FTC, and normal tissues	Tissue	↑ S100A6	SELDI-TOF-MS, Western blotting and IHC	[112]
PTC and other nodular thyroid lesions	Tissue	↑ CaT12	ELISA, IHC, LC-MS-MS	[118]
PTC and normal tissues	Tissue	↑ enolase 1, TPI, cathepsin D, annexin A2, cofilin 1, PCNA, copine 1, HSP27	2DE, LC-MS-MS, Western Blotting,	[4]

PTC and normal tissue	Tissue	↑ ribosomal protein P2	IMS, MALDI-TOF, LC-ESI-MS/MS	[119]
PTC and benign tissues	Cyst fluid	↑ CK19 and S100A13	LC-MS/MS, Western blotting, IHC, and ELISA	[120]
PTC and benign tissues	Tissue	↑ 26 proteins including PRDX and SERPINA1	2-DE, LC-MS/MS Q-TOF	[14]
PTC patients with and without benign background	Tissue and Serum	Tissue: ↑ A1AT, HSP70, ↓ A1AT, PDI and UBE2N Serum: ↑ A1AT, A1B, APOA4 and AHSG	2-DE, LC-MS/MS Q-TOF, and ELISA	[110]
PTC, FTC and normal tissues	Tissue	↑ ANXA1, TMSB10, GAL3, CK19, ICAM1, GALE, CRABP1, FN1, and S100A6; ↓ TPO and DEHAL1	SWATH-MS, iTRAQ-MS, and Western blotting	[113]
PTC GLAG-66-CXCR7-1 cell line and control GLAG-66-1 cells	Cell lines	↑ AHNAK2 and TAGLN2	iTRAQ-coupled 2D LC-MS/MS, and Western blotting	[121]
Patients with PTC/BTG: controls	Urine	PTC: ↑ Gelsolin BTG: ↓ Osteopontin	iTRAQ-coupled 2D LC-MS/MS, and Western blotting	[115]

↑: overexpressed; ↓: underexpressed; PTC: papillary thyroid carcinoma; FTC: follicular thyroid carcinoma; BTG: benign thyroid goitre; cPTC: classical variant PTC; TCV: tall cell variant.

## Conclusion

Existing molecular evidence suggests that PTC, the most prevalent form of thyroid malignancy, is etiopathogenically complex and requires further in-depth investigations. The presently limited genomic, miRNA and proteomic biomarker discovery analyses performed

in PTC, when taken together, also show the need for more future studies so that the findings can eventually be translated into useful clinical applications in the diagnosis and management of the cancer that is rapidly increasing in incidence and recurrence.

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## Competing Interest

The authors have declared that no competing interest exists.

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