

## An update on molecular biology of thyroid cancers

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### Abstract

Differentiated thyroid cancer (DTC) is the most common endocrinological malignancy. There are several histological variants such as papillary and follicular thyroid carcinoma. Many patients with well-differentiated subtypes of DTC are cured by surgery alone or with

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radioiodine, while poorly differentiated types usually have a worse prognosis. The aggressiveness of thyroid tumors is closely linked to specific gene alterations.

Several diagnostic and prognostic molecular markers such as BRAF and RAS point mutations; RET/PTC and PAX8/PPAR $\gamma$  gene rearrangements; MAPK, PI3K, p53, Wnt–beta catenin, HIF1 $\alpha$  and NF-kappaB signaling pathways; microRNA profiles and aberrant methylation have been demonstrated in more than 70% of DTC. Diagnostic use of these molecular markers may be optimized for identifying higher risks of mortality, tumor recurrence and metastatic potential. Understanding the molecular biology of thyroid cancers can be an important avenue for diagnosis and treatment of radioiodine-refractory or inoperable DTC patients with novel molecular targeted therapeutic agents.

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## 1. Introduction

Thyroid cancer is the most common malign endocrine neoplasm originating from follicular or parafollicular thyroid cells. Histopathological classification of thyroid tumors is critical for patient management and for determining the clinical course of the disease. Follicular thyroid cells derived from histological subtypes are follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC), poorly differentiated thyroid carcinoma and anaplastic thyroid carcinoma (ATC). FTC and PTC are classified as differentiated thyroid carcinoma (DTC). Poorly differentiated and ATC also originate from follicular cells, and many cases are believed to develop as a result of dedifferentiation of a well-differentiated papillary or follicular carcinoma. Medullary thyroid cancer (MTC) is derived from parafollicular or C cells which are included in the neuroendocrine tumor family; MTC may be a part of multiple endocrinological malignancy type II (MEN II) [1–3].

In the United States, approximately 23,500 new DTC cases are diagnosed each year and its incidence has continuously increased in the last three decades all over the world except for a few countries (such as Norway and Sweden). Recent reports indicated that there were 3- to 5-fold increases in incidence rates from 1980 to 1997 depending on age, gender, histological type of thyroid cancer, radiation exposure, geographical region and other factors. The highest increase in incidence was found in PTC [4]. Frequent use of sensitive diagnostic techniques such as high-resolution ultrasonography, computerized tomography, magnetic resonance or positron emission tomography may be responsible for incidental detection of thyroid tumors. Additionally, true increases in incidence rates of thyroid cancers can also be explained with increased environmental radiation and use of medical radiation, iodine intake, the Chernobyl disaster, carcinogens, environmental, ethnic and genetic factors or combinations of these factors [4–7].

The most frequent type of thyroid cancer is PTC constituting 75–85% of all cases. Multifocality, lymphatic or local spreading and lymph node metastases are characteristic features of PTC whereas distant metastases by hematogenous spreading are relatively uncommon. The overall 5 and 10-year survival rates of PTC are approximately 97% and 93%, respectively [6,8,9]. In most low risk patients with small tumors, no local or distant metastases and extrathyroidal

infiltration, surgery and post-surgical radioiodine ablation therapy is usually adequate. Moreover, for PTC patients with <1 cm tumor size, surgical treatment alone may be sufficient. Metastatic doses and repeated radioiodine therapy have been used for patients with lymph node or distant metastases and extrathyroidal soft tissue spreading [10–13]. PTCs are the predominant histological type in children with thyroid cancer and in patients with head-and-neck irradiation history. The clinical course of these patients is relatively worse because of the aggressive behavior of these tumor [3,6,8]. PTCs frequently have genetic alterations such as point mutations of BRAF and RAS genes and RET/PTC rearrangements. These genetic alterations are found in more than 70% of the patients and may have prognostic implications [14–17].

FTC is second most common thyroid malignancy and accounts for 10–15% of thyroid cancers [6,8]. FTC tends to metastasize to lung and bone *via* the bloodstream. Prognosis of FTC is worse than PTC especially in patients with distant metastases. The overall 5 and 10-year survival rates for follicular thyroid cancer are 91% and 85%, respectively. Treatment strategy is thyroidectomy followed by ablative or metastatic doses of radioiodine therapy [12,13,18,19]. Approximately 50% of FTC patients have mutations in RAS family genes or PAX-PPAR $\gamma$  rearrangements [14,20,21].

ATC is an uncommon, lethal malignancy of older adults. The mean survival time is usually less than 6 months from the time of diagnosis and this outcome is not altered by current treatments. Patency of the airway is critical for the patient's course due to aggressive local spreading of the primary tumor and airway obstruction is the primary cause of death in most patients [1].

Thyroid follicular cells trap iodine by a Na<sup>+</sup>/I<sup>-</sup> Symporter (NIS), which is an energy-dependent transport system regulated by thyroid stimulating hormone (TSH). Iodine is organified by thyroid peroxidase (TPO) at the apical surface of the thyroid cells and then conjugated to thyroglobulin (Tg). Iodine is also trapped and organified by DTC cells as in thyrocytes and this unique characteristic plays the most important role on treatment and diagnosis of DTC [22–24]. <sup>131</sup>Iodine (<sup>131</sup>I) is a  $\beta$  and  $\gamma$  emitting radionuclide and chemically identical to the non-radioactive form of iodine. Radioiodine is an effective therapeutic and imaging agent for DTCs. Dedifferentiated, poorly differentiated and anaplastic thyroid tumor cell clones have lost the ability to trap iodine. Thus, radioiodine is not effective for detection and therapy of these tumors

[25]. Non-radioiodine avid tumors have aggressive behavior and poor prognosis.

Whereas radioiodine is the primary therapeutic agent for DTC, clinical response of residual or recurrent tumor and distant metastasis is variable. Age, gender, tumor histological type, clinicopathological features, stage of the disease, presence of distant metastasis, ability to trap radioiodine and molecular genetic alterations may be responsible for the aggressive clinical course of the disease. Molecular profiling of the thyroid cancers is important for determination of prognosis, causes of treatment resistance and targeted therapy options especially in radioiodine refractory patients.

In recent years, knowledge about genetic aberrations in thyroid cancers has significantly increased. Accumulating data on the molecular biology of thyroid cancers can be an important avenue for diagnosis, classification and treatment of thyroid cancers. In this comprehensive review article, we update the data generated on molecular biology of thyroid cancers and discuss the translational aspects of thyroid cancer molecular biology.

## 2. Genetic changes in thyroid cancer

PTC and FTC are the most common types among all thyroid malignancies and the development of molecular markers for these tumors is very significant in terms of diagnosis, prognosis, treatment and follow up [26–28]. Four different gene mutations with significant effects on tumor prognosis and diagnosis have been identified. They are BRAF and RAS point mutations, and RET/PTC and PAX8/PPARc rearrangements.

### 2.1. BRAF

BRAF, a member of the RAF protein family, is a serine–threonine kinase. As RAS binds and activates BRAF, it is translocated to the cell membrane and directly associates with the MAPK signaling pathway [14]. BRAF phosphorylates and activates MEK, which in turn activates ERK and all the downstream effector molecules of MAPK. Activated ERK and ELK1 translocate to the nucleus and regulate transcription of genes involved in cell differentiation, proliferation, and survival (Fig. 1) [26,14]. The most common genetic changes in PTC include point mutations of BRAF which are observed in 35–70% of papillary thyroid carcinomas [29,30]. More than 95% of BRAF mutations detected in thyroid cancers are thymine to adenine transversions at position 1799 (T1799A) resulting in the substitution of valine by glutamate at residue 600 (V600E) (Table 1) [31]. This mutation causes constitutive activation of BRAF kinase and, thus, induction of the MAPK signaling pathway, which is responsible for thyroid tumorigenesis [14,31]. In small percentages of PTC, K601E point mutation, small deletions or insertion around codon 600 and AKAP9/BRAF rearrangement are also be observed [30]. There is a direct association between

BRAF mutation and aggressive clinical outcomes such as invasion, metastasis and relapse of PTC [32]. It has also been demonstrated that BRAF mutation is associated with loss of radioiodine avidity in recurrent PTC which cannot be cured [33]. Several studies demonstrated a close association between BRAF mutation and dedifferentiation of PTC [32–34]. All the rearrangements and mutations increase BRAF activity and cause continuous induction of the MAPK signaling pathway.

Effects of BRAF mutations on induction and dedifferentiation, in addition to its effects on tumor aggressiveness, were examined in a transgenic mice model with V600E BRAF. The results revealed that these transgenic animals had PTCs and showed invasion, a common property of aggressive tumors [35]. All these data suggest that mutations in BRAF can be used as a novel marker for the prognosis of thyroid cancer.

### 2.2. RET/PTC rearrangements

RET (rearranged during transfection), a proto-oncogene, encodes for a membrane-bound receptor tyrosine kinase and is highly expressed in calcitonin-producing parafollicular cells (C cells) in the thyroid gland (Table 1) [36]. The activation of the RET gene in follicular cells as a result of a chromosomal rearrangement called RET/PTC plays important roles especially in the pathogenesis of childhood PTCs and PTC arising from radiation exposure [36,37]. RET/PTC rearrangements are characterized by a fusion between the tyrosine kinase domain of the RET gene located on the 3' and 5' termini of various partner genes [38]. Based on the partner genes, at least 10 different types of RET/PTC rearrangements have been identified. Of these, the most common types in PTC are RET/PTC1 and RET/PTC3 [39,40]. The partner genes for RET/PTC1 and RET/PTC3 are the coiled-coil domain containing gene 6, CCDC6 (also called H4/D10S170) and the nuclear receptor co-activator gene 4, NcoA4 (also called RFG/ELE1) genes, respectively [36,41]. Oncogenic activity of RET/PTC is related with the MAPK signaling cascade. In this cascade, RET/PTC first binds to adaptor proteins such as GRB2 and SOS and then activates RAS. Then, RAS activates RAF kinases such as BRAF and the downstream signaling cascade including MEK and ERK [42]. Although the exact role of RET/PTC at the early stage of thyroid tumorigenesis is unknown, a correlation between the presence of RET/PTC and a high growth rate of benign thyroid tumors has been found in a recent study [43]. Therefore, testing for RET/PTC rearrangements by different molecular approaches will be helpful for the diagnosis of thyroid cancer [44].

### 2.3. The MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) pathway plays a major role in terms of regulating cellular events, such as proliferation, survival, and is also known to affect tumorigenesis. Genetic and epigenetic alteration of the RAS–RAF–MEK–MAPK–ERK pathway is the most

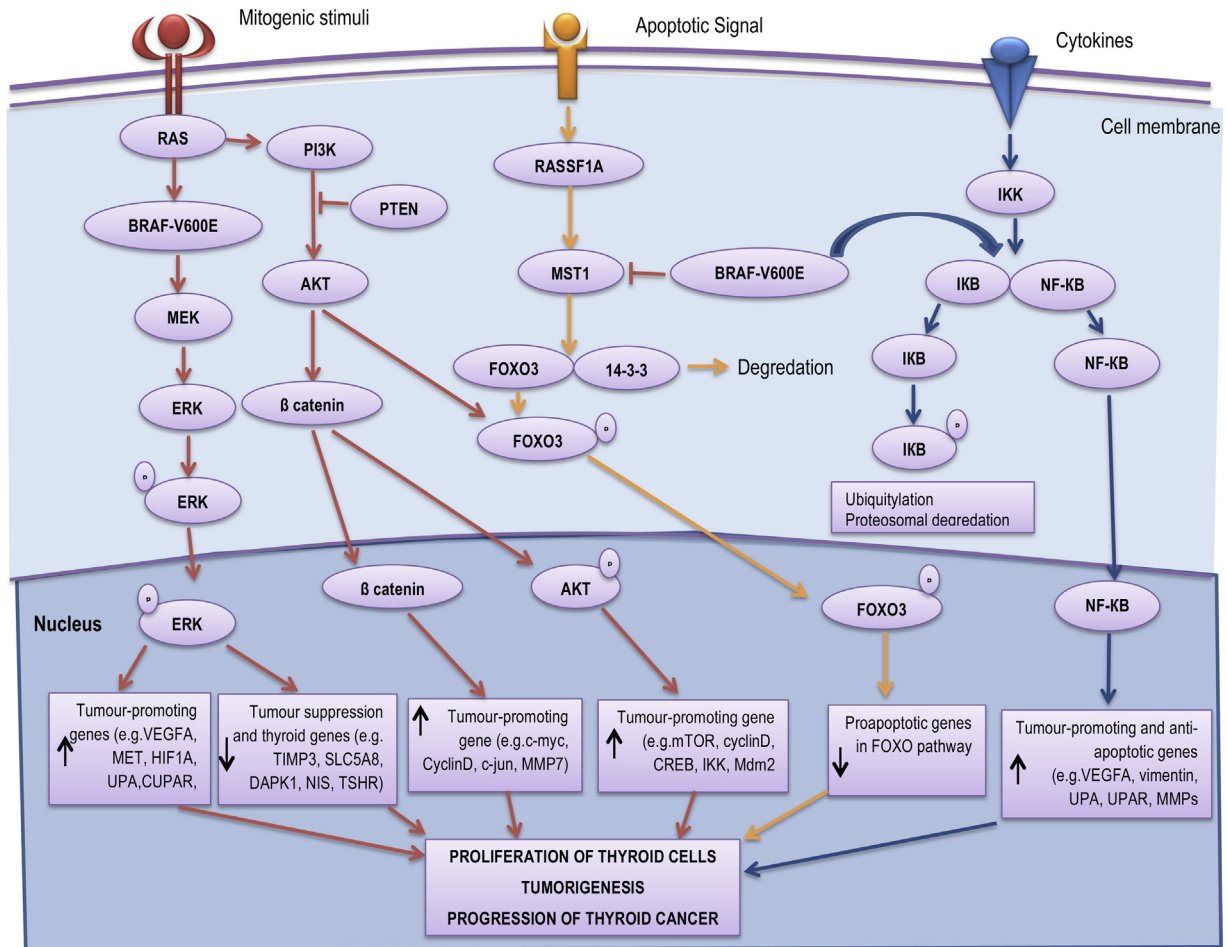


Fig. 1. The signaling pathways in thyroid cancers.

common, and was shown to highly affect thyroid cancer, particularly for PTC (Fig. 1) [45,34].

As mentioned earlier, the MAPK signaling pathway regulates cell proliferation, differentiation and survival by allowing the cells to respond to external stimuli by interaction of cellular tyrosine kinases (such as RET and G-protein-coupled receptors) with hormones, growth factors and cytokines [46]. The MAPK pathway also has a major role in human tumorigenesis. In thyroid cancer, RET/PTC rearrangements and point mutations in RAS and BRAF genes have been shown to activate the MAPK pathway [47–51]. Following the activation of the MAPK pathway, secondary molecular events occur, such as hypomethylation and genome-wide hypermethylation, which augment the tumorigenic activity of this pathway [52]. In addition, upregulation of several oncogenic proteins, such as chemokines [53,54], nuclear factor  $\kappa$ B (NF- $\kappa$ B) [55], vascular endothelial growth factor A (VEGFA) [56], matrix metalloproteinases (MMPs) [53,55,57], MET [58,59], vimentin [60], prokineticin 1 (PROK1; also known as EG VEGF) [61], prohibitin [62], hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) [63], thrombospondin 1 (TSP1) [64], urokinase plasminogen activator (uPA) and

its receptor (uPAR) [65,66] and transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) [67,68] drive cancer cell growth, proliferation and survival, together with tumor angiogenesis, invasion and metastasis.

#### 2.4. The PI3K/AKT signaling pathway

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway has major roles in several cellular events including growth, proliferation and apoptosis [69,70]. Activation of this pathway leads to tumorigenesis including thyroid carcinoma [71,72]. The role of the PI3K/AKT pathway on thyroid tumorigenesis was first revealed by the finding that Cowden's syndrome (which is caused by germline mutations of PTEN) was associated with follicular thyroid adenoma (FTA) and FTC [73]. Activation and increased expression of Akt has been shown to activate the PI3K/Akt pathway and lead to thyroid tumorigenesis (Fig. 1). FTCs have been shown to have higher levels of Akt activation as compared to papillary carcinoma and normal tissues [74,75]. Three types of Akt have been identified: Akt-1, Akt-2 and Akt-3. Activated Akt induces the signaling cascade by phosphorylating

Table 1  
Thyroid tumors with their characteristics and mutational profiles.

Tumor type	Prevalence (% of thyroid cancers)	10-Year survival (%)	Mutations observed and their prevalence	Effects of these mutations on tumor
Papillary carcinoma	80–85	95–98%	BRAF (V600E) 45% RET/PTC 20% RAS 10% TRK <5%	Promoting tumorigenesis, invasion, metastasis, recurrence and mortality
Follicular carcinoma	10–15	90–95	RAS 45% PAX8-PPAR $\gamma$ 35% PIK3CA <10% PTEN <10% BRAF (V600E) <10%	Promoting tumorigenesis, invasion, metastasis, recurrence and mortality
Medullary carcinoma	3–5	60–80	Familial forms of RET >95% Sporadic RET 50%	
Poorly differentiated carcinoma	<2	50	RAS 35% BETA CATENIN 20% TP53 20% BRAF 15% PIK3CA 10% AKT 10%	Promoting tumorigenesis and tumor progression, invasion, metastasis, recurrence and mortality
Anaplastic carcinoma	1–2	<10	TP53 70% BETA CATENIN 65% RAS 55% BRAF 20% PIK3CA 20% PTEN <10% AKT 10%	Promoting tumorigenesis and tumor progression, invasion, metastasis, recurrence and mortality

downstream protein effectors. Upon activation of the pathway, cell proliferation is promoted and apoptosis is inhibited. Akt-1 and Akt-2 were shown to be the most important genes in thyroid cancer [76]. It was previously determined that mutations, or amplification and genomic copy gain of the PI3KCA gene, encoding the catalytic subunit of PI3K, occur in thyroid tumors [77–79]. Decreased expression or inactivation of the tumor suppressor gene product PTEN and activation by RAS oncogenes have also been described to activate the PI3K/Akt signaling pathway and play a role in thyroid tumorigenesis [78–80]. Combinations of some of these PI3K/Akt genetic alterations, as well as combinations of these alterations with BRAF mutation, were shown to be present in more aggressive thyroid tumors such as ATC [78]. Genetic alterations that activate both MAPK and PI3K/Akt pathways were shown to be present in most (81%) ATC cases.

In a transgenic mouse model where a mutant thyroid hormone receptor  $\beta$  gene (TR $\beta$ PV) was knocked-in, the PI3K/Akt pathway was shown to be activated following interaction of TR $\beta$ PV with the p85 $\alpha$  regulatory subunit of PI3K, and spontaneous development of FTCs was observed [81,82]. Several human tumor studies proposed that activation and nuclear localization of Akt1 is involved in the invasiveness and metastasis of FTC induced by the PI3K/Akt pathway [83]. This proposal was supported by the detection of Akt1 mutations in metastatic thyroid cancers [84]. On the other hand, excision of Akt1 was shown to delay and prevent tumor development, angiogenesis, and movement of FTC to distant sites in TR $\beta$ PV mice [85].

## 2.5. RAS

The family of RAS (an abbreviation of RAt sarcoma) oncogenes encodes 21 kDa-G-proteins that transmit signals from the receptors on cell membrane to several types of targets in the cell controlling MAPK and PI3K signaling pathways in thyroid cancer [86,87]. There are three different RAS genes, known as HRAS, KRAS, and NRAS, that pass the signals from cell membrane receptors to their intracellular adaptor molecules (Fig. 1) [86,87]. RAS proteins exist as an active form with guanosine triphosphatase (GTPase) activity and an inactive form that is bound to guanosine diphosphate (GDP). Generally, point mutations in codons 12, 13, and 61 of the RAS oncogene are observed in thyroid cancer. While the point mutations in codons 12 and 13 result in elevated affinity for GTP, point mutation in codon 61 leads to repression of GTPase activity in autocatalysis [86–88]. All of these point mutations fix the RAS protein in activated states and, therefore, result in continuous stimulation of downstream targets of RAS. This, in turn, results in instability of the genome, induction of other mutations, and transformation into malignancy (Table 1) [89]. These mutations are seen at different frequencies in different types of thyroid cancers. While in PTC, RAS mutations were observed in approximately 10% of cases, in ATC and FTC this frequency is 10–20% and 40–50%, respectively [90–93]. RAS mutation in PTC leads follicular variants, and also more encapsulation, and fewer metastases to lymph node [94,95]. In ATCs, RAS mutations are generally

observed in tumors that develop microfollicular pattern [96].

Studies on RAS mutation in different types of thyroid cancer have shown that these mutations are observed in both malignant and benign types of tumors [97]. However, codon 61 point mutations have been especially reported to cause tumor progression in an aggressive manner [98,99]. Studies with transgenic mice demonstrated that RAS mutations resulted in carcinogenesis and hyperplasia in thyroid [100].

## 2.6. PAX8/PPAR $\gamma$ rearrangement

The PAX8 gene encodes a transcription factor required for the generation of thyroid follicular cells and tissue-specific gene expression in thyroid. The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) gene is a member of the nuclear hormone receptor superfamily including retinoic acid, estrogen receptor, androgen receptor, and also thyroid hormone [101]. The PAX8/PPAR $\gamma$  rearrangement occurs as a result of genetic translocation between chromosomes 2 (exon 7, 8 or 9) and 3 (exon 1), t(2;3)(q13;p25) [102]. Although the main mechanism is not yet clear, this fusion results in significant increases in expression of PAX8/PPAR $\gamma$  chimeric protein which inhibits the tumor suppressor activity of PPAR $\gamma$  [103–106]. Deregulation of PAX8 function, essential for differentiation of thyroid cells, and induction of some other genes, that are unrelated to wild type PAX8 or PPAR $\gamma$  genes, may be responsible for generation of thyroid cancer after this rearrangement [107,108]. Generally, PAX8/PPAR $\gamma$  rearrangement is seen in follicular thyroid carcinoma at 30–35% frequency (Table 1) [109,110]. In addition, most studies have shown that this rearrangement is also seen in follicular adenomas at 2–13% frequency, and in papillary carcinomas with follicular variants at 1–5% frequency [111–113]. Rarely, in 1 out of 42 follicular carcinomas, another type of gene rearrangement of PPAR $\gamma$  with CREB3L2 (CREB3L2/PPAR  $\gamma$ ) was observed [114].

## 2.7. p53

The p53 gene encodes a transcription factor that regulates vitally important cellular functions such as cell growth, proliferation, cell cycle, apoptosis and DNA repair [115]. Expression of p53, known as the guardian of the genome, is triggered when the cells are under stress conditions and cell cycle progression is inhibited. Damage to the DNA is then repaired or the cells undergo apoptosis [116]. However, point mutations that inactivate the tumor suppressor gene, TP53, are common events in the initiation of cancer. In thyroid tumors, loss of TP53 gene activity is observed at a later stage. p53 loss is generally observed in 15–30% of poorly differentiated carcinomas, in 60–80% of ATCs, and is extremely rare in DTCs (Table 1) [86,117–120]. p53 inactivation in thyroid cells has been shown to enhance tumor growth and also causes continuous loss of differentiation. In one study, it was observed that the progressive loss of differentiation in

ATC and poorly differentiated carcinomas is controlled by inactivation of the p53 gene [121,122].

The first mouse model of ATC was recently generated based on information provided by research and patient-derived genetic data. It is known that molecular changes that characterize ATC are: inactivation of the TP53 gene or activation of the PI3K cascade, RAS family members, or BRAF [123,124]. Mouse thyroid follicular cells were used in the study for which inactivation of the TP53 gene was combined with constitutive activation of the PI3K signaling cascade, *via* deletion of the PTEN tumor suppressor. The tumors developing in these mice were observed to closely resemble human ATCs, and they were shown to undergo glycolytic shift and consequently respond to glycolytic inhibitor therapy.

It was demonstrated that loss of p53 triggers dedifferentiation of thyroid tumors. PTC developed in transgenic mice with thyroid-specific RET/PTC rearrangements while crossing with p53<sup>-/-</sup> mice, the progeny was developed [125,126]. On the other hand, expression of thyroid specific genes was detected after introduction of wild-type p53 into ATC [127,121].

It is known that p53 mutation is rarely found in thyroid cancer, mainly in undifferentiated tumors, and it is generally involved in the progress of advanced cancer. However, recently, there is evidence indicating that p53 mutation may also be effective in the early stages of thyroid cancer. In these tumors, various inactivating mechanisms acting on the protein and/or its signaling pathway may inhibit wild-type p53 activity [122]. It is assumed that p53 mutations may be an initiator in poorly differentiated or ATC; but it is a late event contributing to the development of transformed phenotype.

## 2.8. WNT–beta catenin pathway

Abnormal activation of the Wnt signaling pathway leads to the development of epithelial tumors. In recent years, the importance of the Wnt/ $\beta$ -catenin pathway in the initiation of thyroid cancer has gained significance. This pathway plays a major role in tumor development and epithelial renewal. In thyroid tumors, the most frequent mutations are found in the scaffold proteins adenomatous polyposis coli (APC) and Axin, as well as in  $\beta$ -catenin (Fig. 1) [128].

Poorly differentiated and ATCs constitute additional genetic changes that are normally not found in well-differentiated cancers. These changes represent late events, which may be involved in the initiation of tumor differentiation. One of these late events is the formation of mutations in the CTNNB1 gene, which encodes  $\beta$ -catenin, a cytoplasmic protein that has a role in cell adhesion and is known to be an important intermediate in the wingless (Wnt) signaling pathway [129,130]. Point mutations occur in exon 3 of the gene these mutations have been found in 66% of ATCs and 25% of poorly differentiated carcinomas, although not in DTCs (Table 1) [131,132].

The direct role of  $\beta$ -catenin in proliferation of undifferentiated thyroid tumor cells has been understood. However

more studies should be undertaken to determine the role of  $\beta$ -catenin in the earlier stages of thyroid tumor progression. The use of *in vivo* models which have a constitutively active Wnt/ $\beta$ -catenin pathway could help to understand the mechanism by which the Wnt pathway promotes tumor development in thyroid cancer.

### 2.9. The NF- $\kappa$ B signaling pathway

The NF- $\kappa$ B pathway is one of the main players in the regulation of inflammatory responses that are involved in induction of cancer [133]. There are significant increases in activity of NF- $\kappa$ B in thyroid cancer cells and tissues [134–136].

Inhibition of NF- $\kappa$ B protein synthesis with specific anti-sense oligonucleotides significantly decreased growth rate of undifferentiated carcinoma cells [136]. This decrease was related to suppressed expression of c-myc, a target for NF- $\kappa$ B118. NF- $\kappa$ B is a transcription factor that induces expression levels of different oncogenes and is upregulated by the MAPK pathway, RET/PTC, RAS and BRAF-V600E in thyroid cancers [137]. Recently, it was shown that proliferative and anti-apoptotic genes were regulated by the NF- $\kappa$ B signaling pathway in thyroid cancer [137,138]. The mechanism of BRAF-V600E induced increases in expression levels of NF- $\kappa$ B and was related with I $\kappa$ B (inhibitor of NF- $\kappa$ B) degradation in thyroid cancer cells [55].

### 2.10. MicroRNAs

Micro-RNAs (miRNAs) are small noncoding RNA genes composed of 21–25 nucleotides. They inhibit expression levels of genes at the transcriptional and posttranslational levels [139]. The aberrant expression of certain miRNAs can induce initiation and/or progression of human cancers [140]. The involvement of miRNAs in proliferation, differentiation and apoptosis have been defined and thus they could be used as biomarkers for diagnostic and therapeutic targets in many cancers including lung, pancreas, breast, and colorectal cancers in addition to thyroid cancers [141–144]. There are several reports displaying changes in miRNA profiles in thyroid cancers as compared to normal thyroid tissues [143,144]. Based on cellular origin and degree of tumor differentiation, different miRNAs are expressed in thyroid cancer [145]. Particularly miR-222, miR-221, and miR-146b have been found to be the most upregulated miRNAs in PTC. It was shown that miRNA-221 was also upregulated in unaffected thyroid tissue of several PTC patients, which was considered as an early event in carcinogenesis [146]. Moreover, the association between increased expression of miR-222, miR-221, and miR-146b and pathological features of PTC has been examined and revealed that expression levels of these miRNAs were strongly related with extrathyroidal invasion. In addition, miR-146b was found to be highly expressed in PTC samples [147]. miR-222, miR-221, and miR-146b have been thought to play crucial roles in PTC initiation

and development. The candidate targets of these miRNAs could be p27kip1, a cell cycle progression inhibitor, and KIT, a receptor tyrosine kinase that plays important roles in cell growth and differentiation [143,148]. Interestingly, high expression levels of miR-146b were found to be correlated with lower overall survival and with the development of drug resistance [149]. A recent study demonstrated that miR-146b could cause thyroid cancer development by regulating TGF- $\beta$  signaling, although the mechanisms of miR-146b-mediated tumor behavior in PTC are largely unknown [150]. There were also significant increases in expression levels of miR-146b, miR-221, miR-222 and miR-135b in samples of PTC patients with extrathyroidal invasion [151].

There was an association between upregulation of miR-193b, miR-2861, miR-1202, and miR-451 and downregulation of miR-let-7i, miR-542-5p, miR-664, and miR-564 with lymph node metastasis [152]. Yu et al. identified the circulating miRNA profiles of PTC patients and showed that there were significant increases in serum let-7e, miR-151-5p, and miR-222 in PTC cases. An association with certain clinicopathological features such as tumor size and metastasis stage and expression levels of these miRNAs was also determined [153]. There were also significant downregulation of expression of certain miRNAs including miR-1, -191, -486, and -451 in PTC [154]. miR-1 silences CXCR4 which plays a major role in lymph node metastasis [155]. Therefore, its downregulation *via* miR-1 could be a potential strategy for overcoming lymph node metastasis. Recently several studies have reported the importance of miRNA deregulation in FTCs, although most of the studies have focused on PTCs. Weber et al. found four miRNAs, miR-192, -197, -328, and -346, that seem to be specific for FTCs since they have not yet been associated with other thyroid cancer types [156]. The targets of miR-197 are ACVR1 and TSPAN3 genes while EFEMP2 is the target of miR-346 [156]. The EFEMP2 gene is thought to have tumor suppressor functions while the ACVR1 and TSPAN3 genes are involved in the control of cell growth and metastatic potential, respectively [157–159]. *In vitro* studies related with these miRNAs showed that their overexpression induced significant cell proliferation whereas inhibition caused growth arrest in human thyroid carcinoma follicular cells [154,156].

In conclusion, miRNA expression profiles demonstrate differences between thyroid tumors and healthy tissues, primary tumors and metastatic tumors, and even between different regions of the same tumor. Therefore, miRNAs could be used as a novel marker for tumor diagnostic purposes.

### 2.11. The HIF1 $\alpha$ pathway

Hypoxia-inducible factor (HIF)-1 $\alpha$ , through binding to HIF1 $\beta$  (known as ARNT), forms HIF1 transcription factor which triggers the expression of genes regulating tumor angiogenesis [160]. It was demonstrated by several groups that HIF1 $\alpha$  is expressed in thyroid tumors, such as ATC, and is not expressed in healthy thyroid tissues [161,162].

A significant increase in expression levels of HIF1 $\alpha$  in BHP10-3SC (mice) and human PTC tissue was determined [163]. HIF1 induces expression of VEGF, a growth factor, and MET, an oncogene, which are also upregulated in thyroid tumors as compared to normal thyroid tissues [162]. On the other hand, PI3K-AKT and MAPK increase expression of HIF1 in thyroid tumor [161,164,165] while a mutation in BRAF (V600E) also affects expression levels of HIF1 $\alpha$  in papillary thyroid cancer [165]. Interestingly, inhibition of expression levels of HIF-1 $\alpha$  and HIF-2 $\alpha$  by GDC-0941 in four different thyroid tumor cells (BcPAP, WRO, FTC133, and 8505c) resulted in inhibition of VEGF secretion [166]. Koperek et al. demonstrated that tumor hypoxia is involved in the development of regional metastases in MTC [167]. All these results suggest that HIF1 can be used as a diagnostic and even prognostic marker for thyroid tumors. More importantly, targeting HIF1 with special inhibitors could be a novel approach for effective treatment of thyroid cancers since it induces angiogenesis and metastasis of thyroid tumors.

### 2.12. Aberrant methylation

Changes in methylation status of the genome are hallmarks of cancers including thyroid cancer [168]. Hypermethylation of many tumor suppressor genes was determined for thyroid cancer by several groups [168]. Aberrant methylation results in downregulation of the genes as it occurs in regulatory sequences. It was determined that the BRAFV600E mutation is associated with hypermethylation of various tumor suppressor genes, such as death-associated protein kinase 1 (DAPK1), retinoic acid receptor- $\beta$  (RAR $\beta$ ), tissue inhibitor of metalloproteinases 3 (TIMP3) and SLC5A8 [169]. Interestingly, Hou et al. demonstrated that BRAF-V600E signaling induced changes in methylation, both hypermethylation and hypomethylation, and methylation regulates high numbers of genes involved in the control of vital important cellular functions in PTC cells [170]. An association between BRAF mutations and aberrant methylation of DNA repair genes, especially the hMLH1 gene was detected which also determines the aggressiveness of PTC [170,171]. These results revealed that changes in methylation levels resulting from BRAF-V600E are important in induction of thyroid cancers.

Methylation of PTEN in regulatory sequences resulting in downregulation of expression of PTEN is also observed in FTC [172–174]. Methylation of PTEN is related with changes in the PI3K–AKT pathway, mutations in RAS, and mutations and amplifications in PIK3CA in thyroid tumors [171]. It was shown that increased activity of the PI3K–AKT pathway induces changes in methylation and downregulates PTEN [172].

Thyroid molecules including NIS, TSH, Tg, TPO receptors, and pendrin are generally lost in thyroid tumors because of aberrant methylation. This aberrant methylation is regulated by the BRAF mutation-promoted MAP kinase signaling

pathway and inhibition of MAP kinase by specific chemicals can reverse this event [168].

Although genetic problems were clearly identified for many thyroid cancers, around 30–35% of DTCs could not be explained by genetic problems. This indicates that changes in epigenetics should be further investigated.

### 3. Molecular targets in histopathological diagnosis and classification of thyroid cancer

Histopathological examination is essential in current clinical practice to classify histological subtypes or variants and to distinguish malign thyroid tumors from benign ones. The histopathological classification recommended by WHO has been used for distinguishing thyroid tumor types in post-operative thyroidectomy materials and the Bethesda system has been used for classifying thyroid cytopathology of fine needle aspiration biopsy (FNAB) samples [175]. For many thyroid tumor types such as follicular patterned tumors (follicular adenoma, FTC, minimally invasive FTC and follicular variant of PTC) and several variants of PTC, histological or cytological criteria are subjective. Thus it is possible to have diagnostic errors, problems and interobserver variability. Furthermore, reliable prognostic markers are still insufficient. The most prominent interobserver variability has been reported in follicular-patterned tumors [175–178]. Final agreement for reported criteria such as capsular or vascular invasion and differential diagnosis of follicular adenoma and carcinoma is very low (27, 20 and 37%, respectively) [177]. Some disagreements between pathologists have also been reported for classification of histological variants of PTC and FNAB [175–178].

Morphological and biological characteristics of FTA and FTCs are very similar and thus it may be impossible to make definitive differential diagnosis by FNAB sampling. Therefore, 10–40% of all FNABs are reported as indeterminate for malignancy. Many immunohistological markers have been improved for more accurate differential diagnosis of thyroid tumors. However they have limited use in routine practice because many of them show overlap between some subtypes of thyroid cancer [179].

Molecular markers hold promise to improve preoperative diagnosis of thyroid cancer. Diagnostic use of gene mutations (BRAF, RAS, RET/PTC, PAX8/PPAR $\gamma$ ), alterations in gene expression profile and miRNA signatures have several advantages in differential diagnosis of thyroid tumors. Identifying specific molecular or genetical alterations offers not only the possibility of distinguishing benign and malign tumors and to classify thyroid malignancies but also to make prognostic stratification. Diagnostic use of mutational markers as single gene analyses or a panel of mutations may help to improve the accuracy of FNAB. RET/PTC, BRAF, NTRK, RAS and PAX8–PPAR $\gamma$  mutations have been frequently explored for diagnosis of thyroid cancer. Gene expression profiling and microRNA studies have identified a variety



of potential molecular markers to help distinguish benign from malignant thyroid neoplasms especially for nodules that are classified as indeterminate for malignancy by FNAB [36,37,45,34,90–93,109–113,178,180–182]. The inability to exclude malignancy in these nodules may often lead to unnecessary surgery or two step surgical procedures. More accurate preoperative diagnosis significantly reduces the frequency of unnecessary surgery and thus patient morbidity.

PTCs are a histologically heterogeneous group of tumors that are classified as classical, follicular, tall-cell, columnar-cell, cribriform-cell, oncocytic (hurtle cell), diffuse sclerosing and solid variants. The role of molecular diagnostic markers on classification of PTC is uncertain. The vast majority of PTCs are characterized by mutations or rearrangements along the MAPK pathway like RET/PTC rearrangements and BRAF mutations.

The prevalence of RET/PTC rearrangements in PTC is variable (0–87%) depending on histological subtype, age, geographical factors, radiation exposure and detection methods but such rearrangements represent most of the cases with no or very low incidence in FTC and ATCs. Initial reports suggested that RET/PTC was specific for PTC [183,184] but later it was also found in some benign lesions [185,186] and diseases such as Hashimoto disease [187]. According to these results, RET/PTC is not a specific PTC marker. However, recent studies suggested that RET rearrangements in benign thyroid nodules could be associated with a higher growth rate of the nodule, thus, RET/PTC positive benign nodules must be subjected to careful follow-up [188–190]. RET/PTC1 and RET/PTC3 are the most common isoforms of RET/PTC, with the first predominant in the classical type of PTC and the second predominant in the solid variant of PTC [180,191]. RET/PTC3 is also predominant among radiation-induced PTCs such as post-Chernobyl PTCs and has more aggressive behavior [192,193]. However, there was no significant correlation found in the occurrence of RET/PTC in patients with and without history of medical radiation [194]. Presence of RET/PTC activation may be a complementary approach in patients reported to have indeterminate cytology by FNAB and with borderline histological features of malignancy in microscopic papillary foci of thyroid nodules [195]. Most studies demonstrated that the sensitivity of molecular assays has improved by combined detection of BRAF and RET/PTC. An increased sensitivity from about 42.3–69% to 56.4–78% has been reported [196–198].

The BRAF point mutation is the most common mutation in PTC (35–70%) and has recently been reported to be associated with poor prognostic features like extrathyroidal soft tissue infiltration, lymph node metastasis, disease aggressiveness, tumor recurrence, loss of radioiodine avidity and, thus, resistance to annual therapeutic methods [29–34,199,200]. Additionally, the BRAF mutation occurs most commonly in aggressive subtypes of PTC [178,201]. Loss of NIS and TPO are early markers of dedifferentiation and they are also responsible for poor prognosis in BRAF positive PTCs [202]. According to these results, the BRAF

mutation is a potential independent prognostic molecular marker for PTC. More extensive surgery, higher doses of radioiodine, lower levels of suppression of TSH and closer monitoring during the follow-up period will be helpful for disease control in patients with BRAF positive PTC.

Because of its high prevalence and specificity to PTC and PTC-derived dedifferentiated tumors, screening the BRAF mutations in FNAB samples may be useful for preoperative diagnosis [203,204]. Recent studies reported that the BRAF mutation is a highly accurate marker of malignancy for thyroid nodules sampled by FNAB [205–209]. However, the most frequent diagnostic problems in FNAB samples are indeterminate follicular tumors and suspicion of malignancy according to the Bethesda system. The diagnostic potential of the BRAF mutation is relatively low for indeterminate follicular neoplasms [210]. Additionally, BRAF mutations demonstrate a high geographical variability and local frequency must be taken into consideration for evaluation of the results. Despite these factors, recent studies reported that 580 of 581 BRAF-positive nodules were PTC and the false positive rate of BRAF mutational analysis was only 0.2% [26,28]. Moreover 15–40% of BRAF-positive FNAB samples were indeterminate by cytology. These reports indicate that BRAF analysis on FNAB samples is a reliable diagnostic marker for PTC. Despite high specificity, analyses of BRAF alone in FNAB samples may miss some thyroid cancers that are negative for BRAF mutation.

In FTC, RAS mutations and a fusion oncogene between PAX8 and PPAR $\gamma$  have been more frequently observed than follicular adenoma. Frequency of RAS mutation, the second most common mutation after BRAF, is about 40–50% in FTCs and 10% in PTCs (follicular variants) [90–93]. The positive predictive value of RAS mutation positivity in FNAB samples for thyroid cancer is about 74–87%. Detection of the RAS mutation is associated with an 87–100% probability of malignancy [28]. Most importantly, RAS-positive thyroid cancers are FTC and the follicular variant of PTC which may cause false negative results in cytological evaluations. These results suggest that the diagnostic value of RAS mutation in FNAB samples is high for DTCs. Total thyroidectomy has been suggested as the initial surgical procedure in patients with thyroid nodules that are positive for the RAS mutation by FNAB due to the high preoperative probability of cancer [32]. PAX8/PPAR $\gamma$  rearrangement is characteristically seen in FTCs and it may be used as a diagnostic marker. However, PAX8/PPAR $\gamma$  mutation is rarely observed in some follicular adenomas. Detection of the PAX8/PPAR $\gamma$  mutation should alert the pathologist to re-examination for histological features of follicular carcinoma such as vascular or capsular invasion [26,28].

Several studies reported that the most effective diagnostic utility can be achieved by a panel of mutations in FNAB samples. Ohori et al. suggested a panel of biomarkers including BRAF-RAS gene mutations and RET/PTC-PAX8-PPAR $\gamma$  gene translocations to optimize FNAB results [211]. This panel includes some of the most frequent and specific

molecular markers identified to date for thyroid cancer and may be used in routine practice. TRK rearrangements may also be added to this panel. Presence of BRAF, RAS, RET/PTC, PAX8–PPAR $\gamma$  and TRK in FNAB samples correlated with malignancy in 97% of cases [196,197,212]. Therefore, detection of any mutation of this panel is a strong predictor for thyroid cancer. The revised American Thyroid Association's (ATA) Management Guideline recommended using a mutational panel for nodules classified as indeterminate by FNAB [213]. Nodules with indeterminate cytology have a 5–10% probability for malignancy and usually undergo repeated FNAB according to classical follow-up protocols [175,214]. Analysis of a panel of mutations is particularly useful for management of patients with indeterminate thyroid nodule that are at the low risk category for thyroid cancer. The most common mutations in these nodules are RAS, BRAF and PAX8–PPAR $\gamma$  rearrangement. Detection of these molecular alterations indicates a high risk category for malignancy. Thus, the patient may be referred to surgery without repeated FNAB. Conversely, the risk of cancer is about 0–7% in thyroid nodules those are negative for all these mutations [196,211]. In this lowest risk group, follow-up is sufficient without the necessity of surgical intervention. As has been recommended in the revised ATA guideline, the use of mutational analyses in FNAB samples is useful for pre-operative evaluation and clinical management of thyroid nodules, especially those which are indeterminate.

In addition to somatic mutations, changes in gene expression profiles detected by microarray techniques and some miRNA markers have been identified and may be of potential use for diagnostic purposes in thyroid cancer [26,32,178]. Upregulation or aberrant expression of HMGA2, MET, TPO, TIMP1, DPP4, TFF3, SERPINA1, TIMP1, FN1, TGFA, CRABP1, FCGBP, EPS8 and PROS1 genes have been detected in malignant thyroid tumors [215–220]. Diagnostic use of expression levels and profiles of multiple genes have been tested and it is reported that the sensitivity and specificity of these techniques in differential diagnosis of malignant and benign thyroid nodules were 92% and 84%, respectively [221]. Mathur et al. have explored the diagnostic utility of combined use of mutational analysis, gene expression profiling and classical cytological evaluation of FNAB samples [222]. This study suggested that combined use of these molecular analyses has improved diagnostic accuracy of FNABs. However, no reliable single marker was identified and diagnostic use of micro-array technology in routine practice on FNAB samples is currently not only feasible but also cost-effective.

There are several studies reporting overexpression of miRNAs including miR-222, miR-221, and miR-146b in PTC and these miRNAs may be of diagnostic potential [143–151]. In a recent study, a panel of seven miRNAs was used in thyroid FNAB samples [223]. When presence of up-regulation of three or more of these miRNAs including miR-222, miR-221, miR-146b, miR-224, miR-155, miR-187 and miR-155 was accepted as a predictor of thyroid cancer, diagnostic accuracy

of FNAB was 98% [223]. This result suggests that diagnostic use of miRNA analysis is feasible and improves diagnostic accuracy of thyroid FNABs in preoperative evaluation. However, their clinical use in routine practice requires additional studies.

#### 4. Targeting molecular pathways for the treatment of thyroid cancer

Currently, novel therapy regimens for thyroid cancer have been developed using therapeutic targets such as BRAF, RET, MEK and AKT, and tested at different stages of clinical trials. The results of these clinical studies give information on therapeutic efficacy of these therapies and their potential use alone or in combination with other treatment strategies for thyroid cancer (Table 2). These novel therapeutic regimens will be useful particularly for the treatment of MTC, non-radioiodine avid DTC's, and aggressive types of thyroid cancer, including poorly differentiated and ATC's.

##### 4.1. Targeting BRAF

Due to the high frequency of BRAF mutations in thyroid cancers and its association with tumor dedifferentiation and resistance to radioiodine therapy, several BRAF inhibitors have been recognized and tested as potential therapeutic agents. Moreover, since BRAF takes place downstream of RET and RAS in the signaling cascade, BRAF inhibitors were suggested to be potentially effective in tumors with other mutations that affect the signaling pathway upstream of BRAF.

A promising BRAF inhibitors is BAY 43–9006, a multikinase inhibitor, which effectively inhibits wild-type BRAF and the kinase activity of mutant V600E BRAF [224]. This agent has also been shown to have potent activity against VEGFR-2, VEGFR-3, FLT-3, c-KIT and PDGFR $\beta$  kinases [225]. In a study, oral administration of BAY 43-9006 resulted in a strong antitumor effect in xenograft models of various types of cancer. The results demonstrated that this effect was through inhibition of MAPK signaling and angiogenesis [225]. In preclinical studies, BAY 43-9006 has been shown to hinder BRAF signaling and inhibit the growth of all thyroid cancer cell lines carrying the mutant *BRAF* [226]. It was also shown to impair the growth of the ATC cell line xenografts in nude mice [228]. Recently, the therapeutic effects of BAY 43-9006 on cells carrying the activated forms of RET and RET/PTC were also demonstrated [227].

BAY 43-9006, another BRAF inhibitor, has also been tested in clinical studies for several types of cancer, including thyroid cancer. In the trial of patients with progressive papillary carcinoma, some patients had minimal or partial response [228,229].

The efficiency of AAL-81 and LBT-613, other inhibitors of RAF kinases, have been tested in thyroid cells in preclinical studies [230]. Both agents were determined to inhibit MAPK

Table 2

Completed/ongoing phase I/II trials with molecular therapy and their published response rates for thyroid cancers (NOR: no objective responses).

Agents	Completed/ongoing phase I, II, III trial	Molecular target	Response rates	Refs.
Everolimus	Phase II completed	mTOR inhibitor	5%	[254]
Lenvatinib	Phase II completed	Tyrosine kinase inhibitor targeting VEGFR1-3, FGFR1-4, RET, KIT and PDGFR $\beta$	50%	[255]
Gefitinib	Phase II completed	Inhibition of EGFR	NOR	[256]
Depsipeptide (DEP)	Phase II completed	Histone deacetylase inhibition	NOR	[257]
Vorinostat	Phase II completed	Histone deacetylase inhibition	NOR	[258]
Axitinib	Phase II completed	Inhibition of VEGFR	30%	[259]
Sorafenib	Phase II completed	Inhibiting Raf kinase, VEGFR, PDGFR, and RET tyrosine kinases	23%	[260]
Suberoylanilide hydroxamic acid	Phase I completed	Inhibits histone deacetylase activity in peripheral-blood mononuclear cells (PBMNCs)	30%	[261]
Motesanib	Phase II completed	Inhibitor of VEGF R, PDGFR and KIT	14%	[262]
Thalidomide	Phase II completed	Inhibit angiogenesis induced by bFGF or VEGF <i>in vivo</i>	18%	[263]
Pazopanib	Phase II completed	Tyrosine-kinase inhibitory	49%	[264]
XL-184	Phase I completed	MET, VEGFR-2 and RET kinase inhibitor	26%	[265]
XL-184	Phase II completed	MET, VEGFR-2 and RET kinase inhibitor	21%	[265]
Imatinib	Phase II completed	Tyrosine-kinase inhibitor	NOR	[266]
Celecoxib	Phase II completed	Selectively COX-2 inhibiting	3%	[267]
Combretastatin A4 phosphate	Phase II completed	Tubulin-binding vascular disrupting agent	NOR	[268]
Sunitinib	Phase II completed	Inhibitory activity against RET, VEGFR, and PDGFR	13%	[269]
Selumetinib (AZD6244)	Phase II completed	RAF/MEK/ERK (MAPK) signaling	3%	[270]
Vandetanib	Phase III completed	Inhibitor of RET kinase, VEGFR and EGFR signaling	37%	[271]
Vandetanib	Phase II completed	Inhibitor of RET kinase, VEGFR and EGFR signaling	20%	[272]
Lenalidomide	Phase II completed	Inhibit tumor necrosis factor production	22%	[273]
Cabozantinib	Phase III completed	Tyrosine kinase inhibitor (TKI) of MET, VEGFR2, RET, MTC	28%	[274]
Thyrotropin	Phase I/II completed	131I uptake and Tg release	NOR	[275]
Fosbretabulin	Phase I completed	Tubulin inhibitory	NOR	[276]
Romidepsin	Phase I/II completed	Histone deacetylase inhibition	NOR	[277]
Doxorubicin and interferon $\alpha$	Phase II completed	Inhibits the enzyme topoisomerase II	6%	[278]
Rosiglitazone	Phase II completed	PPAR $\gamma$	25%	[279]
Cisplatin	Phase II completed	Microtubulin inhibitor	NOR	[264]
Decitabine	Phase II Ongoing	Inhibiting DNA methyltransferase	No study results posted yet	[264]
Temsirolimus + sorafenib	Phase II Ongoing	Inhibiting Raf kinase, VEGFR, PDGFR, RET tyrosine kinases, mTOR	No study results posted yet	[264]
Nintedanib (BIBF1120)	Phase II Ongoing	Inhibits receptors of VEGF, FGF and PDGF	No study results posted yet	[264]
Pazopanib hydrochloride	Phase II Ongoing	Tyrosine kinase inhibitor of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- $\alpha/\beta$ , and c-kit	No study results posted yet	[264]
Motesanib	Phase II completed	Inhibits VEGF	14%	[262]

signaling and growth of human thyroid tumor cell lines and rat thyroid cells carrying the V600E BRAF and RET/PTC1 mutations. The growth of BRAF mutant tumor xenografts in nude mice was also shown to be suppressed [231].

Potential therapeutic targets along the MAPK pathway are also found downstream of BRAF. A non-ATP competitive MEK inhibitor has been designated as CI-1040, and it was shown to inhibit the growth of BRAF mutant xenografts derived from various tumor types [232]. This study has also progressed to clinical trials.

#### 4.2. Targeting RET mutations and RET/PTC

Activated RET kinase has been identified as a target by several small-molecule tyrosine kinase inhibitors in preclinical and clinical studies. ZD6474 is an orally active low

molecular weight receptor tyrosine kinase inhibitor, which was shown to inhibit VEGFR-2 and block RET tyrosine kinase [233]. ZD6474 was demonstrated to block phosphorylation and signaling from RET/PTC3 *in vitro*. In that way, the growth of human papillary carcinoma cell lines harboring RET/PTC1 and RET/PTC3-transformed fibroblasts was arrested in nude mice [234,235].

In a clinical trial with metastatic familial MTC patients, ZD6474 therapy was reported to give some evidence of response [232]. Currently, a multi-center phase II double blinded study is being conducted to compare the efficiency of ZD6474 (ZACTIMA<sup>TM</sup>, AstraZeneca Pharmaceuticals, DE, USA) versus placebo in patients with familial and sporadic MTC.

The antitumor activity of ZD6474 was suggested to be due to a combination of antiangiogenic activity mediated by

blocking VEGFR and anti-RET activity of the agent. Therefore it is important to explore whether the presence of the RET mutation and its type affect the extent of the therapeutic response to ZD6474.

In preclinical studies, it was shown that ZD6474 can inhibit all the mutated variants of RET except for the V804L and V804M mutations [235]. V804 corresponds to the gatekeeper residues of PDGFR, c-KIT, ABL, and EGFR kinases, and mutations at these residues result in resistance to various inhibitors [236]. These results suggest that RET V804L and V804M mutations in medullary carcinomas may show primary resistance to ZD6474 [235].

A multikinase inhibitor SU12248 (Sunitinib) has been demonstrated to effectively block signaling from RET/PTC kinase. Sunitinib was also tested in Phase II clinical trial in radioiodine-refractory, unresectable DTC [237]. Additionally, in preclinical studies, the pyrazolopyrimidine compounds PP1 and PP2, two small-molecule tyrosine kinase inhibitors, have been shown to effectively inhibit RET/PTC signaling *in vivo* and abolish its oncogenic effects in experimental animals [238,239].

#### 4.3. Targeting RAS

Point mutations of RAS occur in both benign and malignant thyroid tumors. In PTCs, RAS mutations are found at relatively low frequencies, in approximately 10% of tumors. In follicular thyroid carcinomas, 40–50% of tumors carry RAS mutations [87,91,96], which may correlate with poor prognosis and tumor dedifferentiation [97,240]. RAS mutations may influence tumor dedifferentiation, as they are found with high frequency in ATCs. *In vitro* studies have provided evidence that the mutant RAS affects chromosomal stability [241,242]. Active RAS may induce thyroid tumorigenesis through the classic MAP kinase pathway or through interaction with the PI3K/Akt pathway. RAS mutations, however, are not specific for thyroid tumors, they can also occur in benign follicular adenomas.

#### 4.4. Targeting PI3K

Previous studies have shown that activation of PI3K signaling is a common feature of many cancers including thyroid cancer [243]. Particularly, FTCs have been identified to have greater levels of Akt activation as compared to PTCs [74,75]. Genetic changes that activate the MAPK and PI3K/Akt pathways were shown to be present in most (81%) ATC cases. Since both the MAP kinase and PI3K pathways are involved in the pathogenesis of ATC, targeting multiple signaling pathways may be a valuable therapeutic approach for thyroid cancer. Downstream signaling relay molecules such as BRAF, AKT, MEK and mTOR are examples of therapeutic targets that are being actively tested for the treatment of thyroid cancer [27]. In a recent study, the activity of the PI3K inhibitor GDC-0941 was assessed on hypoxia-inducible-factor-1 (HIF-1), a transcription factor regulated

by PI3K, for its metastatic behavior of thyroid cancer cells both *in vitro* and *in vivo* [164]. GDC-0941 has been shown to inhibit HIF-1 $\alpha$  and HIF-2 $\alpha$  expression, and HIF activity in thyroid carcinoma cells. *In vivo*, GDC-0941 has been demonstrated to decrease HIF-1 $\alpha$  expression in FTC xenografts, and reduce FTC tumor growth and metastatic lung colonization.

In a transgenic mouse model where a mutant thyroid hormone receptor  $\beta$  gene (TR $\beta$ PV) was knocked-in, the activation of the PI3K/Akt pathway was determined following the interaction of TR $\beta$ PV with the p85 $\alpha$  regulatory subunit of PI3K, and spontaneous development of FTCs was observed [81,82]. Several human tumor studies proposed that activation and nuclear localization of Akt1 is involved in the invasiveness and metastatic behavior of FTC triggered by the PI3K/Akt pathway [75]. This result was supported by the detection of Akt1 mutations in metastatic thyroid cancers [84]. On the other hand, excision of Akt1 was shown to delay and prevent tumor development, angiogenesis, and movement of FTC to distant sites in TR $\beta$ PV mice [85]. In another study it was shown that treatment with LY294002, a PI3K inhibitor, prevents tumor development [244]. Several drugs have been developed to target the PI3K/Akt pathway, and their efficiency for the treatment of cancer is currently assessed in various clinical studies [245].

#### 4.5. Targeting PPAR

To date, there is no definite evidence that PAX8/PPR $\gamma$  rearrangement could be a sign of outcome in FTCs. Therefore, its use as a novel therapeutic target for FTC has been uncertain. However, a number of approved, well-tolerated, orally administered regulators of the PPAR $\gamma$  pathway are available, and are used in the treatment of type 2 diabetes. Currently, some clinical trials are ongoing with PPAR $\gamma$  agonists, alone or in combination with other conventional chemotherapeutic agents [246].

#### 4.6. Targeting NF- $\kappa$ B

The NF- $\kappa$ B pathway has a significant role in the regulation of inflammatory responses that are linked to oncogenesis [247], and increased NF- $\kappa$ B activation has been well documented in thyroid cancer cell lines [134–136]. In one study, it was shown that concurrent suppression of the NF- $\kappa$ B and MEK–ERK pathways through application of NF- $\kappa$ B and MEK inhibitors repressed the proliferation of thyroid cancer cells carrying a BRAF-V600E mutation [248].

#### 4.7. Targeting epigenetic mechanisms

Epigenetic alterations in thyroid tumors include changes that occur in DNA methylation patterns in the CpG islands of promoters of genes important in normal thyrocyte function and increased promoter methylation by DNA methyltransferases (DNMTs) causing gene silencing and dedifferentiation [249,250]. Studies on how posttranslational

modification of histones may influence cancer have recently gained significance. The nucleosome consists of 147 bp of DNA wrapped around an octamer of four core histone proteins (H2A, H2B, H3, and H4) [251]. Methylation, acetylation, phosphorylation, and ubiquitination are included in histone modifications that synergistically act with DNA promoter methylation to regulate gene silencing [252]. It is believed that epigenetic drug targets will play a more significant role in cancer treatment in the near future.

DNMT inhibitors such as 5'-azadeoxycytidine have been tested as "redifferentiation" agents, which allow tumors to regain their sensitivity to conventional radioactive iodine therapy [253]. A summary of ongoing or completed trials of treatment of thyroid cancers with molecular therapy is presented in Table 2.

### 5. Current challenges and future venues for research in molecular therapy of thyroid cancers

In the near future, increased access to array-type and next generation sequencing technologies will provide significant progress in our understanding of the molecular biology of thyroid cancers. Novel information including new mutations, genes and/or signaling pathways that are involved in initiation or progression of thyroid cancers may be an important avenue for diagnosis and prognosis of thyroid cancers. Therefore, determination of nucleotide sequences of genes, rearrangements and expression levels of the genes should become common diagnostic tools.

Targeting certain molecules involved in initiation and progression of thyroid cancers will also be an important research area. However, it is vital to determine whether the genetic differences observed in thyroid cancers correlate with the therapeutic response to specific inhibitors. Acquired and intrinsic drug resistance mechanisms and reversal of drug resistance are also important considerations for a complete cure.

On the other hand, environmental factors and sporadic conditions that induce thyroid cancer need to be addressed in order to clarify the increasing rates of thyroid cancers in various parts of the world in the last three decades. Overcoming these challenges will provide better prevention, diagnosis and therapy of thyroid cancers.

### 6. Conclusion and future directions

The contribution of multiple signaling pathways in the development of aggressive thyroid cancer suggests that it may be necessary to target them simultaneously for successful treatment. Indeed, clinical trials undertaken with single kinase inhibitors in thyroid cancer have generally shown only partial responses. Recently, various preclinical studies testing the combination of MEK or BRAF-V600E inhibitors with AKT, PI3K, or mTOR inhibitors demonstrated that they

inhibit proliferation and induce apoptosis of thyroid cancer cells synergistically [280–282].

Novel and promising treatment strategies for thyroid cancers are based on molecular targeting [283]. New small-molecule protein-kinase inhibitors including axitinib [259], sorafenib [260,284,285], motesanib [262] and pazopanib [264] have shown promise in clinical trials on thyroid cancer. PLX4032 (also known as vemurafenib), a BRAF-V600E-specific inhibitor, has been recently approved by the FDA for the treatment of BRAFV600E-positive melanoma [286].

Targeting multiple signaling pathways in order to restore radioiodine avidity can be considered as another promising area of translational research on thyroid cancer. It was recently demonstrated that simultaneous inhibition of the MAPK, PI3K-AKT and histone deacetylase pathways could stimulate the expression of thyroid iodide-handling genes in thyroid cancer cells as compared to inhibition of one pathway alone [287].

In conclusion, remarkable progress has been made in understanding the molecular biology of thyroid cancer in recent years. This understanding of the molecular pathogenesis of thyroid tumorigenesis has now opened great opportunities for the development of novel molecular-based approaches for the effective management of the diagnostic and therapeutic obstacles of thyroid cancer.

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### Conflict of interest

None of the authors have any interests which might influence the compilation of the current literature in this subject. We apologize to the authors whose valuable studies were not included here due to space limitations and the concentrated scope of the review.

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## References

- [1] DeLellis RA, Lloyd RV, Heitz PU, Eng C. World Health Organization classification of tumors. Pathology and genetics of tumors of endocrine organs. Lyon: IARC Press; 2004.
- [2] Cooper DS, Doherty GM, Haugen BR, et al. (American Thyroid Association Guidelines Taskforce) management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2006;16:109–41.
- [3] Pitoia F, Bueno F, Urciuoli C, Abelleira E, Cross G, Tuttle RM. Outcome of patients with differentiated thyroid cancer risk stratified according to the American Thyroid Association and Latin American Thyroid Society risk of recurrence classification systems. *Thyroid* 2013;23(11):1401–7.
- [4] 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; 2013:1–10.
- [5] Kilfoy BA, Zheng T, Holford TR, et al. International patterns and trends in thyroid cancer incidence, 1973–2002. *Cancer Causes Control* 2009;20:525–31.
- [6] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics 2010. *CA: Cancer J Clin* 2010;60:277–300.
- [7] Kent WDT, Hall SF, Isotalo PA, Houlden RL, George RL, Groome PA. Increased incidence of differentiated thyroid carcinoma and detection of subclinical disease. *Can Med Assoc J* 2007;177:1357–61.
- [8] Jemal A, Murray T, Ward E, et al. 2005 Cancer statistics. *CA Cancer J Clin* 2005;55:10–30.
- [9] Sherman SI. Thyroid carcinoma. *Lancet* 2003;361:501–11.
- [10] Randolph GW, Duh QY, Heller KS, et al. The prognostic significance of nodal metastases from papillary thyroid carcinoma can be stratified based on the size and number of metastatic lymph nodes, as well as the presence of extranodal extension. *Thyroid* 2012;22:1144–52.
- [11] Yu XM, Lloyd R, Chen H. Current treatment of papillary thyroid microcarcinoma. *Adv Surg* 2012;46:191–203.
- [12] Meas T, Vercellino L, Faugeron I, Toubert ME. The 2009 Revised American Thyroid Association Guidelines for Thyroid Cancer: multifocality in T1 tumors in question, for or against a more minimalist approach? *Thyroid* 2013;23(8):1042–3.
- [13] Lee SL. Radioactive iodine therapy. *Curr Opin Endocrinol Diabetes Obes* 2012;19:420–8.
- [14] Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. *Mod Pathol* 2008;21:S37–43.
- [15] Witt RL, Ferris RL, Pribitkin EA, Sherman SI, Steward DL, Nikiforov YE. Diagnosis and management of differentiated thyroid cancer using molecular biology. *Laryngoscope* 2013;123:1059–64.
- [16] Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res* 2003;63:1454–7.
- [17] Fagin JA. Genetics of papillary thyroid cancer initiation: implications for therapy. *Trans Am Clin Climatol Assoc* 2005;116:259–69.
- [18] Luster M, Häscheid H, Freudenberg LS, Verburg FA. Radioiodine therapy of metastatic lesions of differentiated thyroid cancer. *J Endocrinol Invest* 2012;35:21–9.
- [19] Molinaro E, Pieruzzi L, Viola D. Radioiodine post-surgical remnant ablation in patients with differentiated thyroid cancer: news from the last 10 years. *J Endocrinol Invest* 2012;35:16–20.
- [20] Boos LA, Dettmer M, Schmitt A, et al. Diagnostic and prognostic implications of the PAX8-PPAR $\gamma$  translocation in thyroid carcinomas—a TMA-based study of 226 cases. *Histopathology* 2013.
- [21] Goyal N, Setabutr D, Abdulghani J, Goldenberg D. Molecular and genetic markers of follicular-cell thyroid cancer: etiology and diagnostic and therapeutic opportunities. *Adv Exp Med Biol* 2013;779:309–26.
- [22] Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature* 1996;379:458–60.
- [23] Eskandari S, Loo DDF, Dai G, Levy O, Wright EM, Carrasco N. Thyroid Na<sup>+</sup>/I<sup>-</sup> symporter: mechanism, stoichiometry, and specificity. *J Biol Chem* 1997;272:2723–8.
- [24] Regalbuto C, Frasca F, Pellegriti G, et al. Update on thyroid cancer treatment. *Future Oncol* 2012;8:1331–48.
- [25] Mian C, Barollo S, Pennelli G, et al. Molecular characteristics in papillary thyroid cancers (PTCs) with no 131 I uptake. *Clin Endocrinol* 2008;68:108–16.
- [26] Nikiforov YE. Molecular diagnostics of thyroid tumors. *Arch Pathol Lab Med* 2011;135:569–77.
- [27] Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. *Lancet* 2013;381:1058–69.
- [28] Nikiforov YE. Molecular analysis of thyroid tumors. *Mod Pathol* 2011;24:S34–43.
- [29] Xing M. BRAF mutation in thyroid cancer. *Endocr Relat Cancer* 2005;12:245–62.
- [30] Cohen Y, Xing M, Mambo E, et al. BRAF mutation in papillary thyroid carcinoma. *J Natl Cancer Inst* 2003;95:625–7.
- [31] Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. *Nature* 2013;13:184–9.
- [32] Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nature* 2011;7:569–80.
- [33] Legakis I, Syrigos K. Recent advances in molecular diagnosis of thyroid cancer. *J Thyroid Res* 2011; 2011:1–8.
- [34] Xing M. Recent advances in molecular biology of thyroid cancer and their clinical implications. *Otolaryngol Clin N Am* 2008;41:1135–46.
- [35] Knauf JA, Ma X, Smith EP, et al. Targeted expression of BRAFV600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. *Cancer Res* 2005;15(65(10)):4238–45.
- [36] Fagin JA. Challenging dogma in thyroid cancer molecular genetics—role of RET/PTC and BRAF in tumor initiation. *J Clin Endocrinol Metab* 2004;89:4264–6.
- [37] Cahill S, Smyth P, Finn SP, et al. Effect of ret/PTC 1 rearrangement on transcription and post-transcriptional regulation in a papillary thyroid carcinoma model. *Mol Cancer* 2006;5:1–12.
- [38] Santoro M, Dathan NA, Berlingieri MT, et al. Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. *Oncogene* 1994;9(2):509–16.
- [39] Jhiang SM, Sagartz JE, Tong Q, et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. *Endocrinology* 1996;137:375–8.
- [40] Powell Jr DJ, Russell J, Nibu K, et al. The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. *Cancer Res* 1998;58:5523–8.
- [41] Celetti N, Cerrato A, Merolla F, Vitagliano D, Vecchio G, Grieco M. H4(D10S170), a gene frequently rearranged with RET in papillary thyroid carcinomas: functional characterization. *Oncogene* 2004;23:109–21.
- [42] Santoro M, Melillo RM, Fusco A. RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology Prize Lecture. *European Journal of Endocrinology* 2006;155:645–53.
- [43] Sapiro MR, Guerra A, Marotta V, et al. High growth rate of benign thyroid nodules bearing RET/PTC rearrangements. *J Clin Endocrinol Metab* 2011;96(6):E916–9.
- [44] Salvatore G, Giannini R, Faviana P, et al. Analysis of BRAF point mutation and RET/PTC rearrangement refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2004;89(10):5175–80.
- [45] Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer* 2006;6:292–306.
- [46] Robinson MJ, Cobb MH. Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 1997;9(2):180–6.
- [47] Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/

- PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res* 2003;63(7):1454–7.
- [48] Soares P, Trovisco V, Rocha AS, et al. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. *Oncogene* 2003;22(29):4578–80.
- [49] Frattini M, Ferrario C, Bressan P, et al. Alternative mutations of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. *Oncogene* 2004;23(44):7436–40.
- [50] Ciampi R, Nikiforov YE. Minireview: RET/PTC rearrangements and braf mutations in thyroid tumorigenesis. *Endocrinology* 2007;148(3):936–41.
- [51] Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;417(6892):949–54.
- [52] Hou P, Liu D, Xing M. Genome-wide alterations in gene methylation by the BRAF V600E mutation in papillary thyroid cancer cells. *Endocr Relat Cancer* 2007;18:687–97.
- [53] Melillo RM, Castellone MD, Guarino V, et al. The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. *J Clin Invest* 2005;115:1068–81.
- [54] Oler G, Camacho CP, Hojajir FC, Michaluart Jr P, Riggins GJ, Cerutti JM. Gene expression profiling of papillary thyroid carcinoma identifies transcripts correlated with BRAF mutational status and lymph node metastasis. *Clin Cancer Res* 2008;14:4735–42.
- [55] Palona I, Namba H, Mitsutake N, et al. BRAFV600E promotes invasiveness of thyroid cancer cells through nuclear factor  $\kappa$ B activation. *Endocrinology* 2006;147:5699–707.
- [56] Jo YS, Li S, Song JH, et al. Influence of the BRAF V600E mutation on expression of vascular endothelial growth factor in papillary thyroid cancer. *J Clin Endocrinol Metab* 2006;91:3667–70.
- [57] Mesa Jr C, Mirza M, Mitsutake N, et al. Conditional activation of RET/PTC3 and BRAFV600E in thyroid cells is associated with gene expression profiles that predict a preferential role of BRAF in extracellular matrix remodeling. *Cancer Res* 2006;66:6521–9.
- [58] Mesa Jr C, Mirza M, Mitsutake N, et al. Molecular classification of papillary thyroid carcinoma: distinct BRAF RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. *Oncogene* 2005;24:6646–56.
- [59] Kumagai A, Namba H, Mitsutake N, et al. Childhood thyroid carcinoma with BRAF T1799A mutation shows unique pathological features of poor differentiation. *Oncol Rep* 2006;16:123–6.
- [60] Watanabe R, Hayashi Y, Sassa M, et al. Possible involvement of BRAFV600E in altered gene expression in papillary thyroid cancer. *Endocr J* 2009;56:407–14.
- [61] Pasquali D, Santoro A, Bufo P, et al. Upregulation of endocrine gland-derived vascular endothelial growth factor in papillary thyroid cancers displaying infiltrative patterns, lymph node metastases, and BRAF mutation. *Thyroid* 2011;21:391–9.
- [62] Franzoni A, Dima M, D'Agostino M, et al. Prohibitin is overexpressed in papillary thyroid carcinomas bearing the BRAF(V600E) mutation. *Thyroid* 2009;19:247–55.
- [63] Zerilli M, Zito G, Martorana A, et al. BRAF(V600E) mutation influences hypoxia-inducible factor 1 $\alpha$  expression levels in papillary thyroid cancer. *Mod Pathol* 2010;23:1052–60.
- [64] Nucera C, Porrello A, Antonello ZA, et al. B Raf(V600E) and thrombospondin 1 promote thyroid cancer progression. *Proc Natl Acad Sci USA* 2010;107:54–1064.
- [65] Nowicki TS, Kummer NT, Iacob C, et al. Inhibition of uPAR and uPA reduces invasion in papillary thyroid carcinoma cells. *Laryngoscope* 2010;120:1383–90.
- [66] Nowicki TS, Zhao H, Darzynkiewicz Z, et al. Downregulation of uPAR inhibits migration, invasion, proliferation, FAK/PI3K/Akt signaling and induces senescence in papillary thyroid carcinoma cells. *Cell Cycle* 2011;10:100–7.
- [67] Riesco-Eizaguirre G, Rodríguez I, De la Vieja A, et al. The BRAFV600E oncogene induces transforming growth factor  $\beta$  secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. *Cancer Res* 2009;69:8317–25.
- [68] Knauf JA, Sartor MA, Medvedovic M, et al. Progression of BRAF-induced thyroid cancer is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGF $\beta$  signaling. *Oncogene* 2011;30:3153–62.
- [69] Mian C, Barollo S, Pennelli G, et al. Molecular characteristics in papillary thyroid cancers (PTCs) with no <sup>131</sup>I uptake. *Clin Endocrinol* 2008;68(1):108–16.
- [70] Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase-AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489–501.
- [71] Xing M. Genetic alterations in the phosphatidylinositol 3 kinase/Akt pathway in thyroid cancer. *Thyroid* 2010;20:697–706.
- [72] Saji M, Ringel MD. The PI3K Akt-mTOR pathway in initiation and progression of thyroid tumors. *Mol Cell Endocrinol* 2010;321:20–8.
- [73] Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 1997;16:64–7.
- [74] Ringel MD, Hayre N, Saito J, et al. Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 2001;61(16):6105–11.
- [75] Vasko V, Saji M, Hardy E, et al. Akt activation and localisation correlate with tumor invasion and oncogene expression in thyroid cancer. *J Med Genet* 2004;41(3):161–70.
- [76] Ringel MD, Hayre N, Saito J, et al. Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 2001;61:6105–11.
- [77] García-Rostán G, Costa AM, Pereira-Castro I, et al. Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res* 2005;65(22):10199–207.
- [78] Wu G, Mambo E, Guo Z, et al. Uncommon mutation, but common amplifications, of the PIK3CA gene in thyroid tumors. *J Clin Endocrinol Metab* 2005;90(8):4688–93.
- [79] Hou P, Liu D, Shan Y, et al. Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer. *Clin Cancer Res* 2007;13(4):1161–70.
- [80] Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signaling pathway and cancer. *Cancer Treat Rev* 2004;2(30):193–204.
- [81] Suzuki H, Willingham MC, Cheng SY. Mice with a mutation in the thyroid hormone receptor  $\beta$  gene spontaneously develop thyroid carcinoma: a mouse model of thyroid carcinogenesis. *Thyroid* 2002;12:963–9.
- [82] Furuya F, Hanover JA, Cheng SY. Activation of phosphatidylinositol 3 kinase signaling by a mutant thyroid hormone  $\beta$  receptor. *Proc Natl Acad Sci USA* 2006;103:1780–5.
- [83] Vasko V, Saji M, Hardy E, et al. Akt activation and localisation correlate with tumor invasion and oncogene expression in thyroid cancer. *J Med Genet* 2004;41:161–70.
- [84] Ricarte-Filho JC, Ryder M, Chitale DA, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. *Cancer Res* 2009;69:4885–93.
- [85] Saji M, Narahara K, McCarty SK, et al. Akt1 deficiency delays tumor progression, vascular invasion, and distant metastasis in a murine model of thyroid cancer. *Oncogene* 2011;30:4307–15.
- [86] Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *J Clin Invest* 1993;91(1):179–84.
- [87] Suarez HG, du Villard JA, Severino M, et al. Presence of mutations in all three ras genes in human thyroid tumors. *Oncogene* 1990;5:565–70.
- [88] Ezzat S, Zheng L, Kolenda J, Safarian A, Freeman JL, Asa SL. Prevalence of activating ras mutations in morphologically characterized thyroid nodules. *Thyroid* 1996;6:409–16.
- [89] Meinkoth JL. Biology of Ras in thyroid cells. *Treat Res* 2004;122:131–48.

- [90] Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. *Clin Endocrinol (Oxf)* 1999;50:529–35.
- [91] Motoi N, Sakamoto A, Yamochi T, Horiuchi H, Motoi T, Machinami R. Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. *Pathol Res Pract* 2000;196:1–7.
- [92] Manenti G, Pilotti S, Re FC, Della Porta G, Pierotti MA. Selective activation of ras oncogenes in follicular and undifferentiated thyroid carcinomas. *Eur J Cancer* 1994;30A:987–93.
- [93] Namba H, Rubin SA, Fagin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. *Mol Endocrinol* 1990;4:1474–9.
- [94] Adeniran AJ, Zhu Z, Gandhi M, et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. *Am J Surg Pathol* 2006;30:216–22.
- [95] Zhu Z, Gandhi M, Nikiforova MN, Fischer AH, Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. *Am J Clin Pathol* 2003;120:71–7.
- [96] Lemoine NR, Mayall ES, Wyllie FS, et al. High frequency of ras oncogene activation in all stages of human thyroid tumorigenesis. *Oncogene* 1989;4:159–64.
- [97] Basolo F, Pisaturo F, Pollina LE, et al. N-ras mutation in poorly differentiated thyroid carcinomas: correlation with bone metastases and inverse correlation to thyroglobulin expression. *Thyroid* 2000;10(1):19–23.
- [98] Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. *Clin Endocrinol* 1999;50(4):529–35.
- [99] Motoi N, Sakamoto A, Yamochi T, Horiuchi H, Motoi T, Machinami R. Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. *Pathol Res Pract* 2000;196(1):1–7.
- [100] Rochefort P, Caillou B, Michiels FM, et al. Thyroid pathologies in transgenic mice expressing a human activated Ras gene driven by a thyroglobulin promoter. *Oncogene* 1996;12(1):111–8.
- [101] Kroll TG, Sarraf P, Pecciarini L, et al. PAX8-PPARGamma1 fusion oncogene in human thyroid carcinoma. *Science* 2000;289:1357–60.
- [102] Wang Y, Hou P, Yu H, et al. High prevalence and mutual exclusivity of genetic alterations in the phosphatidylinositol-3-kinase/Akt pathway in thyroid tumors. *J Clin Endocrinol Metab* 2007;92(6):2387–90.
- [103] Kroll TG, Sarraf P, Pecciarini L, et al. PAX8-PPAR gamma1 fusion oncogene in human thyroid carcinoma. *Science* 2000;289:1357–60.
- [104] Gregory Powell J, Wang X, Allard BL, et al. The PAX8/PPARGamma fusion oncoprotein transforms immortalized human thyrocytes through a mechanism probably involving wild-type PPARgamma inhibition. *Oncogene* 2004;23:3634–41.
- [105] Tallini G. Molecular pathobiology of thyroid neoplasms. *Endocr Pathol* 2002;13(4):271–88.
- [106] Kroll TG, Sarraf P, Pecciarini L, et al. PAX8-PPAR 1 fusion in oncogene human thyroid carcinoma. *Science* 2000;289(5483):1357–60.
- [107] Giordano TJ, Au AY, Kuick R, et al. Delineation, functional validation, and bioinformatic evaluation of gene expression in thyroid follicular carcinomas with the PAX8-PPARG translocation. *Clin Cancer Res* 1983;12(7 Pt 1):93.
- [108] Reddi HV, McIver B, Grebe SK, Eberhardt NL. The paired box-8/peroxisome proliferator-activated receptor-gamma oncogene in thyroid tumorigenesis. *Endocrinology* 2007;148(3):932–5.
- [109] French CA, Alexander EK, Cibas ES, et al. Genetic and biological subgroups of low-stage follicular thyroid cancer. *Am J Pathol* 2003;162:1053–60.
- [110] Nikiforova MN, Lynch RA, Biddinger PW, 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.
- [111] Dwight T, Thoppe SR, Foukakis T, et al. Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors. *J Clin Endocrinol Metab* 2003;88:4440–5.
- [112] Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8-PPARGamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. *Am J Surg Pathol* 2002;26:1016–23.
- [113] Marques AR, Espadilha C, Catarino AL, et al. Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas. *J Clin Endocrinol Metab* 2002;87:3947–52.
- [114] Lui WO, Zeng L, Rehrmann V, et al. CREB3L2-PPARGamma fusion mutation identifies a thyroid signaling pathway regulated by intramembrane proteolysis. *Cancer Res* 2008;68:7156–64.
- [115] Farid NR, Shi Y, Zou M. Molecular basis of thyroid cancer. *Endocr Rev* 1994;15(2):202–32.
- [116] Segev DL, Umbricht C, Zeiger MA. Molecular pathogenesis of thyroid cancer. *Surg Oncol* 2003;12(2):69–90.
- [117] Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G, Pierotti MA. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. *J Clin Invest* 1993;91(4):1753–60.
- [118] Dobashi Y, Sugimura H, Sakamoto A, et al. Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. *Diagn Mol Pathol* 1994;3(1):9–14.
- [119] Ho YS, Tseng SC, Chin TY, Hsieh LL, Lin JD. p53 gene mutation in thyroid carcinoma. *Cancer Lett* 1996;103(1):57–63.
- [120] Takeuchi Y, Daa T, Kashima K, Yokoyama S, Nakayama I, Noguchi S. Mutations of p53 in thyroid carcinoma with an insular component. *Thyroid* 1999;9(4):377–81.
- [121] Moretti F, Farsetti A, Soddu S, et al. p53 re-expression inhibits proliferation and restores differentiation of human thyroid anaplastic carcinoma cells. *Oncogene* 1997;14(6):729–40.
- [122] Fagin JA, Tang SH, Zeki K, Di Lauro R, Fusco A, Gonsky R. Reexpression of thyroid peroxidase in a derivative of an undifferentiated thyroid carcinoma cell line by introduction of wild-type p53. *Cancer Res* 1996;56(4):765–71.
- [123] Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. *Endocr Relat Cancer* 2009;16:17–44.
- [124] Nikiforov YE. Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. *Endocr Pathol* 2004;15:319–27.
- [125] Xing M. Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. *Thyroid* 2010;20:697–706.
- [126] La Perle KMD, Jhiang SM, Capen CC. Loss of p53 promotes anaplasia and local invasion in ret/PTC1-induced thyroid carcinomas. *Am J Pathol* 2000;157(2):671–7.
- [127] Powell Jr DJ, Russell JP, Li G, et al. Altered gene expression in immunogenic poorly differentiated thyroid carcinomas from RET/PTC3p53<sup>-/-</sup> mice. *Oncogene* 2001;20(25):3235–46.
- [128] Polakis P. The many ways of Wnt in cancer. *Curr Opin Genet Dev* 2007;17:45–51.
- [129] Kraus C, Liehr T, Hülsken J, et al. Localization of the human beta-catenin gene (CTNNB1) to 3p21: a region implicated in tumor development. *Genomics* 1994;23(1):272–4.
- [130] van Hengel J, Nollet F, Bex G, van Roy N, Speleman F, van Roy F. Assignment of the human beta-catenin gene (CTNNB1) to 3p22-p21.3 by fluorescence in situ hybridization. *Cytogenet Cell Genet* 1995;70(1–2):68–70.
- [131] Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL, Tallini G. Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. *Am J Pathol* 2001;158(3):987–96.
- [132] Miyake N, Maeta H, Horie S, et al. Absence of mutations in the beta-catenin and adenomatous polyposis coli genes in papillary and follicular thyroid carcinomas. *Pathol Int* 2001;51(9):680–5.



- [133] Karin M. NF- $\kappa$ B as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 2009;1(5):000141.
- [134] Pacifico F, Mauro C, Barone C, et al. Oncogenic and anti-apoptotic activity of NF- $\kappa$ B in human thyroid carcinomas. *J Bio Chem* 2004;279:5461–9.
- [135] Visconti R, Cerutti J, Battista S, et al. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NF $\kappa$ B p65 protein expression. *Oncogene* 1997;15:1987–94.
- [136] Mitsiades CS, McMillin D, Kotoula V, et al. Antitumor effects of the proteasome inhibitor bortezomib in medullary and anaplastic thyroid carcinoma cells in vitro. *J Clin Endocrinol Metab* 2006;91:4013–21.
- [137] Pacifico F, Leonardi A. Role of NF- $\kappa$ B in thyroid cancer. *Mol Cell Endocrinol* 2010;321:29–35.
- [138] Li X, Abdel-Mageed AB, Mondal D, Kandil E. The nuclear factor  $\kappa$ -B signaling pathway as a therapeutic target against thyroid cancers. *Thyroid* 2012. Epub ahead of print.
- [139] Zhou YL, Liu C, Dai X, Zhang XH, Wang OC. Overexpression of miR-221 is associated with aggressive clinicopathologic characteristics and the BRAF mutation in papillary thyroid carcinomas. *Med Oncol* 2012;29:3360–6.
- [140] Greco A, Borrello MG, Miranda C, Degl’Innocenti D, Pierotti MA. Molecular pathology of differentiated thyroid cancer. *Q J Nucl Med Mol Imaging* 2009;53(5):440–53.
- [141] Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005;435:834–8.
- [142] Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci* 2006;103(7):2257–61.
- [143] Pallante P, Visone R, Ferracin M, et al. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer* 2006;13:497–508.
- [144] Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *J Clin Endocrinol Metab* 2008;93(5):1600–8.
- [145] Schwertheim S, Sheu SY, Worm K, Grabellus F, Schmid KW. Analysis of deregulated miRNAs is helpful to distinguish poorly differentiated thyroid carcinoma from papillary thyroid carcinoma. *Horm Metab Res* 2009;41:475–81.
- [146] He H, Jazdzewski K, Li W, et al. The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci* 2005;102(52):19075–80.
- [147] Chou CK, Chen RF, Chou FF, et al. miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extrathyroidal invasion and the BRAF(V600E) mutation. *Thyroid* 2010;20(5):489–94.
- [148] Wang S, Wu J, Savas L, Patwardhan N, Khan A. The role of cell cycle regulatory proteins, cyclin D1, cyclin E, and p27 in thyroid carcinogenesis. *Hum Pathol* 1998;29(11):1304–9.
- [149] Chou CK, Yang KD, Chou FF, et al. Prognostic implications of miR-146b expression and its functional role in papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2013;98(2):E196–205.
- [150] Geraldo MV, Yamashita AS, Kimura ET. MicroRNA miR-146b-5p regulates signal transduction of TGF- $\beta$  by repressing SMAD4 in thyroid cancer. *Oncogene* 2012;31:1910–22.
- [151] Wang Z, Zhang H, He L, et al. Association between the expression of four upregulated miRNAs and extrathyroidal invasion in papillary thyroid carcinoma. *Onco Targets Ther* 2013;6:281–7.
- [152] Wang Z, Zhang H, Zhang P, Li J, Shan Z, Teng W. Upregulation of miR-2861 and miR-451 expression in papillary thyroid carcinoma with lymph node metastasis. *Med Oncol* 2013;30:577.
- [153] Yu S, Liu Y, Wang J, et al. Circulating MicroRNA profiles as potential biomarkers for diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2012;97(6):2084–92.
- [154] Pallante P, Visone R, Croce CM, Fusco A. Deregulation of microRNA expression in follicular cell-derived human thyroid carcinomas. *Endocr Relat Cancer* 2010;17:F91–104.
- [155] Castellone MD, Guarino V, De Falco V, et al. Functional expression of the CXCR4 chemokine receptor is induced by RET/PTC oncogenes and is a common event in human papillary thyroid carcinomas. *Oncogene* 2004;23:5958–67.
- [156] Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C. A limited set of human microRNA is deregulated in follicular thyroid carcinoma. *J Clin Endocrinol Metab* 2006;91:3584–91.
- [157] Argraves WS, Greene LM, Cooley MA, Gallagher WM. Fibulins: physiological and disease perspectives. *EMBO Rep* 2003;4:1127–31.
- [158] Schulte KM, Jonas C, Krebs R, Roher HD. Activin A and activin receptors in thyroid cancer. *Thyroid* 2001;11:3–14.
- [159] Boucheix C, Duc GH, Jasmin C, Rubinstein E. Tetraspanins and malignancy. *Expert Rev Mol Med* 2001;2001:1–17.
- [160] Choi KS, Bae MK, Jeong JW, Moon HE, Kim KW. Hypoxia-induced angiogenesis during carcinogenesis. *J Biochem Mol Biol* 2003;36:120–7.
- [161] Burrows N, Resch J, Cowen RL, et al. Expression of hypoxia-inducible factor 1 $\alpha$  in thyroid carcinomas. *Endocr Relat Cancer* 2010;17:61–72.
- [162] Scarpino S, Cancellario d’Alena F, Di Napoli A, Pasquini A, Marzullo A, Rucio LP. Increased expression of Met protein is associated with up-regulation of hypoxia inducible factor-1 (HIF-1) in tumor cells in papillary carcinoma of the thyroid. *J Pathol* 2004;202:352–8.
- [163] Mo JH, Choi II, Jeong WJ, Jeon EH, Ahn SH. HIF-1 $\alpha$  and HSP90: target molecules selected from a tumorigenic papillary thyroid carcinoma cell line. *Cancer Sci* 2012;103(3):464–71.
- [164] Burrows N, Babur M, Resch J, et al. GDC-0941 inhibits metastatic characteristics of thyroid carcinomas by targeting both the phosphoinositide-3 kinase (PI3K) and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) pathways. *J Clin Endocrinol Metab* 2011;96:e1934–43.
- [165] Zerilli M, Zito G, Martorana A, et al. BRAF(V600E) mutation influences hypoxia-inducible factor-1 $\alpha$  expression levels in papillary thyroid cancer. *Mod Pathol* 2010;23(8):1052–60.
- [166] Burrows N, Babur M, Resch J, et al. GDC-0941 inhibits metastatic characteristics of thyroid carcinomas by targeting both the phosphoinositide-3 kinase (PI3K) and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) pathways. *Clin Endocrinol Metab* 2011;96(12):E1934–43.
- [167] Koperek O, Bergner O, Pichlhöfer B, et al. Expression of hypoxia-associated proteins in sporadic medullary thyroid cancer is associated with desmoplastic stroma reaction and lymph node metastasis and may indicate somatic mutations in the VHL gene. *J Pathol* 2011;225(1):63–72.
- [168] Xing M. Gene methylation in thyroid tumorigenesis. *Endocrinology* 2007;148:948–53.
- [169] Hu S, Liu D, Tufano RP, et al. Association of aberrant methylation of tumor suppressor genes with tumor aggressiveness and BRAF mutation in papillary thyroid cancer. *Int J Cancer* 2006;119:2322–9.
- [170] Hou P, Liu D, Xing M. Genome-wide alterations in gene methylation by the BRAF V600E mutation in papillary thyroid cancer cells. *Endocr Relat Cancer* 2011;18:687–97.
- [171] Hou P, Ji M, Xing M. Association of PTEN gene methylation with genetic alterations in the phosphatidylinositol 3-kinase/AKT signaling pathway in thyroid tumors. *Cancer* 2008;113:2440–7.
- [172] Alvarez-Núñez F, Bussaglia E, Mauricio D, et al. PTEN promoter methylation in sporadic thyroid carcinomas. *Thyroid* 2006;16:17–23.
- [173] Frisk T, Foukakis T, Dwight T, et al. Silencing of the PTEN tumor-suppressor gene in anaplastic thyroid cancer. *Genes Chromosomes Cancer* 2002;35:74–80.
- [174] Bruni P, Boccia A, Baldassarre G, et al. PTEN expression is reduced in a subset of sporadic thyroid carcinomas: evidence that PTEN growth suppressing activity in thyroid cancer cells mediated by p27kip1. *Oncogene* 2000;19:3146–55.
- [175] Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. *Am J Clin Pathol* 2009;132:658–65.

- [176] Fassina AS, Montesco MC, Ninfo V, Denti P, Masarotto G. Histological evaluation of thyroid carcinomas: reproducibility of the “WHO” classification. *Tumori* 1993;79:314–20.
- [177] Franc B, de la Salmoniere P, Lange F, et al. Interobserver and intraobserver reproducibility in the histopathology of follicular thyroid carcinoma. *Hum Pathol* 2003;34:1092–100.
- [178] Eszlinger M, Krohn K, Hauptmann S, Dralle H, Giordano TJ, Paschke R. Perspectives for improved and more accurate classification of thyroid epithelial tumors. *J Clin Endocrinol Metab* 2008;93:3286–94.
- [179] Faggiano A, Caillou B, Lacroix L, et al. Functional characterization of human thyroid tissue with immunohistochemistry. *Thyroid* 2007;17:203–11.
- [180] Fusco A, Santoro M. 20 years of RET/PTC in thyroid cancer: clinic-pathological correlations. *Arq Bras Endocrinol Metab* 2007;51:731–5.
- [181] Marotta V, Guerra A, Sapio MR, Vitale M. RET/PTC rearrangement in benign and malignant thyroid disease: a clinical standpoint. *Eur J Endocrinol* 2011;165:499–507.
- [182] Clark DP. Molecular diagnostics on thyroid fine-needle aspirations. *Cancer Cytopathol* 2010;25:14–6.
- [183] Fusco A, Grieco M, Santoro M, et al. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. *Nature* 1987;328:170–3.
- [184] Grieco M, Santoro M, Berlingieri MT, et al. PTC is a novel rearranged form of the RET proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* 1990;60:557–63.
- [185] Santoro M, Melillo RM, Fusco A. RET/PTC activation in papillary thyroid carcinoma. *Eur J Endocrinol* 2006;155:645–53.
- [186] Chiappetta G, Toti P, Cetta F, et al. The RET/PTC oncogene is frequently activated in oncocytic thyroid tumors (Hürthle cell adenomas and carcinomas), but not in oncocytic hyperplastic lesions. *J Clin Endocrinol Metab* 2002;87:364–9.
- [187] Rhoden KJ, Unger K, Salvatore G, et al. RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto’s thyroiditis share low-level recombination events with a subset of papillary carcinoma. *J Clin Endocrinol Metab* 2006;91:2414–23.
- [188] Guerra A, Sapio MR, Marotta V, et al. Prevalence of RET/PTC rearrangement in benign and malignant thyroid nodules and its clinical application. *Endocr J* 2011;58:31–8.
- [189] Marotta V, Guerra A, Sapio MR, et al. Growing thyroid nodules with benign histology and RET rearrangement. *Endocr J* 2010;57:1081–7.
- [190] Sapio MR, Guerra A, Marotta V, et al. High growth rate of benign thyroid nodules bearing RET/PTC rearrangements. *J Clin Endocrinol Metab* 2011;96:E916–9.
- [191] Tallini G, Santoro M, Helie M, et al. RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. *Clin Cancer Res* 1998;4:287–94.
- [192] Thomas GA, Bunnell H, Cook HA, et al. High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. *J Clin Endocrinol Metab* 1999;84:4232–8.
- [193] Rabes HM, Demidchik EP, Sidorow JD, et al. Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clin Cancer Res* 2000;6:1093–103.
- [194] Elisei R, Romei C, Vorontsova T, et al. RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated malignant and benign thyroid lesions in children and adults. *J Clin Endocrinol Metab* 2001;86(7):3211–6.
- [195] Fusco A, Chiappetta G, Hui P, et al. Assessment of RET/PTC oncogene activation and clonality in thyroid nodules with incomplete morphological evidence of papillary carcinoma: a search for the early precursors of papillary cancer. *Am J Pathol* 2002;160(6):2157–67.
- [196] Nikiforov YE, Steward DL, Robinson-Smith TM, et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. *J Clin Endocrinol Metab* 2009;94(6):2092–8.
- [197] Cantara S, Capezzone M, Marchisotta S, et al. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. *J Clin Endocrinol Metab* 2010;95:1365–9.
- [198] Pizzolanti G, Russo L, Richiusa P, et al. Fine-needle aspiration molecular analysis for the diagnosis of papillary thyroid carcinoma through BRAFV600E mutation and RET/PTC rearrangement. *Thyroid* 2007;17:1109–15.
- [199] Tufano RP, Teixeira GV, Bishop J, Carson KA, Xing M. BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. *Medicine* 2012;91:274–86.
- [200] Xing M. Prognostic utility of BRAF mutation in papillary thyroid cancer. *Mol Cell Endocrinol* 2010;321:86–93.
- [201] Xing M, Westra WH, Tufano RP, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab* 2005;90:6373–9.
- [202] Romei C, Ciampi R, Faviana P, et al. BRAFV600E mutation, but not RET/PTC rearrangements, is correlated with a lower expression of both thyroperoxidase and sodium iodide symporter genes in papillary thyroid cancer. *Endocr Relat Cancer* 2008;15:511–20.
- [203] Jin L, Sebo TJ, Nakamura N, et al. BRAF mutation analysis in fine needle aspiration (FNAB) cytology of the thyroid. *Diagn Mol Pathol* 2006;15:136–43.
- [204] Cohen Y, Rosenbaum E, Clark DP, et al. Mutational analysis of BRAF in fine needle aspiration biopsies of the thyroid: a potential application for the preoperative assessment of thyroid nodules. *Clin Cancer Res* 2004;10:2761–5.
- [205] Sapio MR, Posca D, Raggioli A, et al. Detection of RET/PTC, TRK and BRAF mutations in preoperative diagnosis of thyroid nodules with indeterminate cytological findings. *Clin Endocrinol (Oxf)* 2007;66(5):678–83.
- [206] Pizzolanti G, Russo L, Richiusa P, et al. Fine-needle aspiration molecular analysis for the diagnosis of papillary thyroid carcinoma through BRAF(V600E) mutation and RET/PTC rearrangement. *Thyroid* 2007;17:1109–15.
- [207] Nikiforova MN, Nikiforov YE. Molecular diagnostics and predictors in thyroid cancer. *Thyroid* 2009;19:61.
- [208] Kim SK, Hwang TS, Yoo YB, et al. Surgical results of thyroid nodules according to a management guideline based on the BRAF(V600E) mutation status. *J Clin Endocrinol Metab* 2011;96:658–64.
- [209] Kim SW, Lee JI, Kim JW, et al. BRAFV600E mutation analysis in fine-needle aspiration cytology specimens for evaluation of thyroid nodule: a large series in a BRAFV600E-prevalent population. *J Clin Endocrinol Metab* 2010;95:3693–700.
- [210] Rowe LM, Bentz BM, Bentz JM. Utility of BRAF V600E mutation detection in cytologically indeterminate thyroid nodules. *Cytojournal* 2006;3:10.
- [211] Otori NP, Nikiforov MN. Contribution of molecular testing to thyroid fine needle aspiration cytology of follicular lesion of undetermined significance/atypia of undetermined significance. *Cancer (Cytopathology)* 2010;118:17–23.
- [212] Moses W, Weng J, Sansano I, et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. *World J Surg* 2010;34:2589–94.
- [213] Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2009;19:1167–214.
- [214] Baloch ZW, LiVolsi VA, Asa SL, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. *Diagn Cytopathol* 2008;36:425–37.

- [215] Lubitz CC, Fahey 3rd TJ. The differentiation of benign and malignant thyroid nodules. *Adv Surg* 2005;39:355–77.
- [216] Prasad NB, Somervell H, Tufano RP, et al. Identification of genes differentially expressed in benign versus malignant thyroid tumors. *Clin Cancer Res* 2008;14:3327–37.
- [217] Belge G, Meyer A, Klemke M, et al. Upregulation of HMGA2 in thyroid carcinomas: a novel molecular marker to distinguish between benign and malignant follicular neoplasias. *Genes Chromosomes Cancer* 2008;47:656–63.
- [218] Chiappetta G, Ferraro A, Vuttariello E, et al. HMGA2 mRNA expression correlates with the malignant phenotype in human thyroid neoplasias. *Eur J Cancer* 2008;44:1015–21.
- [219] Lappinga PJ, Kip NS, Jin L, et al. HMGA2 gene expression analysis performed on cytologic smears to distinguish benign from malignant thyroid nodules. *Cancer Cytopathol* 2010;118:287–97.
- [220] Griffith OL, Melck A, Jones SJM, Wiseman SM. Meta-analysis and meta-review of thyroid cancer gene expression profiling studies identifies important diagnostic biomarkers. *J Clin Oncol* 2006;24:5043–51.
- [221] Chudova D, Wilde JJ, Wang ET, et al. Molecular classification of thyroid nodules using high-dimensionality genomic data. *J Clin Endocrinol Metab* 2010;95:5296–304.
- [222] Mathur A, Weng J, Moses W, et al. A prospective study evaluating the accuracy of using combined clinical factors and candidate diagnostic markers to refine the accuracy of thyroid fine needle aspiration biopsy. *Surgery* 2010;148:1170–6.
- [223] Nikiforova MN, Chiosea SI, Nikiforov YE. MicroRNA expression profiles in thyroid tumors. *Endocr Pathol* 2009;20:85–91.
- [224] Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004;116(6):855–67.
- [225] Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004;64(19):7099–109.
- [226] Salvatore G, De Falco V, Salerno P, et al. BRAF is a therapeutic target in aggressive thyroid carcinoma. *Clin Cancer Res* 2006;12(5):1623–9.
- [227] Carlomagno F, Anaganti S, Guida T, et al. BAY 43-9006 inhibition of oncogenic RET mutants. *J Natl Cancer Inst* 2006;98:326–34.
- [228] Baudin E, Schlumberger M. New therapeutic approaches for metastatic thyroid carcinoma. *Lancet Oncol* 2007;8:148–56.
- [229] Espinosa AV, Porchia L, Ringel MD. Targeting BRAF in thyroid cancer. *Br J Cancer* 2007;96:16–20.
- [230] Ouyang B, Knauf JA, Smith EP, et al. Inhibitors of Raf kinase activity block growth of thyroid cancer cells with RET/PTC or BRAF mutations in vitro and in vivo. *Clin Cancer Res* 2006;12:1785–93.
- [231] Solit DB, Garraway LA, Pratilas CA, et al. BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 2006;439:358–62.
- [232] Herbst RS, Heymach JV, O'Reilly MS, Onn A, Ryan AJ. Vandetanib (ZD6474): an orally available receptor tyrosine kinase inhibitor that selectively targets pathways critical for tumor growth and angiogenesis. *Expert Opin Invest Drugs* 2007;16:239–49.
- [233] Carlomagno F, Vitagliano D, Guida T, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res* 2002;62(24):7284–90.
- [234] Santoro M, Carlomagno F. Drug insight: small-molecule inhibitors of protein kinases in the treatment of thyroid cancer. *Nat Clin Pract Endocrinol Metab* 2006;2(1):42–52.
- [235] Carlomagno F, Guida T, Anaganti S, et al. Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. *Oncogene* 2004;23:6056–63.
- [236] Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. *J Clin Oncol* 2005;23:2556–68.
- [237] Kim DW, Jo YS, Jung HS, et al. An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. *J Clin Endocr Metab* 2006;91:4070–6.
- [238] Carlomagno F, Vitagliano D, Guida T, et al. The kinase inhibitor PP1 blocks tumorigenesis induced by RET oncogenes. *Cancer Res* 2002;62:1077–82.
- [239] Carlomagno F, Vitagliano D, Guida T, et al. Efficient inhibition of RET/papillary thyroid carcinoma oncogenic kinases by 4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). *J Clin Endocrinol Metab* 2003;88:1897–902.
- [240] Garcia-Rostan G, Zhao H, Camp RL, et al. Ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. *J Clin Oncol* 2003;21:3226–35.
- [241] Fagin JA. Minireview: branded from the start-distinct oncogenic initiating events may determine tumor fate in the thyroid. *Mol Endocrinol* 2002;16:903–11.
- [242] Saavedra HI, Knauf JA, Shirokawa JM, et al. The RAS oncogene induces genomic instability in thyroid PCCL3 cells via the MAPK pathway. *Oncogene* 2000;19:3948–54.
- [243] Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol* 2004;22(14):63.
- [244] Furuya F, Lu C, Willingham MC, Cheng SY. Inhibition of phosphatidylinositol 3-kinase delays tumor progression and blocks metastatic spread in a mouse model of thyroid cancer. *Carcinogenesis* 2007;28(12):2451–8.
- [245] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009;8(8):627–44.
- [246] Placzkowski KA, Reddi HV, Grebe SK, Eberhardt NL, McIver B. The role of the PAX8/PPARGgamma fusion oncogene in thyroid cancer. *PPAR Res* 2008;2008:672829.
- [247] Karin M. NF- $\kappa$ B as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 2009;1(5):41.
- [248] Liu D, Xing M. Potent inhibition of thyroid cancer cells by the MEK inhibitor PD0325901 and its potentiation by suppression of the PI3K and NF- $\kappa$ B pathways. *Thyroid* 2008;18(8):853–64.
- [249] Xing M. Minireview: gene methylation in thyroid tumorigenesis. *Endocrinology* 2007;148(3):948–53.
- [250] Akagi T, Luong QT, Gui D, et al. Induction of sodium iodide symporter gene and molecular characterisation of HNF3 $\beta$ /FoxA2, TTF-1 and C/EBP $\beta$  in thyroid carcinoma cells. *Br J Cancer* 2008;99(5):781–8.
- [251] Kouzarides T. Chromatin modifications their function. *Cell* 2007;128(4):693–705.
- [252] Ting AH, McGarvey KM, Baylin SB. The cancer epigenome-components and functional correlates. *Genes Dev* 2006;20(23):3215–31.
- [253] Riesco-Eizaguirre G, Santisteban P. New insights in thyroid follicular cell biology and its impact in thyroid cancer therapy. *Endocr Relat Cancer* 2007;14(4):957–77.
- [254] Lim SM, Chang H, Yoon MJ, et al. A multicenter, phase II trial of everolimus in locally advanced or metastatic thyroid cancer of all histologic subtypes. *Ann Oncol* 2013;24(12):3089–94.
- [255] Ball DW, Sherman SI, Jarzab B, et al. Lenvatinib treatment of advanced RAI-refractory differentiated thyroid cancer (DTC): cytokine and angiogenic factor (CAF) profiling in combination with tumor genetic analysis to identify markers associated with response. *J Clin Oncol* 2012;30(15):5518.
- [256] Pennell NA, Daniels GH, Haddad RI, et al. A phase II study of gefitinib in patients with advanced thyroid cancer. *Thyroid* 2008;18(3):317–23.
- [257] Sherman EJ, Fury MG, Tuttle RM, et al. Phase II study of depsipeptide (DEP) in radioiodine (RAI)-refractory metastatic nonmedullary thyroid carcinoma. *J Clin Oncol* 2009;27(15S):6059.
- [258] Woyach JA, Kloos RT, Ringel MD, et al. Lack of therapeutic effect of the histone deacetylase inhibitor vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma. *J Clin Endocrinol Metab* 2009;94(1):164–70.

- [259] Cohen EE, Rosen LS, Vokes EE, et al. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. *J Clin Oncol* 2008;26(29):4708–13.
- [260] Gupta-Abramson V, Troxel AB, Nellore A, et al. Phase II trial of sorafenib in advanced thyroid cancer. *J Clin Oncol* 2008;26(29):4714–9.
- [261] Kelly WK, O'Connor OA, Krug LM, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol* 2005;23(17):3923–31.
- [262] Sherman SI, Wirth LJ, Droz JP, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. *N Engl J Med* 2008;359(1):31–42.
- [263] Ain KB, Lee C, Williams KD, Phase II. trial of thalidomide for therapy of radioiodine-unresponsive and rapidly progressive thyroid carcinomas. *Thyroid* 2007;17(7):663–70.
- [264] Bible KC, Suman VJ, Molina JR, et al. Efficacy of pazopanib in progressive, radioiodine-refractory, metastatic differentiated thyroid cancers: results of a phase 2 consortium study. *Lancet Oncol* 2010;11(10):962–72.
- [265] Zhang Y, Guessous F, Kofman A, Schiff D, Abounader R. XL-184, a MET, VEGFR-2 and RET kinase inhibitor for the treatment of thyroid cancer, glioblastoma multiforme and NSCLC. *IDrugs* 2010;13(2):112–21.
- [266] de Groot JW, Zonnenberg BA, van Ufford-Mannesse PQ, et al. A phase II trial of imatinib therapy for metastatic medullary thyroid carcinoma. *J Clin Endocrinol Metab* 2007;92(9):3466–9.
- [267] Mrozek E, Kloos RT, Ringel MD, et al. Phase II study of celecoxib in metastatic differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2006;91(6):2201–4.
- [268] Cooney MM, Savvides P, Agarwala S, et al. Phase II study of combretastatin A4 phosphate (CA4P) in patients with advanced anaplastic thyroid carcinoma (ATC). *J Clin Oncol* 2006;24(18).
- [269] Cohen EE, Needles BM, Cullen KJ, et al. Phase 2 study of sunitinib in refractory thyroid cancer. *J Clin Oncol* 2008;26(15):6025.
- [270] Wells Jr SA, Robinson BG, Gagel RF, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol* 2012;30(2):134–41.
- [271] Wells Jr SA, Gosnell JE, Gagel RF, et al. Vandetanib in metastatic hereditary medullary thyroid cancer: follow-up results of an open-label phase II trial. *J Clin Oncol* 2007;25(18):6018.
- [272] Ain KB, Lee C, Holbrook KM, Dziba JM, Williams KD. Phase II study of lenalidomide in distant metastatic, rapidly progressive, and radioiodine-unresponsive thyroid carcinomas: preliminary results. *J Clin Oncol* 2008;26(15):6027.
- [273] Elisei R, Schlumberger MJ, Müller SP, et al. Cabozantinib in progressive medullary thyroid cancer. *J Clin Oncol* 2013;31(29):3639–46.
- [274] Meier CA, Braverman LE, Ebner SA, et al. Diagnostic use of recombinant human thyrotropin in patients with thyroid carcinoma (phase I/II study). *J Clin Endocrinol Metab* 1994;78(1):188–96.
- [275] Mooney CJ, Nagaiah G, Fu P, et al. A phase II trial of fosbretabulin in advanced anaplastic thyroid carcinoma and correlation of baseline serum-soluble intracellular adhesion molecule-1 with outcome. *Thyroid* 2009;19(3):233–40.
- [276] Piekarz R, Luchenko V, Draper D, et al. Phase I trial of romidepsin, a histone deacetylase inhibitor, given on days one, three and five in patients with thyroid and other advanced cancers. *J Clin Oncol* 2008;26(15):3571.
- [277] Argiris A, Agarwala SS, Karamouzis MV, Burmeister LA, Carty SE. A phase II trial of doxorubicin and interferon alpha 2b in advanced, non-medullary thyroid cancer. *Invest New Drugs* 2008;26(2):183–8.
- [278] Kebebew E, Peng M, Reiff E, et al. A phase II trial of rosiglitazone in patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer. *Surgery* 2006;140(6):960–6.
- [279] www.clinicaltrials.gov
- [280] Liu D, Xing J, Trink B, Xing M. BRAF mutation-selective inhibition of thyroid cancer cells by the novel MEK inhibitor RDEA119 and genetic-potiated synergism with the mTOR inhibitor temsirolimus. *Int J Cancer* 2010;127(12):2965–73.
- [281] Jin N, Jiang T, Rosen DM, Nelkin BD, Ball DW. Synergistic action of a RAF inhibitor and a dual PI3K/mTOR inhibitor in thyroid cancer. *Clin Cancer Res* 2013;17(20):6482–9.
- [282] Liu R, Liu D, Xing M. The Akt inhibitor MK2206 synergizes, but perifosine antagonizes, the BRAF(V600E) inhibitor PLX4032 and the MEK1/2 inhibitor AZD6244 in the inhibition of thyroid cancer cells. *J Clin Endocrinol Metab* 2012;97(2):E173–82.
- [283] Xing M. Genetic-targeted therapy of thyroid cancer: a real promise. *Thyroid* 2009;19(8):805–9.
- [284] Kloos RT, Ringel MD, Knopp MV, et al. Phase II trial of sorafenib in metastatic thyroid cancer. *J Clin Oncol* 2009;27(10):1675–84.
- [285] Ahmed M, Barbachano Y, Riddell A, et al. Analysis of the efficacy and toxicity of sorafenib in thyroid cancer: a phase II study in a UK based population. *Eur J Endocrinol* 2011;165(2):315–22.
- [286] Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010;363(9):809–19.
- [287] Hou P, Bojdani E, Xing M. Induction of thyroid gene expression and radioiodine uptake in thyroid cancer cells by targeting major signaling pathways. *J Clin Endocrinol Metab* 2010;95(2):820–8.

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