Signaling pathways in follicular cell-derived thyroid carcinomas (Review)

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Abstract. Thyroid carcinoma is the most common malignant endocrine neoplasia. Differentiated thyroid carcinomas (DTCs) represent more than 90% of all thyroid carcinomas and comprise the papillary and follicular thyroid carcinoma subtypes. Anaplastic thyroid carcinomas correspond to less than 1% of all thyroid tumors and can arise *de novo* or by dedifferentiation of a differentiated tumor. The etiology of DTCs is not fully understood. Several genetic events have been implicated in thyroid tumorigenesis. Point mutations in the *BRAF* or *RAS* genes or rearranged in transformation (RET)/papillary thyroid carcinoma (PTC) gene rearrangements are observed in approximately 70% of papillary cancer cases. Follicular carcinomas commonly harbor *RAS* mutations and paired box gene 8 (PAX8)-peroxisome proliferator-activated receptor γ (PPARγ) rearrangements. Anaplastic carcinomas may have a wide set of genetic alterations, that include gene effectors in the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and/or β-catenin signaling pathways. These distinct genetic alterations constitutively activate the MAPK, PI3K and β-catenin signaling pathways, which have been implicated in thyroid cancer development and progression. In this context, the evaluation of specific genes, as well as the knowledge of their effects on thyroid carcinogenesis may provide important information on disease presentation, prognosis and therapy, through the development of specific tyrosine kinase targets. In this review, we aimed to present an updated and comprehensive review of the recent advances in the understanding of the genetic basis of follicular cell-derived thyroid carcinomas, as well as the molecular mechanisms involved in tumor development and progression.

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1. Introduction

Thyroid carcinoma is the most common type of malignant endocrine neoplasia, accounting for approximately 1% of all new malignant diseases with an annual incidence of 5.9 and 17.3 per 100,000 in men and women, respectively (US 2005-2009) (1,2). Follicular cell-derived thyroid neoplasias include differentiated thyroid carcinoma (DTC), which represents more than 90% of all thyroid malignancies and comprise the papillary and follicular thyroid carcinomas (FTCs). The anaplastic thyroid carcinoma (ATC) corresponds to 1% of all thyroid tumors and can arise *de novo* or by the dedifferentiation of a papillary or follicular tumor (3). Medullary thyroid carcinoma (MTC) is a malignancy arising from the parafollicular C-cells and accounts for approximately 3-8% of all thyroid carcinomas (4).

The etiology of DTC is not yet fully understood. External radiation is the only exogenous factor which has been clearly identified as causing thyroid carcinoma, almost exclusively the papillary form. Iodine excess has been associated with the increase in the incidence of papillary thyroid carcinoma (PTC) (5,6). A number of genetic events have been described in thyroid carcinoma pathogenesis. Papillary carcinomas commonly present genetic alterations that lead to the activation of the mitogen-activated protein kinase (MAPK) pathway (7-9). In follicular carcinomas, the induction of both the MAPK and phosphatidylinositol 3-kinase (PI3K) cascades is frequently observed (10). On the contrary, anaplastic carcinomas harbor a wide set of additive genetic alterations, occurring mainly in the gene effectors of the MAPK, PI3K and β-catenin signaling pathways (11-13). These distinct signaling pathways have been implicated in follicular cell-derived thyroid cancer development and progression (14-16).

In this review, we aimed to present a comprehensive account of the recent advances in the understanding of the signaling pathways in follicular cell-derived thyroid carcinomas, as well as the molecular mechanisms involved in tumor development and progression.

2. Papillary thyroid carcinoma

PTC represents ~80% of all malignant thyroid tumors. The overall incidence of PTC is 7.7 per 100,000 and is increasing, in part due to the increase in the detection of small tumors (16). PTC is often diagnosed at approximately the 5th decade of life and is known to be a slow-growing tumor (17,18). Patients usually present with a palpable nodule and the absence of any other clinical findings is common (3). The majority of patients have a favorable outcome; however, ~10% of the cases have tumor recurrence and metastatic disease (18,19).

Aberrant activation of the MAPK pathway due to mutations or gene rearrangements is the most common genetic event in PTC (7-9). Point mutations in *BRAF* or *RAS* genes and (RET)/PTC or NTRK1 rearrangements are mutually exclusive and identified in more than 70% of PTCs (7-9). The Fig. 1A summarizes the major signaling pathways involved in PTC.

BRAF oncogene. Mutations in the *BRAF* gene are the most common genetic alteration in PTC, occurring in ~45% of cases (6). BRAF is a serine-threonine kinase protein, member of the RAF (v-raf-1 murine leukemia viral oncogene homolog) family, which comprises the serine/threonine-specific kinase effectors of the MAPK cascade (7,20,21). Briefly, the MAPK cascade effects initiate upon RAS activation, which recruits BRAF to the plasma membrane initiating its activation. Once activated, BRAF phosphorylates MEK, which in turn provides the signal to activate the tyrosine, ERK, in the cytosol and nucleus, leading to cell proliferation, migration and survival (22,23) (Fig. 1A). Approximately 95% of all *BRAF* mutations involve a T>A transversion at gene position 1799, resulting in valine to glutamate amino acid substitution at position 600 of the protein (V600E). Other described alterations in the BRAF gene include the A>G transversion at gene position 1801 (K601E), fusion with the A-kinase anchor protein 9 (*AKAP9*) gene and small in-frame insertions or deletions around codon 600 (24-26).

The presence of BRAF mutations in micro-PTC (~40%) and benign tumors (9,27,28) suggests a role of this alteration in the early stages of PTC development. $BRAF^{V600E}$ is an oncogenic protein with markedly elevated kinase activity that overactivates the MAPK pathway (34,35). Studies using $BRAF^{V600E}$-transgenic mice have shown the development of PTC with similar properties to those observed in human *BRAF*-positive PTCs (29), whereas mice with the constitutive or doxycycline-inducible *BRAF*-mutated gene develop infiltrative PTC with a high rate of extrathyroidal structures, vascular invasion and a poorly differentiated aspect (30,31). The induction of $BRAF^{V600E}$ mutation has been shown to abolish the expression of several thyroid-specific genes, radioiodine uptake and cause pronounced hypothyroidism, which may be partially explained by the down-regulation of the thyroid hormone activating type 1 and 2 deiodinases and induction of the thyroid hormone inactivating type 3 deiodinase, as recently described (31,33).

BRAF mutations are typically identified in classical and tall cell variant of PTC and are associated with a more aggressive tumor behavior (9,34,35). The high growth rates observed in $BRAF^{V600E}$ tumors may be explained partially by the MAPK-induced hyperphosphorylation with consequent inhibition of the retinoblastoma (RB) protein, dependent transcription factors (E2F) and p27 of cyclin-dependent kinase (CDK) activity (36). Moreover, the *BRAF* oncogene induces the expression of matrix metalloproteinases (MMPs), a large group of enzymes that regulate cell-matrix composition and are important factors of tumor invasiveness (37-39). Previous studies have suggested that MMP proteins are modulated according to the intensity of MAPK pathway activation and/or signal transducer and activator of transcription (STAT) expression, which may explain the mechanism of induction of these proteins in BRAF-mutated PTCs and the increased propensity of these tumors to invade surrounding tissues (37,40). The BRAF-mutated protein also induces nuclear factor-κB (NF-κB). Thyroid cells (WRO) harboring this oncogene display increased levels of activity in the NF-κB pathway, which results in the upregulation of anti-apoptotic factors and the induction of cell invasion (40).

Recently, a novel inhibitory mechanism that may operate in $BRAF^{V600E}$-induced PTC was shown. The presence of $BRAF^{V600E}$ mutation abolished the macrophage stimulating 1/forkhead box O3 (MST1/FOXO3) pathway transactivation in a thyroid cell line (FRO), resulting in the suppression of p21 and p27 CDK inhibitors and interrupting the apoptotic process. Accordingly, the development of $BRAF^{V600E}$ transgenic mice with the MST1 knockout leads to abundant foci of poorly differentiated thyroid carcinoma and large areas without follicular architecture or colloid formation, suggesting that the activity of the MST1/FOXO3 pathway determines the phenotype of $BRAF^{V600E}$ tumors (41).

RET/PTC rearrangements. The *RET* proto-oncogene, located on chromosome 10q11.2, encodes a tyrosine kinase receptor. The RET protein is usually expressed in cells derived from the neural crest and gain-of-function mutations are associated with MTC (42). In PTC, genomic rearrangements juxtapose the RET tyrosine kinase domain to unrelated genes, thereby creating dominantly transforming oncogenes, denominated RET/PTC. The RET/PTC rearrangements are the 2nd most common genetic alteration described in PTC and observed in ~13-43% of cases, mostly in pediatric cancers or in individuals exposed to ionizing radiation from nuclear accidents (12,43-45). At least 12 types of RET/PTC rearrangements have been reported, all originating from the RET fusion to different partners (44,46). RET/PTC1 comprises up to 60% of the rearrangements and is derived from an intrachromosomal rearrangement (10q), leading to the fusion of the RET tyrosine kinase domain to the *H4* gene (*D10S170*). The RET/PTC1 encodes a 585-amino acid protein with unknown function (47). RET/PTC3 accounts for 20-30% of the rearrangements and is formed by the RET gene fusion with the nuclear receptor coactivator 4 (*NCOA4*) gene (also known as *ELE1, RFG* or *ARA70*) (44,47).

Papillary tumors harboring the RET/PTC1 rearrangement commonly exhibit the classical papillary histology, whereas RET/PTC3 tumors normally present the solid variant (48). RET/PTC tumors tend to be small, with a favorable outcome and usually do not progress to a more aggressive behavior and/

or undifferentiated thyroid carcinoma (9,49,50). This alteration has also been associated with a younger age at diagnosis and a higher rate of lymph node metastasis (9,49). The high prevalence of RET/PTC in occult (42%) or microscopic PTC (77%) as well as in follicular adenoma (45%), may indicate a putative role of this rearrangement during the early stages of PTC development (51,52). Accordingly, studies performed using transgenic mice carrying RET/PTC1 and/or RET/PTC3 have shown that the PTC tumors which develop in these animals are similar to those occurring in humans (53,54).

The RET/PTC-derived mechanisms of tumor induction initiate with the fusion of protein partners, resulting in the ligand-independent autophosphorylation of the RET protein. The RET intracellular domain contains at least 12 autophosphorylation sites, and 11 of them are preserved in the RET/PTC protein (55). The Y1062 and Y1015 RET residues are constitutively phosphorylated and are required for cell transformation (56). These residues are essential binding sites for several proteins, which in turn, lead to the activation of the MAPK and PI3K/AKT signaling pathways and play an essential role in RET/PTC signaling with downstream cellular effects on migration and proliferation (57-59).

Another dysfunctional signaling pathway identified in 65-90% of RET/PTC-positive tumors is β-catenin, which is involved in gene transcription and cell adhesion regulation (60,61). The β-catenin pathway can be directly activated by several mechanisms: via RET tyrosine residue, cAMP response element-binding (CREB), glycogen synthase kinase 3 phosphorylation (GSK3-S) or via effectors of the MAPK and PI3K pathways (61,62). The increase in the free β-catenin protein pool promotes proliferation and invasion, possibly due to the interaction with transcriptional factors, such as the T-cell factor/lymphoid enhancer factor (TCF/LEF), c-Myc (v-myc myelocytomatosis viral oncogene homolog), or cyclin D1 (60,61,63).

RAS oncogene. *RAS* genes (H-*RAS*, K-*RAS*, and N-*RAS*) encode highly related G-proteins which play a central role in intracellular signal transduction by the activation of the MAPK and other signaling pathways, such as PI3K/AKT (see below) (15). *RAS* gene mutations are found in 10-43% of PTCs, particularly in the follicular variant (64-66). The RAS point mutations generally occur in codons 12, 13, or 61 of H-RAS, K-RAS, or N-RAS proteins. RAS-mutated PTC tends to be encapsulated and exhibits a low rate of lymph node metastasis (9,65). However, previous studies have reported that this mutation may also be associated with a more aggressive phenotype and a higher incidence of distant metastasis (66,67). The molecular mechanism proposed for *RAS*-derived tumorigenesis is the constitutive activation of distinct pathways involved in proliferation, differentiation and cell survival processes (66).

NTRK1 rearrangements. The neurotrophic tyrosine kinase receptor, type 1 (*NTRK1*) gene, located on chromosome 1, encodes the high-affinity nerve growth factor (NGF) receptor and is activated through the MAPK pathway (68). NTRK1 rearrangements are usually found in <10% of PTCs and result from the *NTRK1* gene fusion with different partners (69,70,71). Experimental evidence suggests that the *NTRK1* oncogene represents an early event in the process of thyroid carcinogenesis. Transgenic mice carrying *NTRK1* oncogene develop thyroid hyperplasia and PTC (72). Additionally, crossing NTRK1 mice with p27kip1-deficient mice has been shown to increase the penetrance of thyroid cancer and shorten the tumor latency period (73). NTRK1 rearrangements are associated with a younger age at diagnosis and a less favorable outcome (69,70).

3. Follicular thyroid carcinoma

The FTC represents 10-15% of thyroid cancers. These tumors are generally unifocal and present less lymph node involvement (<5%) than PTCs. By contrast, distant metastases, mainly to the lungs and bones, are more frequent at disease presentation (~20%) (4). Although former studies have indicated that FTCs, particularly the invasive form, have a poorer prognosis than PTCs (74,75), a recent study that evaluated more than 1,000 patients did not find differences in tumor-specific survival between PTC and FTC, after controlling for age, primary tumor size, extrathyroidal invasion or distant metastasis at diagnosis (76).

The most common genetic events observed in follicular carcinomas are point mutations in *RAS* genes and the rearrangements between the thyroid-specific transcription factor gene and the peroxisome proliferator-activated receptor gene [paired box gene 8 (PAX8)-peroxisome proliferator-activated receptor γ (PPARγ) rearrangements] (80%). Similarly to what is described in PTC, their oncogenic effects occur through the activation of the MAPK cascade; however, the induction of the PI3K pathway is an important event in follicular pathogenesis (15). Fig. 1B summarizes the major signaling pathways involved in FTC.

RAS oncogene. Activating mutations in the *RAS* gene are observed in 18-52% of follicular carcinomas and are associated with tumor dedifferentiation and a less favorable prognosis (77,78). A number of studies have suggested that *RAS* mutations are an early event in follicular thyroid tumorigenesis, since they are identified in up to 50% of benign follicular tumors (77,79,80,82,83). Studies using transgenic mice carrying the mutated N-*RAS* (Gln61Lys) oncogene demonstrated that these rodents developed follicular adenomas (11%), invasive follicular carcinomas (~40%) and, in certain cases, tumors with a mixed papillary/follicular morphology. Moreover, 25% of these carcinomas displayed large, poorly differentiated areas, with vascular invasion and with lung, bone or liver metastasis (81).

The RAS-mutated protein mediates its effects on cellular proliferation in part by activation of a cascade of kinases: RAF (A-RAF B-RAF and C-RAF), dual-specificity mitogen-activated protein kinases (MEK1/2), extracellular signal-regulated kinases (ERK1/2) and p38 mitogen-activated protein kinase. RAS also activates the PI3K pathway, via a direct interaction with the catalytic subunit of the protein. The PI3K activation leads to the accumulation of the 2nd messenger, phosphatidylinositol 3,4,5-trisphosphate (PIP3), resulting in pyruvate dehydrogenase kinase isozyme 1 (PDK1) and v-akt murine thymoma viral oncogene homolog (AKT) activation (85,86) (Fig. 1B). Previous studies using mice harboring a phosphatase and tensin homolog (*PTEN*) gene deletion and a $KRAS^{G12D}$ mutation, have shown that the separate activation of MAPK or PI3K pathways, is unable to transform thyroid follicular cells;

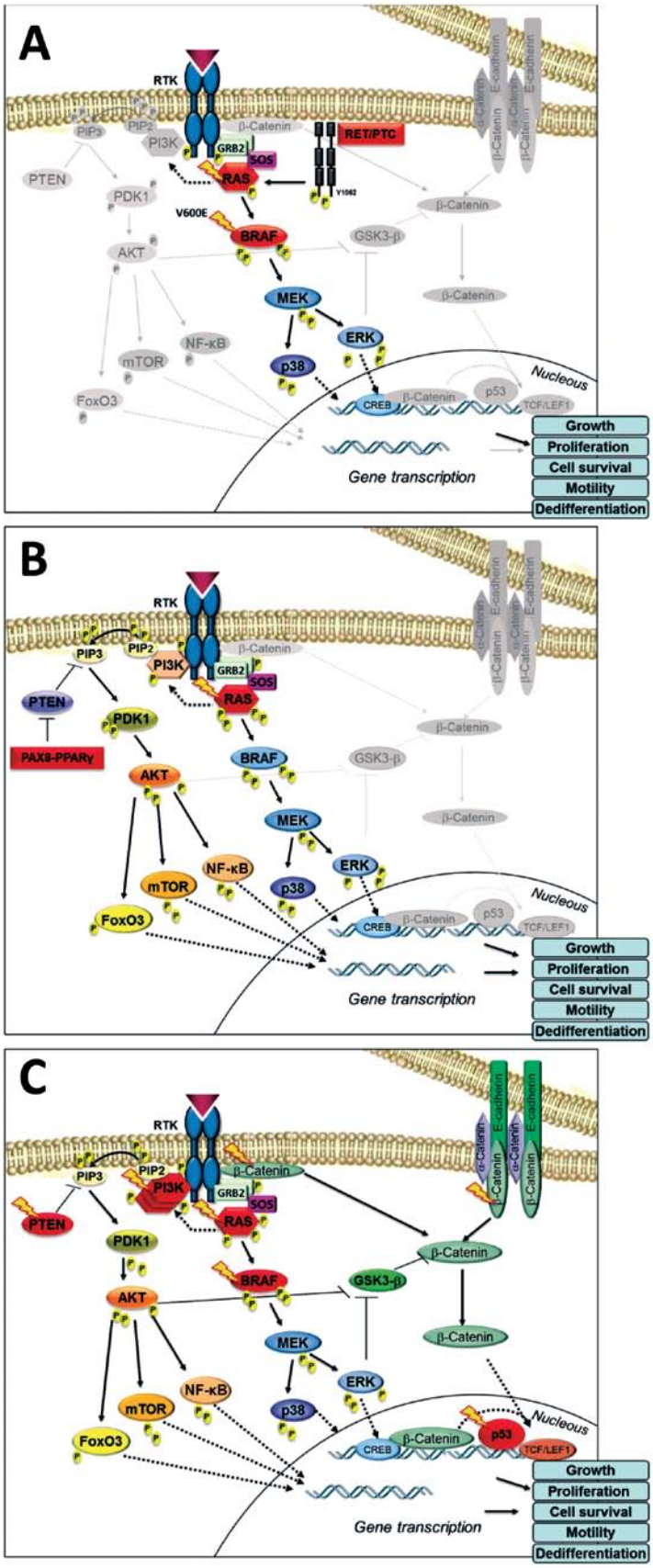

Figure 1 Schematic presentation of the signaling pathways involved in follicular-derived thyroid carcinoma. (A) In papillary thyroid carcinoma, BRAFV600E or *RAS* point mutations, or RET/PTC rearrangement result in a constitutively phosphorylated protein which leads to a potent activation of downstream effectors of the MAPK pathway. (B) In follicular thyroid carcinoma, RAS-mutated protein can mediate its cellular effects either by the activation of the MAPK cascade or the PI3K pathway, while PAX8-PPARγ rearrangement leads to the abrogation of the PTEN inhibitory effect and the PI3K signaling activation. (C) In anaplastic thyroid carcinoma, the MAPK cascade is induced by *RAS* or *BRAF* mutations, while copy gain or mutations of the PI3K and *PTEN* mutations are associated with the constitutive activation of PI3K/AKT pathway. Additionally, β-catenin mutations activate the β-catenin/E-cadherin pathway, whereas *TP53* gene alterations lead to aberrant cell cycle regulation.

however, their simultaneous activation is highly oncogenic, leading to locally invasive follicular carcinomas and distant metastasis (84).

PAX8-PPARγ rearrangements. The thyroid-specific transcription factor (*PAX8*) gene is a critical regulator of thyroid differentiation and growth (87). *PPARγ* is a ligand-dependent nuclear transcription factor highly expressed in adipose tissue, where it plays a critical role in adipocyte differentiation and fat metabolism regulation (88). The PAX8-PPARγ rearrangement arises through a chromosomal translocation, fusing the 5' portion of the *PAX8* gene with the entire coding sequence of the *PPARγ* gene (chromosomes 3p25 and 2q13). It is detected in ~35% of FTCs (10,89,90).

The PAX8-PPARγ rearrangement leads to strong induction of the PPARγ protein and the consequent abrogation of the normal PPARγ function (95,96). Under normal conditions, PPARγ inhibits cell proliferation and induces apoptosis via downstream pathways. The loss of these functions results in uncontrolled cell growth (14). *PPARγ* overexpression abolishes the PTEN-inhibitory effect on immunoactive AKT, which in turn induces the PI3K signaling pathway (58,97). The PAX8-PPARγ rearrangement also activates the MAPK, transforming growth factor β (TGFβ) and Wnt/β-catenin (wingless in *Drosophila*) signaling pathways. The increased expression of the C-terminal binding protein (*CTBP2)* gene has been observed in the PAX8-PPARγ-positive-tumors (95). CTBPs are co-repressor proteins associated with several transcriptional factors involved in Wnt, TGFβ and MAPK signaling activation, thus explaining their major role in follicular tumor development (98).

Patients with FTC harboring the PAX8-PPARγ rearrangement are usually diagnosed at a young age, have a small tumor size and the majority of tumors are overtly invasive at presentation (10,89). These findings, however, were not reproduced in other studies and the impact of PAX8-PPARγ on the biology and behavior of FTCs remains controversial (10,92).

Follicular adenomas have been shown to have lower frequency rates of PAX8-PPARγ rearrangements, suggesting that this chromosomal translocation may be involved in the early phases of the neoplastic process of FTC, possibly even in premalignant lesions (90,91,93). Transfection studies of PAX8-PPARγ in thyroid follicular epithelial cells have demonstrated accelerated growth rates and a lower number of cells in the G0/G1 resting state (14,94).

4. Anaplastic thyroid carcinoma

ATC, also known as undifferentiated thyroid carcinoma, is the most aggressive form of thyroid neoplasia. It can originate *de novo* or represent an advanced stage of follicular cell-derived thyroid tumors (4,99). Anaplastic tumors represent <1% of all thyroid tumors and their annual incidence is ~1-2 cases per 1,000,000 with a higher overall incidence in endemic goiter areas (100,101). The ATC typical presentation is advanced disease at diagnosis. Patients with anaplastic carcinoma usually have widespread local invasion and distant metastases, most frequent in the lung, pleura, bone and brain (100). This tumor has poor or no response to conventional therapeutic modalities. The median survival time after diagnosis is <1 year (102,103). A younger age (<60 years), smaller tumor size (<7 cm) and restricted disease have been associated with a lower mortality rate on multivariate analysis (104).

ATCs have been described as carrying multiple distinct genetic alterations with a high prevalence of mutations in MAPK effectors (13,21). Mutations in the *TP53* gene, β-catenin and PI3K cascade also play a critical role in ATC development, promoting the dedifferentiation of a previously well differentiated thyroid tumor (11,105,106). Fig. 1C summarizes the signaling pathways involved in ATC.

Mutations in gene effectors of the MAPK pathway. MAPK activating genetic alterations have been described to be involved in the development/progression of ATCs. ATC tumors present a significant prevalence of *RAS* (6-55%) and *BRAF* mutations (24-50%) (13,14,107). By contrast, RET/PTC, NTRK and PPARγ-PAX8 rearrangements are rarely observed in these undifferentiated tumors, supporting the hypothesis that DTCs associated with these rearrangements do not usually progress to anaplastic form (108,109).

$BRAF^{V600E}$ mutation is typically found in ATC tumors which contain areas of well-differentiated PTC, but also in poorly differentiated and anaplastic tumor areas. These observations suggest that although this mutation may occur early in tumorigenesis, it is not sufficient to initiate the dedifferentiation process. However, it is conceivable that *BRAF* mutations may predispose to additional genetic alterations which in turn activate more aggressive pathways and lead to dedifferentiation (15,110,111). Of note, $BRAF^{V600E}$ mutation has also been observed in lymph-node metastasis of ATCs (111). Of note, patients with ATCs harboring *BRAF* mutations have a higher mortality rate than those patients presenting with *RAS* or with no identified mutation, indicating a negative prognosis of these genetic alterations during all stages of thyroid cancer progression (13).

RAS mutations are found in a high prevalence in ATCs (6-55%) (13,14,77). A previous study suggested that the RAS effect may be due to the promotion of chromosomal instability, since the expression of constitutively activated RAS destabilizes the genome of PCCL3 thyroid cells, predisposing to large scale genomic abnormalities (112).

Genetic alterations in genes involved in the activation of the PI3K pathway

PIK3CA mutations and copy number gains. The *PIK3CA* gene encodes a catalytic subunit of PI3K and has been described to be mutated in 12-23% of ATC cases, normally restricted to the undifferentiated thyroid components. Previous studies have shown a preferential expression of *PIK3CA* mutations during the later stages of thyroid cancer, suggesting that this event may be more important in ATCs (12-23%) than in DTCs (PTCs, ~2% and FTCs, <10%) (11,106).

PIK3CA copy number gains are the 2nd most frequent event in ATC occurring in ~38-61% of tumors (14,106). Of note, this occurs almost exclusively in the undifferentiated component of the tumor. The copy number gain induces the activation of the PI3K cascade through the enhanced activity of AKT, leading to thyroid cancer progression. Of note, the *PIK3CA* mutations and copy number gain may co-exist with other somatic mutations in ATC, reinforcing the activation of the distinct signaling pathway in these tumors (11).

Table I. Clinical trials and follicular cell-derived thyroid tumors response.

Trade name	Compound	Target	Tumor type	No. of patients	Partial response[a] [% (n)]	Stable disease[b] [% (n)]	Refs.
Sorafenib	BAY 43-9006	BRAF ($BRAF^{V600E}$)	PTC	41	15 (6)	56 (23)	(127)
		VEGFR1-3, PDGFR,	DTC	31	25 (8)	-	(128)
		RET, RET/PTC	DTC	30	23 (7)	34 (10)	(129)
Axitinib	AG-013736	VEGFR1-3, PDGFR,	PTC	30	26 (8)	40 (12)	(131)
		c-Kit	FTC	15	40 (6)	46 (7)	
			ATC	2	50 (1)	-	
Pazopanib	W786034	VEGFR1/2, PDGFR	DTC	39	49 (18)	-	(132)
Motesanib	AMG706	VEGFR1-3, RET, c-kit	DTC	93	14 (13)	67 (62)	(133)
Gefitinib	ZD1839	EGFR	DTC	25	0	12 (3)	(134)
Selumetinib	AZD6244	MEK1/2	PTC (IR)	32	3 (1)	54 (21)	(135)
PLX4032	RG7204	$BRAF^{V600E}$	PTC	3	33 (1)	66 (2)	(130)

DTC, differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma (IR, iodine-131 refractory); FTC, follicular thyroid carcinoma; ATC, anaplastic thyroid carcinoma. [a]Partial response: a decrease of at least 30% in the sum of the largest diameter of target lesions, relative to the corresponding sum at baseline. [b]Stable disease: the absence of shrinkage sufficient for a partial response and the absence of enlargement sufficient for progressive disease, relative to the corresponding sum at baseline.

PTEN gene alterations. *PTEN* is a tumor suppressor gene that antagonizes signaling through the PI3K pathway. Its action occurs by removing a phosphate group from the inositol ring of PIP3, which reduces the downstream activity of the AKT kinase, thereby inducing cell cycle arrest, apoptosis, or both (113). Several genetic alterations in the *PTEN* suppressor gene have been described in ATCs: 12% present a mutated form (106,108), 28% gene silencing (114) and 69% the hypermethylated *PTEN* gene (115). These alterations lead to PTEN inactivation by different mechanisms, with a prominent role in the pathogenesis of follicular epithelium-derived thyroid carcinomas, particularly in the most aggressive or undifferentiated forms (114,115). Moreover, PI3K activation produced by downregulated PTEN has been shown to correlate with regions of tumor invasion and metastasis (58,116). Of note, studies using transgenic mice with a deletion of *PTEN* or *RAS* mutations have shown that the presence of both genetic events is required to trigger this aggressive form of thyroid cancer (84).

TP53 mutations. The *TP53* gene encodes a nuclear protein that can induce cell cycle arrest, senescence and apoptosis in response to various stimuli. Alterations in the p53 pathway may contribute to carcinogenesis, disease progression and resistance to therapy (117). In thyroid tumors, *TP53* mutations are commonly observed in anaplastic carcinomas (~70%) and are rarely described in well-differentiated thyroid carcinomas (0-9%) (12,105,118). This suggests that *TP53* mutations are a late event in tumor progression and that this gene may play a critical role in the transformation of DTC into the anaplastic form (105). The frequent association of p53 inactivation with PI3K activation may contribute to genomic instability, leading cancer cells to become resistant to apoptosis and to escape from any growth restriction. This contributes to a rapidly enlarging neck mass as well as to chemotherapy and radiotherapy resistance commonly observed in these tumors (11).

β-catenin genetic alterations. Genetic alterations in the β-catenin (*CTNNB1*) gene are observed in ~65% of thyroid anaplastic tumors. Gain-of-function mutations can promote β-catenin nuclear translocation which consequently triggers the transcription process (119,120). The expression of E-cadherin, a component of the β-catenin pathway, normally expressed in thyroid tissue, is usually absent in undifferentiated thyroid carcinomas (121). These changes appear to play a pathogenic role in thyroid tumor invasion and regional lymph node metastasis, due to a decrease in intercellular adhesion and enhancement of cell motility (122). The lack of E-cadherin expression is associated with an adverse prognosis for patients with thyroid carcinoma (123).

5. Clinical Implications: Potential therapeutic targets

DTCs demonstrate indolent behavior in the majority of patients and can be effectively treated by surgery followed by radioactive iodine and/or thyroid hormone suppressive therapy (124,125). In patients with metastatic disease, radioactive iodine therapy can be effective in some cases, whereas suppressive thyroid hormone therapy can help to delay the pace of the disease (125,126). Nevertheless, for those patients with metastatic DTC that progresses despite radioiodine and thyroid hormone therapy, no effective treatments are currently available.

Over the last decades, cancer research has been predominantly focused on the genetic alterations and the advances in the understanding of the molecular events involved in differentiated thyroid carcinogenesis have allowed for the development of new therapies designed for patients with metastatic disease refractory to radioactive iodine treatment. Specific tyrosine multikinase inhibitors to target key molecules such as BRAF, RET/PTC rearrangements, vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor recep-

tors (PDGFR) have been evaluated as potential alternatives to DTC treatment. Table I summarizes the results obtained to date in several clinical trials. Phase II studies using BAY 43-9006 (sorafenib) have shown partial response (15-25%) and stable disease (34-56%) in progressive DTC patients and the median progression-free survival was significantly longer in patients harboring *BRAF* mutations (127-129). A recent study using PLX4032, an inhibitor of mutant BRAF, in metastatic melanoma patients evaluated the effect of this drug in 3 PTC patients. The response lasted 8 months in 1 patient (progression-free lasted for 12 months) and stable disease lasted 11 and 13 months in each of the other 2 patients (130). Although these compounds have demonstrated the most impressive clinical responses to date in the treatment of advanced thyroid cancer, the low rate of partial response, the rare report of complete responses and the emergence of eventual progression, point out to the need to develop either more effective single agents or to identify rational combinations of therapeutic targets.

6. Conclusion

Thyroid carcinogenesis consists of a complex process with a large number of molecular alterations among several thyroid neoplasias. The set of genetic alterations observed in follicular-cell derived thyroid carcinomas activates specific pathways, such as the MAPK, PI3K and β-catenin signaling pathways, which have been shown to play an important role in thyroid cancer initiation and progression. The screening for follicular cell-derived specific mutations in association with traditional diagnosis methods has improved the diagnostic accuracy, impacting the prognosis of these tumors. Moreover, the advances in the knowledge of the effects of thyroid oncogenes and related mechanisms of action have allowed for the development of multikinase inhibitor targets, promoting new perspectives on therapy to aggressive thyroid tumors.

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