UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

EXPRESSÃO DA IODOTIRONINA DESIODASE TIPO 3 NO CARCINOMA DIFERENCIADO DE TIREÓIDE

MÍRIAN ROMITTI

Porto Alegre, Março de 2012

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MÍRIAN ROMITTI

Dissertação apresentada ao curso de Pós-Graduação em Ciências Médicas: Endocrinologia, UFRGS como requisito parcial para obtenção do grau de Mestre

Orientadora: Profa. Dra. Ana Luiza Maia

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- Artigo de revisão;

- Artigo original referente ao trabalho de pesquisa propriamente dito; encaminhado para publicação em jornal científico de circulação internacional.

LISTA DE ABREVIATURAS E SIGLAS

AKT - Protein Kinase B

ATC - Anaplastic thyroid carcinoma

BCC - Basal Cell Carcinoma

BRAF - Serine/threonine-protein kinase B-Raf or Raf murine sarcoma viral oncogene homolog B1

CDK - Cyclin-dependent kinase

c-Myc - v-myc myelocytomatosis viral oncogene homolog (avian)

CREB - cAMP response element-binding

CTBP2 - C-terminal binding protein 2

DIO1/D1 - Deiodinase type 1

DIO2/D2 - Deiodinase type 2

DIO3/D3 – Deiodinase type 3

DTC - Differentiated thyroid carcinomas

E2F-1 - Dependent transcription factor

ERK - Extracellular-signal-regulated kinases

FOXO3 - Forkhead box O3

FTC - Follicular thyroid carcinoma

GSK3-S - Glycogen synthase kinase 3 phosphorylation

HCC - Hepatocellular carcinoma

HIF-1 - Hypoxia-inducible transcription factor 1

HRAS: Harvey rat sarcoma viral oncogene homolog

KRAS: Kirsten rat sarcoma viral oncogene homolog

MAPK - Mitogen-activated protein kinase signaling pathway

MMP - Matrix metalloproteinase

MST1 - Macrophage stimulating 1

MTC – Medullary thyroid carcinoma

NFkβ - Nuclear factor kappa β

NRAS - Neuroblastoma RAS viral (v-ras) oncogene homolog

NTRK1 - Neurotrophic tyrosine kinase, receptor, type 1

p21 - Cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1

p27 - Cyclin-dependent kinase inhibitor 1B

PAX8-PPARγ - transcription factor (*PAX8)* gene and peroxisome proliferator-activated receptor (*PPARγ)* gene rearrangement

PDGFR - Platelet-derived growth factor receptor

PDK1 - activation of phosphoinositol-dependent kinase-1

PI3K - Phosphatidylinositol 3-kinase signaling pathway

PIK3CA: phosphoinositide-3-kinase, catalytic, alpha polypeptide

PIP2 - Phosphatidylinositol 4,5 phosphate

PIP3 - Phosphatidylinositol 3,4,5 phosphate

PTC - Papillary thyroid carcinoma

PTEN - Phosphatase and tensin homolog

RB - Retinoblastoma

RET - Rearrangement during transfection

RET-PTC - RET tyrosine kinase domain rearrangement with different partners (table 1)

SB203580 - p38 inhibitor

STAT - signal transducer and activator of transcription

T3 - triiodothyronine

T4 – thyroxine

TCF/LEF - T-cell factor/lymphoid enhancer factor

TGFβ - Transforming growth factor, beta

TNM - Tumor/Node/Metastasis system

TP53 - Tumor protein p53

TPM3 - Non-muscle tropomyosin

U0126 - ERK inhibitor

VEGFR - Vascular endothelial growth factor receptor

Wnt - Wingless-type MMTV integration site family pathway

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PARTE I

SIGNALING PATHWAYS IN FOLLICULAR CELL-DERIVED THYROID CARCINOMAS

SIGNALING PATHWAYS IN FOLLICULAR CELL-DERIVED THYROID CARCINOMAS

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ABSTRACT

Thyroid carcinoma is the most common endocrine malignant neoplasia. Differentiated thyroid carcinomas (DTC), represent more than 90% of all thyroid carcinomas and comprise the papillary (PTC) and follicular thyroid carcinomas (FTC) subtypes. Anaplastic thyroid carcinoma (ATC) corresponds to less than 5% of all thyroid tumors. The etiology of DTC is not fully understood. Several genetic events have been implicated on differentiated thyroid tumorigenesis. Point mutations in *BRAF* and *RAS* genes and RET/PTC rearrangements are observed in about 70% of PTC cases. Follicular carcinomas commonly harbor *RAS* mutations and PAX8-PPARγ rearrangements. Anaplastic carcinomas may harbor a wide set of genetic alterations, as in genes encoding effectors in the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinases (PI3K) and β-Catenin signaling pathways. These distinct genetic alterations are able to activate constitutively several signaling pathways as MAPK, PI3K and β-Catenin, which have been implicated on thyroid cancer development and progression. In this context, the evaluation of the specific oncogenes, as well as the knowledge of their effects on thyroid carcinomas can provide important information about the disease presentation, prognosis and therapy, through the development of specific tyrosine kinase targets. Particularly in this review, we explore the main genetic alterations observed in follicular cell-derived thyroid carcinomas as well as the molecular mechanisms involved in thyroid tumors development and progression.

INTRODUCTION

Thyroid carcinoma is the most common endocrine malignant neoplasia and accounts for approximately 1% of all new malignant diseases (1). Follicular cell-derived thyroid neoplasias, include the differentiated thyroid carcinoma (DTC), which represents more than 90% of all thyroid malignancies and comprise the papillary and follicular thyroid carcinomas and the anaplastic thyroid carcinoma (ATC), which corresponds to 1% of all thyroid tumors. It is suggested that ATC originates de novo or derive from dedifferentiation of a differentiated tumor. Medullary thyroid carcinoma (MTC) is a malignancy arising from the parafollicular C-cells and accounts for approximately 3-8% of all thyroid carcinomas (2, 3).

The etiology of DTC remains not fully understood. External radiation is the only exogenous factor which has been clearly identified to be able to cause thyroid carcinoma, almost exclusively the papillary form. Iodine excess has also implicated with the papillary form (4, 5). On the other hand, several genetic events have been described in thyroid carcinoma pathogenesis (Table 1). Papillary carcinomas commonly present genetic alterations leading to the activation of the mitogen-activated protein kinase (MAPK) pathway. Point mutations in *BRAF* or *RAS* genes and RET/PTC or NTRK1 rearrangements are mutually exclusive and identified in more than 70% of PTCs (6-8). Follicular carcinomas frequently present *RAS* mutations and PAX8-PPARγ rearrangements (9). Anaplastic carcinomas may harbor a wide set of genetic alterations, as in genes encoding effectors in the MAPK, phosphatidylinositol 3-kinases (PI3K) and β-Catenin signaling pathways (10-12). These genetic alterations lead to activation of distinct pathways, as MAPK and PI3K, which have been implicated on thyroid cancer development and progression (13-15).

The purpose of this review is to explore the main genetic alterations observed in follicular cell-derived thyroid carcinomas as well as the molecular mechanisms involved in thyroid tumors development and progression.

1. PAPILLARY THYROID CARCINOMA

Papillary thyroid carcinoma (PTC) represents approximately 80% of all thyroid malignant tumors. The overall incidence of PTC is rising, in part due to the increased detection of very small tumors (16). PTC is often diagnosed around the fifth decade of

life and is known to be a slow-growing tumor (17). Patients usually present a palpable nodule and the absence of any other clinical findings is the rule (3). The majority of patients have a favorable outcome; however, about 10% of the cases have tumor recurrence and metastatic disease (17, 18).

Point mutations in *BRAF* or *RAS* genes, as well as, RET/PTC or NTRK rearrangements are mutually exclusive and found in more than 70% of papillary thyroid carcinomas. These genetic alterations are characterized by the aberrant activation of the MAPK pathway that has been observed in this tumor (6-8). The role of these oncogenes on PTC development and progression, as well as, the signaling pathways activated involved events will be discussed.

1.1 BRAF oncogene

BRAF is a serine–threonine kinase protein, part of the RAF family proteins and comprises intracellular effectors of the MAPK cascade (6, 19, 20). The MAPK cascade effects initiate with 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 cellular effects, as growth, proliferation, migration and survival (21, 22)(Figure 1). Mutations in *BRAF* gene are the most common genetic alteration, occurring in ~45% of PTCs. About 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). Besides $BRAF^{V600E}$, other alterations in *BRAF* gene have been described in a minority of PTCs (1–2%), as K601E (A>G transversion at gene position 1801) point mutation, AKAP9/BRAF rearrangement and small in-frame insertions or deletions around codon 600 (23-25).

BRAF mutations seem to have a role in the early stages of PTC development, due its presence in microPTC (~40%) and also in benign tumors (8, 26, 27). Studies in transgenic mice with $Braf^{V600E}$ showed PTC development with similar properties of that observed in human *BRAF*-positive PTCs (28). Accordingly, findings obtained in mice with a thyroid-specific knock-in of *Braf* gene (LSL-$Braf^{V600E}$/TPO-Cre) demonstrated that Braf activation led to the development of classic infiltrative PTC with high rate of extrathyroidal structures and vascular invasion (29).

BRAF mutations are typically identified in classical and tall cell variant and are associated with a more aggressive tumor behavior (8, 30, 31). Indeed, $BRAF^{V600E}$ is an oncogenic protein with markedly elevated kinase activity that over-activates the MAPK pathway (32, 33). Thus, the high rate of growth observed in $BRAF^{V600E}$ tumors can be explained, in part, by the MAPK-induced hyperphosphorylation with consequent inhibition of the RB (retinoblastoma) protein, dependent transcription factors (E2F) and p27 of cyclin-dependent kinase (CDK) activity, which allow the passage of tumor cells from G1 to S phase of the cell cycle (34, 35).

Moreover, *BRAF* oncogene induced the expression of matrix metalloproteinase (MMP), its enzymatic activity and cell invasiveness (36). The MMPs constitute a large group of enzymes that regulate cell-matrix composition and the enhanced expression of MMPs and its receptors, as observed in PTC samples, is thought to be an important determinant of tumor invasion (37, 38). Moreover, studies suggest that MMP proteins also are modulated accordingly to the intensity of MAPK pathway activation and/or STAT (signal transducer and activator of transcription) expression, explaining in part the mechanism of induction of this proteins in PTCs BRAF-mutated and the ability of these tumors to invade surrounding tissues (36, 39, 40).

BRAF-mutated protein seems also to induce the nuclear factor kappa β (NFkβ) in PTC samples (38). Previous studies performed in thyroid cells (WRO) harboring this oncogene showed an increase in NFkβ pathway activity, resulting in up-regulation of antiapoptotics factors and induction of cell invasion (40).

Recently a novel inhibitory mechanism that might operate in $BRAF^{V600E}$-induced PTC was shown. $BRAF^{V600E}$ presence was able to abolish markedly the macrophage stimulating 1/forkhead box O3 (MST1/FOXO3) pathway transactivation in thyroid cell line (FRO cells), resulting in the suppression of p21 and p27 CDK inhibitors expression, inhibiting the apoptotic processes. Accordingly, the development of $Braf^{V600E}$ transgenic mice with the MST1 knockout, lead to abundant foci of poorly differentiated thyroid carcinoma and large areas without follicular architecture or colloid formation, suggesting that the activity of MST1/FOXO3 pathway might determine the phenotype $Braf^{V600E}$ tumors (41).

1.1.2 RET/PTC rearrangements

RET gene is located in chromosome 10 and a high rate of translocations reflect the frequent structural cytogenetic alterations of this chromosome. RET/PTC rearrangements are observed in about 13-43% of PTCs, mostly in pediatric cancers and individuals exposed to ionizing radiation of nuclear accidents (11, 42-44). At least twelve types of RET/PTC rearrangements have been reported, all originated by the RET fusion to different partners (43, 45) (Table 2). RET/PTC1 comprises up to 60% of the rearrangements and is derived from intrachromosomal rearrangement (10q), fusioning the RET tyrosine kinase domain to a H4 gene (D10S170), encoding an 585–amino acid protein with unknown function (46). RET/PTC3 accounts for 20–30% of rearrangements and is formed by *RET* gene fusion with the *NCOA4* - nuclear receptor coactivator 4 gene (also called *ELE1, RFG* or *ARA70*) (43, 46).

PTCs harboring RET/PTC1 rearrangement commonly exhibit the classical papillary histology whereas RET/PTC3 tumors normally present the solid-variant (47). RET/PTC tumors tend to be small, with favorable outcome and seem to not exhibit a tendency to progress to a more aggressive and/or undifferentiated thyroid carcinoma (8, 48, 49). However, studies also demonstrated that this alteration was associated with younger onset and high rate of lymph node metastasis (8, 48).

The high prevalence of RET/PTC in occult (42%) or microscopic PTC (77%) as well as in the follicular adenomas (45%), might indicate a putative role of this rearrangement in early stage of PTC development (50, 51). Accordingly, studies performed in transgenic mice carrying the Ret/PTC1 and/or Ret/PTC3 observed that the tumors developed in these animals were similar to human PTCs (52, 53).

The RET/PTC oncogene mechanisms of tumor induction, start with the fusion of protein partners, resulting in ligand-independent autophosphorylation of the RET protein. RET intracellular domain contain at least 12 autophosphorylation sites and 11 of which are maintained in RET/PTC protein (54). In RET/PTC rearrangement, both Y1062 and Y1015 RET residues are constitutively phosphorylated and are required for cell transformation (55). These residues are essential binding sites for several proteins, which in turn, lead to the activation of MAPK and PI3K/AKT signaling pathways and seems to play an essential role in RET/PTC signaling with downstream cellular effects on migration and proliferation (56-58).

Another dysfunctional signaling pathway identified in 65 to 90% of RET/PTC PTCs is β-catenin, a multifunctional protein involved in the regulation of gene transcription and cell adhesion (59, 60). The β-catenin pathway can be activated directly by binding with RET tyrosine residue, but also by cAMP response element-binding (CREB), glycogen synthase kinase 3 phosphorylation (GSK3-S) or effectors of MAPK and PI3K pathways (60, 61). The increase in free β-catenin protein pool promotes PTC tumor proliferation and invasion, due to the β-catenin interaction with the transcriptional factors, as T-cell factor/lymphoid enhancer factor (TCF/LEF), c-Myc (v-myc myelocytomatosis viral oncogene homolog) and cyclin D1 (59, 60, 62) (Figure 1)

1.1.3 NTRK1 rearrangements

The *NTRK1* gene, localized in chromosome 1, encodes the high-affinity nerve growth factor (NGF) receptor and is activated through the MAPK pathway (63). NTRK1 rearrangements are usually found in less than 10% of PTC and result of the fusion of the *NTRK1* (neurotrophic tyrosine kinase, receptor, type 1) gene to different partners (64, 65). This oncogene is the result of a somatic rearrangement that fused *TPM3* (non-muscle tropomyosin) gene to the kinase domain of a novel tyrosine-kinase receptor (66). Experimental evidence suggests that *NTRK1* oncogene represents an early event in the process of thyroid carcinogenesis. Transgenic mice carrying *Ntrk1* oncogene develop thyroid hyperplasia and PTC (67). Additionally, crossing Ntrk1 mice with p27kip1-deficient mice increased the penetrance of thyroid cancer and shortened the latency period of tumor (68). NTRK1 rearrangements are described to be associated with younger age at diagnosis and a less favorable outcome (64, 65).

1.1.3 RAS oncogene

RAS genes (H-RAS, K-RAS, and N-RAS) encode highly related G-proteins which play a central role in the intracellular signals transduction by activation of the MAPK and other signaling pathway, such as PI3K/AKT (vide below) (14). Mutations in *RAS* gene are found in 10–43% of PTCs, particularly in the follicular variant (FVPTC) (69-71). Point mutations in *RAS* gene generally occur in codons 12, 13, or 61 of *H-RAS, K-RAS,* and *N-RAS.* Studies have suggested that *RAS* mutation can be an early event in thyroid follicular carcinogenesis, occurring in ~30% of follicular adenomas and that it is

significantly correlated with malignant progression (72, 73). The *RAS*-mutated PTC tumors tend to be encapsulated and to exhibit a low rate of lymph node metastasis (8, 70). However, studies reported that this mutation could be associated with more aggressive phenotype and higher incidence of distant metastasis (71, 74). The molecular basis proposed for tumorigenesis of *RAS,* is the constitutively activation effectors of distinct pathways, as MAPK and PI3K, which are involved in proliferation, differentiation and cell survival processes (71).

2. FOLLICULAR THYROID CARCINOMA

The follicular thyroid carcinoma represents 10-15% of thyroid cancers. These tumors are generally unifocal and present a lower involvement of lymph node (<5%) than PTCs. By contrast, distant metastasis, mainly to lungs and bones are highly frequent at disease presentation (~20%) (3). FTC, especially if invasive has a poorer prognosis than PTC (75, 76). However, a study containing more than 1000 patients did not found differences in cancer-specific survival between PTC and FTC considering the age at diagnosis, primary tumor size and the presence of extrathyroidal invasion or distant metastasis (77).

RAS mutations and PAX8-PPARγ rearrangements are commonly observed in of the follicular thyroid carcinomas (80%) and the pathogenic effects generally occur by the activation of MAPK and PI3K signaling pathways (14).

2.1.1 RAS oncogene

Activating mutations in gene *RAS* are observed in 18–52% of follicular carcinomas and are correlated with tumor dedifferentiation and less favorable prognostic (78, 79). Several studies suggest *RAS* mutations as an early event in follicular thyroid tumorigenesis, since they are identified in up to 50% of follicular benign tumors (78, 80, 81). Studies performed in transgenic mice carrying *N-Ras* (Gln61Lys) oncogene demonstrated that this rodents developed follicular adenomas (11%), invasive follicular carcinomas (~40%) and in some 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 (82).

RAS-mutated gene encode constitutively active G-proteins and results in constitutive stimulation of MAPK, PI3K/AKT and other pathways involved in proliferation, differentiation and cell survival (83-85). As MAPK, PI3K pathway has been proposed to be an essential via in thyroid pathogenesis promoted by *RAS* mutations. PI3K are heterodimeric molecules composed of a regulatory (p110) and a catalytic (p85) subunit and both can activate the PI3K pathway by distinct mechanisms. First, a phosphorylated Y residue on the receptor serves as a docking site for the p85 regulatory subunit of PI3K. Alternatively, RAS-activated is able to induce the membrane translocation and activation of p110 subunit of PI3K. Once activated PI3K converts phosphatidylinositol 4,5 phosphate (PIP2) into phosphatidylinositol 3,4,5 phosphate (PIP3) which results in the membrane localization and activation of phosphoinositol-dependent kinase-1 (PDK1). The primary mediator of this cascade is AKT; it has a number of downstream substrates that can promote cellular effects (86, 87) (Figure 1). Recently, a study using double-mutated mice (Pten$^{-/-}$ and Kras^{G12D}) showed that MAPK and PI3K pathways, isolated, were unable to transform thyroid follicular cells, but their simultaneous activation was highly oncogenic, leading to locally invasive follicular carcinomas and with distant metastasis (85).

2.1.2 PAX8-PPARγ rearrangement

PAX8 gene is a critical regulator of thyroid differentiation and growth (88). In contrast, PPARγ is a ligand dependent nuclear transcription factor highly expressed in adipose tissue, where it plays a critical role in the differentiation and in the regulation of fat metabolism (89). PAX8-PPARγ rearrangement is created by a fusion gene of the promoter 5' coding sequence of the thyroid-specific transcription factor (*PAX8)* gene and most of the coding sequence of the peroxisome proliferator-activated receptor (*PPARγ)* gene (chromosome 3p25 and 2q13) and has been detected in about 35% of FTCs (9, 90, 91).

PAX8-PPARγ rearrangement was demonstrated to be associated with younger age at presentation, smaller size and more frequent vascular invasion of FTC (9, 90). These findings, however, were not reproduced in other studies and the impact of PAX8-PPARγ on the biology and behavior of FTCs is controversial (92, 93).

Follicular adenomas present lower frequency rates of this rearrangement, suggesting that this chromosomal translocation might be involved early in the neoplastic

process of FTC, possibly even at the premalignant levels (91, 92, 94). Transfection studies of PAX8-PPARγ in thyroid follicular epithelial cells, showed accelerated growth rates and lower number of cells in the G0/G1 resting state (13, 95).

PAX8-PPARγ rearrangement leads to strong overexpression of the PPARγ protein and seem to have a dominant negative function with abrogation of the normal PPARγ function (96, 97). In normal conditions, PPARγ acts via downstream pathways to inhibit the proliferation of cell growth and to induce apoptosis. The loss of these functions resulted in uncontrolled cell growth (13).

Since PPARγ also regulates *PTEN* expression, its inhibition increases the levels of immunoactive AKT and consequently induces to PI3K signaling activation (57, 98). Moreover, this rearrangement is also associated with MAPK, TGFβ (transforming growth factor beta) and Wnt/β-catenin (wingless in *Drosophila*), signaling pathways activation. A higher expression of *CTBP2* gene (C-terminal binding protein 2) was observed in the PPARγ-PAX8 positive-tumors (96). CTBPs are co-repressor proteins which mediate effects by its association with several transcriptional factors involved in Wnt, TGFβ and MAPK signaling activation, thus explaning its important role in follicular tumor development and oncogenesis (99).

3. ANAPLASTIC THYROID CARCINOMA

Anaplastic thyroid carcinoma, also called undifferentiated thyroid carcinoma, is the most aggressive form of thyroid neoplasia that can originates de novo or represent an advanced stage of follicular cell-derived thyroid tumors (3, 100). Anaplastic tumors represent less than 5% of all thyroid tumors and its annual incidence is about one to two cases per million with an overall incidence higher in areas of endemic goiter (101, 102). ATC typically present with advanced disease at presentation with little response to conventional therapeutic modalities and a mean survival time of less than 1 year after diagnosis (Kitamura, Shimizu et al. 1999; Smallridge, Marlow et al. 2009). Patients with anaplastic carcinoma usually present with widespread local invasion and a high frequency of distant metastases in lung, pleura, bone and brain (101).

ATCs have been described to carry several distinct genetic alterations with a high prevalence of mutations in MAPK effectors (12, 20). Moreover, *TP53* gene mutations, β-catenin and PI3K cascade alterations, have an relevant incidence and

might play a critical role in ATC development and promoting dedifferentiation of a previously well differentiated thyroid tumor(10, 103, 104).

3.1 Mutations in effectors genes of MAPK pathway

MAPK activating genetic alterations have been described to be involved on the development/progression of ATCs. ATCs tumors present a significant prevalence of *RAS* (12-17%) and *BRAF* mutations (24-50%) (12, 13, 105). However, RET/PTC, NTRK and PPARγ-PAX8 rearrangements are rarely observed in these undifferentiated tumors, supporting the hypothesis that differentiated thyroid cancers associated with these rearrangements do not usually progress to anaplastic form (106, 107).

Nowadays it is proposed that ATC can originate de novo or be the result of DTC malignant progression (2, 108). $BRAF^{V600E}$ mutation is typically found in ATC tumors which contain areas of well-differentiated PTC, but also in poorly differentiated and anaplastic tumor areas, suggesting that it can occurs early in the tumorigenesis and that these events are probably insufficient by themselves to initiate anaplastic dedifferentiation, but may predispose the tumor cells to gain additional genetic alterations that activate other pathways and lead to dedifferentiation (14, 108, 109). Of note, $BRAF^{V600E}$ mutation was also found in lymph-node metastasis of ATCs (109). Interestingly, patients with ATCs harboring *BRAF* mutations had higher mortality than patients presenting *RAS* or with no known mutation, indicating to the negative prognostic of these genetic alterations at all stages of thyroid cancer progression (12).

RAS mutations are found with high prevalence in ATCs (6-55%) (14, 78). Studies suggest that RAS effect might be due to the promotion of chromosomal instability, since expression of constitutively activated RAS destabilizes the genome of PCCL3 thyroid cells, predisposing to large scale genomic abnormalities (110). Moreover, the *RAS*-mutated is known to promote constitutively activation of MAPK and PI3K pathways, implicated in several processes as proliferation, cell survival and dedifferentiation, thus contributing to progression/development of undifferentiated thyroid tumors (83-85).

3.2 Genetics alterations in genes involved in the activation of PI3K pathway

3.2.1 PIK3CA mutations and copy gains

PIK3CA encodes a catalytic subunit of PI3K and has been described be mutated in 12–23% of ATC cases, normally restricted to the undifferentiated thyroid components. Studies showed a preferential expression of *PIK3CA* mutations in the later stages of thyroid cancer, demonstrating a more important role of this event in ATCs than in DTC (PTCs, ~2% and FTCs, <10%) (10, 104).

Copy gains of *PIK3CA* seem to be the most relevant event in ATCs occuring in about 38–61% of the tumors (13, 104). Recently it was demonstrated that change in the gene copy number of *PIK3CA* occurs almost exclusively in the undifferentiated component of the tumor. This mechanism of copy gain results in an increased expression of PI3K, by enhanced activity of AKT which acts on the apoptosis regulation, proliferation and motility of thyroid cells leading to thyroid cancer progression. Of note, the *PIK3CA* mutations and copy gain number may coexist with other somatic mutations in ATC, reinforcing the activation of the distinct signaling pathway in these tumors (10).

3.2.2 PTEN gene alterations

PTEN is a tumor suppressor gene that antagonizes signaling through the PI3K pathway, it action occurs by removing of a phosphate group from inositol ring of PIP3, which reduces the downstream activity of Akt kinase, thereby inducing cell-cycle arrest, apoptosis, or both (111). Several genetic alterations in *PTEN* suppressor gene have been described in ATCs. While 12% of these tumors present this gene mutated (104, 106) 28% present gene silencing (112) and 69% present *PTEN* gene hypermethylated (113). These alterations lead to *PTEN* inactivation, by different mechanisms, with a prominent role in the pathogenesis of follicular epithelium-derived thyroid carcinomas, especially in the most aggressive or undifferentiated forms (112, 113). Moreover, PI3K activation produced by *PTEN* down-regulated has been correlated with regions of tumor invasion and with metastasis (57, 114). Interestingly, transgenic mouse with deletion of *Pten* or *Ras* mutations, showed that these alterations each alone did not caused the development

of thyroid cancer, but simultaneous introduction of both genetic events caused aggressive thyroid cancer (85).

3.3 TP53 mutations

The *TP53* gene encodes a nuclear protein that can induce cell-cycle arrest, senescence and apoptosis in response to diverse stimuli. Alterations in p53 pathway can contribute to carcinogenesis, disease progression and resistance to therapy (115). In thyroid tumors, *TP53* mutations are commonly observed in anaplastic carcinomas (~70%) and is rare or completely absent in well-differentiated thyroid carcinomas (0-9%) (11, 103, 116). It suggests that *TP53* mutation are a late event in the tumor progression and appears to play a critical role in the transformation of differentiated thyroid carcinoma to anaplastic form (103). Moreover, the frequent association related of TP53 inactivation and PI3K activation in some ATC might contribute to the genomic instability, allowing that cancer cells become resistant to apoptosis and allowing an escape from any restriction of growth, what contribute to a rapidly enlarging neck mass as well as to the chemotherapy and radiotherapy resistance commonly observed in these tumors (10).

3.4 β-Catenin genetic alterations

In thyroid tumors, mutations in β-catenin are present in ~65% of anaplastic carcinomas and lead to nuclear localization of β-catenin protein and to transcription activation (117, 118). The expression of E-cadherin, a component of β-catenin pathway, is high in normal thyroid tissue, but decreased in undifferentiated thyroid carcinomas (119). These inhibitory mechanism permit the induction of growth arrest at high cell density and the dysfunction of this system would be contributing to the loss of contact inhibition observed in ATC cells (119). Thus, dysfunctions of both E-cadherin expression and β-catenin mutations appear to be associated with a pathogenic role in thyroid tumors invasion and in regional lymph node metastasis, by decreases intercellular adhesion and enhances motility (120), and analysis of survival showed that the lack of E-cadherin expression represents an adverse prognostic factor for thyroid carcinomas (121).

CLINICAL IMPLICATIONS OF GENETIC ALTERATIONS ANALYSIS AND POTENTIAL THERAPEUTIC TARGETS

MAPK signaling pathway activation is a hallmark in thyroid tumors pathogenesis. Multikinase or tyrosine kinase inhibitors have been investigated as potential drugs for thyroid aggressive tumor therapy. Several preclinical studies and clinical trials are ongoing to establish its effects on DTCs (122, 123). Specific tyrosine multikinase inhibitor with potent activity against BRAF, RET (Rearrangement during transfection), RET/PTC rearrangement, VEGFRs (Vascular endothelial growth factor receptors), PDGFR (Platelet-derived growth factor receptors) and other receptors have been tested. These studies have demonstrated generally, disease stabilization and/or partial tumor response in DTCs (122-124). Interestingly, data of trial with sorafenib reported that the median progression-free survival was significantly longer in PTC patients harboring *BRAF* mutations (125). These initial results provide important possibilities in the treatment of aggressive DTC, however further study with longer follow-up times are need to validate the multikinase inhibitors effects in the thyroid tumors.

CONCLUSION

Thyroid carcinogenesis consists in a complex process with a wide number of molecular alterations identified among the several types of thyroid neoplasias. Since some thyroid oncogenes were found exclusively and with high frequency in malignant thyroid tumors and provides prognosis information, the screening for mutations of cytopathology after fine-needle aspiration of thyroid nodules, associated with traditional diagnosis methods can improve the diagnostic accuracy of these tumors. The evaluation of the specific oncogenes, as well as, the knowledge of their effects on thyroid carcinomas can provide important information about the disease presentation and prognosis. Furthermore, as demonstrated here, the set of genetic alterations observed in thyroid carcinomas is able to activate specific pathways of MAPK and PI3K signaling pathways which have been demonstrated to exercise an important role on thyroid cancer initiation and progression. The advances on knowledge of mechanisms of action of

these oncogenes, have allowed the development of specific multikinase targets, permitting new perspectives on therapy to aggressive thyroid tumors.

FIGURE

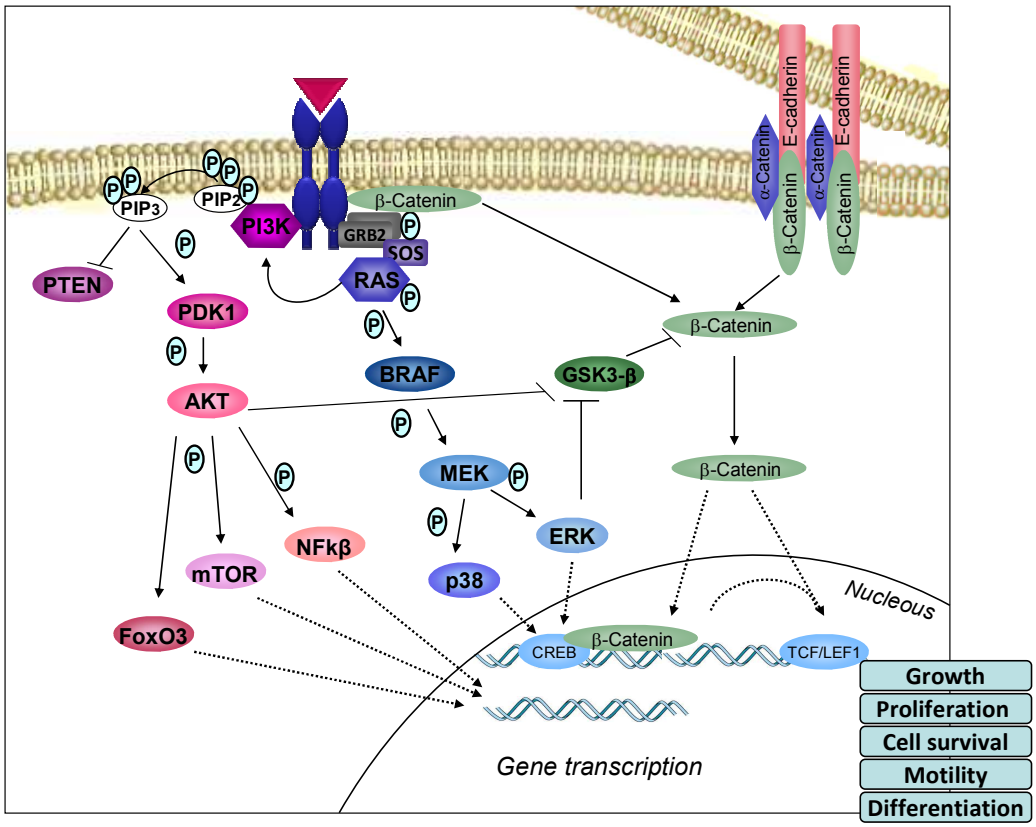

Figure 1 Schematic presentation of PI3K, MAPK and β-catenin signaling pathways in physiological conditions. Activation of receptor protein tyrosine kinases results in autophosphorylation on tyrosine residues and in activation of several effectors of MAPK, PI3K and β-catenin cascades. Once activated, these pathways mediate the activation and inhibition of several targets, resulting in distinct cellular effects.

TABLES

Table 1 Description of the oncogenic genetic alterations frequencies observed in the follicular-derived thyroid carcinomas

	PTC (%)	FTC (%)	ATC (%)
***BRAF* mutations**	~45	_	24-50
***RET/PTC* rearrangement**	13-43	_	_
***NTRK1* rearrangement**	<10	_	_
***RAS* mutations**	10–43	18–52	6-55
***PAX8-PPARγ* rearrangement**	_	~35	_
β-Catenin	_	_	65
PI3KCA			
Mutations	~2	<10	12–23
Copy gain	–	–	38–61
PTEN			
Mutation	–	–	12
Silencing	–	–	28
Hypermethylation	–	–	69
***TP53* mutations**	0-9	0-9	~70

Table 2 RET/PTC rearrangements subtypes

RET/PTC chromossomal rearrangements	
RET/PTC1	Intrachromosomal rearrangement (10q11.2-10q21): RET tyrosine kinase domain (TK) fusion with the 5' terminal region of H4 gene
RET/PTC2	Chromossomal translocation t(10;17)(q11.2;q23): RET domain with PRKR1A (regulatory subunit type Iα of protein kinase A)
RET/PTC3	Intrachromosomal rearrangement: RET domain fusion **(exon 12)** with NCOA4 gene – (the nuclear receptor coactivator 4) / (ELE1/RFG/ARA70)
RET/PTC4	Intrachromosomal rearrangement: RET domain fusion **(exon 11)** with the nuclear receptor coactivator 4 gene - NCOA4 (ELE1/RFG/ARA70)
RET/PTC5	Chromossomal translocation t(10;14)(q11.2;q32): RET domain fusion with GOLGA5/RFG5 gene (like golgi autoantigen glogin subfamily A)
RET/PTC6	Chromossomal translocation t(7;10)(q32;q11.2): RET domain fusion with HTIF-1 (human transcription intermediary factor 1)
RET/PTC7	Chromossomal translocation t(1;10)(p13;q11.2): RET domain fusion with TRIM33 gene (Ectodermin/RFG7/Ectodermin/HTIFγ)
RET/PTC8	Chromossomal translocation t(10;14)(q11.2;q22.1): RET domain fusion with KTN1 gene (kanectin)
RET/PTC9	Chromossomal translocation t(10;18)(q11.2;q21-22): RET domain fusion with RFG9
ELKS/RET	Chromossomal translocation t(10;14)(q11.2;p13) : RET domain fusion with the 5' portion of ELKS gene (Rab6-interacting/CAST family member 1)
PCM1/RET	Chromossomal translocation t(8;10)(p21-22;q11.2) : RET domain fusion with PCM1 gene (pericentriolar material 1)
RFP/RET	Chromossomal translocation t(6;10) (p21.3;q11.2): RET domain fusion with RFP gene (RET finger protein)

REFERENCES

1. Hundahl SA 1998 Perspective: National Cancer Institute summary report about estimated exposures and thyroid doses received from iodine 131 in fallout after Nevada atmospheric nuclear bomb tests. CA Cancer J Clin. **48**:285-298.

2. Wiseman SM, Loree TR, Rigual NR, et al. 2003 Anaplastic transformation of thyroid cancer: review of clinical, pathologic, and molecular evidence provides new insights into disease biology and future therapy. Head Neck. **25**:662-670.

3. DeLellis R, Lloyd R, Heitz P, Eng C 2004 World Health Organization classification of tumours. Pathology and genetics of tumours of endocrine organs. . IARC Press, Lyon.

4. Harach HR, Ceballos GA 2008 Thyroid cancer, thyroiditis and dietary iodine: a review based on the Salta, Argentina model. Endocr Pathol. **19**:209-220.

5. Nikiforov YE 2010 Is ionizing radiation responsible for the increasing incidence of thyroid cancer? Cancer. **116**:1626-1628.

6. Kimura ET, Nikiforova MN, Zhu Z, et al. 2003 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. **63**:1454-1457.

7. Frattini M, Ferrario C, Bressan P, et al. 2004 Alternative mutations of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene. **23**:7436-7440.

8. Adeniran AJ, Zhu Z, Gandhi M, et al. 2006 Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol. **30**:216-222.

9. Nikiforova MN, Lynch RA, Biddinger PW, et al. 2003 RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab. **88**:2318-2326.

10. Garcia-Rostan G, Costa AM, Pereira-Castro I, et al. 2005 Mutation of the PIK3CA gene in anaplastic thyroid cancer. Cancer Res. **65**:10199-10207.

11. Kondo T, Ezzat S, Asa SL 2006 Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer. **6**:292-306.

12. Ricarte-Filho JC, Ryder M, Chitale DA, et al. 2009 Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res. **69**:4885-4893.

13. Liu Z, Hou P, Ji M, et al. 2008 Highly prevalent genetic alterations in receptor tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated protein kinase pathways in anaplastic and follicular thyroid cancers. J Clin Endocrinol Metab. **93**:3106-3116.

14. Nikiforov YE 2008 Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol. **21 Suppl 2**:S37-43.

15. Paes JE, Ringel MD 2008 Dysregulation of the phosphatidylinositol 3-kinase pathway in thyroid neoplasia. Endocrinol Metab Clin North Am. **37**:375-387, viii-ix.

16. Davies L, Welch HG 2006 Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA. **295**:2164-2167.

17. Franceschi S, Boyle P, Maisonneuve P, et al. 1993 The epidemiology of thyroid carcinoma. Crit Rev Oncog. **4**:25-52.

18. Pacini F, Cetani F, Miccoli P, et al. 1994 Outcome of 309 patients with metastatic differentiated thyroid carcinoma treated with radioiodine. World J Surg. **18**:600-604.

19. Cohen Y, Rosenbaum E, Clark DP, et al. 2004 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. **10**:2761-2765.

20. Xing M 2005 BRAF mutation in thyroid cancer. Endocr Relat Cancer. **12**:245-262.

21. Gutkind JS 2000 Regulation of mitogen-activated protein kinase signaling networks by G protein-coupled receptors. Sci STKE. **2000**:re1.

22. McKay MM, Morrison DK 2007 Integrating signals from RTKs to ERK/MAPK. Oncogene. **26**:3113-3121.

23. Ciampi R, Knauf JA, Kerler R, et al. 2005 Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. J Clin Invest. **115**:94-101.

24. Carta C, Moretti S, Passeri L, et al. 2006 Genotyping of an Italian papillary thyroid carcinoma cohort revealed high prevalence of BRAF mutations, absence of RAS mutations and allowed the detection of a new mutation of BRAF oncoprotein (BRAF(V599lns)). Clin Endocrinol (Oxf). **64**:105-109.

25. Hou P, Liu D, Xing M 2007 Functional characterization of the T1799-1801del and A1799-1816ins BRAF mutations in papillary thyroid cancer. Cell Cycle. **6**:377-379.

26. Lupi C, Giannini R, Ugolini C, et al. 2007 Association of BRAF V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. J Clin Endocrinol Metab. **92**:4085-4090.

27. Basolo F, Torregrossa L, Giannini R, et al. 2010 Correlation between the BRAF V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. J Clin Endocrinol Metab. **95**:4197-4205.

28. Knauf JA, Ma X, Smith EP, et al. 2005 Targeted expression of BRAFV600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. Cancer Res. **65**:4238-4245.

29. Franco AT, Malaguarnera R, Refetoff S, et al. 2011 Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. Proc Natl Acad Sci U S A. **108**:1615-1620.

30. Xing M, Westra WH, Tufano RP, et al. 2005 BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab. **90**:6373-6379.

31. Santarpia L, Sherman SI, Marabotti A, et al. 2009 Detection and molecular characterization of a novel BRAF activated domain mutation in follicular variant of papillary thyroid carcinoma. Hum Pathol. **40**:827-833.

32. Davies H, Bignell GR, Cox C, et al. 2002 Mutations of the BRAF gene in human cancer. Nature. **417**:949-954.

33. Wan PT, Garnett MJ, Roe SM, et al. 2004 Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. **116**:855-867.

34. Motti ML, De Marco C, Califano D, et al. 2007 Loss of p27 expression through RAS-->BRAF-->MAP kinase-dependent pathway in human thyroid carcinomas. Cell Cycle. **6**:2817-2825.

35. Knauf JA, Fagin JA 2009 Role of MAPK pathway oncoproteins in thyroid cancer pathogenesis and as drug targets. Curr Opin Cell Biol. **21**:296-303.

36. Mesa C, Jr., Mirza M, Mitsutake N, et al. 2006 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. **66**:6521-6529.

37. Ahmed M, Uddin S, Hussain AR, et al. 2011 FoxM1 and Its Association with Matrix Metalloproteinases (MMP) Signaling Pathway in Papillary Thyroid Carcinoma. J Clin Endocrinol Metab. **97**:E1-E13.

38. Bommarito A, Richiusa P, Carissimi E, et al. 2011 BRAFV600E mutation, TIMP-1 upregulation, and NF-kappaB activation: closing the loop on the papillary thyroid cancer trilogy. Endocr Relat Cancer. **18**:669-685.

39. Overall CM, Lopez-Otin C 2002 Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer. **2**:657-672.

40. Palona I, Namba H, Mitsutake N, et al. 2006 BRAFV600E promotes invasiveness of thyroid cancer cells through nuclear factor kappaB activation. Endocrinology. **147**:5699-5707.

41. Lee SJ, Lee MH, Kim DW, et al. 2011 Cross-regulation between oncogenic BRAF(V600E) kinase and the MST1 pathway in papillary thyroid carcinoma. PLoS One. **6**:e16180.

42. Fugazzola L, Pilotti S, Pinchera A, et al. 1995 Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. Cancer Res. **55**:5617-5620.

43. Nikiforov YE 2002 RET/PTC rearrangement in thyroid tumors. Endocr Pathol. **13**:3-16.

44. Zhu Z, Ciampi R, Nikiforova MN, et al. 2006 Prevalence of RET/PTC rearrangements in thyroid papillary carcinomas: effects of the detection methods and genetic heterogeneity. J Clin Endocrinol Metab. **91**:3603-3610.

45. Tallini G, Asa SL 2001 RET oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol. **8**:345-354.

46. Grieco M, Santoro M, Berlingieri MT, et al. 1990 PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell. **60**:557-563.

47. Nikiforov YE, Rowland JM, Bove KE, et al. 1997 Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res. **57**:1690-1694.

48. Tallini G, Santoro M, Helie M, et al. 1998 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. **4**:287-294.

49. Smyth P, Finn S, Cahill S, et al. 2005 ret/PTC and BRAF act as distinct molecular, time-dependant triggers in a sporadic Irish cohort of papillary thyroid carcinoma. Int J Surg Pathol. **13**:1-8.

50. Viglietto G, Chiappetta G, Martinez-Tello FJ, et al. 1995 RET/PTC oncogene activation is an early event in thyroid carcinogenesis. Oncogene. **11**:1207-1210.

51. Sugg SL, Ezzat S, Rosen IB, et al. 1998 Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. J Clin Endocrinol Metab. **83**:4116-4122.

52. Jhiang SM, Sagartz JE, Tong Q, et al. 1996 Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology. **137**:375-378.

53. Powell DJ, Jr., Russell J, Nibu K, et al. 1998 The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. Cancer Res. **58**:5523-5528.

54. Kawamoto Y, Takeda K, Okuno Y, et al. 2004 Identification of RET autophosphorylation sites by mass spectrometry. J Biol Chem. **279**:14213-14224.

55. Salvatore D, Barone MV, Salvatore G, et al. 2000 Tyrosines 1015 and 1062 are in vivo autophosphorylation sites in ret and ret-derived oncoproteins. J Clin Endocrinol Metab. **85**:3898-3907.

56. Knauf JA, Kuroda H, Basu S, Fagin JA 2003 RET/PTC-induced dedifferentiation of thyroid cells is mediated through Y1062 signaling through SHC-RAS-MAP kinase. Oncogene. **22**:4406-4412.

57. Vasko V, Saji M, Hardy E, et al. 2004 Akt activation and localisation correlate with tumour invasion and oncogene expression in thyroid cancer. J Med Genet. **41**:161-170.

58. Melillo RM, Castellone MD, Guarino V, et al. 2005 The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. J Clin Invest. **115**:1068-1081.

59. Gujral TS, van Veelen W, Richardson DS, et al. 2008 A novel RET kinase-beta-catenin signaling pathway contributes to tumorigenesis in thyroid carcinoma. Cancer Res. **68**:1338-1346.

60. Castellone MD, De Falco V, Rao DM, et al. 2009 The beta-catenin axis integrates multiple signals downstream from RET/papillary thyroid carcinoma leading to cell proliferation. Cancer Res. **69**:1867-1876.

61. Pradeep A, Sharma C, Sathyanarayana P, et al. 2004 Gastrin-mediated activation of cyclin D1 transcription involves beta-catenin and CREB pathways in gastric cancer cells. Oncogene. **23**:3689-3699.

62. Peifer M, Polakis P 2000 Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. Science. **287**:1606-1609.

63. Djakiew D, Delsite R, Pflug B, et al. 1991 Regulation of growth by a nerve growth factor-like protein which modulates paracrine interactions between a neoplastic epithelial cell line and stromal cells of the human prostate. Cancer Res. **51**:3304-3310.

64. Bongarzone I, Vigneri P, Mariani L, et al. 1998 RET/NTRK1 rearrangements in thyroid gland tumors of the papillary carcinoma family: correlation with clinicopathological features. Clin Cancer Res. **4**:223-228.

65. Musholt TJ, Musholt PB, Khaladj N, et al. 2000 Prognostic significance of RET and NTRK1 rearrangements in sporadic papillary thyroid carcinoma. Surgery. **128**:984-993.

66. Martin-Zanca D, Mitra G, Long LK, Barbacid M 1986 Molecular characterization of the human trk oncogene. Cold Spring Harb Symp Quant Biol. **51 Pt 2**:983-992.

67. Russell JP, Powell DJ, Cunnane M, et al. 2000 The TRK-T1 fusion protein induces neoplastic transformation of thyroid epithelium. Oncogene. **19**:5729-5735.

68. Fedele M, Palmieri D, Chiappetta G, et al. 2009 Impairment of the p27kip1 function enhances thyroid carcinogenesis in TRK-T1 transgenic mice. Endocr Relat Cancer. **16**:483-490.

69. Esapa CT, Johnson SJ, Kendall-Taylor P, et al. 1999 Prevalence of Ras mutations in thyroid neoplasia. Clin Endocrinol (Oxf). **50**:529-535.

70. Zhu Z, Gandhi M, Nikiforova MN, et al. 2003 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. **120**:71-77.

71. Santarpia L, Myers JN, Sherman SI, et al. 2010 Genetic alterations in the RAS/RAF/mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways in the follicular variant of papillary thyroid carcinoma. Cancer. **116**:2974-2983.

72. Wynford-Thomas D 1993 Molecular genetics of thyroid cancer. Trends Endocrinol Metab. **4**:224-232.

73. Vasko V, Ferrand M, Di Cristofaro J, et al. 2003 Specific pattern of RAS oncogene mutations in follicular thyroid tumors. J Clin Endocrinol Metab. **88**:2745-2752.

74. Hara H, Fulton N, Yashiro T, et al. 1994 N-ras mutation: an independent prognostic factor for aggressiveness of papillary thyroid carcinoma. Surgery. **116**:1010-1016.

75. Passler C, Scheuba C, Prager G, et al. 2004 Prognostic factors of papillary and follicular thyroid cancer: differences in an iodine-replete endemic goiter region. Endocr Relat Cancer. **11**:131-139.

76. Gulcelik MA, Gulcelik NE, Kuru B, et al. 2007 Prognostic factors determining survival in differentiated thyroid cancer. J Surg Oncol. **96**:598-604.

77. Verburg FA, Mader U, Luster M, Reiners C 2009 Histology does not influence prognosis in differentiated thyroid carcinoma when accounting for age, tumour diameter, invasive growth and metastases. Eur J Endocrinol. **160**:619-624.

78. Lemoine NR, Mayall ES, Wyllie FS, et al. 1989 High frequency of ras oncogene activation in all stages of human thyroid tumorigenesis. Oncogene. **4**:159-164.

79. Garcia-Rostan G, Zhao H, Camp RL, et al. 2003 ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. J Clin Oncol. **21**:3226-3235.

80. Namba H, Rubin SA, Fagin JA 1990 Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol. **4**:1474-1479.

81. Bond JA, Wyllie FS, Rowson J, et al. 1994 In vitro reconstruction of tumour initiation in a human epithelium. Oncogene. **9**:281-290.

82. Vitagliano D, Portella G, Troncone G, et al. 2006 Thyroid targeting of the N-ras(Gln61Lys) oncogene in transgenic mice results in follicular tumors that progress to poorly differentiated carcinomas. Oncogene. **25**:5467-5474.

83. Barbacid M 1990 ras oncogenes: their role in neoplasia. Eur J Clin Invest. **20**:225-235.

84. Malumbres M, Barbacid M 2003 RAS oncogenes: the first 30 years. Nat Rev Cancer. **3**:459-465.

85. Miller KA, Yeager N, Baker K, et al. 2009 Oncogenic Kras requires simultaneous PI3K signaling to induce ERK activation and transform thyroid epithelial cells in vivo. Cancer Res. **69**:3689-3694.

86. Vojtek AB, Der CJ 1998 Increasing complexity of the Ras signaling pathway. J Biol Chem. **273**:19925-19928.

87. Krasilnikov MA 2000 Phosphatidylinositol-3 kinase dependent pathways: the role in control of cell growth, survival, and malignant transformation. Biochemistry (Mosc). **65**:59-67.

88. Damante G, Tell G, Di Lauro R 2001 A unique combination of transcription factors controls differentiation of thyroid cells. Prog Nucleic Acid Res Mol Biol. **66**:307-356.

89. Desvergne B, Wahli W 1999 Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. **20**:649-688.

90. Kroll TG, Sarraf P, Pecciarini L, et al. 2000 PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. Science. **289**:1357-1360.

91. Cheung L, Messina M, Gill A, et al. 2003 Detection of the PAX8-PPAR gamma fusion oncogene in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab. **88**:354-357.

92. Marques AR, Espadinha C, Catarino AL, et al. 2002 Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab. **87**:3947-3952.

93. Lacroix L, Mian C, Barrier T, et al. 2004 PAX8 and peroxisome proliferator-activated receptor gamma 1 gene expression status in benign and malignant thyroid tissues. Eur J Endocrinol. **151**:367-374.

94. Klemke M, Drieschner N, Belge G, et al. 2011 Detection of PAX8-PPARG fusion transcripts in archival thyroid carcinoma samples by conventional RT-PCR. Genes Chromosomes Cancer.

95. Gregory Powell J, Wang X, Allard BL, et al. 2004 The PAX8/PPARgamma fusion oncoprotein transforms immortalized human thyrocytes through a mechanism probably involving wild-type PPARgamma inhibition. Oncogene. **23**:3634-3641.

96. Lui WO, Foukakis T, Liden J, et al. 2005 Expression profiling reveals a distinct transcription signature in follicular thyroid carcinomas with a PAX8-PPAR(gamma) fusion oncogene. Oncogene. **24**:1467-1476.

97. Reddi HV, McIver B, Grebe SK, Eberhardt NL 2007 The paired box-8/peroxisome proliferator-activated receptor-gamma oncogene in thyroid tumorigenesis. Endocrinology. **148**:932-935.

98. Farrow B, Evers BM 2003 Activation of PPARgamma increases PTEN expression in pancreatic cancer cells. Biochem Biophys Res Commun. **301**:50-53.

99. Chinnadurai G 2002 CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol Cell. **9**:213-224.

100. Neff RL, Farrar WB, Kloos RT, Burman KD 2008 Anaplastic thyroid cancer. Endocrinol Metab Clin North Am. **37**:525-538, xi.

101. Ain KB 1998 Anaplastic thyroid carcinoma: behavior, biology, and therapeutic approaches. Thyroid. **8**:715-726.

102. Giuffrida D, Gharib H 2000 Anaplastic thyroid carcinoma: current diagnosis and treatment. Ann Oncol. **11**:1083-1089.

103. Nikiforov YE 2004 Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. Endocr Pathol. **15**:319-327.

104. Hou P, Liu D, Shan Y, et al. 2007 Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer. Clin Cancer Res. **13**:1161-1170.

105. Nikiforova MN, Kimura ET, Gandhi M, et al. 2003 BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab. **88**:5399-5404.

106. Costa AM, Herrero A, Fresno MF, et al. 2008 BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma. Clin Endocrinol (Oxf). **68**:618-634.

107. Sobrinho-Simoes M, Maximo V, Rocha AS, et al. 2008 Intragenic mutations in thyroid cancer. Endocrinol Metab Clin North Am. **37**:333-362, viii.

108. Begum S, Rosenbaum E, Henrique R, et al. 2004 BRAF mutations in anaplastic thyroid carcinoma: implications for tumor origin, diagnosis and treatment. Mod Pathol. **17**:1359-1363.

109. Santarpia L, El-Naggar AK, Cote GJ, et al. 2008 Phosphatidylinositol 3-kinase/akt and ras/raf-mitogen-activated protein kinase pathway mutations in anaplastic thyroid cancer. J Clin Endocrinol Metab. **93**:278-284.

110. Saavedra HI, Knauf JA, Shirokawa JM, et al. 2000 The RAS oncogene induces genomic instability in thyroid PCCL3 cells via the MAPK pathway. Oncogene. **19**:3948-3954.

111. Sansal I, Sellers WR 2004 The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol. **22**:2954-2963.

112. Frisk T, Foukakis T, Dwight T, et al. 2002 Silencing of the PTEN tumor-suppressor gene in anaplastic thyroid cancer. Genes Chromosomes Cancer. **35**:74-80.

113. Hou P, Ji M, Xing M 2008 Association of PTEN gene methylation with genetic alterations in the phosphatidylinositol 3-kinase/AKT signaling pathway in thyroid tumors. Cancer. **113**:2440-2447.

114. Ringel MD, Hayre N, Saito J, et al. 2001 Overexpression and overactivation of Akt in thyroid carcinoma. Cancer Res. **61**:6105-6111.

115. Petitjean A, Mathe E, Kato S, et al. 2007 Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. **28**:622-629.

116. Ito T, Seyama T, Mizuno T, et al. 1992 Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res. **52**:1369-1371.

117. Cerrato A, Fulciniti F, Avallone A, et al. 1998 Beta- and gamma-catenin expression in thyroid carcinomas. J Pathol. **185**:267-272.

118. Garcia-Rostan G, Tallini G, Herrero A, et al. 1999 Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. Cancer Res. **59**:1811-1815.

119. Motti ML, Califano D, Baldassarre G, et al. 2005 Reduced E-cadherin expression contributes to the loss of p27kip1-mediated mechanism of contact inhibition in thyroid anaplastic carcinomas. Carcinogenesis. **26**:1021-1034.

120. Naito A, Iwase H, Kuzushima T, et al. 2001 Clinical significance of E-cadherin expression in thyroid neoplasms. J Surg Oncol. **76**:176-180.

121. von Wasielewski R, Rhein A, Werner M, et al. 1997 Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. Cancer Res. **57**:2501-2507.

122. Cohen EE, Rosen LS, Vokes EE, et al. 2008 Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. J Clin Oncol. **26**:4708-4713.

123. Gupta-Abramson V, Troxel AB, Nellore A, et al. 2008 Phase II trial of sorafenib in advanced thyroid cancer. J Clin Oncol. **26**:4714-4719.

124. Bible KC, Suman VJ, Molina JR, et al. 2010 Efficacy of pazopanib in progressive, radioiodine-refractory, metastatic differentiated thyroid cancers: results of a phase 2 consortium study. Lancet Oncol. **11**:962-972.

125. Brose MS, Nutting CM, Sherman SI, et al. 2011 Rationale and design of decision: a double-blind, randomized, placebo-controlled phase III trial evaluating the efficacy and safety of sorafenib in patients with locally advanced or metastatic radioactive iodine (RAI)-refractory, differentiated thyroid cancer. BMC Cancer. **11**:349.

Parte II

INCREASED TYPE 3 DEIODINASE EXPRESSION IN PAPILLARY THYROID CARCINOMA IS ASSOCIATED WITH AGGRESSIVE TUMOR BEHAVIOR

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INCREASED TYPE 3 DEIODINASE EXPRESSION IN PAPILLARY THYROID CARCINOMA IS ASSOCIATED WITH AGGRESSIVE TUMOR BEHAVIOR

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Abstract

Background: Thyroid hormone regulates a wide range of cellular activities, including the balance between cell proliferation and differentiation. The thyroid hormone-inactivating type 3 deiodinase (*DIO3*, D3) has been shown to be reactivated in human neoplasias. Here, we evaluated *DIO3* expression in human papillary thyroid carcinoma (PTC).

Methods: Tumor and surrounding normal thyroid tissue were collected from 26 unselected PTC patients. Clinical data were retrospectively reviewed in medical records. *DIO3* mRNA levels were measured by Real-Time PCR and D3 activity by paper-descendent chromatography. Studies of *DIO3* gene regulation were performed in a human PTC-derived cell line (K1 cells). $BRAF^{V600E}$ mutation was identified in DNA from paraffin-embedded tissues by direct sequencing. Immunohistochemistry analyses were performed using a specific human D3 antibody.

Results*:* Increased D3 activity was detected in all 26 PTC samples analyzed as compared with adjacent thyroid tissue. The augment in D3 activity were paralleled by increased DIO3 mRNA levels (~5 fold). In PTC-derived cells, *DIO3* transcripts were further up-regulated by the transforming growth factor β (TGFβ1). Interestingly, preincubation with mitogen-activated protein kinase (MAPK) cascade inhibitors U0126 (ERK pathway) and SB203580 (p38 pathway) decreased *DIO3* mRNA levels and blocked the TGFβ1-induced increase in *DIO3* transcripts, suggesting that D3-induction might be mediated through the MAPK signaling pathway. Accordingly*, DIO3* mRNA and activity levels were significantly higher in $BRAF^{V600E}$ mutated samples (P=0.001). Increased D3 activity was correlated with tumor size (r=0.68, P=0.003), and associated with lymph node (P=0.03) or distant metastasis (P=0.006) at diagnosis. Conversely, decreased levels of the thyroid hormone activating type 2 deiodinase (*DIO2*) gene were observed in PTC which might contribute to further decreases in intracellular thyroid hormone levels. Increased D3 expression was also observed in follicular thyroid carcinoma but not in medullary or anaplastic thyroid carcinoma samples.

Conclusions: These results indicate that the malignant transformation of thyroid follicular cell towards PTC promotes opposite changes in *DIO3* and *DIO2* expression by pre-transcriptional mechanisms. The association between increased levels of D3 activity and advanced disease further supports a role for intracellular T3 concentration on the thyroid tumor cell proliferation or/and dedifferentiation.

Introduction

Thyroid cancer is the most common malignancy of the endocrine system accounting for approximately 1% of all human cancers. The papillary thyroid cancer (PTC) is the most frequent histotype and accounts for 85-90% of all thyroid malignancies (1). The majority of patients with PTC have a favorable outcome. However, 5-20% will develop tumor recurrence while 10% will have distant metastasis (1, 2). Genetic alterations leading to the activation of the mitogen-activated protein kinase (MAPK) signaling pathway are a hallmark of PTC. The most common is a thymine (T) to adenine (A) transversion at nucleotide position 1799 (T1799A) of *BRAF* gene, resulting in a valine-to-glutamate substitution at residue 600 ($BRAF^{V600E}$) and occurring in ~50% of cases (3-5). *RET/PTC* rearrangement and *RAS* gene mutations are responsible for ~20% and 10-15% of cases, respectively (6). The presence of *BRAF* mutations has been inconsistently associated with aggressive tumoral behavior and poor outcome (7-10).

Thyroid hormone influences a wide variety of biological events including the balance between proliferation and differentiation. The activation of the pro-hormone T4 (thyroxine) into the biologically active hormone T3 (triiodothyronine) is catalyzed by type 1 (D1) and type 2 (D2) iodothyronine deiodinases via outer-ring deiodination (11). In contrast, type 3 iodothyronine deiodinase (D3) catalyzes the inactivation of both T4 and T3 via inner-ring deiodination. D2 plays a critical role in providing local T3 to regulate intracellular T3 concentration and also seems to be an important source for circulating T3 (11). The main physiological role of D3 is protecting tissues, particularly those in the developing fetus, from inappropriate high levels of T3.

Although the role of deiodinases in neoplasias has not been fully understood, studies focusing on *DIO1* and *DIO2* genes have reported changes on the expression of these enzymes in benign and malignant tumors (12, 13). *DIO1* and *DIO2* mRNA levels are diminished or unaltered in the majority of thyroid neoplasias, with the exception of augmented D2 activity in follicular (FTC) and medullary thyroid carcinomas (MTC) (14, 15). D3 has been shown to be reactivated in human benign neoplasias such as hepatic haemangiomas as well as in malignant brain tumors (16). More recently, the increased D3 expression has been described in human malignant basal cell carcinoma (BCC). Interestingly, augmented D3 activity resulted in increased levels of cyclin D1 and cell proliferation whereas *Dio3* knockdown caused a 5-fold reduction in the growth

of tumor xenografts (17). These findings suggest a role for D3 in cell proliferation and might indicated that the local hypothyroidism, due to the augmented D3 activity, could favor tumor cell growth.

Here we have investigated the expression of D3 in human PTC. Increased levels of D3 activity were observed in all PTC samples analyzed. The induced *DIO3* expression occurred at transcriptional level and PTC tumors carrying the $BRAF^{V600E}$ mutation presented the highest levels of D3 activity. We observed a positive correlation between tumor size and D3 activity. Moreover, increased D3 activity in tumor samples were associated with advanced disease at diagnosis.

Material and Methods

Patients and tissues

Tumor and surrounding normal thyroid tissue were collected from 26 unselected patients diagnosed with PTC attending the Endocrine or Head and Neck Surgery Divisions at Hospital de Clínicas de Porto Alegre, Brazil. Sample tissues were immediately frozen in liquid nitrogen and stored at -70° until analysis. Surgery was independently indicated by attending physicians. Tumors were histological classified according to WHO recommendations (18). The clinical stage was determined by the Tumor/Node/Metastasis (TMN) system (19). Clinical data were retrospectively reviewed in medical records. The study was approved by the Ethical Committee of the Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Immunohistochemistry studies

Eight PTC (randomly selected from the initial 26 patients), 4 FTC, 10 MTC and 1 anaplastic thyroid carcinoma (ATC) samples were used for immunohistochemistry studies. Immunohistochemistry analyses were performed on 6 μm sections of previously formalin-fixed and paraffin-embedded tissuesThe routine immunohistochemical technique comprised deparaffination and rehydration, antigenic recovery, inactivation of endogenous peroxidase, and blockage of unspecific reactions. D3 antibody 676 was kindly provided by Dr. Domenico Salvatore (University of Naples). Primary antibodies were incubated overnight at a temperature of 4°C, at dilutions of 1:400 for D3 (the same antibody concentration was also used for positive control) followed by subsequent incubation with biotinilated secondary antibody, streptavidin horseradish

peroxidase conjugate (LSAB; Dako Cytomation Inc, Carpinteria, CA, USA), and diaminobenzidine tetrahydrochloride (Kit DAB; Dako Cytomation Inc, Carpinteria, CA, USA). Placenta tissue was used as a positive control. The negative control was obtained by omission of the primary antibody. The slides were examined using an Olympus BX51 microscope with an Olympus QColor 5 camera. The QCapturePro software was used to capture the images.

Cell Culture

Studies to evaluate *DIO3* expression were performed in a human PTC-derived cell line that harbors $BRAF^{V600E}$ mutation (K1 cells, purchased from HPA Culture Collections, UK). Cells were grown in DMEM:Ham's F12:MCDB105 (2:1:1) plus 2mM glutamine and 10% fetal bovine serum. Cells were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air, and the culture medium was changed 3 times a week.

To evaluate the *DIO3* regulation, K1 cells were incubated for 18–24h in the appropriate medium above plus 0.1% BSA and 10-200nM T3, 100nM selenium, 1mM dibutyril cAMP (Bu) 2cAMP (DBT), 20ng/ml TGFβ1 or 20uM iopanoic acid as indicated. Additional studies evaluating *DIO3* gene expression were performed on the presence of 5 uM MAPK pathway inhibitors (U0126 - ERK1/2 kinase inhibitor and SB203580 - p38 inhibitor). Controls were incubated with medium + vehicle (2% DMSO). At the appropriate times, cells were harvested and processed for total RNA extraction or measurement of D3 activity. All reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA) or Invitrogen Life Technologies (Carlsbad, CA, USA). All experiments were performed in triplicate in at least two independent experiments.

Assay of D3 activity

D3 activity in tissue samples and cells was determined using paper chromatography as previously described (20, 21). Tissue or cells were individually homogenized and sonicated with 10mM tris-HCl, 0.25 sucrose buffer (pH 7.5) and 10mM dithiothreitol (DTT). Protein concentration was quantified by Bradford assay using BSA as a standard. The homogenates were incubated for an hour with 200000cpm ^{125}I labeled T3, 2nM T3, 20mM DTT and 1mM propiltiouracil (PTU) in order to inhibit any D1 activity. The addition of 200nM of T3 completely abolished D3 activity in all samples. The reaction was stopped by adding 200ul ethanol 95%, 50ul NaOH 0.04N and 5mg PTU. Deiodination was determined based on the amount of ^{125}I -3,3´-T2

produced after separation of reaction products by paper chromatography. Results were expressed as the fraction of T2 counts minus the nonspecific deiodination (always <1.5%), obtained with the saturating concentration of T3 (200nM). D3 activity was expressed as femtomoles T3 per minute per milligram protein (fmol/mg.prot.min). The quantity of protein assayed was adjusted to ensure that less than 30% of the substrate was consumed. All experiments were performed in triplicate in at least two independent experiments.

Real-time PCR

Total RNA was extracted from normal, tumor tissue or cells using Trizol Reagent and cDNAs generated were used in a real-time PCR with a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Standard curves representing 5-point serial dilution of cDNA of the experimental and control groups were analyzed and used as calibrators of the relative quantification of product generated in the exponential phase of the amplification curve. The r2 was greater than 0.99, and the amplification efficiency varied between 80% and 100%. Samples were measured by relative quantification (change in expression in the experimental group versus control; untreated versus treated cells). The data generated by ABI Prism 7500 system SDS software (Applied Biosystems, Foster City, CA, USA) were then transferred to an Excel spreadsheet (Microsoft), and the experimental values were corrected to that of the cyclophilin A standard. The oligonucleotides used were the following: human DIO2 gene, 5′-ACTTCCTGCTGGTCTACATTGATG- 3′ and 5′-CTTCCTGGTTCTGGTGCTTCTTC-3′; human DIO3 gene, 5′-TCCAGAGCCAGCACATCCT-3′ and 5′- ACGTCGCGCTGGTACTTAGTG- 3′; cyclophilin A (internal control), 5′-GTCAACCCCACCGTGTTCTTC- 3′ and 5′-ACTTGCCACCAGTGCCATTATG-3′.

BRAF^{V600E} *Mutation Analysis*

Of the initial group of 26 patients, 17 paraffin-embedded formalin-fixed tissue blocks were available for analysis. The DNA was extracted from 10μm slides of paraffin-embedded tissue blocks, using the Magnesil Genomic Fixed Tissue System (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. The *BRAF* exon 15 was amplified by PCR using specific oligonucleotides:

5’ACCTAAACTCTTCATAATGCTTGCT3’and3’CTGATTTTTGTGAATACTGGGA ACT5’. To PCR amplification we used 100–300 ng/ml of DNA in a reaction mix (25 µl) containing 20 mM Tris–HCl, pH 8.0, 50 mM KCl, 2 mM MgCl2, 0.2 mM dNTPs, 0.2 mM of each primer, and 1.25 U Platinum Taq DNA Polymerase (Invitrogen Life Technology, Carlsbad, CA, USA). $BRAF^{V600E}$ mutation genotyping was performed using direct sequencing. For sequencing, PCR products were purified using the GFX PCR DNA purification kit (GE Healthcare, Buckinghamhire, UK) and submitted to direct sequencing using the Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Data were expressed as median and interquartile interval (P25-P75) for clinical features unless otherwise indicated. D3 activity was expressed as media ± standard deviation (SD). Statistical analysis was performed using Mann-Whitney test or Pearson’s correlation coefficient. Statistical significance was assumed for P value < 0.05.

Results

Type 3 deiodinase is induced in follicular cell-derived tumors

The clinical and oncological characteristics of the 26 PTC patients included in this study are detailed in table 1 and were as follows: the mean age was 38.73±16.18 years and 76.9% were female. The median size tumor was 2.5 cm (0.7-10.5), nine patients (34.6%) had lymph node metastasis while eight (30.7%) presented distant metastasis at diagnosis. All patients presented normal serum TSH levels (0.4-4 UI/L) at surgery.

D3 activity was significantly increased in PTC samples as compared with the surrounding thyroid normal tissue (P<0.0001, Figure 1A). The augment in D3 activity were paralleled by increases in *DIO3* mRNA levels (~5 fold, P<0.0001, figure 1B), indicating that the *DIO3* induction occurs at transcriptional level. Further analysis by immunohistochemistry assay with a specific D3 antibody demonstrated that D3 is almost undetectable in normal follicular thyroid tissue whereas a strong positive staining was observed in PTC cells (Figures 1C and 1D).

D3 expression in a human PTC-derived cell line

To further evaluate *DIO3* expression in PTC, we undertook studies in K1 cells, a human PTC-derived cell line. *DIO3* transcripts and activity were readily detected in K1 cells. Additional studies demonstrated that D3 activity was stimulated by selenium (100nM), T3 (10 nM) or DBT (1mM). Conversely, D3 activity was abolished by higher T3 doses (200nM) or iopanoic acid (20uM) (Figure 2A).

Next we investigated the role of TGFβ on *DIO3* expression in PTC since it has been previously implicated in *DIO3* regulation in other tissues (22). Addition of TGFβ1 (20ng/ml) to K1 cell culture medium promoted a ~5-fold induction in *DIO3* mRNA levels (Figure 2B). Interestingly, preincubation with mitogen-activated protein kinase (MAPK) cascade inhibitors U0126 (ERK1/2 kinase inhibitor) and SB203580 (p38 pathway) decreased *DIO3* mRNA levels and blocked the TGFβ1-induced increase in *DIO3* transcripts, suggesting that D3-induction might be mediated through the MAPK signaling pathway (Figure 2B).

Type 3 deiodinase activity is induced in BRAF ^{V600E} mutated samples

Next we investigated the role of $BRAF^{V600E}$ mutation on *DIO3* expression. Of the initial group of 26 PTC patients, 17 PTC samples were available for analysis. Seven (41.2%) out of the 17 PTC samples analyzed were positive for $BRAF^{V600E}$ mutation. A significant increase in D3 activity was observed in BRAF-mutated as compared with negative samples (P=0.009, Figure 3A). As expected, the increase in D3 activity was paralleled by an augment in *DIO3* mRNA levels (Figure 3B; P=0.001). No differences in gender, age, tumor size, and local or distant metastasis were observed between patients with or without BRAF mutation (all P>0.5).

D3 activity is associated with advanced disease at diagnosis

Previous studies indicated that D3 induction is associated with tumor cell proliferation, thus we sought to evaluate whether the increase in *DIO3* expression in PTC would be associated with disease presentation at diagnosis. Indeed, we observed that increased D3 activity was positively correlated with tumor size (r= 0.68; P=0.003, Figure 4A). Moreover, increased levels of D3 activity in tumor samples were associated

with lymph node (P=0.03) or distant metastasis at diagnosis (P=0.006; Figure 4B), indicating that higher D3 activity might be associated with metastatic disease.

DIO2 expression is down regulated in papillary thyroid tumors

Previous studies in BCC have shown that the *DIO3* and *DIO2* genes are regulated in an opposite fashion. Consistently, we observed a significant decrease in *DIO2* mRNA levels in PTC sample as compared to the surrounding tissue (0.18±0.02 vs. 0.85±0.003 AU, respectively; P=0.001).

DIO3 expression in other malignant thyroid neoplasias

We next evaluated the D3 expression in other histological types of thyroid carcinomas. Figure 5 shows the immunohistochemical analysis of a panel comprising representative samples of normal thyroid cells, PTC, FTC, hereditary or sporadic MTC and ATC. As already shown, D3 staining is barely visible in the normal thyroid tissue (Figure 5A) while we observe a strong staining in PTC tumor (Figure 5B). D3 staining is also readily detected in FTC samples, although weaker than in PTC (Figure 5C). In contrast, D3 protein was barely detectable in a MTC sample of thyroid tissue of a patient with hereditary or in sporadic MTC (figure 5D and 5E). No D3 staining was observed in an ATC sample from a 40 year-old-man who underwent total thyroidectomy due to a 8x6.5x4cm fast growing neck mass (Figure 5F).

Discussion

In the present study we evaluated the *DIO3* expression in human PTC. *DIO3* transcripts and D3 activity were increased in all PTC samples analyzed. D3-induction is likely to be mediated through the MAPK pathway, which is constitutively activated in this tumor. Accordingly, the highest levels of D3 activity were observed in PTC samples harboring $BRAF^{V600E}$ mutation. Increased D3 activity was positively correlated with tumor size and associated with disseminated disease at diagnosis. In contrast, *DIO2* transcript levels were significantly decreased in PTC as compared to surrounding thyroid tissue. The reduction of D2 activity might contribute to further decrease in

intracellular thyroid hormone levels. Taken together, these findings add support for a role of intracellular hypothyroidism in the tumor cell proliferation and/or differentiation.

The ontogenic profile of *DIO3* gene shows that D3 activity is present at high levels in developing organs and almost undetectable in most mature tissues. In adults, D3 is predominantly expressed in brain and skin (23, 24). Recently, a previously unrecognized role of D3 has been documented in a number of pathological states, unraveling an important role of D3 in both health and disease (16, 21, 25). Moreover, a potential role of D3 in tumorigenesis has been postulated since induction of *DIO3* gene has been observed in several benign and malignant tumors and cell lines (17, 25, 26).

Here we have demonstrated that *DIO3* expression is induced in human PTC. D3-induction occurs at transcriptional level and it is likely to be driven by the constitutive activation of the MAPK pathway, which has been previously implicated in D3 up-regulation in other pathological conditions (22, 27-29). Consistently with this hypothesis, we show that samples carrying the $BRAF^{V600E}$ mutation display the highest levels of *DIO3* mRNA and activity (Figure 3B). Based on these observations, we speculate that BRAF-induced increase in *DIO3* expression might be involved in the development of aggressive PTC tumors and severe hypothyroidism in thyroid-specific knock-in *Braf* mice (*LSL-*$Braf^{V600E}$*/TPO-Cre*) (30). Other potential inducer of *DIO3* expression in PTC might be the TGFβ, which is overexpressed in thyroid tumors and implicated on cell dedifferentiation and proliferation (31-33). TGFβ induces *DIO3* gene via cooperative effects of Smads and MAPK-dependent pathway. Interestingly, recent studies show that mice with thyroid-specific expression of oncogenic *Braf* (Tg-Braf) developed PTCs that were locally invasive and had well-defined foci of poorly differentiated thyroid carcinoma. This effect seems to be mediated by oncogenic *Braf* induction of TGFβ secretion, which in turn might mediate, effects as cell migration, invasiveness and epithelial–mesenchymal transition (34).

A potential role of intracellular thyroid hormone levels on tumorigenesis has long been speculated. It has been demonstrated that thyroid hormone is able to block oncogenic Ras-mediated proliferation and transcriptional induction of cyclin D1 in neuroblastoma cells by interfering specifically with the activity of the MAPK pathway and cyclic AMP response element (CRE)-mediated transcription (35). More recently, studies performed in BCC demonstrated that D3-induction reduces intracellular thyroid hormone levels and promotes the malignant keratinocytes proliferation (17). The role of D3 was demonstrated by silencing of *DIO3* gene that resulted in reduction of both

proliferation and cyclin D1 levels, whereas the reintroduction of *DIO3* gene in D3-depleted cells reversed these effects. As suggested by the authors, the putative effect of D3 on diminishing microenvironment T3 could provide an advantage for tumor cell proliferation (17). Consistently with these observations, we observed a positive correlation between increased levels of D3 activity and tumor size in human PTC samples. Immunohistochemical staining of D3 protein was also detected in FTC, but, surprisingly, it was nearly undetectable in MTC and ATC samples. These findings were somehow unexpected since the hypoxia-inducible transcription factor (HIF)-1 has previously been shown to be a potent inducer of the *DIO3* gene (36). Of note, highest levels of HIF-1 have been shown in ATC samples whereas in differentiated PTCs and FTCs its expression was variable (37).

Another interesting observation of this study was the association between increased levels of D3 activity in thyroid tumor samples and metastatic disease at diagnosis. PTC samples from patients with lymph node or distant metastasis displayed significantly higher levels of D3 activity than those samples obtained from patients with intra-thyroidal disease (Figure 4B). This association was independent of the presence of *BRAF* mutation, which has been associated with aggressive tumoral behavior by some studies. One possible explanation for this observation could be an effect of the enhanced D3 activity in the regulation/reactivation, probably through hypothyroidism, of genes responsible for tumor invasiveness and metastasis development. Indeed, several previous studies have demonstrated an association between hypothyroidism and advanced disease. Xenograft tumors of hepatocellular carcinoma (HCC) or breast cancer cells implanted into hypothyroid nude mice were more aggressive and presented a higher rate of distant metastasis than those tumors-induced into euthyroid mice (38). Additionally, a case-control study observed that individuals with long term hypothyroidism presented a twofold higher risk for HCC development when compared with individuals with no history of thyroid disorders (39). Of interest, a recent study using a human and mouse HCC model has identified a cluster of microRNAs (miRNA) involved in up-regulation of DLK1-DIO3 genomic imprinted region. The authors show that overexpression of DLK1-DIO3 miRNAs cluster was associated with higher metastatic rate and poor overall survival in HCC patients (40).

An opposite pattern between increased *DIO3* and diminished *DIO2* gene expression was observed in PTC samples. Changes on the deiodinase balance with dual convergent mechanisms, transcriptional up-regulation of *DIO3* and posttranscriptional

down regulation of *DIO2*, were also demonstrated in BCC (17). The reduction of D2 activity might contribute with D3 induction to further extend the local hypothyroidism. Interestingly, the *DIO2* down regulation seems to be a characteristic feature of papillary carcinomas, since high levels of *DIO2* expression has been reported in FTC and MTC (15, 41).

In conclusion, we have demonstrated that *DIO3* expression is up regulated in PTC. Remarkable, PTC samples harboring $BRAF^{V600E}$ mutation display the highest levels of D3 activity. Increased D3 activity was associated with tumor size and disease spread. These findings further support the concept that increased thyroid hormone inactivation, and consequent intracellular hypothyroidism, might be associated with the degree of tumor cell dedifferentiation and enhancement of tumor invasiveness.

Declarations of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

1. Franceschi S, Boyle P, Maisonneuve P, et al. 1993 The epidemiology of thyroid carcinoma. Crit Rev Oncog. **4**:25-52.

2. Pacini F 2003 Role of percutaneous ethanol injection in management of nodular lesions of the thyroid gland. J Nucl Med. **44**:211-212.

3. Fagin JA 2005 Genetics of papillary thyroid cancer initiation: implications for therapy. Trans Am Clin Climatol Assoc. **116**:259-269; discussion 269-271.

4. Trovisco V, Soares P, Preto A, et al. 2007 Molecular genetics of papillary thyroid carcinoma: great expectations. Arq Bras Endocrinol Metabol. **51**:643-653.

5. Kimura ET, Nikiforova MN, Zhu Z, et al. 2003 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. **63**:1454-1457.

6. Fagin JA, Mitsiades N 2008 Molecular pathology of thyroid cancer: diagnostic and clinical implications. Best Pract Res Clin Endocrinol Metab. **22**:955-969.

7. Nikiforova MN, Kimura ET, Gandhi M, et al. 2003 BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab. **88**:5399-5404.

8. Xing M 2005 The T1799A BRAF mutation is not a germline mutation in familial nonmedullary thyroid cancer. Clin Endocrinol (Oxf). **63**:263-266.

9. Fugazzola L, Mannavola D, Cirello V, et al. 2004 BRAF mutations in an Italian cohort of thyroid cancers. Clin Endocrinol (Oxf). **61**:239-243.

10. Puxeddu E, Susta F, Orvietani PL, et al. 2007 Identification of differentially expressed proteins in papillary thyroid carcinomas with V600E mutation of BRAF. Proteomics Clin Appl. **1**:672-680.

11. Maia AL, Kim BW, Huang SA, et al. 2005 Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. J Clin Invest. **115**:2524-2533.

12. Huang Y, Prasad M, Lemon WJ, et al. 2001 Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci U S A. **98**:15044-15049.

13. Murakami M, Araki O, Hosoi Y, et al. 2001 Expression and regulation of type II iodothyronine deiodinase in human thyroid gland. Endocrinology. **142**:2961-2967.

14. Kim BW, Daniels GH, Harrison BJ, et al. 2003 Overexpression of type 2 iodothyronine deiodinase in follicular carcinoma as a cause of low circulating free thyroxine levels. J Clin Endocrinol Metab. **88**:594-598.

15. Meyer EL, Goemann IM, Dora JM, et al. 2008 Type 2 iodothyronine deiodinase is highly expressed in medullary thyroid carcinoma. Mol Cell Endocrinol. **289**:16-22.

16. Dentice M, Ambrosio R, Salvatore D 2009 Role of type 3 deiodinase in cancer. Expert Opin Ther Targets. **13**:1363-1373.

17. Dentice M, Luongo C, Huang S, et al. 2007 Sonic hedgehog-induced type 3 deiodinase blocks thyroid hormone action enhancing proliferation of normal and malignant keratinocytes. Proc Natl Acad Sci U S A. **104**:14466-14471.

18. Hedinger C, Williams ED, Sobin LH 1989 The WHO histological classification of thyroid tumors: a commentary on the second edition. Cancer. **63**:908-911.

19. Greene FL, Sobin LH 2002 The TNM system: our language for cancer care. J Surg Oncol. **80**:119-120.

20. Galton VA, Schneider MJ, Clark AS, St Germain DL 2009 Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. Endocrinology. **150**:2957-2963.

21. Wajner SM, Goemann IM, Bueno AL, et al. 2011 IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. J Clin Invest. **121**:1834-1845.

22. Huang SA, Mulcahey MA, Crescenzi A, et al. 2005 Transforming growth factor-beta promotes inactivation of extracellular thyroid hormones via transcriptional stimulation of type 3 iodothyronine deiodinase. Mol Endocrinol. **19**:3126-3136.

23. Huang TS, Chopra IJ, Beredo A, et al. 1985 Skin is an active site for the inner ring monodeiodination of thyroxine to 3,3',5'-triiodothyronine. Endocrinology. **117**:2106-2113.

24. Bates JM, St Germain DL, Galton VA 1999 Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. Endocrinology. **140**:844-851.

25. Huang SA, Tu HM, Harney JW, et al. 2000 Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med. **343**:185-189.

26. Nauman P, Bonicki W, Michalik R, et al. 2004 The concentration of thyroid hormones and activities of iodothyronine deiodinases are altered in human brain gliomas. Folia Neuropathol. **42**:67-73.

27. Pallud S, Ramauge M, Gavaret JM, et al. 1999 Regulation of type 3 iodothyronine deiodinase expression in cultured rat astrocytes: role of the Erk cascade. Endocrinology. **140**:2917-2923.

28. Zrouri H, Le Goascogne C, Li WW, et al. 2004 The role of MAP kinases in rapid gene induction after lesioning of the rat sciatic nerve. Eur J Neurosci. **20**:1811-1818.

29. Lamirand A, Pallud-Mothre S, Ramauge M, et al. 2008 Oxidative stress regulates type 3 deiodinase and type 2 deiodinase in cultured rat astrocytes. Endocrinology. **149**:3713-3721.

30. Franco AT, Malaguarnera R, Refetoff S, et al. 2011 Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. Proc Natl Acad Sci U S A. **108**:1615-1620.

31. Matsuo SE, Fiore AP, Siguematu SM, et al. 2010 Expression of SMAD proteins, TGF-beta/activin signaling mediators, in human thyroid tissues. Arq Bras Endocrinol Metabol. **54**:406-412.

32. Pisarev MA, Thomasz L, Juvenal GJ 2009 Role of transforming growth factor beta in the regulation of thyroid function and growth. Thyroid. **19**:881-892.

33. Matoba H, Sugano S, Yamaguchi N, Miyachi Y 1998 Expression of transforming growth factor-beta1 and transforming growth factor-beta Type-II receptor mRNA in papillary thyroid carcinoma. Horm Metab Res. **30**:624-628.

34. Knauf JA, Sartor MA, Medvedovic M, et al. 2011 Progression of BRAF-induced thyroid cancer is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGFbeta signaling. Oncogene. **30**:3153-3162.

35. Garcia-Silva S, Aranda A 2004 The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. Mol Cell Biol. **24**:7514-7523.

36. Simonides WS, Mulcahey MA, Redout EM, et al. 2008 Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. J Clin Invest. **118**:975-983.

37. Burrows N, Resch J, Cowen RL, et al. 2010 Expression of hypoxia-inducible factor 1 alpha in thyroid carcinomas. Endocr Relat Cancer. **17**:61-72.

38. Martinez-Iglesias O, Garcia-Silva S, Regadera J, Aranda A 2009 Hypothyroidism enhances tumor invasiveness and metastasis development. PLoS One. **4**:e6428.

39. Hassan MM, Kaseb A, Li D, et al. 2009 Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. Hepatology. **49**:1563-1570.

40. Luk JM, Burchard J, Zhang C, et al. 2011 DLK1-DIO3 genomic imprinted microRNA cluster at 14q32.2 defines a stemlike subtype of hepatocellular carcinoma associated with poor survival. J Biol Chem. **286**:30706-30713.

41. Souza Meyer E, Dora J, Wagner M, Maia A 2005 Decreased type 1 iodothyronine deiodinase expression might be an early and discrete event in thyroid cell dedifferentiation towards papillary carcinoma. Clin Endocrinol **62**:672-678.

Table 1: Clinical and Oncological Characteristics of Patients with Papillary Thyroid Carcinoma

Patient no./	**Sex**	**Age (years)**	**Size (cm)**	**Histology**	**Stage (TNM)**	**Invaded organ**	**BRAF^{V600E} Mutation**
1	M	50	1.5	Classic type	III	Cervical lymph nodes	-
2	M	22	1.3	Classic type	II	Cervical lymph nodes; Lung	-
3	M	22	2.3	Follicular variant	I	Cervical lymph nodes; Lung	-
4	F	35	1.8	Classic type	I		-
5	F	33	1.0	Classic type	I	Cervical lymph nodes	-
6	F	21	0.8	Classic type	I	Cervical lymph nodes	-
7	F	27	6.4	Classic type	II	Cervical lymph nodes; Lung	+
8	M	48	0.9	Classic type	I		NA
9	F	36	2.5	Follicular variant	I		NA
10	F	53	0.8	Classic type	I	Cervical lymph nodes	+
11	F	41	2.9	Classic type	I		+
12	F	53	2.5	Classic type	III	Cervical lymph nodes	+
13	F	47	2.3	Classic type	II		-
14	F	53	2.5	Classic type	III	Cervical lymph nodes	+
15	M	50	8.0	Classic type	III	Cervical lymph nodes	+
16	M	61	1.5	Classic type	IV	Cervical lymph nodes; Lung	-
17	F	69	7.5	Classic type	IV	Cervical lymph nodes	-
18	F	61	8	Classic type	IV	Cervical lymph nodes; Lung	-
19	F	30	1	Follicular variant	I		NA
20	F	42	8	Classic type	I	Cervical lymph nodes; Conjunctive tissue and skeletal muscle	+
21	F	10	10.5	Classic type	I	Cervical lymph nodes	-
22	F	18	3.5	Classic type	I		NA
23	F	55	3	Follicular variant	IV	Skeletal muscle of the neck	NA
24	F	30	2.5	Classic type	I		NA
25	F	11	6.0	Classic type	I	Cervical lymph nodes; Lung	NA
26	F	29	0.7	Follicular variant	II		NA

*TNM: Tumor/Node/Metastasis system

** Positive (+), Negative (-), NA (Not available)

Figures

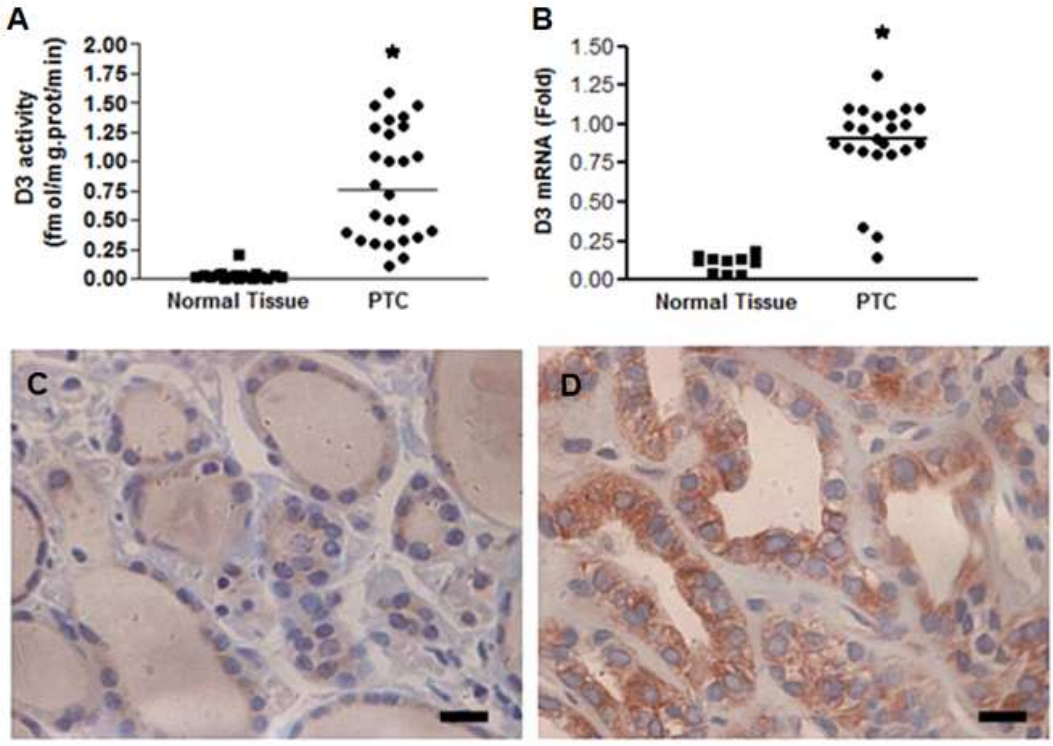

Figure 1 D3 activity (A) and *DIO3* mRNA levels (B) are markedly increased in human papillary thyroid carcinoma (PTC) as compared with surrounding normal thyroid tissue. Representative micrographs of D3 brown-dark immunohistochemical staining: Normal thyroid tissue was nearly undetectable for D3 (magnification 400×) (C), whereas PTC tissue showed a strong positive staining (magnification 400×) (D). *P<0.0001

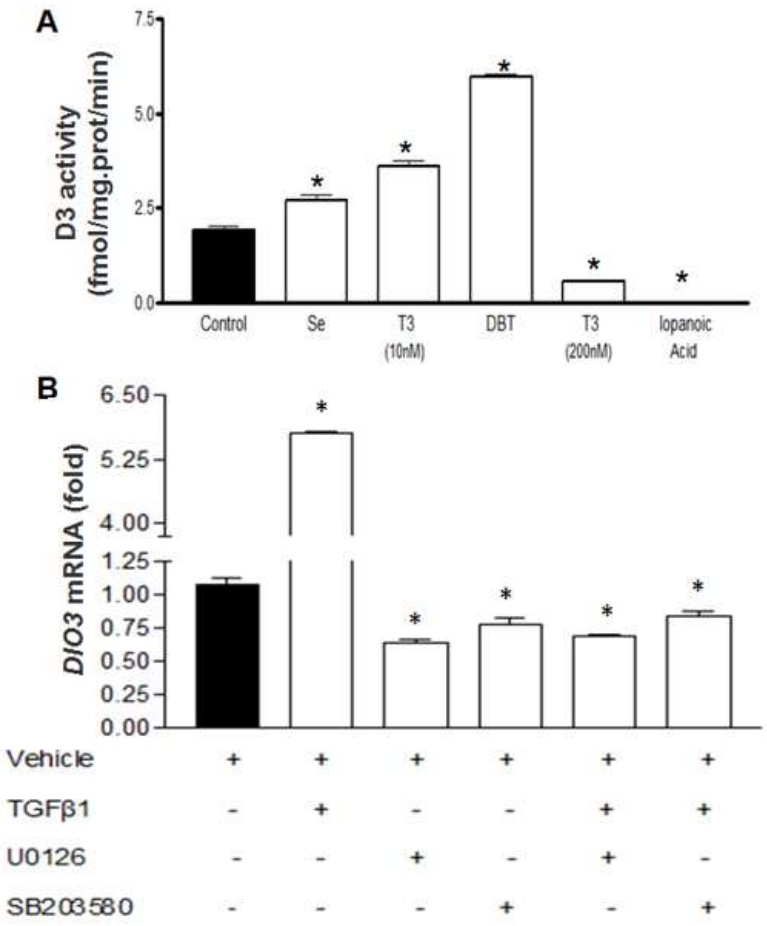

Figure 2 Regulation of *DIO3* expression in human PTC-derived cell line. (A) D3 activity is induced in K1 cell line, by 100nM selenium (Se), 10nM T3, 1mM DBT, 20 ng/ml TGFβ whereas inhibited by 200nM T3 or iopanoic acid, *P<0.0001. (B) Changes elicited by TGFβ1 (20ng/ml) and MAPK inhibitors (5uM of U0126 (ERK) and SB203580 (p38) in K1 cells expressing D3. *DIO3* mRNA levels of control cells were set as 1. Data are mean ± SD of at least three independent experiments. *P<0.001 versus control

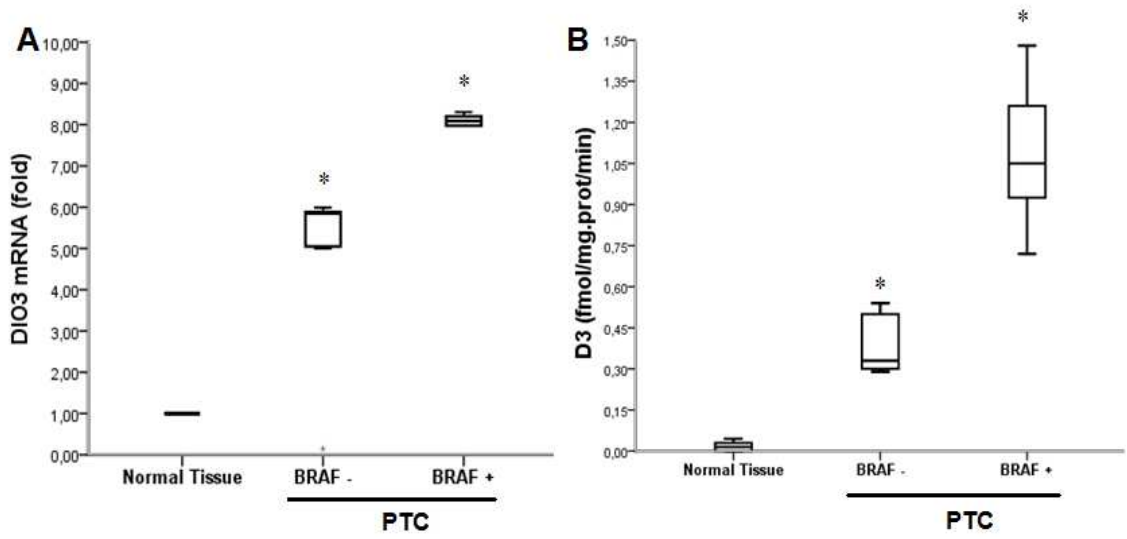

Figure 3 Human PTC samples harboring $BRAF^{V600E}$ mutation display higher levels of *DIO3* mRNA (A) and activity (B) as compared with those samples negative for this mutation. *P=0.001

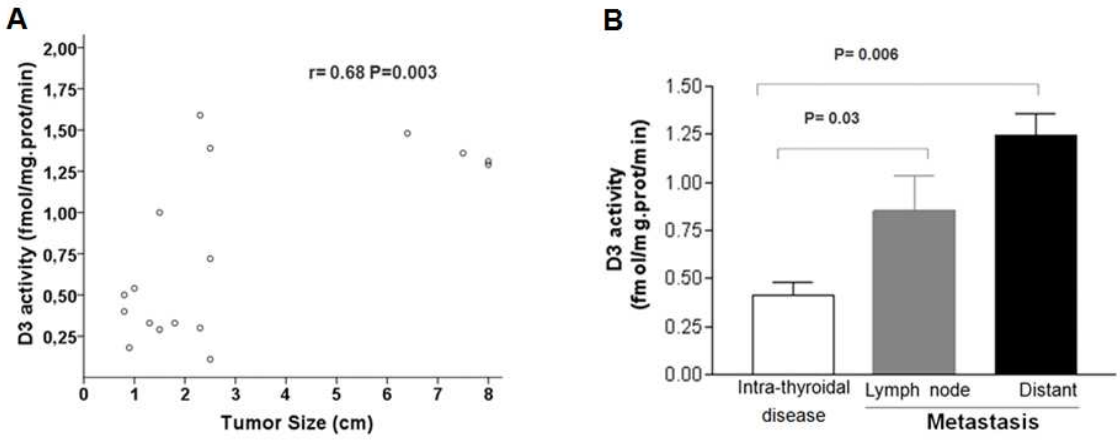

Figure 4 D3 activity levels correlate with tumor size (r=0.68, P=0.003) (A). PTC samples from patients with lymph node (P=0.03) or distant metastasis (P=0.006) display higher levels of *DIO3* mRNA and activity than PTC samples obtained from patients with intra-thyroidal disease (B).

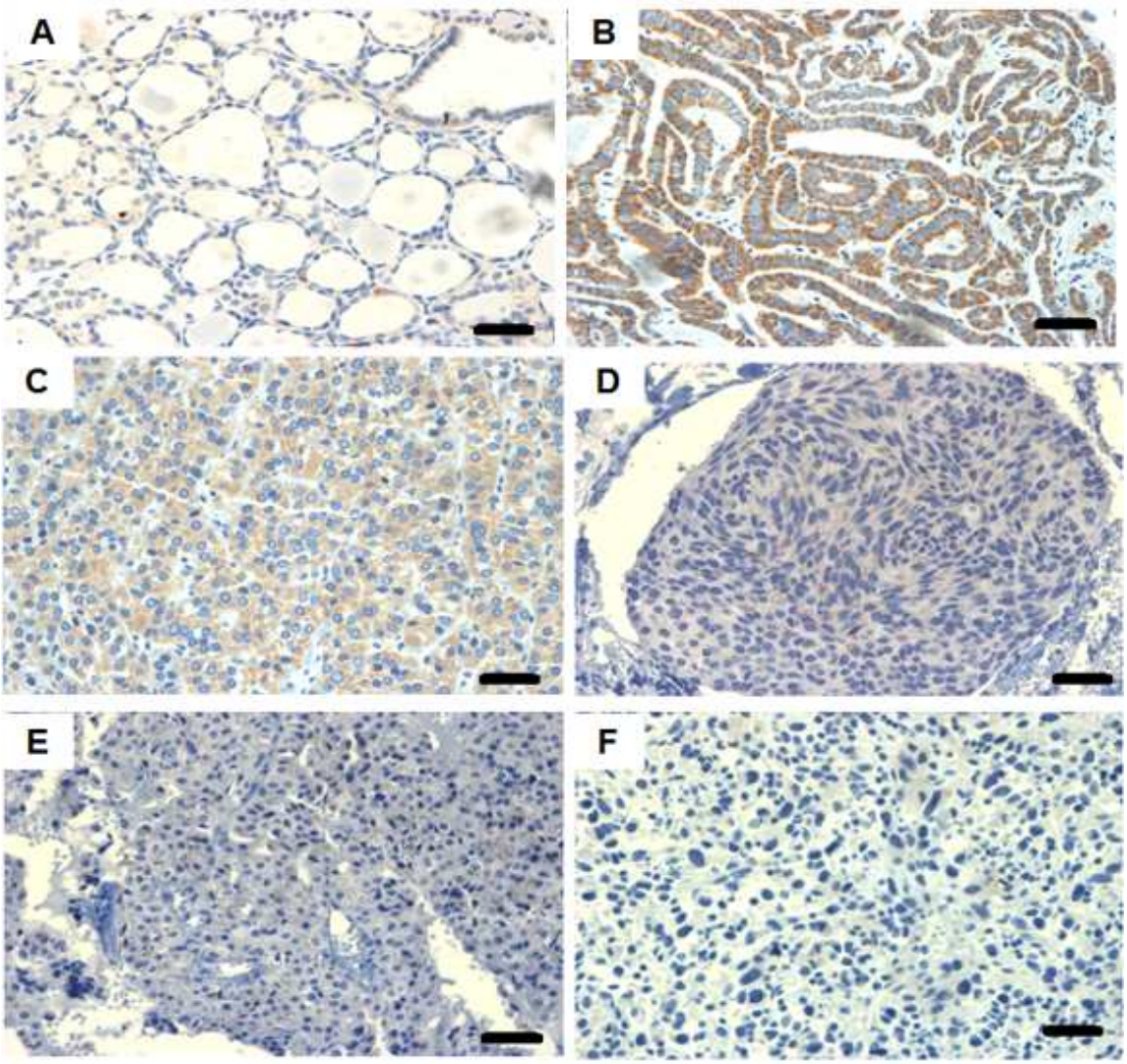

Figure 5 Panel demonstrating D3 immunostaining of representative human thyroid malignant neoplasias. A nearly undetectable D3 expression was observed in thyroid tissue (A) whereas papillary thyroid carcinoma display diffuses cytoplasmic positivity to D3 (B). D3 staining is also easily demonstrable in FTC, although weaker than that observed in PTC (C). D3 protein is barely detectable in sporadic (D) as well as in hereditary MTC (E), No D3 staining is present in ATC (F). * Black bars represent 400x magnification.

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