Molecular Genetics of Papillary Thyroid Carcinoma – Great Expectations...

ABSTRACT

Papillary thyroid carcinoma (PTC) is the most prevalent type of endocrine cancer and, in recent epidemiological surveys, one of the types of human cancer whose incidence is growing. Despite the favourable outcome and long survival rates of most patients, some tumours display an aggressive behaviour and may progress to the highly aggressive and lethal, anaplastic thyroid carcinoma. In recent years, several progresses have been made on the molecular characterization of PTC, in general, and in the genetic alterations underlying the histotype diversity of this type of cancer, in particular. This holds true regarding alterations on nuclear DNA as well as mitochondrial DNA. In this review we have summarized the most recent findings in the genetic characterization of PTC, giving a particular emphasis to the genotype-phenotype associations, the prognosis implications, and the diagnostic and therapeutic value of the newly identified genetic markers. **(Arq Bras Endocrinol Metab 2007;51/5:643-653)**

Keywords: Thyroid; Papillary thyroid carcinoma; Oncogene

RESUMO

Genética Molecular do Carcinoma Papilífero de Tireóide – Grandes Esperanças...

O carcinoma papilífero de tireóide (CPT) é o tipo mais prevalente de câncer endócrino e, em pesquisas epidemiológicas recentes, um dos tipos de câncer humano cuja incidência vêm crescendo. A despeito do prognóstico favorável e da longa taxa de sobrevivência da maioria dos pacientes, alguns tumores mostram um comportamento agressivo e podem progredir para o altamente agressivo e letal carcinoma anaplásico de tireóide. Recentemente, vários progressos foram feitos quanto à caracterização molecular do CPT, em general, e às alterações genéticas subjacentes à diversidade histológica desse tipo de câncer, em particular, particularmente com respeito às alterações dos DNAs nuclear e mitocondrial. Nesta revisão, nós sumarizamos os achados mais recentes da caracterização genética do CPT, dando ênfase particular às associações genótipo-fenótipo, às implicações prognósticas e ao valor diagnóstico e terapêutico dos marcadores genéticos recentemente identificados. **(Arq Bras Endocrinol Metab 2007;51/5:643-653)**

Descritores: Tireóide; Carcinoma papilífero de tireóide; Oncogenes

THYROID CANCER IS THE most prevalent type of endocrine cancer with incidence rates of 4 and 12 per 100,000 in men and women, respectively (1). Papillary thyroid cancer (PTC) represents virtually 80% of all thyroid cancers and recent epidemiological surveys indicate thyroid cancer as the type of human cancer displaying the highest growing incidence in USA [6.3 percent annual increase in the 1997–2003 period (1)]. The observation that the

revisão

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incidence increase was not accompanied by a rise in deaths from the condition (0.5 per 100,000) and that nearly half the tumours are 1 centimetre or less, led some authors to suggest that better, high-tech diagnostic tests are picking up very small tumours, most of which pose no long-term threat (2). The detection of such small cancers presents a dilemma for physicians since, as stressed by Mazzaferri (3), they are not always benign. Despite the generally favourable prognosis of patients with papillary microcarcinomas, "cancer-related mortality rates may be as high as 1.0%, the rate of distant metastases as high as 2.5%, and rates of lymph node recurrence as high as 5%" (3,4). These figures, together with the fact that some cases of conventional PTC have an aggressive behaviour and may even progress to anaplastic thyroid carcinoma (ATC) (5,6), highlight the need of more powerful indicators for diagnosis and prognosis in PTC.

The essential diagnostic criteria in PTC still relies in the cytological nuclear features: large, irregular, "grooved", pale staining and with "ground glass" appearance (5,7). Whenever unequivocal nuclear features are lacking, one can use additional morphological features such as papillary architecture, stromal reaction, diffuse growth and/or the presence of psammoma bodies (8).

At present, the "*in diebus illis*" of the pathologist resides in the differential diagnosis of encapsulated lesions with some of the aforementioned nuclear characteristics and a follicular pattern of growth. This diagnostic dilemma and the aforementioned prognostic difficulties justify the great expectations that were put in the molecular characterization of PTC. In this review we will discuss whether or not they were (or will be) fulfilled.

DNA CONTENT, CYTOGENETIC AND GENETIC STABILITY OF PTC

In thyroid tumours there is no correlation between aneuploidy and malignancy and thus it cannot be used as a distinctive criterion. Aneuploidy can indeed be present in benign and malignant thyroid lesions; PTC, in particular, usually displays a diploid or near diploid DNA content (9). Despite this, an aneuploid DNA content is more frequently found in metastising/more aggressive PTC than in conventional PTCs (10,11).

In general, PTC is cytogenetically characterized by normal karyotypes or, whenever abnormal, by simple cytogenetic alterations. Structural chromosomal alterations involve more frequently chromosomes 1, 3, 7, and 10. Remarkably, some cases of the follicular variant of PTC (FVPTC) are characterized by chromosomal aberrations commonly found in follicular thyroid adenomas (FTA) or follicular thyroid carcinomas (FTC), such as t(2;3) and gains of chromosomes 3, 5, 7, 9, 12, 14, 17, and 20 (12). These findings suggest that there are cytogenetic changes preferentially associated with FVPTC and that FVPTC is closer to the FTC and FTA group of lesions than to classical PTC (12,13). Another signal of the genetic stability of PTC lies in the rare microsatellite instability in these carcinomas (14).

Taking the apparent genetic stability of PTC together with the frequent occurrence, in young patients, of cases of multicentric PTC, it is tempting to consider that PTC tumourigenesis reflects the end product of few carcinogenic events (8,15).

The indolent growth of PTC is reflected in the very low rates of proliferative activity, evaluated both by immunohistochemical markers (i.e. MIB-1) and by cytometric assessment of the S-phase (9,16).

RET/PTC AND *NTRK1* REARRANGEMENTS

Genetic rearrangements are frequently detected in haematological and mesenchymal neoplastic diseases at variance with their rarity in carcinomas. Thyroid cancer is one of the few types of carcinoma having such genetic events, but the reason for this singularity is still unclear. The chromosomal rearrangements so far detected in PTC involve the tyrosine kinase (TK) growth factor receptors RET and NTRK1 (also known as TRKA) (17), which play a role in the regulation of growth, differentiation and programmed cell death of neurons in the peripheral and the central nervous system (18).

The rearrangements of *RET* (*RET*/PTC) and *NTRK1* involve their fusion to heterologous genes (19) and result in chimeric proteins that have been extensively studied in *in vitro* models using several *RET*/PTC and *NTRK1* (TRKT1) chimeric transcripts. Such studies have shown that the tumourigenic properties of RET/PTC and TRKT1 result from the aberrant and persistent activation of their tyrosine kinase domain (17).

RET normal expression and kinase activity is restricted to a subset of cells derived from embryonic neural crest cells (20). Consistent with this, wild-type *RET* is expressed at high levels in parafollicular C-cells, but its expression in follicular thyroid cells remains disputable (19).

RET gene is located in chromosome 10 and its rearrangements reflect the frequent structural cytogenetic alterations of this chromosome. *RET*/PTC

rearrangements can be either paracentric rearrangements, as with *H4* or *ELE1* genes (RET/PTC1 and RET/PTC3, respectively), or reciprocal translocation, as the one involving *PRKAR1A* gene, encoding RI-α on 17q23 (RET/PTC2). RET/PTC1, 2, and 3 are the most frequent alterations involving *RET* proto-oncogene in PTC but at least 15 different types have been identified to date (21).

Somatic rearrangements of the *RET* proto-oncogene have been detected in 3–60% of sporadic PTC (19,21). The prevalence of *RET*/PTC in PTC varies significantly in different studies, probably reflecting the different methodologies and the geographic sampling, as well as the histological composition of the series (see below). In most series dealing with sporadic PTC *RET*/PTC1 is the most common type, comprising up to 60–70% of the rearrangements, whereas *RET*/PTC3 accounts for 20–30% (16,19,22,23). At variance with this, in Chernobyl-related thyroid cancers, *RET*/PTC3 rearrangements are the most frequent (19,24-26), at least for the "first wave" of cancers arising in this setting, since *RET*/PTC1 seems to predominate in cases with a longer latency period [for a revision see (27)]. Other rare types of *RET*/PTC rearrangements appear to be mainly associated with radiation exposure (19).

The existence of a precursor lesion of PTC was a longstanding question and the detection of *RET*/PTC rearrangements in inflammatory lesions of the thyroid has recently fuelled this issue (28). Using highly sensitive methods (real-time PCR), some authors described *RET*/PTC rearrangements in up to 95% of cases of Hashimoto's thyroiditis (HT) (29,30). However, other groups were unable to reproduce these data using a similar methodology (28,31). It remains to be clarified if the use of very sensitive methods highlights "spurious" genetic alterations with low biological potential, or if HT is a real "pre-neoplastic" entity as it has been suggested by some authors (29,30).

Oncogenic rearrangements of *NTRK1* gene are also found in PTC. The *NTRK1* gene, localized in chromosome 1 (again, one of the chromosomes with more frequent structural alterations in PTC), codes for the high-affinity nerve growth factor (NGF) receptor, and its activation has been reported to elicit the activation of the RAF-MEK-ERK pathway (32). *NTRK1* rearrangements are rare, usually found in less than 10% of cases of sporadic PTC (33-35). In 2004, Frattini and co-workers (36) studied *NTRK1* gene rearrangement by RT-PCR and found the expression of *NTRK1* TK domain, which is suggestive of oncogenic rearrangement in three out of 55 PTC cases (5.5%).

NTRK1 cell signalling is modulated by the presence of p75 (NTR) (32). In contrast to NTRK1, p75 (NTR) is able to bind all neurotrophins but lacks intrinsic tyrosine kinase activity (18). Although initially described as a low affinity receptor, p75 has the same affinity for NGF as does NTRK1, and when co-expressed with NTRK1 enhances its ability to bind and to respond to neurotrophins. p75 also modulates/enhances the specificity of other TRKs for their preferred ligands (18).

The detection of *NTRK1* rearrangements in PTC and the observation that the thyroid targeted expression of the rearranged NTRK1 chimeric protein (TRKT1) in transgenic mice leads to the development of PTC (37), support the involvement of NTRK1 and of p75 (NTR) in the etiopathogenesis of PTC. Recently we have observed neoexpression of p75, particularly in conventional PTC (38). We also shown that the cellular localization of p75 appears to be related to the presence of the $BRAF^{V600E}$ mutation; the biological significance of this finding remains to be clarified (38).

BRAF MUTATIONS

BRAF is one of the three members of the conserved RAF family of serine/threonine kinases – ARAF, BRAF, and CRAF – which are critical effectors of the canonical MAPK pathway RAF-MEK-ERK. This pathway is critical in the transduction of signals by growth factors, hormones and cytokines, being involved in the regulation of cell proliferation, differentiation and apoptosis (39,40).

RAF genes have been described as proto-oncogenes because RAFs are the immediate downstream effectors of RAS oncoproteins and because their initial description was its oncogenic viral form *v-raf* (41). Yet the ultimate evidence was the finding of activating mutations in *BRAF* gene in a wide panel of human cancers, most prominently in cutaneous melanoma (63–66%) (42,43) but also in serous ovarian carcinoma (33–40%) (42,44) and colorectal carcinoma (11–20%) (45,46). Of notice, *BRAF* mutations were mostly non coexistent with *RAS* mutations and the great majority of the mutations (~90%) were of a single type: the 1799T-A transition, leading to the substitution of a valine by a glutamic acid at the position 600 (V600E) (42).

Within thyroid neoplasias, *BRAF* mutations (V600E) were frequently detected in sporadic PTC (29–83%) (47-50), ranking as the major genetic alteration of this type of human cancer. *BRAF* mutations are

almost always exclusive to the relatively rare *RAS* genes mutations and also to *RET* (RET/PTC) and *NTRK1* rearrangements, altogether accounting for about 70% of PTC cases (33,36,47,48,51). The mutually exclusive oncogenic activation of RET/PTC, RAS and BRAF in PTC supports the existence of a linear oncogenic signalling pathway involving RET/PTC-RAS-BRAF-MEK-ERK in these tumours, a concept further reinforced by functional experiments *in vitro* (52,53).

Following the detection of *RET*/PTC rearrangements in HT, *BRAF* mutations were also screened in these lesions (54-56). *BRAF* mutations were solely detected by Kim and co-workers (56) and only in cases in which the HT areas coexisted with *BRAF*-mutated PTC areas. Hence, as the hypothesis of contamination with PTC-positive cells cannot be excluded, these studies do not demonstrate the pre-neoplastic role of HT.

BRAF mutations were found to be rare in childhood PTC in the post-Chernobyl setting (57-60). As in the sporadic cases, *BRAF* gene alterations do not coexist with the highly frequent *RET*/PTC rearrangements (58,59). It has been suggested that the ionizing radiation contributes to the occurrence of PTC primarily by the induction of double-strand DNA breaks and their subsequent illegitimate recombination, thus leading to *RET*/PTC (and *NTRK1*) rearrangements (57,61). High prevalences of *RET*/PTC rearrangements and reciprocal low prevalences of *BRAF* mutations are also hallmarks of sporadic childhood and young PTC (16,54,58,60,62-64). Taking these facts together with the above discussed *RET*/PTC 'waves' in post-Chernobyl PTC (first *RET*/PTC3, and then *RET*/PTC3) (27,65), it is possible that a third wave of *BRAF*-mutated PTC cases in the Chernobyl setting, thus recapitulating the sequence observed in sporadic PTC.

RAS MUTATIONS

Three human *RAS* genes – N*RAS*, H*RAS*, and K*RAS* – are frequently involved in human tumorigenesis (about 15% of all human tumours harbour mutations in these genes) (66). The *RAS* mutations found in tumours typically occur in codons 12, 13 or 61 of any of the three genes and produce constitutively active RAS proteins. RAS proteins are key intracellular signal transducers that can activate several downstream pathways, namely the classical RAF-MEK-ERK pathway (40).

RAS genes mutations are particularly prevalent in FTA and FTC and, less frequently, in PTC (67). Their prevalence in PTC varies widely, depending on the studied series (0–16%) (67-70). The mutations associated to PTC (and to thyroid lesions in general) predominantly involve codons 61 of N*RAS* and, to a less extent, of H*RAS* (13,67,71,72) (see below the genotype-phenotype correlations with regard to *BRAF* and *RAS* mutations).

PAX8/PPARγ REARRANGEMENTS

Translocations involving chromosomes 2 and 3 and LOH in chromosome 3 have been detected in FTC cases (73), suggesting the putative existence of a tumour suppressor gene at 3p25 locus. Kroll and co-workers (74) showed that the translocation t(2;3) (q13;p25) results in the fusion of the DNA-binding domains of the thyroid transcription factor PAX8 (2q13) to the A to F domains of peroxisome proliferator-activated receptor γ (PPARγ1)(3p25). The tumourigenic effect of this event appears to be due to the loss of proper PAX8 and PPARγ transcriptional function in the rearranged PAX8/PPARγ form, as well as of the remnant normal PAX8 and PPARγ proteins due to a dominant-negative effect (74,75).

In the initial description, *PAX8/PPARγ* mRNA and protein were detected in FTC but not in FTA, PTC, or multinodular hyperplasias, and it was thus advanced as a marker of FTC (74). However, the genetic alteration was later on also described in a number of FTA cases (76-78). This ruled out the possibility of considering *PAX8/PPARγ* rearrangements as a molecular indicator of malignancy.

Recently, we reported for the first time the *PAX8/PPARγ* fusion gene in a relatively high percentage of cases of FVPTC (37.5%) (13) (see below the genotype-phenotype meaning of this finding).

So far it is not known if *PAX8/PPARγ* rearrangements are prevalent events in poorly differentiated thyroid carcinoma (PDTC) and ATC and, hence, if they may be used as progression markers of follicular lesions (FTA, FTC and FVPTC) towards more aggressive forms of thyroid neoplasias. It remains also to be confirmed the advanced association of *PAX8/PPARγ* rearrangements with vascular invasiveness in FTC (77).

ALTERATIONS IN MITOCHONDRIAL GENES IN PTC

We agree with the new WHO classification of thyroid tumours in which oncocytic (Hürthle cell) tumours are considered as variants of their non-oncocytic counterparts (e.g. oncocytic variant of PTC and oncocytic variant of FTC) instead of constituting a category by itself (7,8).

The mitochondrial DNA (mtDNA) is small (16.569bp) and encodes 13 essential components of the cellular energy-production apparatus, being absolutely vital for life.

The high copy number of mtDNA and the cytoplasmic location of the mitochondria contribute to its high mutation rate, about 10 to 20 times higher than that of the nuclear DNA (nDNA) [for a thorough review see (79)].

Alterations of mtDNA have been demonstrated in various types of human cancer including thyroid tumours, and include large deletions, missense mutations, frameshift mutations and small deletions/insertions, but the role of mtDNA somatic mutations in tumourigenesis has not been yet fully understood (80-82).

Yeh and co-workers (83) identified three somatic mtDNA mutations in PTC in a series of 21 thyroid tumours with different histotypes and suggested that somatic mtDNA alterations may be involved in thyroid tumourigenesis. Despite their interesting results, the authors only studied a small series of tumours with an overrepresentation of PTC. These results have been, in part, confirmed in a study by our group in a much larger series of thyroid tumour (84). We found that FTC and PTC carried a significantly higher prevalence of non-silent point mutations in complex I genes than FTA (84). We have also found large deletions of mtDNA in all types of tumours, with a striking prominence for Hürthle cell tumours (up to 16%) of mtDNA common deletion independently of the histological variant (84). The same held true to sequence variants of *ATPase 6* gene which were significantly more prevalent in patients with Hürthle cell tumours than in patients with non-Hürthle cell neoplasms (84).

Mutations and sequence variants in mtDNA complex I genes appear to be more frequent in malignant than in benign thyroid tumours, suggesting a role in tumour progression of these alterations. The mtDNA common deletion and *ATPase 6* variants are more frequent in Hürthle cell tumours than in non-Hürthle cell tumours, appearing to be involved with Hürthle cell transformation rather than with tumourigenesis (84). With regard to mtDNA alterations, PTCs do not differ from FTCs, i.e. apparently no specific mtDNA alterations are associated with the papillary histotype (84).

Alterations in *GRIM-19* gene, a nuclear gene encoding a complex I mitochondrial protein, have been recently found in Hürthle cell tumours (85). GRIM-19 is one of several proteins associated with retinoid-interferon-induced mortality (GRIM); it is considered as a cell death regulator that promotes apoptosis, a negative regulator of cell growth, and it is also involved in mitochondrial metabolism (86). *GRIM-19* mutations were detected in three cases of Hürthle cell variant of PTC and in one case of Hürthle cell variant of FTC, whereas no mutations were detected in any non-Hürthle cell carcinoma (85). The detection of *RET*/PTC1 in one of the cases of Hürthle cell variant of PTC with a *GRIM-19* mutation suggests that *GRIM-19* mutations may play a role together with the oncogenic activation in tumour development. Although a larger series of benign and malignant thyroid tumours with and without Hürthle cell features needs to be studied, it seems that *GRIM-19* alterations, like mtDNA alterations, are associated with Hürthle cell features and not with specific histotypes. This finding suggests that *GRIM-19* mutations may serve as a predisposing alteration for the occurrence of tumours with cell oxyphilia. Other alterations, such as *RET*/PTC rearrangements or *BRAF* mutations, appear to be necessary in Hürthle cell tumours, like in any non-Hürthle cell variant of PTC, for the cancer development (48,87).

PHENOTYPE-GENOTYPE RELATIONSHIP IN THYROID NEOPLASIA

PTC represent a heterogeneous group of tumours, comprising several histological variants that share the peculiar and diagnostic nuclear features of PTC (5,7,88). Despite their common classification as 'PTC', convincing molecular evidence has appeared in the last years that support the previous histological distinction of some PTC variants. The most frequent *RET*/PTC rearrangement, *RET*/PTC1, is related to PTC cases displaying the conventional histotype (16,22,23,64), whereas *RET*/PTC3 is associated with the solid variant of PTC (19,24,25). *RET*/PTC3 was also reported to occur frequently in cases of the tall cell variant of PTC (89).

Regarding *BRAF* mutations, the most frequent $BRAF^{V600E}$ mutant form is almost exclusively detected in PTC cases with a papillary or mixed papillary/follicular architecture (irrespectively of the variant being conventional, tall cell, oxyphilic or microcarcinoma) (13,36,54,64,90-92), whereas the less frequent and less reported $BRAF^{K601E}$ form (~7%) is exclusively detected in cases of FVPTC (54,91).

RAS mutations are also closely associated with the tumour's histotype, being rarely detected in conventional PTC (0–16%) and frequently detected in FVPTC (25–100%) (13,64,71,72).

Finally, the relatively high frequency of *PAX8/PPAR*γ rearrangements in FVPTC, in conjunction with the aforementioned data on *RET*, *BRAF*

and *RAS* alterations, reinforce the assumption that some FVPTC cases share some of the molecular features of follicular tumours (FTA and FTC), constituting a sort of intermediate category between conventional PTC and FTC (13).

There is a particular type of thyroid tumour that usually occurs in the context of Familial Adenomatous Poliposys (FAP) and that is considered by most authors as a variant of PTC – the so-called cribiform-morular variant of PTC (93,94). The molecular link between FAP and the cribiform morular variant of PTC is the *APC* gene, since germline and/or somatic alterations have been detected in FAP-associated, as well as in sporadic thyroid carcinomas with a cribiform-morular appearance (95,96). Moreover, the *APC* deleterious mutations related to these tumours are associated with the 5' region of exon 15 and are unfrequent in the considered hotspot mutation region of *APC* (97). The search for germline *APC* mutations in apparently sporadic cases of the cribiform-morular variant of PTC is a must; this approach is of clinical relevance, as these cases may represent an occult FAP condition (93,94).

A last point to stress another type of genotype-phenotype correlation: Hürthle cell tumours display similar mitochondrial alterations (proteins encoded by mtDNA and/or nDNA) regardless of the specific thyroid tumour histotype (PTC or FTC or even FTA) (see above). In this setting one is referring to a mitochondria-rich phenotype rather than to the oncogenic-related tumour phenotype. It remains to be clarified weather or not it will be advantageous to treat patients with Hürthle cell carcinomas targeting both the specific oncogenes involved in the tumour or the mithocondrial abnormalities.

DIAGNOSTIC AND PROGNOSTIC USEFULNESS OF THE SEVERAL PTC-ASSOCIATED GENETIC ALTERATIONS

FNA is the best tool for the early diagnosis of thyroid lesions. As the PTC diagnosis relies on the typical nuclear features, cases with less than typical PTC nuclei in FNA samples, as it frequently occurs in FVPTC, may be misdiagnosed as FTA or as follicular tumour.

The diagnosis of FTC cannot be usually made by FNA since it depends on the observation of capsular or vascular invasion (5,7). All these limitations led to the assumption that good ancillary tools would be very useful for the FNA diagnosis of difficult thyroid tumours.

PAX8-PPARγ rearrangements and *RAS* genes mutations are quite prevalent in cases of FVPTC (13,64,71). Yet, such genetic events are similarly frequent in the lesions we would like to distinguish from PTC – e.g. FTC and, more importantly, FTA (13,67,74,78,98). Thus, the identification of *PAX8-PPARγ* rearrangements and/or *RAS* mutations in FNA samples does not provide useful diagnostic improvements in difficult, concrete, follicular patterned thyroid tumours.

The accurate detection of both *RET*/PTC rearrangements and *BRAF* gene mutations in thyroid FNA samples has proved useful in certain situations (99-104). The detection of *RET*/PTC rearrangements or of *BRAF* mutations (V600E or K601E) will lead to an unequivocal diagnosis of PTC and will therefore allow a better clinical approach in such cases (105). The strong association of $BRAF^{V600E}$ mutation and, to a lesser extent, *RET*/PTC1, to PTC cases displaying typical papillary or mixed papillary-follicular architecture limits, however, their screening usefulness since those are the easiest cases to diagnose by FNA.

PTCs rarely cause the death of the patients (106,107) as they usually respond very well to the surgical removal of the tumour followed by radioactive iodine treatment. Yet, some cases have an aggressive behaviour and some may progress to the highly aggressive and lethal, ATC (5,6). The identification of the PTCs with guarded prognosis would have major clinical relevance and that is the reason why so many groups have been trying to find genetic markers with clinical significance for the outcome of the patients.

Most studies published so far have focused on the clinical significance of *RET*/PTC rearrangements and, particularly, of mutant *BRAF* forms. The presence of *RET*/PTC rearrangements have been associated in some studies with lymph node metastatization (34,62,64,70) while other groups did not find this association (16,108,109). Of the several clinicopathological parameters analysed, *RET*/PTC positive cases have only been convincingly associated with younger age at diagnosis (16,34,62-64). RET/PTC3 seems to be associated with tumours with a guarded prognosis but larger series with longer follow-up are needed to clarify this issue (5,7).

Also controversial has been the putative association of *BRAF* mutations with poor prognosis parameters in PTC. The majority of the groups advanced that PTCs bearing these mutations are prone to a more aggressive clinical behaviour (64,90,110-112). The disclosure of *BRAF* mutations in PDTC and ATC, particularly in cases apparently derived from pre-existent PTC, provided additional support to their prognostic meaning (90,113-115).

Some studies have described a statistically significant association of *BRAF* mutations to older age at diagnosis (54,64,90), male gender (112,116), extrathyroid extension (64,90,110), lymph node metastases (110), distant metastases (111), higher tumour staging (64,90,110,111), and recurrence (110,112). Other groups did not find any of the aforementioned associations (33,51,92,117,118). Moreover, given the strict relationship of *BRAF* mutations to PTCs with predominant papillary architecture, the aforementioned relationship of *BRAF* mutations to poor prognosis indicators may be biased by the constitution of each series with regard to the histological types of PTC. For instance, the tall cell variant of PTC, accepted by some groups as being more aggressive than conventional PTC (119-121), has particularly high frequency of *BRAF* mutations (36,64,90). The alleged histological bias is well shown in the study by Xing and co-workers (110), in which the associations of *BRAF* mutations to extrathyroid extension, lymph node metastasis and high tumour staging were lost upon the stratification of the series by histotype.

Finally, it remains to be verified the implications of *PAX8/PPARγ* in the behaviour of the tumours. We have found a significant association between *PAX8/PPARγ* rearrangements and multifocality and vascular invasiveness in FVPTC, suggesting that the rearrangement confers a higher invasive potential (13). A strong association with vascular invasiveness was described in FTC harbouring these genetic alterations (77). Besides the association with invasiveness, it remains to be demonstrated if cases of FTC or FVPTC positive for *PAX8/PPARγ* are more prone to give rise to blood born/haematological metastases (13).

NEW THERAPEUTIC APPROACHES TARGETING GENETIC ALTERATIONS IN PTC

The treatment of patients with PTC has usually an excellent outcome, leading to a much lower risk for death or disease recurrence than other human malignancies. Initially PTC is treated by surgery (total or partial thyroidectomy), followed by radioiodine (^{131}I) treatment (122). Radioiodine therapy is an extremely effective treatment in well-differentiated thyroid carcinomas (FTC and PTC), being, at present, the only effective treatment in patients with distant metastases (122).

Although the majority of tumours respond well to radioiodine therapy, there are PTCs that are resistant to this classical therapy, namely those that are inoperable and have lost radioiodine avidity. Until recently, there were few clinical trials available for patients with iodine nonresponsive thyroid cancers. The molecular alterations that have been identified in PTC and thought to be important in oncogenesis, namely *RET*/PTC rearrangement and *BRAF*^{V600E} mutation raised the possibility of targeting these molecules as a potential therapeutic approach.

Several inhibitors targeting different kinases are available. BAY 43-9006, a bi-aryl urea (also known as Sorafenib), first designed as a RAF1 kinase inhibitor (123), inhibits *in vitro* both wild-type and V600E-mutant BRAF (124). In addition, BAY 43-9006 has significant activity against VEGFR2 and 3, PDGFRβ, Flt-3, c-Kit and RET. BAY 43-9006 has been approved by the Food and Drug Administration (FDA) for therapy of renal cell carcinoma despite the absence of *BRAF* mutations in this subset of tumours, and it is under evaluation for melanoma and thyroid cancer treatment, the two human malignancies which harbour the highest percentage of *BRAF* mutations. With regard to thyroid tumours, it was observed that BAY 43-9006 inhibits growth of thyroid carcinoma derived cell lines (125).

Two potent RAF kinase inhibitors, NVP-AAL881-NX and NVP-LBT613-AG-8, have been tested *in vitro* and *in vivo* against a panel of thyroid cancer cell lines, and both drugs were found to inhibit proliferation and to induce cell death (126).

Recently, new data concerning the requirement of the Hsp90 chaperone for the stability of BRAF^{V600E} rendered the possibility of targeting BRAF^{V600E} by the use of Hsp90 inhibitors, such as 17-AAG, but further studies are needed (127,128).

Small molecules of various chemical classes have been reported to inhibit RET (two pyrazolopyrimidines – PP1 and PP2 –, a 2-indolinone – RPI-1 – and two indolocarbazole derivates – CEP-701 and CEP-751); for the moment, ZD6474 is the only one in an advanced phase of clinical trials (129).

Loss of radioiodine uptake is related to low or absent Natrium Iodine Symporter (NIS) protein, and this observation led to the attempt of therapeutically inducing an increased expression of NIS. It has been shown in clinical trials that retinoic acid analogues act by increasing NIS expression and promoting higher ^{131}I uptake (122).

PAX8/PPARγ rearrangement have been found in a subset of FVPTC (13), PPARγ agonists, as rosiglitazone, are already in phase II clinical trials in patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer in an attempt to induce radioiodine uptake (130). Other PPARγ agonists, like

troglitazone and the new, high affinity, RS5444, have been shown to inhibit *in vitro* the proliferation in thyroid carcinoma derived cell lines (131,132).

Summing up, the available compounds targeting different molecules that are thought to be involved in PTC carcinogenesis represent promising drugs that are being evaluated in the treatment of patients with thyroid cancer. These drugs vary in their specificity and have been tested with relative success in preclinical studies and in early clinical trials (133). The challenge now is to progress in the validation of the actual targets of the drugs and in the exploitation of the possibility of using combined therapy in patients with progressive thyroid cancer.

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