New Drugs in Thyroid Cancer

ABSTRACT

This review is focused on "new drugs" that might be developed for thyroid cancer treatment. Thyroid cancer is frequently associated to the activation of specific protein (RET, BRAF) and lipid [PI(3)K] kinases. There is good evidence that these genetic lesions are causative events in thyroid cancer initiation or progression. Therefore, novel compounds able to target these kinases might be useful for thyroid cancer treatment. The power of this approach is witnessed by the examples of BCR-ABL, c-KIT and EGFR inhibitors in the treatment of chronic myelogenous leukemia (CML), gastro-intestinal stromal tumors (GIST) and non-small cell lung carcinoma (NSCLC). **(Arq Bras Endocrinol Metab 2007;51/5:857-861)**

Keywords: Thyroid cancer; Lipid kinases; *RET*; *BRAF*; Cancer therapy

RESUMO

Novas Drogas em Câncer de Tiróide.
Esta revisão focaliza as "novas drogas" que estão sendo desenvolvidas para o tratamento do câncer de tiróide. O câncer de tiróide está freqüentemente associado à ativação específica de quinases protéicas (RET, BRAF) e lipídicas [PI(3)K]. Há uma boa evidência de que essas lesões genéticas são eventos causativos de iniciação e progressão do câncer de tiróide. Assim sendo, novos compostos capazes de atuar nestas quinases podem ser úteis no tratamento do câncer de tiróide. A capacidade deste procedimento pode ser constatada pelos exemplos dos inibidores de BCR-ABL, c-KIT e EGFR no tratamento da leucemia mielóide crônica (LMC), dos tumores do estroma gastrointestinal (GIST) e carcinoma de células não pequenas do pulmão (NSCLC). **(Arq Bras Endocrinol Metab 2007;51/5:857-861)**

Descritores: Câncer de tiróide; Cinases de lípides; *RET*; *BRAF*; Terapia do câncer

GENETIC LESIONS FOUND IN THE DIFFERENT THYROID CARCINOMA SUBTYPES

RECENT DISCOVERIES HAVE provided greater understanding of the molecular basis of thyroid cancer; these advances are being exploited to provide targeted drugs and new therapeutic approaches (1-3). There are four major types of thyroid carcinoma: papillary (PTC), follicular (FTC), anaplastic (ATC), and medullary (MTC) (1).

PTC accounts for about 80% of thyroid cancer cases (1,2). As shown in table 1, and detailed in the next sections, PTC is characterized by rearrangements of the RET (RET/PTC) receptor tyrosine kinase (RTK) (2,3) or by activating point mutations in the BRAF serine/threonine kinase (4,5). More rarely, PTC features rearrangements of the NTRK1 RTK (6) and activating mutations or amplification of PIK3CA, the catalytic subunit of the phosphatidylinositol-3-kinase [PI(3)K] (7,8). Activating point mutations in the RAS oncogenes are limited to the PTC follicular variant (2). Scanty informations are available on the involvement

perspectiva

MASSIMO SANTORO
ALFREDO FUSCO

Dipartimento di Biologia e Patologia Cellulare e Molecolare e/o Istituto di Endocrinologia ed Oncologia Sperimentale del CNR, Facoltà di Medicina e Chirurgia di Napoli, Università degli Studi di Napoli "Federico II", and NOGEC (Naples Oncogenomic Center) – CEINGE, Biotecnologie Avanzate-Napoli, & SEMM – European School of Molecular Medicine – Naples, Italy.

Recebido em 12/02/07
Aceito em 19/03/07

of tumor-suppressor genes in PTC. TP53 mutations are rare (2); hemizygous deletion of the PTEN phosphatase occurs in a fraction of PTC (9) (table 1).

FTC (about 10% of thyroid cancer cases) is frequently associated with activating mutations in the RAS oncogenes (2) or gene rearrangements between the PAX8 transcription factor and the peroxisome proliferator-activated receptor γ (PPARγ) (10). In FTC, PIK3CA amplification or mutation is likely more common than PTC (8), while TP53 and PTEN alterations are quite rare (2) (table 1).

ATC accounts for 2–5% of all thyroid cancers (1). ATC has a dismal prognosis, virtually all patients dying from their disease within a few months. A distinctive genetic feature of ATC is the high prevalence of TP53 mutations (2). Also PTEN, PIK3CA and CTNNB1 (β-catenin) genetic alterations are more prevalent in ATC than in the other thyroid cancer subtypes (2,9). RET, NTRK1 and PPARγ rearrangements are very rare or absent in ATC (2). Instead, ATC shares BRAF and RAS mutations with differentiated thyroid carcinomas (table 1), suggesting that these particular oncogenic lesions may drive tumor progression (2,4,5). Importantly, BRAF or RAS mutations often co-exist with PIK3CA alterations in ATC (8). These findings support a model whereby ATC may develop secondary to the cooperation of multiple lesions in oncogenes (like RAS, BRAF and PIK3CA) and tumor-suppressors (like PTEN and TP53).

MTC (about 5% of thyroid cancers) is sporadic in about 75% of cases and inherited in about 25% of the cases as a component of the multiple endocrine neoplasia type 2 (MEN 2A, MEN 2B, FMTC) syndromes. RET mutation is the only genetic lesion consistently associated to MTC. This alteration is present in virtually all familial cases and up to 50% of the sporadic cases (table 1) (3). A recent paper reported a high prevalence of TP53 mutations in MTC (11).

NOVEL MOLECULAR TARGETS FOR THE TREATMENT OF THYROID CARCINOMA

The elucidation of the signal-transduction pathways that drive neoplastic transformation has led to novel rationally designed cancer therapeutics ("targeted therapy") (12). Several compounds directed against different molecular pathways are being tested in patients with advanced thyroid carcinoma. These include PPARγ agonists as well as proteasome, histone deacetylase, and Hsp90 (heat shock protein 90) inhibitors (http://www.thyroidtrials.org and www.cancer.gov/clinicaltrials).

Since specific kinases play a key role in the formation of different types of cancers and since kinases are "druggable" proteins, anti-neoplastic targeted therapy approaches are being often directed against oncogenic kinases. This concept may be applied to thyroid carcinoma, as well. Different strategies have been devised to intercept protein kinases in tumours; two of them, small-molecules and monoclonal antibodies (mAb), are being rapidly translated to the clinic. Among the kinase-targeted oncology drugs that have received regulatory approval so far 3 are mAbs and 5 are small-molecules (12). Small-molecules are low molecular weight organic compounds that obstruct kinase

Table 1. Major structural genetic alterations in thyroid cancer.

Tumor type	Gene alteration			
	Oncogene	Molecular lesion (%)* **	Tumor suppressor	Molecular lesion (%)* **
PTC	RET	r (13–43%)[2]	PTEN	hemiz del (20–30%)[9]
	NTRK1	r (5–13%)[2]		pm (2%)[9]
	BRAF	pm (29–69%)[2]		
	PIK3CA	pm (3%)[7], ampl (12%)[8]		
	RAS	(pm, 0–21%)[2]		
FTC	PAX8-PPARγ	r (25–63%)[2]	PTEN	hemiz del (20–30%)[9]
	RAS	pm (40–53%)[2]		pm (7%)9
	PIK3CA	pm (6%)[8], ampl (28%)[8]		
ATC	RAS	pm (20–60%)[2]	PTEN	hemiz del (60%)[9]
	BRAF	pm (10–35%)[2]	TP53	various (67–88%)[2]
	PIK3CA	pm (12–23%)[7,8],ampl		
	CTNNB1	(42%)[8]		
		pm (66%)[2]		
MTC	RET	pm (familial > 95%;	TP53	various (50%)[11]
		sporadic 30–50%)[3]		

* pm, point mutation; r, rearrangement; ampl, amplification; del, deletion; hemiz del, hemizygous deletion
** References are given as superscript numbers

activity by binding to the catalytic domain, often the ATP binding pocket, of the kinase. The paradigm of imatinib (Gleevec) for BCR-ABL-positive chronic myelogenous leukaemia (CML) and for stem cell growth factor receptor (c-KIT)-positive gastrointestinal stromal tumours (GIST) and of EGFR inhibitors in EGFR-mutation positive non-small cell lung carcinomas (NSCLC) has exemplified the power of this approach (12).

The selection of the target kinase is a critical point of the use of small-molecules for cancer therapy. Thus, kinase gain-of-function and dependence of the cancer cell on the pathogenetic kinase are the best available indicators of candidates who would benefit from treatment with these inhibitors (12). In this context, the RET, BRAF and PI(3)K kinases appear to be rational molecular targets for the treatment of thyroid cancer. In fact, different types of thyroid cancer feature activating mutations in these kinases and there is evidence that in vivo manipulation of the corresponding pathways (RET and BRAF transgenic mice and PTEN null mice) causes experimental thyroid tumors (2,3).

THE RET RECEPTOR

The RET protein is a transmembrane RTK (3). One particular autophosphorylation site, Y1062 (tyrosine 1062), is essential for RET function during normal development as well as for RET oncogenic effects (figure 1). Intriguingly, Y1062 connects RET to BRAF and PI(3)K signaling, thereby linking the major kinases involved in thyroid cancer formation. Once phosphorylated, Y1062 acts as a multidocking site, recruiting a number of PTB (phosphotyrosine binding) domain-containing protein adaptors (like Shc, FRS2, IRS1/2) that, in turn, mediate RET coupling to downstream effectors (3).

RET is involved in the formation of both PTC and MTC. In PTC, chromosomal inversions or translocations cause the fusion of the RET kinase-encoding domain with the 5¢-end of heterologous genes. The resulting chimeric sequences are called "RET/PTC" (table 1) (2,3). Point mutations in RET, at the germline level in (virtually all) MEN 2 patients and, at the somatic level, in about half of the sporadic cases, characterize MTC. Most MEN 2B patients carry the M918T mutation in the RET kinase domain. In > 90% of MEN 2A and FMTC cases, mutations affect one of the five cysteines in the extracellular cysteine-rich domain of RET (COSMIC: Catalogue of Somatic Mutations in Cancer database).

In this scenario, RET appears to be a promising target for the molecular therapy of thyroid cancer. Particularly, since there is no systemic treatment for MTC, these patients might benefit of novel treatments based on RET inhibition (13). Some compounds exert a RET inhibitory effect (3). Two of them, ZD6474 (zactima) and BAY 43-9006 (sorafenib), obstruct RET with an inhibitory concentration 50 (IC_{50}) in the nM range (14,15) and are currently undergoing clinical evaluation in MTC patients (http://www.can-

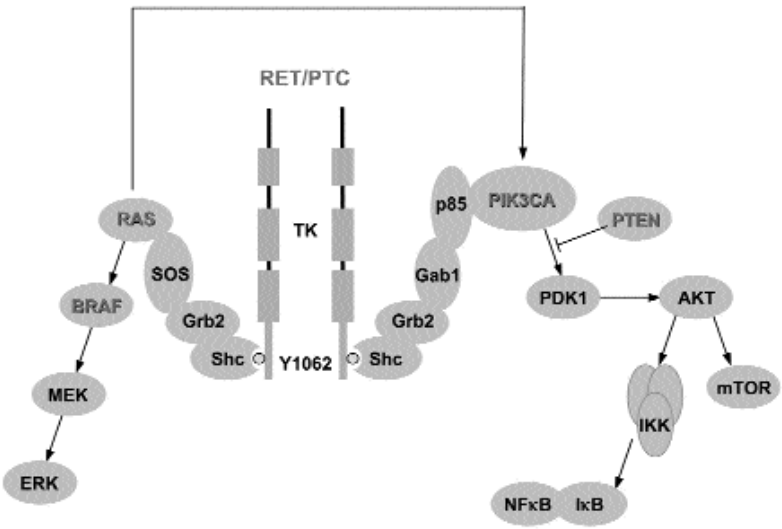

Figure 1. The network of RET/PTC-mediated signaling events. Major signaling pathways include the RAS/BRAF/ERK and PIK3CA signaling cascades. In red are highlighted those proteins that are targeted by mutations in the different types of thyroid cancer. RET/PTC, BRAF, MEK, PIK3CA, AKT, mTOR, and IKK represent potential targets for the development of new anti-thyroid cancer drugs.

cer.gov/clinicaltrials). Both are multi-kinase inhibitors (BAY 43-9006 also inhibits RAF, see below), able to target other kinases besides RET; in particular, both inhibit efficiently vascular endothelial growth factor receptor (VEGFR). Such ability may be an advantage of these drugs that can simultaneously attack neoplastic and endothelial cells. The X-ray structure of ZD6474-RET(TK) complex has recently proved that the compound docks into the ATP-binding pocket of the RET kinase (16). Being exposed on the cell surface, oncogenic RET oncoproteins might also be attacked with specific mAbs in MTC cells.

THE BRAF/ERK SIGNALING PATHWAY

RAF family members (RAF-1, BRAF, and ARAF) are part of the RAS/RAF/MEK/ERK signaling module. RAF proteins are activated through binding to RAS small GTPases in their GTP-bound state. Once activated, RAF phosphorylates MEK (MAPK kinase), which in turn phosphorylates and activates ERK (figure 1).

Oncogenic conversion of BRAF is highly prevalent in PTC. It also occurs in a significant fraction of ATC (table 1) (2,4,5). A glutamate for valine substitution at residue 600 (V600E) accounts for more than 90% of BRAF mutations in thyroid carcinomas. Several observations link BRAF mutations with an aggressive PTC phenotype: i) the correlation of BRAF mutations with extrathyroidal invasion, cancer recurrence and loss of radioiodine uptake; ii) the high prevalence of BRAF mutations in the aggressive tall-cell PTC variant; iii) the formation of undifferentiated tumors in BRAF-transgenic mice (2,4,5).

Thus, BRAF might be an appealing target for the treatment of aggressive PTC and ATC. Various BRAF inhibitors have been identified (17,18). BAY 43-9006 (sorafenib) has reached the clinical testing stage. BAY 43-9006 is a multi-target compound, able to inhibit many tyrosine kinases besides RAF. As a monotherapy, it showed only modest activity against melanoma, another tumor often carrying BRAF mutations. It is possible that in vivo the compound is not potent enough, or that cancer cells 'escape' when BRAF is blocked (18). Other BRAF drugs (like CHIR-265 and SB-590885) are being developed (18).

BRAF can also be blocked by intercepting the downstream signaling cascade with the use of MEK inhibitors. Among them, PD0325901 and ARRY-142886 (AZD6244), two non-ATP competitive inhibitors, are undergoing early clinical testing (17,18). Both of them are more potent than CI-1040, already tested with modest results in cancer patients (17). It should be noted that despite the strong rationale for developing "MAPK-pathway" inhibitors, these compounds might have relevant toxicity given the widespread expression of components of this pathway.

THE PI(3)K-AKT SIGNALING PATHWAY

The phosphoinositide-3-OH kinase [PI(3)K] pathway is another signaling cascade that emerged as important in thyroid cancer formation. Once activated by RTKs or RAS (figure 1), PI(3)K catalyzes the conversion of phosphatidylinositol (4,5)-biphosphate (PIP-2) into phosphatidylinositol (3,4,5)-triphosphate (PIP-3). PIP-3 activates the AKT (PKB) kinase that, in turn, regulates cell proliferation, survival, and size. This process is counteracted by the PTEN (phosphatase and tensin homologue) tumor suppressor, which dephosphorylates PIP-3 (18) (figure 1).

PI(3)K signaling is hyperactivated in a high proportion of thyroid carcinomas. This may occur directly (mainly in ATC) by PIK3CA mutations or PTEN deletion and, indirectly, by RAS- or RET-mediated activation (table 1). Therefore, the PI(3)K pathway may represents another attractive target for small molecule inhibitors for the treatment of thyroid cancer (19). Agents that target PI(3)K, AKT and other downstream components of the pathway are being developed. One AKT inhibitor, KP372-1, blocked proliferation and induced apoptosis of thyroid cancer cells (19). CCI-779 (temsirolimus) and RAD001 (everolimus), two derivatives of the macrolide antibiotic rapamycin, inhibit mTOR (mammalian target of rapamycin), a kinase that acts downstream from AKT (figure 1). They have broad antitumor activity and are under advanced clinical study (20).

CONCLUDING REMARKS

At least three kinases — RET, BRAF, and PI(3)K — can be considered suitable targets for small-molecule inhibitors in the treatment of thyroid cancer. Various agents targeting directly these kinases or, indirectly, their downstream effectors are being tested in preclinical models and in thyroid cancer patients. The potential role of additional kinases in the molecular therapy of thyroid cancer needs also to be considered. These include several RTKs overexpressed (EGFR, FGFRs, IGFR, HGFR) or, rarely, rearranged (NTRK1) in thy-

roid cancer, angiogenic receptors (VEGFR and PDGFR), and several serine/threonine kinases like IKK (NFκB activator) involved in signal transduction or cell cycle progression (Aurora B). Rational combinatorial approaches with drugs that target multiple pathways will need to be studied in the future. In particular, because RTK-RAS-BRAF-ERK and RTK-PI(3)K/AKT signaling both contribute to thyroid cancer formation, it might be interesting to test the efficacy of combination of agents to block simultaneously these two pathways.

ACKNOWLEDGMENTS

We thank members of our laboratory for their comments and advice. We apologize to the many colleagues whose work we have not cited because of space limits. This work was supported by the Italian Association for Cancer Research (AIRC).

REFERENCES

1. DeLellis RA, Williams ED. Thyroid and parathyroid tumors. In: World Health Organization Classification of Tumours. **Pathology and Genetics. Tumours of Endocrine Organs**. Geneva: WHO Press, **2004** pp. 51-6.
2. Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. **Nat Rev Cancer 2006**;6:292-306.
3. Santoro M, Carlomagno F. Drug insight: Small-molecule inhibitors of protein kinases in the treatment of thyroid cancer. **Nat Clin Pract Endocrinol Metab 2006**;2:42-52.
4. Xing M. BRAF mutation in thyroid cancer. **Endocr Relat Cancer 2005**;12:245-62.
5. Groussin L, Fagin JA. Significance of BRAF mutations in papillary thyroid carcinoma: prognostic and therapeutic implications. **Nat Clin Pract Endocrinol Metab 2006**;2:180-1.
6. Pierotti MA, Greco A. Oncogenic rearrangements of the NTRK1/NGF receptor. **Cancer Lett 2006**;232:90-8.
7. Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, et al. Mutation of the PIK3CA gene in anaplastic thyroid cancer. **Cancer Res 2005**;65:10199-207.
8. Hou P, Liu D, Shan Y, Hu S, Studeman K, Condouris S, et al. Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/akt pathway in thyroid cancer. **Clin Cancer Res 2007**;13:1161-70.
9. Eng C. PTEN: one gene, many syndromes. **Hum Mutat 2003**;22:183-98.
10. Mclver B, Grebe SK, Eberhardt NL. The PAX8/PPAR γ fusion oncogene as a potential therapeutic target in follicular thyroid carcinoma. **Curr Drug Targets Immune Endocr Metabol Disord 2004**;4:221-34.
11. Pavelic K, Dedivitis RA, Kapitanovic S, Cacev T, Guirado CR, Danic D, et al. Molecular genetic alterations of FHIT and p53 genes in benign and malignant thyroid gland lesions. **Mutat Res 2006**;599:45-57.
12. Sebolt-Leopold JS, English JM. Mechanisms of drug inhibition of signalling molecules. **Nature 2006**;441:457-62.
13. Leboulleux S, Baudin E, Travagli JP, Schlumberger M. Medullary thyroid carcinoma. **Clin Endocrinol (Oxf) 2004**;61:299-310.
14. Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. **Cancer Res 2002**;62:7284-90.
15. Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, et al. BAY 43-9006 inhibition of oncogenic RET mutants. **J Natl Cancer Inst 2006**;98:326-34.
16. Knowles PP, Murray-Rust J, Kjaer S, Scott RP, Hanrahan S, Santoro M, et al. Structure and chemical inhibition of the RET tyrosine kinase domain. **J Biol Chem 2006**;281:33577-87.
17. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. **Nat Rev Cancer 2004**;4:937-47.
18. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. **Nature 2007**;445:851-7.
19. Shinohara M, Chung YJ, Saji M, Ringel MD. AKT in thyroid tumorigenesis and progression. **Endocrinology 2007**;148:942-7.
20. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. **Nat Rev Cancer 2004**;4:335-48.

Address for correspondence:

Massimo Santoro
Dipartimento di Biologia e Patologia Cellulare e Molecolare
Università di Napoli Federico II
via S Pansini 5, 80131 Naples, Italy
Fax: (+39-081) 746-3037
E-mail: masantor@unina.it