New Perspectives on the Treatment of Differentiated Thyroid Cancer

perspectiva

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ABSTRACT

Even though differentiated thyroid carcinoma is a slow growing and usually curable disease, recurrence occurs in 20–40% and cellular dedifferentiation in up to 5% of cases. Conventional chemotherapy and radiotherapy have just a modest effect on advanced thyroid cancer. Therefore, dedifferentiated thyroid cancer represents a therapeutic dilemma and a critical area of research. Targeted therapy, a new generation of anticancer treatment, is planned to interfere with a specific molecular target, typically a protein that is believed to have a critical role in tumor growth or progression. Since many of the tumor-initiation events have already been identified in thyroid carcinogenesis, targeted therapy is a promising therapeutic tool for advanced thyroid cancer. Several new drugs are currently being tested in *in vitro* and *in vivo* studies and some of them are already being used in clinical trials, like small molecule tyrosine kinase inhibitors. In this review, we discuss the bases of targeted therapies, the principal drugs already tested and also options of redifferentiation therapy for thyroid carcinoma. **(Arq Bras Endocrinol Metab 2007;51/4:612-624)**

Keywords: Thyroid cancer; Redifferentiation therapy; Targeted therapy; Tyrosine kinase inhibitors

RESUMO

Novas Perspectivas no Tratamento do Carcinoma Diferenciado da Tireóide.

Apesar de o carcinoma diferenciado da tireóide ser considerado uma doença de curso indolente e geralmente curável, recorrência tumoral ocorre em aproximadamente 20 a 40% e desdiferenciação celular, em até 5% dos casos. A quimioterapia convencional e a radioterapia apresentam apenas um modesto efeito sobre o câncer de tireóide avançado. Dessa forma, o carcinoma da tireóide desdiferenciado representa um dilema terapêutico e uma importante área de pesquisa. A terapia direcionada, uma nova geração de tratamento para o câncer, tem como objetivo interferir com um alvo molecular específico, geralmente uma proteína considerada fundamental para o crescimento e progressão tumoral. Como muitos eventos iniciadores do processo de carcinogênese tireoideana já foram identificados, a terapia direcionada representa uma promissora opção terapêutica para o carcinoma da tireóide avançado. Várias drogas novas estão em estudos *in vitro* e *in vivo* e algumas já estão sendo testadas em estudos clínicos, como as pequenas moléculas inibidoras de tirosina cinase. Nesta revisão, as bases moleculares da terapia direcionada, as principais drogas utilizadas e as opções terapêuticas de rediferenciação do carcinoma da tireóide serão discutidas. **(Arq Bras Endocrinol Metab 2007;51/4:612-624)**

Descritores: Câncer da tireóide; Rediferenciação tumoral; Terapia direcionada; Inibidor de tirosina cinase

THYROID CARCINOMA IS the most prevalent endocrine malignancy, and accounts for just 1% of all human cancers. Approximately 90% of non-medullary thyroid malignancies are well-differentiated thyroid carcinomas, which are classified as papillary or follicular based on histopathological criteria.

Even though differentiated thyroid carcinomas (DTC) are slow growing and usually curable by the combination of surgery, radioiodine ablation and thyroid stimulating hormone (TSH) suppressive therapy, recurrence occurs in 20–40% of patients (1,2). During tumor progression, cellular dedifferentiation occurs in up to 5% of cases and is usually accompanied by more aggressive growth, metastatic spread and loss of iodide uptake ability, making the tumor resistant to the traditional therapeutic modalities. Conventional chemotherapy and radiotherapy have modest, if any effect on advanced thyroid cancer (3), which is responsible for the vast majority of deaths attributed to thyroid cancer. Therefore, advanced thyroid cancer represents a therapeutic dilemma and is considered a critical area of research.

Several new drugs are currently being tested in *in vitro* and *in vivo* studies and some of them are already being used in clinical trials (4,5). In this review, we discuss the bases of targeted therapies for differentiated thyroid carcinoma, which correspond to drugs that act on molecules involved in neoplastic transformation and tumor progression (table 1). Other types of drugs are being tested for their ability to impair further loss of cell differentiation during tumor progression and reprogram cell differentiation. Special interest on redifferentiation therapy for thyroid cancers has been given, since the re-induction of functional NIS protein expression allows the use of radioiodine, a well-known efficient therapy for thyroid cancer.

OVERVIEW OF GENETIC ALTERATIONS IN DIFFERENTIATED THYROID CANCER

Like other cancers, thyroid carcinomas are characterized by genetic alterations that result in deregulated cell proliferation and death, together with tissue invasion ability. The different morphologic subtypes of thyroid cancer correlate with specific genetic alterations. However, mutations in different genes can be involved in the development of a specific histopathological tumor subtype. As a result, the microscopic phenotype of a tumor does not necessarily correspond to a specific genetic alteration.

In papillary thyroid carcinoma, the most prevalent thyroid cancer subtype, mutations in two protein kinases, RET and BRAF, are responsible for the vast majority of the cases (6,7). Also, activating point mutations in RAS occur in about 10% of cases, mainly in the follicular variant (8). Rarely, papillary carcinoma is associated with NTRK1 (neurotrophic tyrosine receptor kinase) rearrangement (9) or germline mutation in PTEN (phosphatase and tensin homologue deleted on chromosome ten), which is related to familial non-medullary thyroid carcinoma (10). PTEN has also a possible role in sporadic thyroid cancer, since a high frequency PTEN promoter hypermethylation is detected in sporadic tumors (11).

Follicular thyroid cancer accounts for about 10% of all thyroid cancers and 10 to 50% (12-14) of cases are associated with mutations in RAS oncogene. Chromosomal imbalances are frequent in follicular neoplasm, with gains at chromosomes 7 and 5 and deletions at 3p. In some of these tumors, somatic rearrangement results in the fusion of PAX-8 to PPARγ1 (peroxisome proliferator-actived receptor gamma 1). The PAX-8 PPARγ1 fusion oncogene appears to act through a dominant negative effect on the transcriptional activity of the wild-type PPARγ1. The frequency of rearrangement in follicular thyroid carcinoma is estimated in 30% and is not found in classic papillary carcinomas (15). However, this rearrangement has also been recently described in the follicular variant of papillary carcinoma (16). PAX-8 PPARγ1 fusion and RAS gene activation rarely occur in the same tumor.

Table 1. Possible targets in thyroid cancer.

Target	Studies in thyroid cancer
RET	Clinical (MTC); Pre-clinical (PTC)
BRAF	Pre-clinical
RAS	Pre-clinical and clinical
MET	No
NTRK	No
PI3 kinase	Pre-clinical (MTC)
AKT	Pre-clinical
mTOR	No
VEGFR	Pre-clinical and clinical
EGFR	Pre-clinical and clinical
FGFR	Pre-clinical
IGF-1R	Pre-clinical

AKT: protein kinase B, EGFR: epidermal growth factor receptor, FGFR: fibroblast growth factor receptor, IGF-1R: insulin growth factor 1 receptor, MET: hepatocyte growth factor receptor precursor, MTC: Medullary thyroid cancer, mTOR: mammalian target of rapamycin, NTRK: neurotrophic tyrosine receptor kinase, PI3K: phosphatidylinositol-3-kinase, PTC: papillary thyroid cancer.

In addition to these well-established genetic causes of thyroid cancer, overexpression and activation of a variety of tyrosine kinase receptors, mutations in the p53 gene, DNA hypermethylation, leading to silencing of tumor suppressor genes, activation of PI3K (phosphatidylinositol-3-kinase), Wnt signalling and expression of angiogenic factors and receptors are found in thyroid cancer progression.

TARGETED THERAPY

Conventional chemotherapy acts through inducing toxic effects on dividing cells, resulting in damage of tumoral cells, but also of normal tissues. Therefore, side effects like myelosuppression, alopecia and gastrointestinal symptoms are frequent. The optimum goal of anticancer therapy is the discovery of drugs that specifically kill malignant cells and cause no or little side effects. Targeted therapy refers to a new generation of cancer drugs designed to interfere with a specific molecular target, typically a protein that is believed to have a critical role in tumor growth or progression. Therefore, the objective of this therapy is to disrupt pathways that are inappropriately activated in cancer cells. This type of treatment has been applied specially to oncogenic protein kinases (17).

Antisense drugs, monoclonal antibodies and small-molecule drugs are examples of therapy intercepting important molecules in tumors and are being used in clinical trials. Targeted therapy is a promising therapeutic tool for thyroid cancer because many of the tumor-initiation events have already been identified.

Antisense drugs are small synthetic single-stranded DNA sequences of 13-25 oligonucleotides complementary to a particular targeted mRNA. When hybridised to the corresponding mRNA, RNAse H recognises the complex and cleaves the mRNA, leaving the antisense drug intact. These substances also interfere with ribosomal assembly, blocking gene expression and inhibiting protein synthesis. Systemic treatment with antisense drugs is generally well tolerated. Side effects are dose-dependent and include thrombocytopenia, hypotension, fever, complement activation, prolonged partial thromboplastin time, asthenia, and increased concentration of hepatic enzymes (18).

In 1998, the first antisense drug, Fomivirsen (Vitravene®), was approved by the US Food and Drugs Administration (FDA) for the treatment of retinitis by cytomegalovirus in patients with AIDS. The first generation of antisense drugs is 2'-deoxyribophosphorothioate antisense oligonucleotides. More recently, second-generation drugs, in which several nucleotides on each end are chemically modified to 2'-methoxyethoxyribophosphorothioate nucleotides, are in clinical trials. These new drugs are designed to enhance resistance to degradation by exonucleases and improve tissue pharmacokinetics, allowing less frequent dosing schedules. Second generation drugs are also more amenable to subcutaneous and oral delivery than first generation ones (19).

Small-molecules inhibitors and monoclonal antibodies are designed to intercept protein kinases in tumors. Most small-molecule kinase inhibitors obstruct the binding of ATP to the ATP pocket within the catalytic domain and are known as ATP mimetics. Other compounds target regions outside the ATP-biding site of the enzyme, for example the substrate-biding domain. These drugs obstruct autophosphorylation and signal transduction downstream from the targeted kinase. The most notable initial success of small molecule kinase inhibitor has been obtained with Imatinib (Gleevec®) in chronic myelogenous leukemia (CML), where BCR-ABL translocation results in expression of a fusion protein with constitutive activation of Abl kinase. This event promotes unregulated proliferation of haematopoietic cell clone. Imatinib is a relative selective inhibitor of Abl kinase and induces remission in up to 90% of patients with CML in chronic phase and a durable response in a high proportion of patients (20). This drug is also effective in patients with gastrointestinal stromal tumors and dermatofibrosarcoma protuberans, which are associated with activating mutation of tyrosine kinase receptors: C-KIT and platelet-derived growth factor receptor-β (PDGFR-β). Recent clinical studies suggest that Imatinib might also be effective in glioblastoma multiform (21) and malignant gliomas (22) by inhibiting PDGFR tyrosine kinase.

The clinical responses to kinase inhibitors occur in tumors that harbour activating mutations in protein kinases that drive cell transformations and cancer progression or in genes that indirectly lead to the activation of these kinases. However, the degree of response is probably related to the stage of the disease. Satisfactory results could occur when the drug inhibits the activity of a gene product believed to be an early event in tumorigenesis when the disease is not advanced. Nevertheless, taking into consideration the rapid process of tumor progression, interfering with initial events might not be sufficient in the majority of tumors and combination therapies to block multiple pathways may be necessary (23).

THERAPEUTIC TARGETS IN DIFFERENTIATED THYROID CANCER

RET oncogene

RET encodes a transmembrane tyrosine kinase receptor, member of a cell-surface complex that binds ligands of the glial-derived neutrophic factor (GDNF) family (24). These ligands bind RET in conjunction with co-receptors, trigging autophosphorylation and intracellular signalling with stimulation of the Ras/ERK and PI3 kinase/V-Akt cascades (figure 1).

In papillary thyroid cancer, the genetic hallmarks are chromosomal inversions or translocations that cause recombination of the intracellular kinase-encoding domain of RET with the 5' end of heterologous genes. The resulting chimeric sequence is called RET/PTC and is found in around 30% of cases and in over 60% of post-Chernobyl thyroid cancers (9). There are 12 rearrangements described and RET/PTC1 and 3 are the most prevalent variants.

RET/PTC recombination promotes RET expression in the cytoplasm of follicular cells, deletion of negative regulatory domains and a ligand-independent dimerization, resulting in a constitutive activation of RET. The finding that RET-PTC transgenic mice develop papillary thyroid cancer confirms that this oncogene can initiate thyroid carcinogenesis (25). In humans, it has also been demonstrated that RET/PTC is an early event of thyroid tumorigenesis, since it is frequently found in microcarcinoma (26). Therefore, RET is a logical target for selective inhibition in both medullary and papillary thyroid cancer that present RET oncogenic activation.

Several drugs have already been tested in preclinical studies (table 2). One of these compounds, the 4-anilinoquinazoline ZD6474 (Zactima), is being tested in patients with medullary thyroid cancer (27). This drug is an orally available small molecule inhibitor, originally developed as an anti-angiogenic agent that acts through the inhibition of the vascular endothelial growth factor receptor (VEGFR). Zactima also inhibits the epidermal growth factor receptor (EGFR), TIE-2 and RET kinase. Phase II assessment of Zactima is now in progress in a variety of tumor types in single and combination regimens.

In papillary thyroid cancer, it was demonstrated that RET/PTC3-transformed cells treated with Zactima lose the proliferative autonomy and show morphological reversion. Zactima also prevented the growth of human PTC cell lines that carry RET/PTC1 rearrangements and blocked anchorage-independent growth of RET/PTC3-transformed NIH3T3 fibroblasts and the formation of tumors after injection of NIH-RET/PTC3 cells into nude mice (28). More recently, it was observed that the administration of ZD6474 led to 90% reduction of cell number in pap-

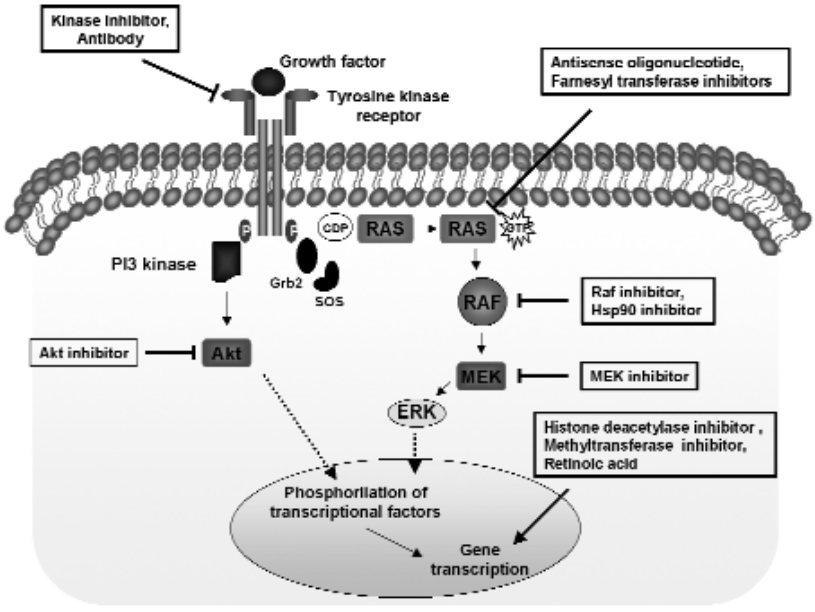

Figure 1. Scheme of principal targets and potential therapeutic options.

Table 2. Targeted therapy for thyroid cancer.

Drug	Target	Mechanism of Action
Zactima (ZD6474)	RET, VEGFR, EGFR	Kinase Inhibitor
Pyrazolopyrimidine (PP1, PP2)	RET, SRC family kinase	Kinase Inhibitor
Iodocarbazole (CEP-701, CEP-751)	RET, NTRK	Kinase Inhibitor
2-indolinone (RPI-1)	RET	Kinase Inhibitor
Benzamide (STI571)	RET, ABL, C-KIT, PDGFR	Kinase Inhibitor
Sorafenib (BAY43-9006)	BRAF, CRAF, VEGFR, RET, PDGFR	Kinase Inhibitor
Geldanamycin and derivatives	BRAF, CRAF, EGF-R, Her-2, AKT, p53, cdk4	Hsp90 inhibitor
Macrolide radicicol and derivatives	BRAF, CRAF, EGF-R, Her-2, AKT, p53, cdk4	Hsp90 inhibitor
Purine-scaffold derivatives	BRAF, CRAF, EGF-R, Her-2, AKT, p53, cdk4	Hsp90 inhibitor
ISIS 5132	CRAF	Antisense oligonucleotide
Tipifarnib (R115777)	RAS	Farnesyl transferase inhibitor
L-778123	RAS	Farnesyl transferase inhibitor
Lonafarnib (SCH66336)	RAS	Farnesyl transferase inhibitor
BMS-214662	RAS	Farnesyl transferase inhibitor
FTI-277	RAS	Farnesyl transferase inhibitor
L744832	RAS	Farnesyl transferase inhibitor
ISIS2503	RAS	Antisense oligonucleotide
CI-1040 (PD184352)	MEK1, MEK2	Kinase Inhibitor
PD0325901	MEK1, MEK2	Kinase Inhibitor
ARRY-142886	MEK1, MEK2	Kinase Inhibitor
LY294002	PI3 kinase	Kinase Inhibitor
KP372-1	AKT	Kinase Inhibitor
CCI-779	mTOR	Kinase Inhibitor
RAD001	mTOR	Kinase Inhibitor
Vatalenib (PTK787)	VEGFR, PDGFR, C-KIT	Kinase Inhibitor
AG013736	VEGFR, PDGFR, C-KIT	Kinase Inhibitor
NVP-AEE788	VEGFR, EGFR	Kinase Inhibitor
Gefitinib (Iressa)	EGFR	Kinase Inhibitor
Erlotinib (OSI-774)	EGFR	Kinase Inhibitor
Lapatinib (GW-572016)	EGFR, Her-2	Kinase Inhibitor
Canertinib (CI-1033)	EGFR, Her-2, Her-3, Her-4	Kinase Inhibitor
Mab 4253	EGFR	Antibody
NVP-ADW742	IGF-1R	Kinase Inhibitor
PD173074	FGFR4	Kinase Inhibitor

AKT: protein kinase B, EGFR: epidermal growth factor receptor, FGFR4: fibroblast growth factor receptor 4, IGF-1R: insulin growth factor 1 receptor, mTOR: mammalian target of rapamycin, NTRK: neurotrophic tyrosine receptor kinase, PDGFR: platelet-derived growth factor receptor, PI3K: phosphatidylinositol-3-kinase, SRC: protein kinase SRC, VEGFR: vascular endothelial growth factor receptor.

illary thyroid cancer cell lines (TPC1). Thus, targeting RET oncogene with Zactima might offer a potential treatment strategy for papillary carcinomas sustaining oncogenic activation of RET.

Other inhibitors of RET kinase have been tested in thyroid cancer cell lines, like pyrazolopyridines, PP1 and PP2. These compounds prevented the growth of two human papillary thyroid carcinoma cell lines that carry spontaneous RET/PTC1 rearrangements (29,30). Indolocarbazole derivatives, CEP-701 and CEP-751, inhibit RET in medullary thyroid cancer cells, but these compounds have not been tested in papillary thyroid cell lines (31).

BAY 43-9006 (Sorafenib), a multikinase inhibitor, was found to be a potent RET kinase inhibitor and was tested in medullary (TT) and papillary (TPC1) thyroid cancer cell lines (32). Recently, the inhibitory effect of SU11248, another multikinase inhibitor, was evaluated by quantification of RET/PTC autophosphorylation. This compound effectively inhibited the kinase activity of RET/PTC3 and represents another potential therapeutic tool for RET-positive thyroid tumors (33).

BRAF

BRAF belongs to the RAF family of serine/threonine kinases, and corresponds to the predominant isoform found in thyroid follicular cells. Raf proteins (A, B and CRAF) are components of the RAF-MAPK kinase-ERK (RAF-MEK-ERK) intracellular signalling pathway.

Point mutations in BRAF gene are the most common genetic lesions found in papillary thyroid cancer, 36–69% of the cases (7,34), and there is practically no overlap between RET/PTC, BRAF and RAS mutations. This suggests that mutations at more than one of theses sites are unlikely to confer additional biologic advantage.

BRAF represents an attractive target for treatment of papillary thyroid cancer due to the relative high prevalence of activating point mutations in this gene, its association with more aggressive cancer behavior and mainly because BRAF mutation is also a possible tumor initiating event in thyroid carcinogenesis (35). Several RAF small molecule kinase inhibitors or antisense oligonucleotides are being tested (table 2).

Sorafenib (Bay 43-9006) is a novel oral multitarget inhibitor that reached clinical testing. Sorafenib inhibits RAF kinase and tyrosine kinase activity of PDGFRα, VEGFR-2, VEGFR-3, FLT-3 and c-KIT. This drug has antitumor effects in colon, pancreas and breast cell lines and in colon, breast and non-cell lung xenograft models. More recently, Salvatore et al. (35) investigated the effect of chemical BRAF blockade by Sorafenib on anaplastic and poorly differentiated thyroid carcinoma cell lines. Sorafenib reduced the phosphorylation of MEK1/2, p44/p42 MAPK and p90RSK and proliferation rate in these cells. Nude mice injected with anaplastic cells (ARO) and treated with Sorafenib had significantly smaller tumors than control mice, with large areas of necrosis. This last effect is probably related to the block of VEGFR, preventing tumor neovascularization.

Therapy with Sorafenib is licensed for advanced renal cell carcinoma. Phase III trials in melanoma and advanced hepatocellular carcinoma, and phase II trials in multiple tumor types, including thyroid cancer, are currently ongoing (23). Sorafenib and other similar small molecule inhibitors represent a promising molecular therapy for advanced papillary and anaplastic thyroid carcinoma with BRAF mutation.

The antisense oligonucleotides designed to inhibit c-RAF, ISIS 5132, has been demonstrated to inhibit the growth of several malignant cells. Initial phase I studies demonstrated minimal side effects, with more than 50% reduction of Raf activity. In phase II trials in patients with colorectal, lung, prostate and ovarian cancer no objective clinical response was observed (18). Although this compound may be active against thyroid cancer, this has not been tested yet.

The multichaperone heat shock protein (Hsp) 90 complex mediates the maturation and stability of a variety of proteins, many of which are crucial in oncogenesis, including epidermal growth factor receptor (EGF-R), Her-2, AKT, BRAF, CRAF, p53, and cdk4. Inhibition of Hsp90 function disrupts the complex and leads to degradation of these proteins in a proteasome-dependent manner. This results in interruption of many signal transduction pathways essential for tumor progression and survival (36). Numerous classes of Hsp90 inhibitors have recently been developed, such as the geldanamycin and derivatives 17-AAG (17-allylamino-17-demethoxygeldanamycin) and 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin); the macrolide radicicol and derivatives; purine-scaffold derivatives; pyrazoles; and shepherdins that bind to the N-terminal high-affinity ATP-binding domain of Hsp90. Other inhibitors have recently been shown to bind to the C-terminal dimerization domain of Hsp90, such as cisplatin and novobiocin, or modify Hsp90 postranslationally, such as histone deacetylase or proteasome inhibitors (37). The most advanced compound, 17-AAG, is in phase I/II clinical trials. Hsp90 inhibitors represent a novel target for cancer therapy and might be beneficial in the molecular treatment of thyroid cancer (4).

RAS

Ras is a GTP-binding protein involved in proliferation, differentiation and cell survival (figure 1). In thyroid cancer, activation of Ras occurs through activating mutations in genes encoding RAS or through activation of upstream regulators. Mutations in all the three *RAS* oncogenes (H-*RAS*, K-*RAS*, and N-*RAS*) have being reported in thyroid cancer since 1988.

The presence of mutant RAS in microfollicular adenomas supported the concept that *RAS* oncogene activation could be an early event in follicular thyroid tumorigenesis. Therefore, Ras may be considered a target for anti-cancer therapy for follicular and also papillary thyroid carcinoma (figure 1).

ISIS2503 was designed to target Ras pathways. It is a first-generation of antisense oligonucleotides that selectively inhibits the expression of H-RAS. In human xenograft models, ISIS2503 inhibited colon, pancreas and lung tumors. In phase I trial, this drug caused no dose-limiting toxicity. Phase II trials have been conducted in patients with advanced pancreatic and colorectal carcinomas (18).

Translocation of Ras to the cytoplasmatic membrane is an important step in its activation. Studies have shown that farnesylation of Ras is the first obligatory step in a series of post-translational modifications leading to membrane association, which, in turn, determines the switch from an inactive to an active form. Farnesyl transferase inhibitors are anticancer agents that were designed to block the post-translational attachment of the prenyl moiety to the C-terminal cysteine residue of Ras and thus inactivate it. Currently, several inhibitors have been developed and are in clinical trials, like R115777 (Tipifarnib), L-778123, SCH66336 (Lonafarnib), BMS-214662, FTI-277 and L744832. L-778123 and BMS-214662 have been evaluated in a phase I study that involved patients with advanced solid carcinomas, including patients with thyroid cancer (table 2). Although phase I clinical trials confirmed low toxicity (except for L778123), no improvement in overall survival has been reported in phase II and III trials in patients with malignant glioma, advanced colorectal, urothelial, lung and pancreatic cancer. Therefore, farnesyl transferase inhibitors failed as single anticancer agent for most solid cancers, but are promising in hematological malignancies (38).

MEK

Given the central role of the ERK/MAPK pathway in mediating growth-promoting signals for a diverse group of upstream stimuli, inhibitors of MEK, as a key central mediator, could have significant clinical benefit in the treatment of several cancers, including thyroid cancer (figure 1). CI-1040 (PD184352) is an orally active, highly specific, small-molecule inhibitor of MEK1/MEK2, and thereby effectively blocks the phosphorylation of ERK and the continued signal transduction through this pathway (table 2). Antitumor activity has been reported in preclinical models, particularly for pancreas, colon and breast cancers. In phase I studies, this drug has been shown to be well tolerated, with safety and pharmacokinetic profiles that permit continuous daily dosing. Recently, it was demonstrated that mutation of BRAF is associated with enhanced and selective sensitivity to MEK inhibition when compared to either "wild-type" cells or cells harbouring a RAS mutation (39). New inhibitors, like PD0325901 and ARRY-142886, have reached the clinical trial stage (40).

VEGF receptor

Angiogenesis is a crucial step in tumor progression and is greatly dependent on pro-angiogenic factors produced by cells undergoing hypoxia or mechanical compression. Vascular endothelial growth factor (VEGF) appears to be the most prominent growth factor involved in tumor angiogenesis and presumably in tumor growth and haematogeneous spread of tumor cells. VEGF belongs to the platelet-derived growth factor (PDGF) superfamily and consists of VEGF-A, -B, -C, -D, -E and the placenta growth factor (PIGF).

Studies have demonstrated that VEGFR is overexpressed in thyroid cancer (41) and VEGF-C immunoreactive protein is correlated with papillary lymph node metastases (42). Serum VEGF is significantly elevated in patients with papillary thyroid cancer compared with the control group (43), especially in patients with metastatic differentiated thyroid cancer but not in those with poorly differentiated thyroid cancer metastases (44).

Most VEGFR kinase inhibitors under investigation block multiple kinases not involved in angiogenesis, resulting in diverse side effects. Newer drugs are being developed to selectively inhibit a small group of protein kinases, with fewer side effects (table 2).

NVP-AEE788 (AEE788), a novel dual specific EGFR and VEGFR kinase inhibitor, reduced follicular thyroid cancer cell growth *in vitro* and the phosphorylation status of EGFR, VEGFR, and two downstream targets, AKT and mitogen-activated protein kinase. AEE788 alone and, to a greater extent, AEE788 plus paclitaxel suppressed tumor growth in nude mice (45). PTK787/ZK222584 (PTK/ZK, Vatalanib), a specific oral blocker of VEGF-receptor tyrosine kinases, caused 41.4% reduction in volume of human follicular thyroid tumor xenografts implanted into nude mice. Immunohistochemistry revealed a significant decrease in neoangiogenesis, expression of extracellular matrix protein and no compensatory overexpression of VEGF protein was detectable (46). These results showed that VEGF receptor blockade is a rational approach to the therapy of thyroid cancer alone or in combination with external radiation for poorly differentiated and radioiodine-resistant thyroid cancers.

AG013736 is an oral inhibitor of the tyrosine kinase portion of the VEGF, PDGF receptors and c-kit. This inhibitor of angiogenesis is undergoing phase II study for thyroid cancer unresponsive to radioiodine therapy (47).

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is a member of Erb family of receptors that is abnormally activated in many epithelial tumors. Four structurally related receptors are part of this family: ErbB1 (Her1 or EGFR), ErbB2 (Her2/neu), ErbB3 (Her3) and ErbB4 (Her4). After binding to a ligand, the activa-

tion of EGFR tyrosine kinase triggers pathways that lead to cell cycle progression and apoptosis. The major downstream signaling route of EGFR family is Ras-Raf-MAPK cascade. Another important route is the phosphatidylinositol-3-kinase pathway.

EGFR and Her2/neu have been implicated in thyroid cancer and overexpression of EGFR by papillary thyroid carcinoma has been associated with a worse prognosis (48). Therefore, EGFR seems to be an adequate therapeutic target. Two main anti-EGFR strategies are currently in clinical development: monoclonal antibodies that are directed to the ligand-binding extracellular domain, causing receptor internalization, and low molecular tyrosine kinase inhibitors that compete with ATP at the tyrosine kinase portion.

Mab 4253, an anti-EGFR-antibody, was tested in papillary thyroid carcinoma cell line (ONCO-DG-1); however, this drug alone was not effective enough for therapeutical use (49). More recently, papillary and follicular carcinoma cell lines were treated with EGF and the EGFR tyrosine kinase inhibitor AG1478. EGF stimulated invasion by thyroid cancer cells up to sevenfold, a process that was antagonized completely by AG1478, suggesting that this drug may be effective for aggressive thyroid carcinomas treatment (50). Geftinib (ZD1839), the first commercially available EGFR tyrosine kinase inhibitor, which is now registered for use in second and third line therapy for advanced lung cancer (51), is undergoing phase II testing in patients with iodine-refractory advanced thyroid carcinoma (47). However, in another study, the presence of EGFR-activating tyrosine kinase domain mutations was detected in just two out of 62 histological specimens (3.2%). Therefore, just a small minority of thyroid cancer patients may benefit from EGFR inhibitors, but additional preclinical evidence of efficacy is needed (52).

Other kinases as potential therapeutic targets

The NTRK1 gene encodes the high affinity receptor for Nerve Growth Factor. Somatic rearrangements of NTRK1 generate TRK oncogenes with constitutive tyrosine kinase activity and are detected in 3 to 12% of papillary thyroid carcinomas (9,53).

Tyrosine kinase inhibitor CEP-701 blocks the NTRK1/NGF receptor and limits the invasive capability of prostate cancer cells *in vitro* (54). This drug might be beneficial in the treatment of patients with papillary thyroid cancer with rearrangements of NTRK1, although it has not been tested.

Fibroblast growth factor (FGF) comprises a large family of heparin-binding growth factors. These ligands signal through four tyrosine kinases, FGFR1-4. Overexpression of FGFR has been identified in various malignancies, including thyroid cancer. FGFR2 is the only receptor consistently expressed in normal thyroid tissue and reduced in thyroid cancer. FGFR1 and 3 are expressed in most well-differentiated thyroid cancers and FGFR4 is expressed predominantly in advanced thyroid cancer. The administration of PD173074, an inhibitor of FGFR4 tyrosine kinase, resulted in significant reduction of aggressive cell line (MRO) growth in xenografted mice (55).

Insulin-like growth factor-I (IGF-I) is a potent growth factor. It was observed that IGF-I receptor staining was more intense in aggressive than indolent thyroid tumors (56) and that the addiction of NVP-ADW742, a small molecule inhibitor of IGF-I receptor type 1, caused a cytotoxic effect in thyroid cancer cell lines (57).

The activation of the Akt protein kinase B (Akt/PKB), a serine/threonine kinase, appears to play an important role in apoptosis, proliferation, cell cycle progression, cytoskeleton stability and motility and energy metabolism. Akt is either constitutively activated by the PI3 kinase pathway or the genetic loss of PTEN expression. The role of Akt in thyroid cancer was first recognized when loss of PTEN expression was identified as the cause of Cowden syndrome, an autosomal disease in which more than 50% of patients develop thyroid neoplasia. Subsequently, the importance of Akt in sporadic follicular thyroid cancer was demonstrated by the finding of increased expression of Akt in thyroid tumor sample compared to normal tissue. KP372-1, an inhibitor of Akt, suppressed Akt activity, cell proliferation and induced apoptosis in thyroid cancer cell line (58).

Several phase I and phase II clinical studies with rapamycin-like drugs have been conducted to inhibit mTOR (mammalian target of rapamycin) and have demonstrated antitumor activity in various types of refractory neoplasms. Inhibition of the PI3 kinase with LY294002 may also lead to a reduction in tumor growth. However, until now this inhibitor was only tested in medullary thyroid carcinoma cell line, and a dose-dependent decrease in cellular proliferation was observed (59).

TRIGGERS OF APOPTOSIS AS ANTICANCERTHERAPY

Apoptosis is a programmed process that leads to cell death initiated via intracellular or extracellular stimuli. Several drugs have been designed to induce apoptosis in cancer cells.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a cytokine member of the TNF family that triggers apoptosis in many human cancer cells, but not in normal cells. TRAIL activates the caspase pathway through two of its receptors: TRAIL-R1 and TRAIL-R2. Park et al. (60) observed that the majority of thyroid cancer cell lines tested were resistant to TRAIL, and growth inhibition was less than 20%. However, pretreatment with troglitazone, cycloheximide, and paclitaxel enhanced TRAIL-induced cell death significantly.

TRM-1 and HGS-ETR2 are human monoclonal agonistic antibodies specific for TRAIL-R1 and TRAIL-R2, respectively. These antibodies can induce cell death in a variety of cultured cells and may have therapeutic value (61,62). However, these compounds have not yet been tested in thyroid cancer.

RE-INDUCING TUMOR IODIDE UPTAKE – DIFFERENTIATING THERAPY

The ability of thyroid to concentrate iodine permits the use of radioactive isotopes of iodine for diagnosis and therapy of benign and malignant thyroid diseases. In the case of DTC, the ability of tumor cells to accumulate iodine allows investigation of cancer relapse by whole body scanning and treatment of cervical remnant, loco regional and distant metastasis with radioiodine.

After the cloning of NIS gene (63), the understanding of mechanisms underlying iodine uptake modulation in thyroid neoplasia was facilitated. Although reduced expression of NIS has been demonstrated and suggested to be responsible for the impaired iodine uptake ability (64-66), immunohistochemistry study verified that, instead, in some thyroid cancer samples NIS is over expressed (67,68). However, in these tumors NIS localization was mostly intracellular, highlighting the importance of understanding the molecular mechanism involved in targeting NIS to plasma membrane (69).

The crucial role of radioiodine therapy in the course of thyroid carcinoma stimulated studies to investigate possible drugs that could act by enhancing functional NIS expression and iodine accumulation.

Lithium has been used for many years for mood disorders therapy. This drug can cause goiter in some patients and inhibit the release of iodine from the thyroid cells. Studies in DTC suggest that lithium therapy (300 mg three times a day or 10 mg/kg/day) may be a potential adjuvant to radioiodine therapy, since it can increase the uptake of iodine and prolong its retention in follicular cells (70-72). However, its possible role as adjuvant to redifferentiating agents has not been evaluated so far.

Histone deacetylase inhibitors can alter transcription of DNA into mRNA, via modification of the level of relaxation of histona-DNA complex. By unclear mechanisms, these inhibitors can induce cell cycle arrest and differentiation. The use of FR901228 in 2 follicular and 2 anaplastic carcinoma cell lines increased not only histona acetylation and expression of both Tg and NIS mRNA, but also iodine accumulation, suggesting induction of functional NIS (73). Study with suberoylanilide hydroxamic acid, another histone deacetylase inhibitor, demonstrated that this drug can inhibit the growth of several anaplastic and papillary thyroid cancer cell lines (74). Valproic acid, a widely used anticonvulsant, is also able to inhibit histone deacetylase, promoting differentiation, NIS expression, iodide uptake, and growth suppression of poorly differentiated thyroid cancer cell lines (75,76). Nevertheless, no clinical trials have been published to confirm safety and efficacy of these drugs.

Aberrant methylation of gene promoter regions, resulting in loss of gene expression, plays an important role in human tumorigenesis, including thyroid tumor. Several tumor suppressor genes are aberrantly methylated in thyroid cancer, like PTEN and RASSF1A genes in follicular thyroid carcinomas (77). In papillary thyroid carcinoma, methylation of TIMP3, SLC5A8 and DAPK are significantly associated with several aggressive features, including extrathyroidal invasion, lymph node metastasis, multifocality and advanced tumor stages. Methylation of these genes was also significantly associated with BRAF mutation (78). Methylation of thyroid-specific genes, such as NIS and TSH receptor, is also common in thyroid cancer. Methylation, and hence silencing of these thyroid-specific genes, may be a cause for the failure of clinical radioiodine treatment of thyroid cancer. Blockers of methyltransferase have been used to induce reexpression of tumor suppressor genes and other genes important for facilitating therapy or reducing proliferation. In 7 human thyroid carcinoma cell lines lacking NIS mRNA, treatment with 5-azacytine or sodium butyrate was able to restore NIS expression in 4 and also iodide transport in 2 cell lines (79).

Phenylbutyrate and its metabolite phenylacetate have been evaluated as an anti-neoplastic agent in several preclinical studies. Although the mechanisms of action of this drug are not clear, phenylacetate seems to have cytostatic effect in some cell lines, including leukemic cells, malignant glioma and prostate cancer. Recently, a phase I dose escalation clinical trial of phenylbutyrate sodium was conducted in 21 patients with advanced solid malignancies. Administration of phenylbutyrate in a twice-daily infusion demonstrated to be safe (80). In human thyroid cell lines, phenylac-

etate decreases TSH and non-TSH induced growth and increases radioiodine concentration (81). Phenylacetate also inhibited the secretion of vascular endothelial growth factor from the thyroid cancer cell lines (82).

Retinoic acids (RAs) are biologically active metabolites of vitamin A, which regulate growth and differentiation of many cell types by binding to specific nuclear receptors (83). Different analogues of RA have been used for treatment and chemoprevention of some malignancies, such as acute promyelocytic leukemia (84) and skin carcinoma (85,86). Recent studies with RAs have shown that these drugs can induce *in vitro* re-differentiation of thyroid carcinoma cell lines, as suggested by increased expression of some thyroid specific proteins (87-91), and by increment of cellular radioiodine uptake (91,92). More recently, RA was shown to decrease in vitro VEGF accumulation and reduce microvessels density in experimental undifferentiated thyroid cancer cell line, suggesting that reduced angiogenesis may be an important mechanism responsible for the therapeutic effect of RA in thyroid carcinoma (93).

In different clinical studies, the administration of RA was able to re-stimulate iodide uptake in 20 to 50% of cases (94-97). The dose recommended is 1.0 to 1.5 mg/kg/day of isotretinoin for at least 5 weeks. Initially, RA was considered a promising option for dedifferentiated thyroid cancer, with low rate of side effects, especially when compared with cytotoxic drugs. However, tumor regression or, at least, its stabilization is seen in just 20% (95,97) or less (98,99). Therefore, an indiscriminate use of isotretinoin in all patients with untreatable thyroid cancer cannot be recommended. It is crucial to determine a possible predictive factor of response to this therapy. Cell lines expressing both RARβ and RXRγ demonstrated significant growth suppression when treated with retinoids, whereas cell lines lacking these isoforms were unaffected. So, these isoforms seem to predict response to retinoid therapy in thyroid cancer cell lines (100). In a preliminary clinical study, the possible correlation between clinical response to RA and tumor expression of RARβ was also identified (101).

OTHER THERAPIES

Cyclooxigenase-2 inhibitor

Activation of cyclooxigenase-2, which is overexpressed in many cancers, including thyroid carcinoma (102), promotes tumor initiation and progression. A phase II trial of high dose of celecoxib was conducted in 32 patients with advanced thyroid cancer. This selective COX-2 inhibitor failed to stop the progression of the disease and 3 patients were off the study due to toxicities (103).

Thiazolidinedione and derivatives

Actually, PPARγ is considered a tumor suppressor gene. In follicular thyroid carcinomas, the PAX8/PPARγ fusion oncogene appears to suppress the activity of the wild-type gene. The antitumor effect of PPARγ agonists is likely to be through transactivating genes that regulate cell proliferation, apoptosis, and differentiation. Park et al. (104) investigated the antiproliferative and redifferentiation effects of troglitazone in 6 human thyroid cancer cell lines: TPC-1 (papillary), FTC-133, FTC-236, FTC-238 (follicular), XTC-1 (Hurthle cell), and ARO82-1 (anaplastic) cell lines. Troglitazone inhibited cell growth, downregulated expression of CD97, a dedifferentiation marker, in FTC-133 cells, and upregulated NIS mRNA in TPC-1 and FTC-133 cells. PPARγ overexpression was not a prerequisite for treatment response in this study.

A novel high-affinity PPARγ agonist (RS5444) demonstrated growth inhibition of anaplastic thyroid carcinoma cell line. RS5444 plus paclitaxel demonstrated additive antiproliferative activity in cell culture and minimal ATC tumor growth *in vivo* (104).

CONCLUSIONS

In summary, although many new approaches for advanced thyroid cancer are emerging, a significant clinical impact on survival by the use of these drugs is still lacking. The new concept of molecular signature has recently been introduced in the literature and corresponds to the knowledge of the exact pathways that are responsible for neoplastic transformation and tumor progression. In the near future, apart from the histopathological subtype of tumors, it will be absolutely necessary to develop diagnostic tools for their molecular classification, so that a better therapeutic option can be used. Thus, the identification of patients who are likely to benefit from each therapeutic option is highly important. The overexpression or activation of a specific target is not always a guarantee for satisfactory response due to tumor progression, and drug resistance is also a possible problem. Inhibition of kinase may induce cytostatic effects rather than cell death in monotherapy, and therefore multidrug therapy based on synergism by combining multiple small molecule kinase inhibitors, anti sense oligonucleotides, antibodies, chemotherapy and/or radiotherapy are more likely to be effective.

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