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How molecular pathology is changing and will change the therapeutics of patients with follicular cell-derived thyroid cancer

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ABSTRACT

Well-differentiated thyroid carcinomas comprise two well-defined histological types: papillary and follicular (PTCs and FTCs, respectively). Despite being derived from the same cell (thyroid follicular cell), these two types of tumour accumulate distinct genetic abnormalities during progression. The molecular pathology of thyroid cancer is now better understood because of our ability to identify RET/PTC rearrangements and BRAF mutations in the aetiopathogenesis of the large majority of PTCs and the high prevalence of RAS mutations and PAX8/PPARγ rearrangements in follicular patterned carcinomas (FTCs and follicular variant of PTCs). This review summarises most of the molecular alterations currently used as targets for new biological treatments and looks at some of the changes that are already occurring or may occur in the treatment of patients with thyroid cancer. For simplicity, the review is divided up according to the major genetic alterations identified in well-differentiated thyroid carcinomas (RET/PTC rearrangements, BRAF mutations, RAS mutations and mitochondrial DNA deletions and mutations) and their respective treatments.

Thyroid cancer is the most common type of endocrine neoplasia and is mostly due to tumours derived from follicular cells.[1] C-cell-derived thyroid medullary carcinoma represents ~5% of clinically evident thyroid carcinomas.[1] In this review, we concentrate on the changes in treatment of patients with well-differentiated follicular cell-derived carcinomas, which represent ~85% of all thyroid carcinomas.[1]

Follicular cell oncogenesis presents multiple discrete stages ranging from common benign follicular adenomas (FTAs) to the less common, highly aggressive, poorly differentiated thyroid carcinomas and undifferentiated (anaplastic) thyroid carcinomas.[1] Between these two ends of the spectrum is the common well-differentiated thyroid carcinomas, which comprise two histological types: papillary and follicular (PTC and FTC, respectively). The essential diagnostic criteria differ between the two; in PTCs, they are cytological, based on the presence of typical nuclear features (large, pale staining, "ground glass" and irregular, "grooved" nuclei), whereas the diagnosis of FTC rests on the histological demonstration of capsular and/or vascular invasiveness.[1]

The two types of well-differentiated thyroid carcinoma (WDTC) accumulate distinct genetic abnormalities during tumour progression. In PTCs, somatic rearrangements of the RET proto-oncogene[2–4] and $BRAF^{V600E}$ mutations[5 6] are the most common events. In contrast, FTCs have a different genetic profile: they are characterised by RAS mutations[7 8] and PAX8/PPARγ rearrangement.[9 10] The follicular variant of PTC (FVPTC) shares some of the molecular features of follicular tumours (FTA and FTC), namely a high frequency of RAS mutations and PAX8/PPARγ rearrangements,[11] whereas a less common and less often reported $BRAF^{K601E}$ form (~7%) is detected in cases of FVPTC.[12] These observations reinforce the assumption that some FVPTC cases are an intermediate category between conventional PTC and FTC.[11]

The behaviour of WDTCs is typically indolent, and they can be effectively treated by surgery followed by radioiodine therapy. However, tumours that lose differentiation and therefore the ability to trap radioiodine do not respond to radioiodine treatment and carry a less favourable prognosis. Patients with such tumours are obvious candidates for alternative approaches such as molecular targeted therapy.

The clinical use of pathway-targeted drugs (mainly tyrosine kinase inhibitors (TKIs)) in patients with thyroid cancer still does not rely on the genetic background of each concrete tumour,[13–15] being mainly based on observations in in vitro models. The situation will be improved substantially after the conclusion of meta-analyses of ongoing clinical trials and by the exploitation of other molecular and/or other metabolic pathways and the utilisation of treatment combinations.

For simplicity, this review has been divided according to the major genetic alterations identified in WDTCs. In each section, the use of new drugs designed to target the inhibition of specific cellular pathways is discussed (a summary is given in table 1). Although we acknowledge the putative importance of relatively unspecific treatments that have been used successfully in other tumour models and are also thought to be useful in thyroid carcinomas (eg, anti-angiogenesis drugs and drugs targeting growth factors/growth factor receptors), we decided to restrict the discussion to mechanisms considered to be the hallmarks of WDTCs.

RET/PTC REARRANGEMENTS AS A THERAPEUTIC TARGET

RET encodes a membrane receptor tyrosine kinase that signals through a ligand–co-receptor–RET complex. The formation of ligand–co-receptor–RET complexes results in RET dimerisation and triggers autophosphorylation at intracellular

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Table 1 Summary of studies using new compounds that target key molecular pathways in follicular cell-derived thyroid cancer models

Compound	Trade name	Structure	Targets	Clinical trials	References
PP1, PP2	Zaleplon	Pyrazolopyrimidine	RET	–	21
ZD6474	Vandetanib	Anilinoquinazoline	RET, VEGFR, EGFR	Phase II	22
RPI-1	–	Indolinone	RET, MET	–	23, 32
SU11248, SU5416	Sunitinib	Butanedioic acid	VEGFR-2, PDGFR, c-KIT, RET, CSF-1R	Phase II	26, 35
ZD1839	Gefitinib	Anilinoquinazoline	EGFR	Phase II	36
BAY43-9006	Sorafenib	Bis-aryl urea	RAF-1, BRAF, VEGFR-2/-3, PDGFR-B, Flt-3, c-KIT, RET	Phase II	14, 28, 30, 31, 60, 62, 63
CI-1040 (PD184352)	–	Benzhydroxamate ester	MEK	–	59
AAL881 LBT-613	–	Isoquinolines	RAF-1, BRAF, VEGFR-2	–	74
17-AAG, 17-DMAG	Tanespimycin	Benzoquinones	Heat shock protein 90	Phase II	76, 77
AMG706	Motesanib diphosphate	Diphosphate salt	VEGFR, PDGFR, KIT, RET	Phase II	29
AG-013736	Axitinib	Benzamide	RET, VEGFR, PDGFR, c-KIT	Phase II	15
R115777	Zarnestra, tipifarnib	Quinolinone	Farnesyltransferase	Phase I (in conjugation with topotecan)	96, 97

EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

tyrosine residues. Tyrosine phosphorylation of intracellular target proteins activates several downstream pathways, which include mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)1/2, phosphatidylinositol 3-kinase, c-Jun N-terminal kinase, p38, ERK5 and cAMP-responsive element-binding protein.

In the thyroid gland, wild-type RET is expressed at high levels in parafollicular C-cells, but not in follicular cells; this finding is consistent with its role in the development and function of neural crest-derived cell lineages.[16] Activating point mutations of RET in C-cells are responsible for sporadic and familial medullary thyroid carcinomas and for the inherited cancer syndromes MEN2A and MEN2B (for a review, see de Groot *et al*[17]).

In sporadic PTCs, three major rearrangements involving the RET gene, RET/PTC1, 2 and 3, have been identified, leading to the presence of a constitutively activated RET tyrosine kinase domain in the cytoplasm of follicular cells. The prevalence of somatic rearrangements of the RET proto-oncogene varies from 3% to 60% in different series of sporadic PTCs.[18] RET/PTC1 is the most common type, comprising up to 60–70% of the rearrangements, whereas RET/PTC3 accounts for 20–30% of positive cases. Other novel and rare types of RET/PTC are usually associated with radiation exposure. RET/PTC rearrangements, especially RET/PTC1, seem to be more common in tumours with a pure, or predominantly papillary, growth pattern.[18 19] The prognostic significance of RET/PTC in PTCs remains controversial. Some groups have suggested an association between RET/PTC and more aggressive tumours,[20] whereas others have proposed that tumours that harbour RET/PTC display slow growth and do not progress to poorly differentiated and undifferentiated thyroid carcinomas.[2–4] In PTCs, almost no overlap exists among mutations in RET/PTC, RAS or BRAF; it is thus tempting to conclude that thyroid cell transformation into PTC takes place through constitutive activation of effectors along the RET/PTC–RAS–BRAF signalling pathway.[5 6]

Oncogenic forms of RET found in PTCs are targets of potential therapeutic interest. Various compounds have been reported to inhibit oncogenic RET (mutated or rearranged) (table 1), including PP1 and PP2,[21] vandetanib (ZD6474),[22] RPI-1,[23] CEP-701,CEP-751,[24] imatinib,[25] sunitinib (SU5416, SU11248),[26] gefitinib,[27] sorafenib (BAY 43-9006),[28] motesanib (AMG706)[29] and axitinib (AG013736).[15]

The mechanism of action of small-molecule TKIs is based on the principle that sterically blocking the ATP-binding pocket results in impaired phosphorylation activity, inhibits signal transduction, and prevents activation of intracellular signalling pathways relevant to tumour growth and angiogenesis.

The pyrazolopyrimidines, PP1 and PP2, and the 4-anilinoquinazoline, vandetanib, inhibit RET rearrangement-derived oncoproteins with a half-maximal inhibitor concentration (IC_{50}) below 100 nM. These molecules were shown to inhibit RET enzymatic activity and phosphorylation of downstream targets, such as ERK1/2. Vandetanib has been found also to inhibit RET signalling in two human PTC cell lines and to reduce tumorigenicity of RET/PTC-transformed fibroblasts injected into nude mice.[21] Vandetanib blocks in vivo phosphorylation and signalling mediated by RET/PTC3 oncoprotein of an epidermal growth factor (EGF)-activated receptor/RET chimeric receptor. Furthermore, it blocks anchorage-independent growth of RET/PTC3-transformed NIH3T3 fibroblasts and the formation of tumours after injection of NIH3T3-RET/PTC3 cells into nude mice.[22]

Although sorafenib (BAY 43-9006) was designed originally as a RAF inhibitor[30] (see below), preclinical studies have shown that it can inhibit the kinase activity and signalling of wild-type and oncogenic RET. Sorafenib inhibited oncogenic RET kinase activity at an IC_{50} of 50 nM or less in NIH3T3 cells. It arrested the growth of NIH3T3 and RAT1 fibroblasts transformed by oncogenic RET and thyroid carcinoma cells that harbour rearranged RET alleles. These inhibitory effects paralleled a decrease in RET phosphorylation.[28] Finally, PTC cells carrying the RET/PTC1 rearrangement were found to be more sensitive to sorafenib than PTC cells carrying a BRAF mutation.[31] There is an ongoing phase II clinical trial using sorafenib in patients with advanced thyroid cancer (see below).[14]

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RPI-1 is a 2-indolinone derivative initially shown to inhibit RET/PTC1 activity with an IC_{50} of 27–42 μM. It selectively inhibited the anchorage-independent growth of NIH3T3-transformed cells expressing the RET/PTC1 gene, and the transformed phenotype of NIH3T3-RET/PTC1 cells reverted to a normal fibroblast-like morphology. In these cells, the constitutive tyrosine phosphorylation of RET/PTC1, of the transducing adaptor protein, shc, and of a series of coimmunoprecipitated peptides was substantially reduced.[23] Activation of c-Jun N-terminal kinase 2 and AKT (acutely transforming retrovirus AKT8 in rodent T cell lymphoma) was abolished, thus supporting the drug inhibitory efficacy on downstream pathways. In addition, cell growth inhibition was associated with a reduction in telomerase activity by nearly 85%.[32]

Sunitinib was initially described as a TKI that targets vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs)[33] and has also been found to inhibit c-KIT.[34] It is now approved for the treatment of gastrointestinal stromal tumour and renal cell carcinoma. In vitro kinase assays showed that sunitinib inhibited the phosphorylation by RET/PTC3 of a synthetic tyrosine kinase substrate peptide in a dose-dependent manner. RET/PTC-mediated Y705 phosphorylation of signal transducer and activator of transcription (STAT) 3 was inhibited by addition of sunitinib, and the inhibitory effects of sunitinib on the tyrosine phosphorylation and transcriptional activation of STAT3 correlated very closely with decreased autophosphorylation of RET/PTC. Sunitinib caused complete morphological reversion of transformed NIH3T3-RET/PTC3 cells and inhibited the growth of TPC-1 cells with an endogenous RET/PTC1.[26] Treatment of two patients with progressive metastatic thyroid carcinomas (a PTC and a FTC) showed sustained clinical responses to sunitinib over a period of 4 years.[35]

Gefitinib was initially approved for non-small cell lung cancer, as it targets oncogenic EGFR. In vitro data suggest that EGFR contributes to RET kinase activation, signalling and growth stimulation. Conditional activation of RET/PTC oncoproteins in thyroid PCCL3 cells markedly induced expression and phosphorylation of EGFR, which was mediated in part through MAPK signalling.[27] RET and EGFR were found to coimmunoprecipitate. Ligand-induced activation of EGFR resulted in phosphorylation of a kinase-dead RET, and this effect was entirely blocked by EGFR kinase inhibitor. Gefitinib also inhibited cell growth induced by various constitutively active mutants of RET in thyroid cancer cells as well as in NIH3T3 cells.[27] This evidence has provided a biological basis for clinical evaluation of gefitinib in thyroid cancer. The results obtained in a phase II trial showed no objective responses among 25 patients with thyroid cancer treated with gefitinib.[36]

BRAF MUTATIONS AS A THERAPEUTIC TARGET

BRAF, together with ARAF and CRAF, constitute the RAF family of serine/threonine kinases. RAF proteins are intermediate members of the canonical MAPK/ERK pathway.[37] This pathway links extracellular signals to the cell, ultimately controlling cellular processes such as proliferation, differentiation, survival and apoptosis.[38] BRAF activation is accomplished by GTP-bound RAS. Active BRAF then phosphorylates MAPK/ERK kinase (MEK)1 and MEK2, which in turn activate ERK1 and ERK2, respectively. Such activation results in ERK translocation to the nucleus, where they trigger a multiplicity of regulatory proteins.[39]

More than 80% of activating BRAF mutations consist of a T to A transversion at nucleotide 1799, which leads to replacement of valine with glutamic acid at position 600.[40] Mutant BRAF is capable of stimulating ERK activity in vivo, independently of RAS, and shows high transforming capacity.[41] This evidence boosted BRAF to the category of a classical oncogene.

$BRAF^{V600E}$ mutation is the most prevalent oncogenic event in thyroid carcinoma and is tightly linked to PTC, which is the most common form of thyroid cancer.[1 5 6 12 42] In PTCs, $BRAF^{V600E}$ mutation frequencies range between 29% and 69%.[18 43 44] The nature and frequency of BRAF mutations were later found to be associated with different subtypes of PTC, ranging from very high prevalences in PTCs with an exclusive or predominantly papillary growth pattern to a much lower prevalence in FVPTCs (0–12%).[12 37 45–48] A different activating BRAF mutation, K601E, was almost exclusively found in cases of FVPTC.[5 49 50] Finally, another type of BRAF mutation, $BRAF^{VK600\text{-}1E}$, has been reported in a case of solid variant PTC, as well as in some metastases of conventional PTCs.[51] The $BRAF^{V600E}$ mutation is also present in some poorly differentiated thyroid carcinomas and in 20–30% of anaplastic thyroid carcinomas.[48 52 53] In anaplastic thyroid carcinomas, BRAF mutations are restricted to cases in which a well-differentiated PTC counterpart is presented, suggesting that BRAF mutations may play a role in the progression of thyroid carcinomas.[47]

BRAF mutations are associated with older age of patients,[47 48] extrathyroidal extension,[48 54] higher tumour staging[54] and tumour recurrence. Furthermore, it has been advanced that BRAF mutation is a negative prognostic marker, which may reflect, at least in part, the diminished radioiodine avidity of cells carrying such a mutation.[54] The prognostic significance of BRAF mutation is more difficult to prove if one takes into account the influence of other clinicopathological factors, namely the papillary or follicular growth pattern of the carcinomas.[48 55]

The role of mutant BRAF in thyroid cancer pathogenesis has been addressed in several studies. Targeted expression of $BRAF^{V600E}$ in thyroid cells of mice resulted in development of PTC lesions that could further progress to poorly differentiated carcinomas.[56] Taken together, the data indicate that BRAF and/or its downstream effectors are logical targets for the treatment of late-stage PTCs and poorly differentiated/undifferentiated carcinomas displaying the BRAF mutation (table 1).

Several strategies for reducing BRAF production or its activation have been reported, using either silencing techniques or small-molecule kinase inhibitors such as sorafenib.[57]

RNA interference methods that suppress the expression of oncogenic $BRAF^{V600E}$ cause inhibition of the MAPK signalling cascade and growth of human anaplastic thyroid carcinoma cell lines.[58] In $BRAF^{V600E}$-harbouring PTC cells, BRAF knockdown by RNA interference induced a decrease in proliferation and abrogated cell transformation and in vivo tumorigenicity.[58]

Protein kinase inhibitors such as the BRAF-targeted and multi-targeted kinase inhibitor, sorafenib, are currently the most promising agents for targeting BRAF activity. Sorafenib is a bis-aryl urea initially designed to target RAF-1,[30] which was found to have strong activity against BRAF and angiogenesis-related receptor tyrosine kinases such as VEGFR-2 and VEGFR-3, PDGFR-β, Flt-3 and c-Kit.[60]

Inhibition of $BRAF^{V600E}$ in several tumour cell lines (melanoma, breast, pancreas and colon) by sorafenib resulted in disruption of the MAPK–ERK pathway and inhibition of cell proliferation. Such effects prevented growth of colon tumour xenografts harbouring activating K-RAS and $BRAF^{V600E}$ mutations.[60] Besides kinase inhibition, the mechanism by which sorafenib controls tumour growth seems to depend on blocking

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angiogenesis through induction of apoptosis in the tumour vasculature.[61]

The anti-tumour effects of sorafenib have also been demonstrated in thyroid cancer models, inhibiting proliferation of anaplastic cell lines.[62] This effect is apparently independent of the presence of a $BRAF^{V600E}$ mutation and seems to result from blocking angiogenesis through disruption of VEGFR signalling.[62]

Four phase I studies using oral sorafenib as a single agent have been completed to date. An encouraging safety profile has been found, with the most common secondary effects being diarrhoea, fatigue, rash, palmar–plantar erythema, musculoskeletal pain and weight loss.[14 63] Currently, phase III clinical trials are being performed in patients with melanoma and advanced hepatocellular carcinoma, and phase II trials in patients with thyroid cancer.[64] Recently, a phase II study of sorafenib in patients with metastatic, iodine-refractory thyroid cancer (without molecular characterisation) showed that sorafenib has significant anti-tumour activity with an overall clinical benefit rate of ~80% and progression-free survival of 79 weeks, without significant toxic effects.[14] A potential problem of sorafenib is the multiplicity of targets; one cannot predict whether the activity of sorafenib is due to BRAF inhibition or disruption of any of its other multiple targets, such as VEGFR. Moreover, even though the array of inhibitory effects that occur in tumour cells may seem desirable, some of these effects may just as easily be toxic to the patient.[57]

Reports of acquired TKI drug-related resistance have been increasing, especially in lung cancer and leukaemia.[65–67] To overcome a similar effect, it has been suggested that sorafenib should be administered concomitantly with drugs that target other components of the MAPK–ERK cascade such as MEK, once this is the direct and main effector of BRAF.[68] MEK phosphorylation can be inhibited by the compound CI-1040 (PD-184352), producing inhibition of colon carcinoma growth in mice[69] and regression of melanoma-derived pulmonary metastases.[70] A recent study by Liu *et al*[59] showed that CI-1040 inhibits proliferation and induces cell cycle arrest of thyroid cancer cells, specifically in those harbouring $BRAF^{V600E}$ and RAS mutations, showing that MEK inhibition could be of particular importance in the therapeutic approach to thyroid cancer. CI-1040 has reached the clinical testing stage and is currently in phase II trials for patients with lung, colon, breast and pancreatic cancer. Sorafenib is also being tested in combination with other cytotoxic chemotherapeutic agents, such as doxorubicin, and has been used in combination with carboplatin to treat patients with melanoma.[71 72] So far, phase II trials have shown no improvement in the survival of these patients.[73]

Two other small-molecule inhibitors of RAF kinase, AAL881 and LBT-613, have also been tested for anti-tumour effects in anaplastic thyroid carcinoma cell lines and xenografts that harbour $BRAF^{V600E}$ mutations or RET/PTC rearrangements. Both compounds were capable of inhibiting MAPK activation, arresting the cell cycle in G1 phase and inhibiting growth of tumour xenografts.[74]

Another targeting strategy relies on interfering with BRAF protein stability by inhibiting chaperone and heat shock protein (Hsp) 90, to which BRAF binds.[75] Inhibition of Hsp90 by the benzoquinone, geldanamycin, and its less toxic analogues, 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-*N,N*-dimethylethylenediaminegeldanamycin (17-DMAG), causes disruption of the $BRAF^{V600E}$–Hsp90 complex, leading to its proteasome-dependent degradation.[76 77] 17-AAG has reached clinical testing and is currently in phase I/II.[78]

RAS MUTATIONS AS A THERAPEUTIC TARGET

RAS proteins are signal-switch molecules, which regulate cell fates by coupling receptor activation to downstream effector pathways that control diverse cellular responses such as proliferation, differentiation and survival.[79 80] Overall, mutated RAS alleles are found in ~30% of all human cancers.[81] When mutated, the RAS genes produce a protein that remain locked in an active state (bound to GTP), thereby relaying uncontrolled proliferative signals. In thyroid tumours, RAS gene mutations are particularly prevalent in FTAs and FTCs and less common in PTCs (for reviews, see Sobrinho-Simoes *et al*[18] and Kondo *et al*[82]). Their prevalence in PTCs varies widely from series to series, being relatively rare (0–16%) in conventional PTCs[7 8 83] and much more common (>25%) in FVPTCs.[11 84–86] RAS mutations are also common in poorly differentiated (55%) and anaplastic carcinomas (52%).[87] In the latter types of thyroid cancer, a significant association between RAS mutations and poor survival has been found, leading to the suggestion that RAS mutation may be considered a marker of aggressive behaviour.[87]

The relationship between RAS activation and chromosomal instability in thyroid tumours[88] has been recently reinforced by the finding of a significant association between H-RAS 81 T–C polymorphism, together with increased p21 (which is the active form of RAS), and the occurrence of aneuploidy.[89] In thyroid oncology, the correlation between aneuploidy and prognosis is not as clear as in other tumour models, but several studies have shown that the presence of aneuploidy is an adverse prognostic factor in thyroid carcinomas,[90 91] making further studies on H-RAS isoforms promising.

The result of activation of oncogenic RAS in thyroid cells is still debatable. Some studies have shown that RAS activation induces proliferation without loss of differentiation,[92] whereas others have shown that a high level of RAS expression induces both growth and loss of differentiation,[93] the dedifferentiation being dependent on the level of RAS expression.[93] It has also been reported that, in thyroid cells, RAS overexpression inhibits thyroid transcription factor 1 (TitF1) and PAX8 activity,[93] but the exact mechanism of this inhibition is not yet understood.

To the best of our knowledge there are no studies using RAS proteins as a direct molecular therapeutic target, but some ongoing studies are using different molecular approaches in an attempt to target the RAS pathway.

Post-translation modifications are crucial to the localisation of RAS proteins to the correct subcellular compartment and to their normal function. These post-translational modifications include prenylation, proteolysis, carboxymethylation and palmitoylation.[94 95] The crucial role of prenylation in the process turns the enzymes that catalyse the post-translational processing of RAS prime targets for drug design. One approach was to use farnesyltransferase inhibitors (FTIs), which simulate the CAAX motif to compete with RAS for its post-translational processing enzymes, thus blocking the first step of RAS modification and thereby inhibiting its activity.[96] Although very promising, both N-RAS and K-RAS were shown to become geranylgeranylated at their C-termini after FTI treatment, which rendered them refractory to inactivation by FTIs.[97] Meanwhile, there are ongoing clinical trials combining FTIs (R115777) with topotecan (a chemotherapy agent that is a topoisomerase 1 inhibitor) in patients with advanced solid tumours, previously treated or beyond standard treatment of clinical benefit (table 1).[98]

The problems with the FTIs forced the development of alternative strategies for blocking RAS function. Recent studies in mice suggested that RAS transformation is impaired in

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protease (RAS converting enzyme)-deficient animals.[99] This protease is responsible for the removal of the AAX.peptide, a critical step in the correct localisation of RAS. Elimination of RAS function by homologous gene recombination or antisense RNA has shown that expression of activated RAS is necessary for maintenance of the transformed phenotype of tumour cells.[100–102]

Mutant RAS oncogenes produce novel proteins that are processed and displayed through HLA molecules on tumour cells. Therefore, mutant RAS proteins are an attractive target for vaccine therapy, and there are ongoing clinical trials using this approach.[103 104]

MITOCHONDRIAL MUTATIONS AND DELETIONS AND METABOLIC PATHWAYS

Although the vast majority of human genes are located in the nucleus, there is one vital set of genes that reside in the cytoplasm, mitochondrial DNA (mtDNA). mtDNA is located in the mitochondria, which are double-membrane organelles responsible for producing most of the cellular ATP by oxidative phosphorylation (OXPHOS) in an oxygen-dependent process.[105–110] In addition to OXPHOS, cells can also produce ATP through glycolysis, which takes place in the cytosol and does not require O_2. OXPHOS is more efficient at generating ATP than glycolysis and therefore it is the preferred process, provided that there is enough O_2 available. Whenever there is a decrease in O_2 levels, there is a shift from OXPHOS to glycolysis and the ATP is generated mainly through glycolysis (Pasteur effect).[111] In the first half of the 20th century, Otto Warburg[112] made an outstanding discovery: cancer cells prefer to metabolise glucose by glycolysis, not using OXPHOS, even in the presence of O_2 (Warburg effect or aerobic glycolysis). He further hypothesised that this phenomenon was attributable to irreversible damage to OXPHOS in cancer cells.[112] The Warburg effect has since been demonstrated in different types of tumour, and the concomitant increase in glucose uptake has been exploited clinically for the detection of tumours by fluorodeoxyglucose positron emission tomography.[113] Although aerobic glycolysis has now been generally accepted as a metabolic hallmark of cancer, its cause and its relationship to cancer progression is still unclear.

One hypothesis to explain the above metabolic shift in cancer cells is related to defects in OXPHOS that push cancer cells towards glycolysis. In the past 10 years, mutations in mtDNA-encoded OXPHOS genes have been shown in most types of human tumour, including thyroid tumours.[114–124]

In 2000, Yeh *et al*[124] screened 25% of the entire mtDNA and reported the presence of point mutations in three out of 13 PTCs (23%). The prevalence of mtDNA mutations was also assessed by Maximo *et al*[125] in a series of 66 thyroid tumours, through direct sequencing of ~70% of the mitochondrial genome. They detected numerous mutations in all genes that encode OXPHOS proteins (except ATPase8), as well as three mutations in three tRNAs.[125] Combining the results of Yeh *et al*[124] and Maximo *et al*,[125] it appears that alterations in mtDNA genes affecting complex I may increase susceptibility to thyroid tumorigenesis. This assumption was later confirmed by several groups.[114–116]

Additional evidence for the involvement of OXPHOS complex I in thyroid tumorigenesis was provided by Maximo *et al*,[126] who analysed a nuclear gene, GRIM-19, that encodes a mitochondrial complex I protein,[127] in oncocytic and non-oncocytic thyroid tumours. They identified three GRIM-19 missense somatic mutations in three oncocytic cell thyroid tumours, as well as a germline mutation in an oncocytic cell thyroid tumour arising in a thyroid with multiple oncocytic cell nodules.[126] No mutations were detected in any of the 20 non-oncocytic cell carcinomas tested, nor in any of the 96 blood donor samples. It was proposed that such mutations may be involved in the genesis of sporadic or familial oncocytic cell thyroid tumours through the dual function of GRIM-19 in mitochondrial metabolism (as part of OXPHOS complex I) and cell death (being involved in retinoic acid-induced and interferon β-induced apoptosis).[126]

Classical oncogenes and tumour suppressor genes such as RAS and p53, involved in thyroid tumorigenesis, may also drive metabolic changes and promote glycolysis.[128–130] The altered metabolism of cancer cells may confer a selective advantage for survival and proliferation in the unique tumour microenvironment, an adaptation in which the hypoxia-inducible factor may play a central role.[128 131]

Although the cause of the metabolic shift toward glycolysis is not yet clear, the Warburg effect may at least be one "Achilles' heel" of cancer cells, as the glycolytic phenotype appears to be the common denominator of diverse molecular abnormalities. Understanding this phenomenon and its targeting may facilitate the treatment of cancer in several organs including the thyroid.[132–136]

The decreased efficiency of oncocytic cells with regard to iodine uptake and hormone synthesis explains the poor responsiveness to radioiodine therapy of oncocytic cell tumours. It has thus been proposed that the treatment of WDTCs with oncocytic cell features may benefit from the discovery of drugs that reverse the Warburg effect.[119 137]

Take-home messages

- The increasing knowledge of the molecular pathways involved in thyroid carcinogenesis provides alternative therapeutic strategies to the current standard treatments (thyroid ablation and radioiodine therapy).
- The hallmarks of well-differentiated thyroid carcinoma (WDTC), such as RET/PTC rearrangements, BRAF and RAS mutations, as well as metabolic defects that are common to most human cancers, are obvious candidates for molecular-targeted intervention.
- Drugs that target such molecular pathways could be useful to treat highly aggressive forms of thyroid cancer such as undifferentiated cancers, particularly those that harbour common genetic defects to WDTC (eg, BRAF mutations).
- Current drugs show promising results in vitro, but most fail to prevent cancer progression in clinical trials, also because of tumour-acquired drug resistance.
- The most promising approaches rely on targeting multiple oncogenic events. For this, it will be necessary to use in vitro cell-based screens and then validate the combinations found in realistic animal model systems.

CONCLUDING REMARKS

As recently stressed by Pfister and Fagin,[13] for many years human thyroid cancers have received very little attention with regard to the use of novel treatments. As reported here, this situation is rapidly changing, partly because many of the molecular pathways involved in thyroid carcinogenesis have now been revealed, providing new therapeutic targets, and

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partly because of the extension to thyroid cancer of drugs developed for the treatment of other cancer types.

We are convinced that the current trend of using massive high-throughput approaches to disclose new targets will not be fruitful unless we can integrate the huge amount of available information into a system biology frame. We also think that metabolic approaches via mitochondria and other more biology-driven targets may prove useful, especially if it proves possible to integrate these approaches in an organismal biology model of cancer development. Finally, we believe that, regardless of the approach used in the treatment of radioiodine-resistant thyroid cancers, it will be necessary to use, together with molecular signatures, in vitro cell-based screens and then to validate the combinations thus found in realistic animal model systems.

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How molecular pathology is changing and will change the therapeutics of patients with follicular cell-derived thyroid cancer

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