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Molecular pathology of thyroid cancer: diagnostic and clinical implications

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Abstract

There is now a reasonably good understanding of the key oncogenic events involved in the initiation and progression of thyroid cancer. Many of these are characteristic of certain tumor types, and their presence conveys diagnostic and prognostic information. It is not yet clear how this information will be applied to clinical practice. Based on preclinical evidence, mutations of genes encoding certain kinases may also predict response to specific tyrosine kinase inhibitors, although this has not yet been explored systematically in clinical trials.

Keywords

thyroid cancer; pathogenesis; oncogene; RET; BRAF; RAS; molecular diagnostics; PTEN; PI3 kinase

The genotype of cancers determines their biological behavior and in some cases their response to specific therapies. This has also been demonstrated in thyroid cancers. This chapter will focus on a discussion of the most significant oncogenic defects found in the different thyroid malignancies, and draw from clinical and experimental evidence to explain their role in disease pathogenesis. Some of these cancer genes provide important prognostic information, an issue that will be highlighted since tumor genotyping may soon become a useful tool for management of patients with this disease. We will focus on thyroid cancers as opposed to benign thyroid disease, with the exclusion of medullary thyroid cancer which is discussed elsewhere in this volume. Readers are also referred to a more extensive review on this topic that was published recently.(1)

PAPILLARY THYROID CANCER (PTC)

PTCs represent approximately 80% of all thyroid malignancies. The overall incidence of this particular form of thyroid cancer is rising, for reasons that remain unclear but that in part may reflect earlier diagnosis.(2) As a rule these are slow-growing tumors that lead to progressive disease relatively infrequently. The pathological diagnosis of PTC relies primarily on the nuclear morphology of the cells. Different variants are described, depending on certain

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combinations of growth patterns, cell types and stromal reactions. Some of these, such as the tall-cell or the follicular variants, are associated with particular biological, clinical and prognostic features that will not be described here in detail, but that have been comprehensively summarized elsewhere.(3)

Molecular genetics of papillary thyroid cancer

The RET fusion oncogenes—Many of the tumor-initiating genetic events associated with PTC are known. Notable among them are the *RET/PTC* oncogenes, which play a causative role in the pathogenesis of a significant proportion of PTCs.(4) The *RET* gene encodes the signaling subunit of a receptor complex for ligands of the glial-derived neurotrophic factor family (GFL).(5) *RET* is normally expressed at very low levels in thyroid follicular cells. Chromosomal rearrangements linking the promoter and N-terminal domains of unrelated gene(s) to the C-terminal kinase domain of *RET* result in the aberrant production of chimeric forms of the receptor (RET/PTC) in thyroid cells that are constitutively active. Twelve forms of *RET/PTC* have been reported so far, and these vary according to the identity of the upstream fusion partner of *RET*.(6)

RET/PTC rearrangements are particularly common in PTCs from individuals exposed to ionizing radiation and in pediatric cancers.(7) In sporadic PTCs from adult patients, the frequency of *RET* rearrangements is lower (~20%) and appears to vary in different geographical regions. RET/PTC1 and RET/PTC3 are the most common RET oncoproteins, and they are formed by fusion of *RET* to the *H4* (*D10S170*) or the *NCOA4* (*ELE1*) genes, respectively. Multiple lines of evidence suggest that *RET/PTC* rearrangements may be a very early event in thyroid cancer development. Thus, there is a high prevalence of RET/PTC expression in occult or microscopic PTC.(8,9) Moreover, the disease can be recreated in transgenic mice by over-expression of either RET/PTC1(10,11) or RET/PTC3(12) in thyroid cells. In addition, exposure of cell lines(13) and fetal thyroid explants(14) to ionizing radiation results in expression of RET/PTC within hours, supporting a direct role for radiation in the illegitimate recombination of *RET*. If RET/PTC oncoproteins are responsible for tumor initiation in these cancers, then all cells in the tumor clone should harbor the recombination. However, recent studies indicate that at least some thyroid cancers are oligoclonal for RET/PTC,(15,16) suggesting that the recombination may have arisen later in tumor progression or as a result of distinct clonal events.

Numerous studies have examined whether papillary thyroid cancers with *RET/PTC* rearrangements have peculiar pathological or clinical characteristics. In post-Chernobyl pediatric cancers RET/PTC1 was found to be associated with classical PTC, whereas RET/PTC3 was seen in solid-variant PTCs.(7) Although there are indications from the literature that PTCs harboring RET/PTC oncogenes may have a more favorable outcome,(17,18) the data are confounded by the different methodologies used to detect RET in some of the studies, and the small number of cases analyzed in most reports. Nevertheless, it is reasonably clear that RET/PTC rearrangements are present in only a comparatively small fraction of poorly differentiated thyroid cancers,(17,19) suggesting that this oncoprotein may not confer a high risk of disease progression.

Like other tyrosine kinase receptor oncoproteins, RET/PTC engages a complex network of signaling pathways. The transforming properties of RET are abrogated in vitro by replacement of the Y1062 residue in the RET kinase domain, which is a multi-docking site for Shc, ShcC, IRS1/2, FRS2, DOK1/4/5, and Enigma.(20,21) Formation of these protein complexes at the level of Y1062 leads to stimulation of the Ras/ERK and PI3K cascades. Recent genetic evidence has focused attention on the role of the Ras/Raf/MAP kinase pathway in PTC development, because of the observation that activating point mutations of *BRAF* and *RAS* are also highly prevalent and mutually exclusive with *RET* in PTC. Altogether, mutation of either

RET, *TRK*, *NRAS*, *HRAS*, *KRAS* or *BRAF* is seen in ~70% of PTCs, with little or no overlap between them.(22–24)

RAS oncogenes in PTC—The four classical Ras proto-oncogenes encoding Hras, KrasA, KrasB and Nras belong to an extended family of small G proteins. Ras proteins are plasma membrane GTPases activated by growth factor receptors, non-receptor tyrosine kinases, and to lesser extent G-protein-coupled receptors, leading to activation of downstream effector pathways. The molecular basis for oncogenesis by Ras is due to mutations that favor their constitutively active, GTP-bound conformation. As will be discussed in the corresponding section, *RAS* mutations are highly prevalent in follicular adenomas and carcinomas. However, they are also seen in PTC(25,26) with an overall prevalence of 10–15%, particularly in follicular variant PTCs.(27) There are some indications that PTCs with *RAS* mutations may have a more aggressive clinical course, but this has not been proven conclusively. Although mutations of all *RAS* genes have been reported in thyroid cancer, the most commonly observed are in codon 61 of *NRAS* and to a lesser extent in *HRAS*.(28)

The BRAF oncogene and its role in thyroid cancer—There are three isoforms of the serine–threonine kinase Raf in mammalian cells: ARaf, BRaf, and CRaf or Raf1. CRaf is expressed ubiquitously, whereas BRaf is expressed at higher levels in hematopoietic cells, neurons and testis.(29) BRaf is also the predominant isoform in thyroid follicular cells (Zhang L and Fagin J, unpublished). Although all Raf isoforms activate MEK, they are differentially activated by oncogenic Ras. In addition, BRaf has higher affinity for MEK1 and 2 and is more efficient in phosphorylating MEKs than other Raf isoforms.(30) Interestingly, activation of ERK by mitogens is not affected in fibroblasts from C-Raf$^{-/-}$ mice, which is consistent with other reports indicating that in cells that express BRaf, this isozyme is the major activator of MEK.(31) This is also the case in thyroid cells.(32)

The *BRAF*^{T1799A} mutation is the most common genetic change in PTC. As in melanomas, >95% of the mutations result from T-to-A transversions at position 1799, resulting in a valine-to-glutamate substitution at residue 600 (V600E). The oncogenic mutations in the activation loop or the P loop of Braf disrupt the interaction between both lobes of the protein and destabilize the inactive conformation of the kinase. Most (but not all) known oncogenic BRaf substitutions allow the formation of new interactions that fold the kinase into a catalytically competent structure.(33)

In addition to point mutations, *BRAF* can also be activated by rearrangement. This novel mechanism of *BRAF* activation occurred via paracentric inversion of chromosome 7q, resulting in the in-frame fusion between exons 1–8 of the *AKAP9* gene and exons 9–18 of *BRAF*. The fusion protein contains the protein kinase domain and lacks the autoinhibitory amino-terminal portion of BRAF. AKAP9–BRAF has elevated kinase activity and transforming activity comparable with the most common BRAF^{V600E} oncoprotein. *AKAP9–BRAF* was found in 11% of PTCs that developed 5–6 years after exposure to ionizing radiation, and in 0–1% of tumors without radiation history or those that developed 9–12 years after exposure.(34) Thus, *AKAP9–BRAF* is found primarily in tumors associated with recent history of radiation exposure. Thus, prior exposure to ionizing radiation during childhood predisposes to development of PTC with *RET* rearrangements and, to a lesser extent, with *NTRK* or *BRAF* intrachromosomal inversions. By contrast, point mutations of *BRAF* are rare in this population, as well as in PTCs of children, with an overall prevalence of 4–6%.(35)

Apart from PTC, *BRAF* mutations are not found in any other form of well-differentiated follicular neoplasm.(22) The overall prevalence of *BRAF* mutations in PTC is ~45%.(22,24, 36–41) *BRAF* mutations can occur early in tumor development, as they are present in microscopic PTCs.(37) Although the majority of PTCs with *BRAF* mutations have a classical

histological appearance, tall-cell-variant PTCs, regarded as more aggressive, have a particularly high prevalence of *BRAF* mutation.(37) Most(37,42) but not all(43) studies show that PTCs with *BRAF* mutations present more often with extrathyroidal invasion and at a more advanced stage. A meta-analysis of data from numerous studies strongly supports the negative prognostic implications of *BRAF* in thyroid cancer,(44) including a greater frequency of neck lymph-node and distant metastases.

Human papillary thyroid cancers with *BRAF* mutations exhibit a marked decrease in expression of genes required for thyroid hormone biosynthesis, including thyroid peroxidase, thyroglobulin, and the sodium iodide symporter (NIS).(45–48) There are also indications that NIS may be mis-localized away from the plasma membrane in tumors expressing oncogenic BRAF.(48) This would predict that tumors with *BRAF* mutations would be more likely to become refractory to radioactive iodine treatment, which appears to be the case.(42)

A mouse model of PTC induced by thyroid-specific over-expression of oncogenic Braf recapitulates virtually all aspects of the disease, including the profound decrease in expression of genes involved in iodine metabolism and thyroid hormonogenesis.(49) It remains to be seen if this process can be reversed, at least in part, with compounds that interfere with Braf signaling, primarily via MEK and ERK. There is clear evidence from preclinical studies that selective MEK inhibitors preferentially inhibit growth of thyroid cancer cell lines with *BRAF* mutations,(50) but whether these compounds can reverse the impairment in iodine transport in a clinically relevant manner remains to be seen. The role of kinase inhibitors in thyroid cancer therapeutics will be discussed elsewhere in this volume.

Research agenda

there is a need to know whether there is a clinical benefit to be derived from routinely genotyping PTC, for example to guide the approach to therapy; for instance:

- should tumors with *BRAF* mutation have more extensive primary surgery?
- should the approach to radioiodine ablation or therapy differ in PTCs with *BRAF* mutation?
- studies are also needed to determine whether there is a genotype-specific response to the use of specific kinase inhibitors in the treatment of PTCs with specific oncogene mutations

FOLLICULAR THYROID CANCER (FTC)

Follicular carcinomas account for 10–15% of thyroid cancers. They are usually unifocal, and have a much lower frequency of lymph-node involvement (<5%) than PTC. By contrast, distant metastases, primarily to lung and bone, are comparatively more frequent at presentation (~20%).(3)

Molecular genetics of follicular thyroid cancers

PAX8–PPARγ rearrangements—About 35% of FTCs are associated with the interchromosomal translocation t(2,3)(q13;p25), which fuses promoter elements of the gene encoding paired box 8 (*PAX8*) with most of the coding sequence of the peroxisome proliferator-activated receptor γ (*PPARγ*) gene. Initially this fusion protein was thought to be unique to FTC, and to a lesser extent oncocytic (Hurthle-cell) carcinomas;(51) however, later studies have proved that it is also found in a subset of follicular adenomas,(52,53) although at a lower frequency.

The functional consequences of expression of Pax8–PPARγ are not fully understood. In the original report, Kroll et al found that the fusion protein could not activate transcription of reporter genes in response to thiazolidinedione ligands, and instead functioned as a dominant negative inhibitor of wild-type PPARγ-induced transactivation.(51) This concept was supported in a subsequent study performed in an immortalized human thyroid cell line (Nthy-ori 3-1) in which the fusion protein induced cell growth and transformation, and interfered with the function of wild-type PPARγ. The biological effects of the fusion protein were recapitulated by treating the parental line with PPARγ-specific inhibitors.(54) These data suggest that the primary mechanism of transformation is through loss of function of the wild-type nuclear receptor. These findings were challenged by Au et al, who found that PAX8–PPARγ disrupts normal transcriptional regulation of genes regulated by PAX8, as well as PPARγ, but does so in a context-dependent fashion, so that some genes are regulated by the fusion protein while others are not.(55) This is supported by expression-profiling studies of human thyroid cancers with the *PAX8–PPARγ* translocation, in which several genes that are transcriptional targets of PPARγ were found to be up-regulated compared to FTCs not harboring the rearrangement.(56)

As will be discussed below, mutations of *RAS* are highly prevalent in FTC. Nikiforova et al found that *RAS* and *PAX8–PPARγ* recombinations are mutually exclusive, and therefore may represent distinct molecular pathways of FTC development.(57) The impact of *PAX8–PPARγ* on the biology and behavior of FTCs is controversial.(57–59)

RAS mutations in FTC—*RAS* mutations are found in 20–50% of follicular thyroid cancers (60) with the following relative frequency: *NRAS* >*HRAS* >*KRAS* (http://www.sanger.ac.uk/genetics/CGP/cosmic/, August 2008). The presence of *RAS* mutations in both follicular adenomas and carcinomas has been taken as evidence that Ras activation may be an early step in thyroid carcinogenesis, although this has not been conclusively proved. This is supported by the fact that the disease can be recapitulated in mice with targeted over-expression of *N-RAS*^{Q61K}.(61) One of the characteristic features that distinguish follicular neoplasms from PTC is that the former have a high prevalence of aneuploidy, whereas PTCs are usually diploid or near-diploid. Oncogenic Ras activation has been shown to promote genomic instability in thyroid cells in vitro.(62,63) However, although a correlation between *RAS* mutations and aneuploidy has been demonstrated in colorectal tumors,(64) no such association has been found in thyroid neoplasms…(1) However, a polymorphism in the *HRAS* gene (T81C) did correlate strongly with aneuploidy in follicular tumors, which the authors proposed could be due to increased expression of the proto-oncogene.(65)

Research agenda

in view of the complex way in which *PAX8–PPARγ* interferes with the transcriptional activity of wild-type PPARγ (and wild-type Pax8), is there a role for thiazolidenediones in the treatment of FTC that express the fusion protein?

do FTCs with *RAS* mutations become dependent on the activity of the oncoprotein for viability, and if so, which is the critical signaling pathway downstream of Ras that mediates the effect, and can this be targeted with specific kinase inhibitors?

HÜRTHLE OR ONCOCYTIC CELL CANCER (HCC)

Although this tumor has been considered by some as a distinct clinical entity, the current WHO/AIRC (Italian Association for the Research against Cancer) classification describes HCC as a variant of FTC(3) characterized by the aberrant accumulation of mitochondria. HCCs have distinct biological and clinical behaviors. They are more frequently refractory to treatment with

radioactive iodine, and have a greater predisposition to develop lymph-node metastases than FTCs.(1)

Molecular genetics of HCC

The pathogenetic hallmark of this disease is the presence of mitochondrial protein defects encoded by both mitochondrial (mtDNA)(66–68) and nuclear genes.(69,70) A recently published study in which the entire mitochondrial genome was sequenced in a large number of oncocytic thyroid tumors and appropriate controls clearly showed that disruptive mutations in complex-I-subunit mtDNAs are highly prevalent in these neoplasms.(67) The mutations are present in most or all mitochondria in these tumors, likely resulting in a profound disruption of oxidative phosphorylation. Somatic mutations of *GRIM-19*, a nuclear gene encoding a protein involved in complex-1 mitochondrial function and in cell death, were observed in two Hürthle-cell PTCs, and a *GRIM-19* germline mutation was found in a patient with familial HCC. Although mitochondrial defects are a peculiar feature of oncocytic tumors, there is some evidence suggesting that dysfunction of the mitochondrial respiratory chain (MRC) may be acquired during thyroid-cell transformation. For instance, conditional activation of oncogenic BRAF results in down-regulation of a cluster of enzymes in complex 1 of the MRC in thyroid cells,(71) suggesting that their functional impairment may be an important event in thyroid tumorigenesis.

POORLY DIFFERENTIATED CARCINOMA (PDTC) AND ANAPLASTIC CARCINOMA (ATC)

PDTCs have a degree of severity intermediate between that of differentiated and anaplastic carcinomas, and are characterized by an infiltrative pattern of growth, necrosis, a higher mitotic index, and vascular invasion.(3,72) ATCs, also called undifferentiated thyroid carcinomas, are highly malignant tumors composed of undifferentiated cells retaining markers of an epithelial lineage. They are usually large, markedly invasive, and consist of admixtures of spindle, epithelioid and pleomorphic giant cells.(3) The fact that ATCs are massively infiltrated with macrophages may contribute in part to their heterogeneous appearance.(73)

Molecular genetics of PDTC and ATC

Mutations of genes encoding effectors in the MAP kinase pathway—There is a high prevalence of mutations of *RAS* genes in PDTC (40–55%);(19,74,75) by contrast, *BRAF* mutations are less frequent (12–17%).(19;74) Patients with PDTC harboring *BRAF* mutations have a higher cause-specific mortality than those with *RAS* or with no known mutation,(19) attesting to the negative prognostic impact of *BRAF* at all stages of progression of thyroid cancer.

In contrast, *BRAF* mutations are the predominant oncogenic defect within the MAP kinase pathway in ATC, with a frequency of 25–50%.(19,74,76–78) *RET/PTC*, *NTRK* and *PAX8–PPARγ* are only rarely seen in PDTC or ATC, supporting the notion that differentiated thyroid cancers associated with these rearrangements do not usually progress to PDTC or ATC.(1, 74)

Mutations of genes encoding effectors in the phosphatidyl inositol-3′-kinase (PI3K) pathway—The PTEN/PI3K/Akt pathway regulates fundamental cellular processes, including glucose metabolism, cell survival, cell cycle progression, adhesion, and motility. Activating mutations of the gene encoding the catalytic subunit p110α of a class-1A PI3K were first reported in colorectal, ovarian, brain, gastric and breast cancers, with the majority of the mutations occurring within the helical and kinase domains of the protein.(79,80) Garcia-Rostan et al reported a relatively low prevalence of *PIK3CA* mutations in well-differentiated PTCs

(2%) and FTCs (15%), and a higher prevalence in ATCs (23%). *PIK3CA* mutations coexisted with *RAS* or *BRAF* mutations in these cancers. Copy gains of *PIK3CA* have also been reported in well-differentiated PTC and in ATC, although it is not clear whether these are of pathogenetic significance or a result of widespread chromosomal imbalances.(78,81,82) The serine–threonine kinase AKT1 is a key effector of PI3K. Recently, a somatic mutation in *AKT1* (E17K) has been detected in breast, colorectal, lung and ovarian cancers.(83) The E17K change lies within the pleckstrin homology domain of the protein, resulting in constitutive AKT1 activation, and induces leukemia in mice. *AKT1* (E17K) mutations are found in PDTC, and do not overlap with PIK3CA mutations, suggesting that they represent alternative mechanisms to activate the pathway in advanced forms of the disease. They are commonly associated with *BRAF* mutations in PDTC.(19)

Cowden disease is an autosomal dominant syndrome caused by germline loss-of-function mutations of *PTEN*, which leads to hamartomas of the skin, intestine, breast, and thyroid, and increased risk of developing breast, thyroid, and endometrial carcinomas.(84) *PTEN* encodes a lipid phosphatase that removes a phosphate group from the inositol ring of PIP3, hence inhibiting the PI3K pathway and preventing Akt activation. Benign and malignant thyroid lesions occur in 50–75% of patients affected by the syndrome,(85,86) with a lifetime risk of thyroid cancer of approximately 10%. Loss of *PTEN* activity also plays a role in sporadic thyroid carcinomas. Intragenic *PTEN* mutations are rare, particularly in well-differentiated thyroid cancers,(87,88) yet loss of heterozygosity (LOH) at the *PTEN* locus is seen in up to 25% of follicular adenomas.(88) Furthermore, decreased expression of the PTEN protein, possibly occurring through promoter hypermethylation,(89) is common in ATC and less frequent in FTC and PTC samples.(88) Also, inappropriate subcellular compartmentalization of PTEN, favoring cytoplasmic sequestration instead of nuclear localization, may interfere with its activity.(90)

Taken together, the activation of the PTEN–PI3K–AKT pathway appears to play a prominent role in advanced forms of the disease, and hence likely represents an important focus for development of targeted therapies.

p53 mutations in PDTC and ATC—The *TP53* gene encodes a nuclear protein that induces cell-cycle arrest, senescence, and/or apoptosis in response to diverse noxious stimuli. A defective p53 pathway can contribute to carcinogenesis, disease progression, and resistance to anticancer therapy. In thyroid carcinomas, *p53* mutations are rare or completely absent (0–9%) in well-differentiated carcinomas,(91,92) including those that are radiation-related,(93) but common in poorly differentiated (17–38%) and particularly in anaplastic carcinomas (67–88%).(91,92,94) Therefore, it appears that in the course of thyroid carcinogenesis, *TP53* mutations are a late event and are associated with tumor progression and a more aggressive phenotype, highlighting the opportunity for p53-based therapy for poorly differentiated and anaplastic thyroid carcinomas in the future.

BETA-CATENIN AND THE APC PATHWAY IN THYROID CANCER

The β-catenin protein, encoded by *CTNNB1*, functions in both cell adhesion and transcription. In normal cells, β-catenin is mostly bound to cell membrane cadherins at adherens junctions, thus fulfilling its essential role in cell adhesion, and remaining sequestered from the nucleus and its growth-promoting role.(95) Also β-catenin is a critical component of the Wnt signaling pathway, which is crucial during embryonal development and is also activated in various human cancers. The cellular abundance of β-catenin is constitutively down-regulated by proteasomal degradation. A multicomponent complex that includes APC (encoded by the *APC* gene, which is inactivated in familial adenomatous polyposis) binds β-catenin and recruits two kinases, glycogen synthase kinase-3 (GSK) and casein kinase I, which phosphorylate β-

catenin and target it for polyubiquitination and degradation by the proteasome. The Wnt signaling pathway stabilizes β-catenin by inhibiting its phosphorylation by GSK-3 and its subsequent proteosomal degradation, allowing β-catenin to translocate to the nucleus and function as a transcriptional Wnt effector.

The nuclear localization and transcriptional growth-promoting activity of β-catenin can be enhanced when its binding to cadherin is abolished (e.g. due to decreased cadherin expression), or when GSK3β-axin-APC-mediated targeting for degradation is defective (e.g. due to inactivating mutations in *APC*, or by *CTNNB1* mutations that disrupt phosphorylation sites and lead to protein stabilization), or by an overactive Wnt pathway.(96) Germline mutations in the *APC* gene are responsible for familial adenomatous polyposis (FAP) and its variant, Gardner syndrome, which confers a markedly increased risk of development of PTC.(97)

E-cadherin expression is high in normal thyroid tissue, but decreased in undifferentiated thyroid carcinomas.(98) Mutations of β-catenin, leading to nuclear localization of the protein, are present in poorly differentiated and anaplastic carcinomas (up to 25% and 65% respectively) but not in well-differentiated tumors.(99,100) Thus, both loss of E-cadherin expression and acquisition of β-catenin mutations appear to be associated with thyroid carcinoma dedifferentiation and disease progression.

CONCLUSION

Use of molecular genetic information for preoperative diagnosis

As some of the most prevalent thyroid oncogenes are found exclusively (i.e. *BRAF*, *RET/PTC*) or with high frequency (*RAS*, *PAX8–PPARγ*) in malignant tumors, several groups have explored whether screening for mutations improves the diagnostic accuracy of cytopathology after fine-needle aspiration of thyroid nodules.(101–106) Although molecular diagnostics is controversial in this setting,(107) the preponderance of the evidence suggests that it could become a useful adjunct to traditional morphological cytopathology, at least in selected cases. Other approaches, such as immunohistochemical staining for proteins expressed preferentially in cancer (e.g. galactin 3), have also shown significant potential.(108,109)

Expression profiling with oligonucleotide microarrays can discriminate between different tumor types with great accuracy by taking advantage of the combinatorial power of globally analyzing patterns of gene expression. Subsets of genes with the greatest discriminatory power can then be selected for development of diagnostic tools. This has been applied to thyroid nodule cytopathology by several groups, with promising results.(110–115)

At this point it is not clear which of these different approaches, if any, will be applied in clinical practice. In general, DNA-based assays will likely be more robust, but any of these methods could theoretically be optimized and used with some success.

Use of molecular genetic information for therapeutic decisions

We alluded to the fact that important prognostic information can be obtained from knowing the specific genetic mutations associated with thyroid cancer. The role of *BRAF*, in particular, is receiving considerable attention, but at this time we cannot provide firm recommendations on how this information should be used in practice. Similarly, several trials of multi-kinase inhibitors in advanced thyroid cancer have recently been reported.(116–118) Based on information from other cancer types, where genetic information predicts the response to specific kinase inhibitors – e.g. *EGFR* and *KRAS* mutations as positive and negative predictors, respectively, of response to gefinitib or erlotinib in non-small-cell lung cancer(119) – it is likely that this will also be the case in thyroid cancers, but the trials published so far were not designed to address these questions.(120)

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