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MOLECULAR MARKERS OF AGGRESSIVENESS OF THYROID CANCER

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Abstract

Purpose of review—Review recent progress at defining molecular markers that predict the biological behavior of thyroid cancer.

Recent findings—Thyroid cancer behavior is defined by the effects of the initiating oncogene as well as secondary events in tumor cells and the tumor microenvironment that are both genetic and epigenetic. These events together play an important role in regulating thyroid cancer behavior. Over the past several years, there has been intense focus on identifying molecular markers to better predict the aggressiveness of thyroid cancers and also define therapeutic targets. The results of recent articles in this area of work are summarized with a focus of differentiated follicular-cell derived forms of thyroid cancer.

Summary—Clinical staging predicts tumor behavior in many cases, but does not allow for true "personalization" of initial therapy or identify potential therapeutic targets for patients with progressive disease that does not respond to standard therapies. Recent data point to several new opportunities to refine thyroid cancer treatment based on molecular information. Several highlighted articles have begun to apply this information with clinical intent.

Keywords

BRAF; PI3 Kinase; tyrosine kinase inhibitors; microRNA; methylation

INTRODUCTION

Thyroid cancer incidence has been increasing due in part to the wide availability of thyroid ultrasound and ultrasound-guided fine needle aspiration of non-palpable thyroid nodules [1]. While the mortality rate from thyroid cancer has been stable, the absolute number of individuals who die from thyroid cancer has been increasing annually and the number of patients with larger tumors is also increasing [2]. The identification of markers that predict the behavior of thyroid cancer for individuals is crucial to avoid over-treating the majority of patients with more benign forms of the disease and under-treating the minority of patients with more aggressive forms of thyroid cancer. A more refined approach toward "personalized" medicine based on the molecular characteristics of thyroid cancers may also allow for better selection

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of patients for clinical trials using targeted therapies. In this review, the recent advances in identifying molecular predictors of aggressive thyroid cancer behavior will be summarized.

CLINICAL STAGING AND IMAGING IN PREDICTING THYROID CANCER BEHAVIOR

Clinical staging systems are used to define cancer prognosis and thereby aid in decision-making [3]. Thyroid cancer presents several unique challenges for staging systems designed to identify groups of patients by risk of death from disease. These include the relative rarity of deaths due to thyroid cancer over a 5-10 year follow-up, differences in survivorship based on patient age, and differences in the biological behaviors of thyroid cancer histological subtypes. Unlike many other cancers, most patients with residual or recurrent thyroid cancer live for decades with relatively stable persistent disease, including patients with distant metastases. Because patients with progressive metastatic disease have a poor prognosis, identifying predictors of disease progression and potential new therapeutic targets is critical.

Several pathological features have been shown to predict aggressive thyroid cancer behavior, including large tumor size, gross tumor invasion, the presence of bulky distant metastases, and tumor dedifferentiation. However, in the absence of these features, prognosticating outcomes in thyroid cancer is difficult, even for patients with widely metastatic disease. Several radiographic features help stratify patients with distant metastases. Several authors have demonstrated for example that patients with large pulmonary nodules, skeletal, or central nervous system metastases, or metastases that do not demonstrate iodine uptake typically have an aggressive clinical course [4,5]. In addition, the presence of ^{18}FDG PET-positive distant metastases has also been associated with a more aggressive clinical behavior [6]. The recent expansion of knowledge regarding the molecular pathogenesis of thyroid cancer and the associations between particular genetic and epigenetic abnormalities with a more aggressive course may allow a more refined approach to predicting tumor behavior and/or response to therapy.

CANDIDATE GENE AND PATHWAY IDENTIFICATION OF MARKERS OF BIOLOGICAL BEHAVIOR IN THYROID CANCER

There are several approaches to identifying molecular markers of aggressive tumor behavior ranging from analysis of candidate genes or pathways to global analysis of gene copy number and expression, or a combination of both. In this section, recent data evaluating several important candidate pathways will be reviewed. There are other pathways not included in this review that have also been studied.

GENETIC CHANGES IN THE RAF/MEK/ERK PATHWAY AS MARKERS OF PAPILLARY THYROID CANCER BEHAVIOR

Papillary thyroid cancer (PTC) is the most common histological subtype of thyroid cancer. *RET/PTC* genetic rearrangements that result in constitutive signaling through the Ret tyrosine kinase are the most common oncogenes in radiation-exposure related and childhood PTCs. These mutations result in an identifiable gene expression profile [7] and have been associated with a more benign clinical course [8]. By contrast, the most common identified somatic gene mutations in adults with PTC are activating mutations in *BRAF* and these have largely been associated with a more aggressive clinical course. *BRAF* encodes a serine-threonine kinase that is a critical signaling node in the RAS-MEK-ERK cascade. Mutations in *BRAF* are not generally found simultaneously in the same tumors that harbor *RET/PTC* rearrangements or *RAS* mutations. *BRAF* V600E and *RET/PTC* result in uncontrolled activation of the MEK-ERK signaling cascade [9,10]. Expression of RET/PTC 1, 3, and BRAF V600E all reduce thyroid

cell differentiation including iodine uptake, induce malignant transformation of thyroid cells, and cause thyroid cancer in vivo; recent data has suggested this may be a more prominent effect with expression of BRAF V600E [11]. In both cases, these effects have been shown to be dependent on signaling through MEK [12] leading to a model in which. constitutive activation of the ERK pathway is the primary pathway that leads to PTC [13].

Overall, the frequency of activating *BRAF* mutations in PTC ranges from ~20-80% depending on the study population, with an overall frequency of ~40% when combining studies [14]. Mutations in *BRAF* have been described both in microPTCs less than 1 cm in size (~30%), and in PTCs with classical and tall cell variant (TCV) histologies, with a particularly high incidence in the latter. They are less common in follicular variant of PTC. Indeed, most, but not all studies demonstrate that PTCs that harbor an activating mutation in *BRAF* are associated with a more aggressive tumor staging and/or clinical course [14]. This has been reaffirmed with recent data from several populations [15-17]. In further support of the association between *BRAF* mutation and an aggressive course in PTC is the high rate (>70%) of *BRAF* mutations in tumor tissue of patients enrolled in a recent clinical trial for treatment- resistant differentiated thyroid cancer [18]

The combination of an association with a more aggressive course and a reduced tendency to concentrate radioiodine have led some authors to recommend more aggressive surgical intervention, such as routine central neck dissections at the time of total thyroidectomy, for patients with PTCs harboring activating mutations in *BRAF*, even if the tumors are very small [19]. In support of this approach, one recent study demonstrated on multivariate analysis the detection of a V600E *BRAF* mutation on fine needle aspiration (FNA) was associted aggressive findings on surgical pathology, including nodal metastases and local invasion [20]. Whether or not more extensive surgery is beneficial for patients with *BRAF* mutation-positive microcarcinomas will require robust clinical trials that balance a potential reduction in tumor recurrence versus a possible increase in surgical complications. Now that mutation screening using clinically dispensable FNA material has been shown to be feasible in clinical practice [20,21], the role of genetic testing in improving outcomes for patients with PTCs can be rigorously tested in clinical trials. In addition to a role in personalizing initial therapy, the presence of a *BRAF* mutation may predict response to particular pharmacological agents, such as BRAF or MEK inhibitors. Indeed, preclinical studies with MEK inhibitors showed that cell lines with activating BRAF mutations had better responses than those with RAS mutations [22,23]. A clinical trial using a specific inhibitor of MEK in PTC is currently ongoing but has not yet been completed.

Finally, it is important to recognize that a variety of non-gene mutation alterations can also lead to activation of the RAS/RAF signaling cascade. Epigenetic regulation of gene expression through hypermethylation of CPG islands, alterations in histone modifications, and expression of microRNAs all have been shown to be abnormal in thyroid cancers. For example, a hypermethylation-mediated reduction in expression of the tumor suppressor *RASSF1A* that negatively regulates RAS/RAF signaling has been identified in thyroid cancer [24,25]. These events, as well as silencing of DNA mismatch repair genes [26], may represent an additional effect of a *BRAF* mutation providing a further upregulation of the signaling cascades in tumors already harboring a *BRAF* mutation. Thus, a more comprehensive interrogation of this critical signaling pathway may uncover additional events that may also serve as markers of aggressive tumor behavior.

GENETIC CHANGES IN THE PHOSPHOTIDYLINOSITOL 3 OH KINASE (PI3K) PATHWAY AS MARKERS OF TUMOR BEHAVIOR

Over the past several years, there has been increasing recognition that activation of PI3K signaling is an important event in thyroid cancer progression. This pathway was initially

identified as a potential oncogenic pathway in thyroid cancer when inactivation of the PTEN tumor suppressor gene, which negative regulates PI3K, was identified as the cause of Cowden's syndrome which includes benign and malignant thyroid tumors, particularly follicular tumors, in its phenotype [27]. AKT, a key signaling kinase in the PI3K signaling cascade, was subsequently found to be overactivated in the majority of sporadic follicular and papillary thyroid cancers with enhanced activity in regions of local invasion [28,29]. Several potential genetic causes for this activation have been identified in addition to mutations in *PTEN*, including a high frequency of mutations in the gene encoding catalytic subunit of P110 alpha subunit of PI3K (*PIK3CA*), amplification of the *PIK3CA* gene, mutations in the pleckstrin homology domain of *AKT1*, and loss of *PTEN* expression through epigenetic regulation [Reviewed in [30]]. Mutations and epigenetic modifications in the PI3K pathway are more common in FTCs than PTCs, consistent with clinical findings in Cowden's syndrome, and they also occur commonly in poorly differentiated and anaplastic thyroid cancers suggesting a role in FTC tumorigenesis and tumor progression [31,32]. In the poorly differentiated thyroid cancers, PI3K pathway alterations do not appear to be mutually exclusive of RAS/RAF/ERK pathway-activating mutations, suggesting they may represent a later step in thyroid cancer progression. Several groups have recently reported that poorly differentiated thyroid cancers often have genetic and/or epigenetic alteration in both the MEK/ERK and PI3K pathways, suggesting that the combination imparts a particularly aggressive tumor behavior [33-35]. Similar to inhibitors of RAS-RAF signaling, a variety of inhibitors of PI3K signaling are in development, including some, such as inhibitors or mammalian target of rapamycin (mTOR) that are approved for clinical use in the United States. Thus, this marker of aggressive thyroid cancer behavior may also have therapeutic targeting benefit.

GENETIC CHANGES IN ADDITIONAL PATHWAYS AS MARKERS OF TUMOR BEHAVIOR

A variety of other signaling pathways have been studied in thyroid cancer and appear to play a role in either/or aggressive tumor behavior or dedifferentiation. For example, mutations in p53 are more frequent in poorly differentiated thyroid cancers. More recent data has implicated activation of Wnt signaling [36], FGF receptors and melanoma associated antigen-3 (MAGE-3) [37,38], S1104a [39], RhoB [40], c-MET [41], Polo-like kinase 1[42] and a variety of other signaling molecules and tyrosine kinase receptors in progressive thyroid cancer. In addition, phase 2 clinical trials using a variety of tyrosine kinase inhibitors have resulted in some partial remissions in patients with metastatic thyroid cancer and induced stable disease in others. These compounds inhibit some unique and some common targets, such as the VEGF receptor, that may be involved in thyroid cancer progression [43]. Determining the precise mechanisms of actions of these compounds may help identify critical regulators of thyroid cancer progression.

It is also important to recognize the potential role of the tumor microenvironment in cancer progression. Data from many solid tumor models have implicated the cells and stroma in the microenvironment as important determinants of tumor progression [44]. One recent manuscript in thyroid cancer identified a potentially important histological association between a high preponderance of tumor associated macrophages (TAM) and anaplastic thyroid cancer [45]. Whether the presence of TAMs identified prospectively in differentiated forms of thyroid cancer, or in their metastases, indicates a more aggressive behavior is not certain.

IDENTIFICATION OF MARKERS OF AGGRESSIVE THYROID CANCER BEHAVIOR BY GLOBAL ANALYSIS

A number of groups have used global methods to identify genes and pathways that may be involved in aggressive tumor behavior. Several recent examples will be described in the following section. For example, Vasko *et al* identified unique signaling networks in the invasive fronts of large grossly invasive PTCs [46]. In this study, gene networks predicted to

initiate epithelial-to-mesenchymal transition (EMT) were identified in the invasive fronts, and higher levels of vimentin expression, a marker of EMT, were associated with increased frequency of tumor invasion and nodal metastases in a validation series. Several independent studies have reported an association with other EMT-related features, such as loss of E-cadherin expression or beta-catenin, APC, and Axin mutations in anaplastic thyroid cancer [reviewed in [47]] suggesting that EMT may be important in tumor progression.

Siraj et. al. performed oligonucleotide microarrays on a series of PTCs and normal tissue and then performed tumor microarrays in a second large group of PTCs as a validation series and identified c-MET as an important overexpressed receptor in the tumors that predicted aggressive biological behavior [41]. In a similar earlier study, Wreesman *et al*. reported that *MUC1*, a gene that encodes a protein involved in cell-matrix interactions, was upregulated in TCV tumors and its gene locus was similarly associated with a gain of copy number in comparative genome hybridization studies [48]. Overexpression of this gene was also associated with aggressive tumor behavior. Recent results evaluating the association between MUC1 expression and PTC aggressiveness have been inconsistent [39,49,50]. Cerutti *et al*. [51] performed serial analysis of gene expression (SAGE) on normal tissue, primary thyroid cancer, and nodal metastases from a group of patients and identified two genes, *LIMD2* and *PTPRC* that were consistently upregulated in nodal metastases suggesting they may be markers or functional regulators of nodal metastases in PTC.

The presence of altered gene copy numbers has been globally studied in thyroid cancer using array comparative genomic hybridization (array CGH). Using this method, several loci encoding genes known to be abnormal in anaplastic thyroid cancer including CCND1 which encodes the cell cycle regulatory protein cyclin D1 [52]. Rodrigues *et al*. [53] performed a study in which they analyzed a group of aneuploid PTCs by array CGH and oligonucleotide microarray an analyzed the data as a function of oncogene expression as well as by the presence or absence of distant metastases or by tumor-specific survival. In this study, they identified the discoid domain receptor family member 2 gene (*DDR2*) as being uniquely overexpressed in patients with distant metastases and in association with death from thyroid cancer. Finally, similar to the work of other groups, they identified gene profiles associated with expression of specific thyroid oncogenes, suggesting important areas of commonality between data sets from different groups.

Finally, over the past several years, there has been an interest in defining the role of microRNAs (miR) in tumorigenesis and cancer progression. MiRs have been implicated as predisposing genes for thyroid cancer [54] and as genes that modify important proteins in thyroid cancer progression such as c-KIT and p27 [54-56]. Particular miR profiles have been identified that associate with specific thyroid oncogenes or histological thyroid cancer types [57-59]. More recently, analysis of thyroid cancer-related miR expression levels has been explored as a molecular diagnostic test on FNA samples [58,60]. The association or role of miRs in thyroid cancer progression is uncertain; however, based on recent data from other solid tumors, such a relationship appears likely and therefore raises their potential use as predictors of thyroid cancer behavior.

CONCLUSION: FUTURE OF MOLECULAR MARKERS OF THYROID CANCER BEHAVIOR

Based on the rate of new publications in the area of molecular abnormalities in thyroid cancer, it seems likely that a growing amount of data will be disclosed that will establish molecular markers of thyroid cancer progression. These studies must be merged with high-quality clinical databases that utilize the most modern methods and definitions to define tumor progression. Targets are most attractive if they also have functional relevance for thyroid cancer behavior

as they may also represent potential therapeutic targets. Studies will need to be performed that clearly establish if molecularly-driven approaches to determining clinical management improve patient outcomes beyond standard approaches. The role of the microenvironment in the local and metastatic sites may also hold clues that may lead to new therapeutic approaches. Finally, with the large amount of array CGH, gene expression, and miR expression data in the public domain, efforts will need to be made to identify areas of commonality between these data sets using robust bioinformatics approaches. Further advancing this field is necessary to "personalize" the therapeutic approach to minimize patient risk from over-treatment and also to identify individuals appropriate for aggressive early intervention and/or treatments with specific targeted therapies in the future.

Acknowledgments

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