4. MOLECULAR EVENTS IN FOLLICULAR THYROID TUMORS

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INTRODUCTION

The analyses of human thyroid tumor tissues have proven informative in identifying key molecular events in epithelial neoplasia. The thyroid gland gives rise to a variety of epithelial tumors that differ markedly in their biologic patterns. The accessibility of thyroid tumors provides a tractable opportunity to define mechanisms of epithelial cell transformation in a spectrum of related cancers.

Two primary issues must be considered when investigating molecular genetic alterations within human thyroid tumor groups. The first is tumor classification. Thyroid tumors are classified predominantly on the basis of morphologic features interpreted by pathologists. Morphologic features provide initial biologic and clinical information but they have been defined somewhat non-specifically in retrospective series. Thus, thyroid tumor diagnosis can be imprecise [1–4] and can create confusion when correlating molecular genetic alterations with clinical and pathologic features. Mutations that predominate in one thyroid tumor group may be identified in others and the distinction as to whether such tumors are misclassified or contain additional alterations is difficult to ascertain. A second important issue relates to mutation detection. Polymerase chain reaction-based amplification and sequencing of nucleic acids from fresh or fast-frozen tissues are most often employed. Such assays are exquisitely sensitive and prone to cross-contamination, particularly when poorly preserved or archival tissues are used. Polymerase chain reaction can even detect genetic alterations within a minute sub-fraction of tumor cells. The biologic significance of this is often unclear. Tissue

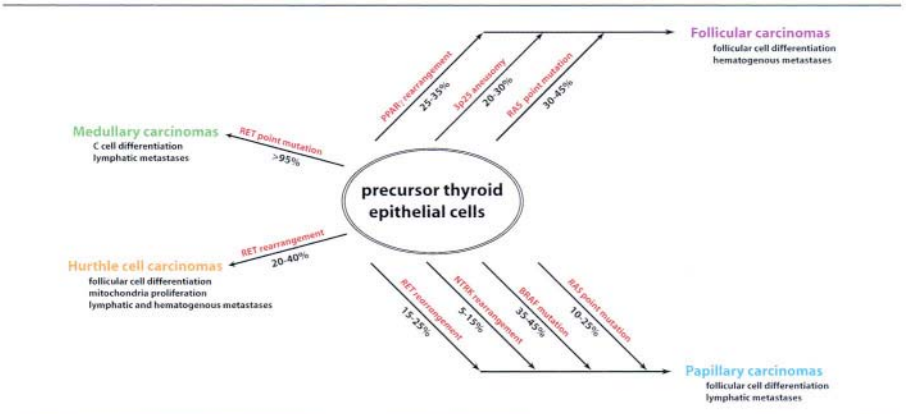

Figure 1. Histologic-Molecular Model of Thyroid Cancer Formation. Four main types of thyroid carcinoma with distinct biologic features are recognized. A subset of each type may progress to poorly differentiated and/or clinically aggressive forms. Genetic alterations that characterize these pathways and sub-pathways are shown.

composition must also be documented rigorously because thyroid tumor resections contain admixtures of tumor, normal thyroid, lymphoid, reactive and stromal elements. All such factors must be considered or erroneous results will be obtained [5–7].

This chapter begins with a histologic-molecular model of thyroid cancer formation and discusses known mutations and emerging biologic and clinical correlates in follicular thyroid tumors. A summary and comparison of thyroid carcinomas with the acute myeloid leukemias follows.

A histologic-molecular model of thyroid cancer

A model that encompasses histologic, molecular, and biologic facets of thyroid cancer formation is shown in Figure 1. At least four sub-types of thyroid cancer with distinct characteristics are recognized. Tumors within each group may progress to poorly differentiated, metastatic, and/or anaplastic forms. The thyroid carcinoma model seems unique relative to other carcinomas in several respects. First, distinct gene mutations define separate pathways of oncogenesis within the thyroid. This is different than a single linear genetic pathway envisioned commonly for other carcinomas such as those arising in the colon [8] and exocrine pancreas [9, 10]. Second, both thyroid specific and non-thyroid specific mutations characterize different thyroid carcinoma subgroups. One particularly interesting class of thyroid-specific mutations is the chromosomal rearrangements that encode gene fusions [11, 12]. Gene fusions been identified infrequently in carcinomas even though they are common in blood cell and soft connective tissue cancers [13]. Third, thyroid cancer mutations correlate with specific biologic properties. For example, *RET* and $PPAR\gamma$ rearrangements characterize papillary [14] and follicular [12] thyroid carcinomas that tend to spread via regional lymphatics or blood vessels, respectively. Distinct *RET* germ line point mutations identify different familial medullary thyroid carcinoma patients with propensities for poor

outcome and/or concomitant non-thyroid disease [15]. Thus, mutation staus provides predictive biologic information in thyroid cancer and thus may augment our current morphology-based classification and treatment schemes. Even so, it must be kept in mind that a combination ofcellular events, not single gene alterations, determines overall thyroid cancer biology. Thyroid tumors with apparently identical single gene mutations but distinct patterns of growth and/or prognoses have been reported [16–21].

PPARγ rearrangements

Somatic rearrangements in the gene encoding the nuclear receptor *PPARγ* have been identified in thyroid cancers with follicular cell differentiation, frequent encapsulation, vascular invasion and capsular penetration. These are follicular thyroid carcinomas (Figure 1). The discovery of *PPARγ* rearrangements resulted from mapping [12] of a chromosomal translocation, t(2;3)(q13;p25), which had been identified in follicular thyroid tumors [12, 22–27]. The t(2;3) rearrangement juxtaposes the promoter region and 5′ coding sequence of the *PAX8* gene on chromosome 2 with most of the coding sequence ofthe *PPARγ* gene on chromosome 3 and results in expression of a chimeric *PAX8-PPARγ* transcription factor (Figure 2).

PAX8-PPARγ is a thyroid-specific mutation and one member of a family of *PPARγ* rearrangements in follicular carcinomas. Another follicular carcinoma translocation, t(3;7)(p25;q31) [28], fuses the promoter and 5′ coding sequence of a novel transcription factor gene termed *CREB3L2* or *BBF2H7* [29] on chromosome 7 with most of the coding sequence of *PPARγ* (Figure 2). PAX8-PPARγ and CREB3L2-PPARγ (Figure 2) contain identical PPARγ sequences that include wild-type PPARγ DNA binding, ligand binding, RXR dimerization, and transactivation domains [30]. Additional putative *PPARγ* rearrangements have been detected in other follicular carcinomas [12, 22, 31, 32].

PPARγ rearrangements have been identified in 25–35% of follicular carcinomas based on studies using pathologically well-defined tissues [32–38]. *PPARγ* rearrangements [32] or *RAS* gene point mutations but not both [33] are detected early in low stage follicular carcinomas, suggesting the existence of sub-pathways of oncogenesis in follicular carcinoma (Figure 1). Such a model is further supported by distinct patterns of galectin-3 and HBME-1 protein expression in *PPARγ* rearrangement- versus *RAS* mutation-positive follicular carcinomas [33] and by an additional genetic subset of follicular carcinomas (25%) that possess 3p25 aneusomy in the absence of *PPARγ* rearrangement [32].

The mechanisms through which *PPARγ* rearrangements deregulate thyrocyte growth are being investigated and aberrations in transcription (Figure 3) and other cell functions may be involved. PAX8-PPARγ stimulates proliferation, inhibits apoptosis, and induces anchorage independent growth of human thyroid cells [39], supporting a primary role for PAX8-PPARγ in follicular cell transformation. PAX8-PPARγ also transforms NIH3T3 mouse fibroblasts in colony assays [39], demonstrating that PAX8-PPARγ can alter both thyrocyte and non-thyrocyte growth functions. PAX8-PPARγ has little ability to stimulate transcription from *PPARγ* response elements *in vitro* and also inhibits transcription mediated by wild-type *PPARγ* [12, 39], activities that fit

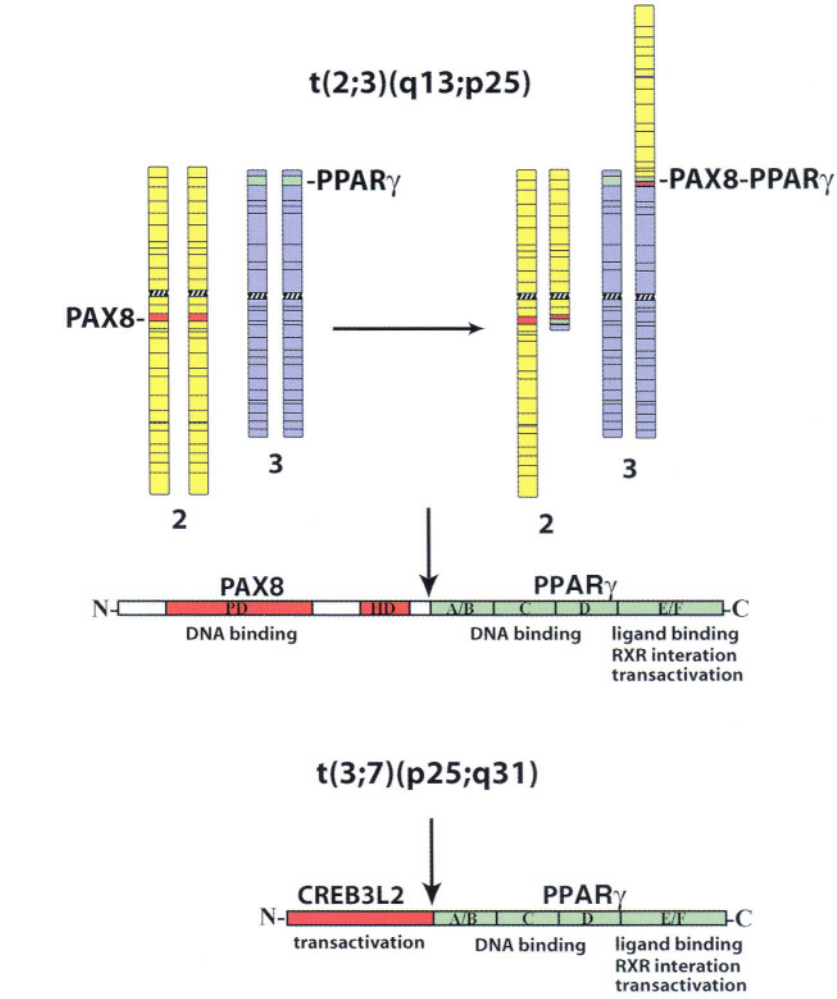

Figure 2. PPARγ Gene Rearrangements in Follicular Thyroid Carcinoma. The breakpoints of two chromosomal rearrangements, t(2;3)(q13;p25) and t(3;7)(p25;q31), have been cloned from human follicular thyroid carcinomas. Each rearrangement encodes a chimeric fusion protein that contains identical domains (A-E) of the PPARγ nuclear receptor.

well with the known tumor suppressor-like effects of wild-type PPARγ in a variety of epithelial cells [40–44]. In general, wild-type PPARγ stimulation inhibits thyroid cell growth [45, 46] and a reduction in PPARγ expression has also been noted in a significant subgroup of thyroid cancers without *PPARγ* rearrangement [32, 38]. The retinoblastoma tumor suppressor protein and cell cycle regulators may be involved [45, 47, 48].

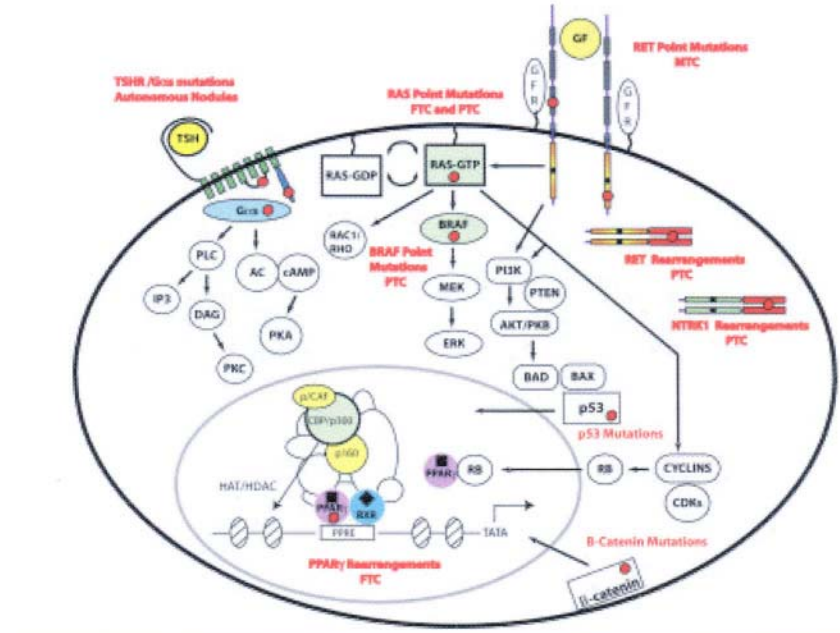

Figure 3. Molecular Pathways in Follicular Thyroid Tumors. Schematic representation of major molecular pathways involved in follicular thyroid tumors. Some, but not all, components and inter-connections of these pathways are indicated. Mutations are note in red and by red dots. Abbreviations: TSHR, thyroid stimulating hormone receptor; Gαs, guanine nucleotide stimulatory factor α; PLC, phospholipase C; IP3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; AC, adenyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; RAC1/RHO, rac1/rho GTP binding proteins; GFR, growth factor receptor; GF, growth factor; RET, ret tyrosine receptor kinase; NTK1, ntrk1 tyrosine receptor kinase; GTP, guanine diphosphate; GTP, guanine triphosphate; RAS, ras GTP binding protein; BRAF, braf serine/threonine kinase; MEK, mitogen activated protein kinase kinase; ERK, extracellular signal regulated kinase (mitogen activated protein kinase); PI3K, phosphoinositol-3-kinase; PTEN, pten dual specificity phosphatase; AKT, akt serine/threonine kinase; PKB, protein kinase B; BAD and BAX, proapoptotic bcl-2 family members; p53, p53 tumor suppressor protein; RB, rb retinoblastoma tumor suppressor protein; CDKs, cyclin-dependent kinases; PPARγ, peroxisome proliferator-activated receptor γ; RXR, retinoid X receptor; p/CAF, CBP/p300, p160, nuclear receptor co-activators; HAT, histone acetyl transferase; HDAC, histone deacetylase complex. TATA, tata box.

Although inhibition of wild-type PPARγ by PAX8-PPARγ appears to be functionally important, the CREB3L2-PPARγ fusion protein appears to exhibit little inhibitory activity [30], suggesting that other mechanisms are also critical. PAX and CREB3L2 rearrangements have been noted in other cancers, supporting the idea that contributions of these domains in PAX8-PPARγ and CREB3L2-PPARγ are functionally important. For example, the *PAX3* and *PAX7* genes are rearranged in alveolar rhabdomyosarcoma [49–51] and *CREB3L2* is rearranged in fibromyxoid sarcoma [29]. Wild-type PAX8, a transcription factor required for normal thyroid follicular cell development [52], also possesses transforming activities *in vitro* [53].

Follicular adenomas with *PAX8-PPARγ* rearrangement have been identified at apparent lower frequency than in follicular carcinomas [33, 34, 36] and it seems most reasonable to consider these early (precursor/in situ) follicular carcinomas [32] unless genetic and/or clinical distinctions from the follicular carcinomas can be documented. *PPARγ* rearrangements are expected in at least some follicular adenomas because differential diagnosis of follicular adenomas from carcinomas is not precise. The possibility that *PPARγ* rearrangements mark a subset of follicular carcinomas, some even before histologic evidence of invasiveness is apparent, suggests that molecular analyses of fine needle aspiration biopsies may be useful to detect these follicular cancers [54]. However, the exact diagnostic utility of PPARγ rearrangements in diagnosis will not be clear until the biologic and molecular relatedness of follicular carcinomas and adenomas with *PPARγ* rearrangement is better defined. Papillary (follicular variant) and Hurthle cell carcinomas with *PPARγ* rearrangement have been observed infrequently [32, 34, 55], suggesting that these thyroid cancers arise via alternate transformation pathways (Figure 1).

Clinical and pathological characteristics of follicular carcinoma patients with *PPARγ* rearrangements have been examined. Follicular carcinomas with *PPARγ* rearrangement tend to have well-defined foci of vascular invasion and capsular penetration but not lymph node metastases [32, 33]. They also tend to present at younger patient age than follicular carcinomas without *PPARγ* rearrangement [32, 33] and progress and metastasize in some cases [23, 35]. Even so, few *PPARγ* rearrangements have been detected in anaplastic thyroid carcinomas [34, 35], which are highly aggressive cancers thought to arise from follicular and papillary carcinomas. Further studies are required to define the biologic characteristics and patterns of progression of follicular thyroid tumors with *PPARγ* rearrangement.

RET rearrangements

Somatic rearrangements in the gene encoding the *RET* receptor tyrosine kinase have been identified in a subset of thyroid cancers that exhibit follicular cell differentiation, characteristic papillary and/or nuclear morphologies, and a propensity for lymph node metastases. These are papillary thyroid carcinomas (Figure 1). Interestingly, the *RET* gene plays a fundamental role in multiple thyroid cancers. Whereas rearrangements of *RET* characterize papillary thyroid carcinomas [11, 14], germ-line *RET* point mutations characterize medullary thyroid carcinomas arising in the multiple endocrine neoplasia type 2 [56–59] and family medullary thyroid carcinoma syndromes. Thus, different *RET* mutations (rearrangements or point mutations) arising in different cellular contexts (follicular or C cell lineages) promote formation of different thyroid cancers (Figure 1). The *RET* rearrangements are discussed in detail in Chapter 12.

RET rearrangements in papillary carcinoma are thyroid-specific mutations and most often result from para-centric chromosomal inversions. For example, the *RET* gene at chromosome 10q11 is recombined frequently with other 10q loci such as *H4* in PTC1 [60] and *ELE1* in PTC3 [61, 62]. Several less frequent reciprocal translocations involving *RET* and other chromosomal loci have been described, particularly in papillary carcinoma patients exposed to radiation in the Chernobyl accident [63–65]. All

known *RET* rearrangements result in expression of cytoplasmic, chimeric fusion proteins that contain the intracellular tyrosine kinase domain of RET fused to domains of non-RET (termed RET fusion genes or RFG) genes. The extracellular cadherin-like, cysteine-rich, and transmembrane domains of RET are not retained in the RFG-RET fusion proteins.

Experiments expressing RFG-RET fusion proteins in thyroid cell lines support a central role of the RAS-BRAF-MEK-ERK pathway in neoplastic transformation of follicular cells into papillary carcinomas (Figure 3). The RFG-RET fusion proteins stimulate follicular cell proliferation and inhibit differentiation [66–70]. Apoptosis may also be altered [71]. These biologic effects are mediated by ligand-independent dimerization [72, 73], cytoplasmic relocation [73], and constitutive activation of the RET tyrosine kinase. Adaptor molecules such as Shc, Frs2, Enigma, and Grb proteins interact with RET proteins [69, 74–78] and stimulate downstream RAS-BRAF-MAPK-ERK and other signal transduction pathways.

Transgenic mouse lines engineered to express RFG-RET fusion proteins in the thyroid document their ability to promote formation of papillary carcinoma-like tumors *in vivo* [79–82]. However, these transgenic lines do not all develop thyroid tumors with high penetrance or short latency and few, if any, develop tumors that metastasize without co-expression of additional mutations, arguing that multiple alterations are required for expression of the full papillary carcinoma phenotype [66, 67, 83].

RET rearrangements have been detected in 15–25% of papillary carcinomas and have been considered specific based on RTPCR and Southern blot experiments [70, 84–91]. *RET* rearrangements appear to arise early in papillary carcinoma because they are most common in low stage and the occult/micropapillary tumors [89, 92–94]. Papillary carcinomas with *RET* rearrangements may also present at younger patient age than papillary carcinomas without *RET* rearrangements [87, 95, 96], in a manner that resembles $PPAR\gamma$ rearrangements in follicular carcinoma. Other strong clinicopathologic correlates of *RET* rearrangement include classic papillary (not follicular variant) morphology [97, 98] and the presence of lymph node spread [86, 87, 96, 98]. The ELE1-RET (PTC3) fusion protein may be more frequent in the aggressive tall cell [17] and solid [16, 20] papillary carcinoma subtypes. A significant fraction of papillary carcinomas with *RET* alterations appear to progress to poorly differentiated thyroid carcinoma [99] but few *RET* rearrangements have been detected in anaplastic thyroid cancers [84, 89].

A few recent reports have noted *RET* rearrangements, somewhat unexpectedly, in benign and malignant Hurthle cell tumors [18, 19] and in thyroid hyalinizing trabecular adenomas [100, 101]. These Hurthle cell carcinomas with *RET* rearrangements appear to have increased tendency for lymphatic spread [102], supporting a biologic connection to papillary carcinoma as well. Thus, one intriguing possibility is that the Hurthle cell tumors with *RET* rearrangement are actually papillary carcinomas with additional morphologic and perhaps biologic features. An alternate possibility that must be excluded is that the *RET* rearrangements are present in a small fraction of the tumor cells because a only combined high cycle RTPCR and nucleotide probe hybridization have so far demonstrated their presence.

NTRK1 rearrangements

Somatic rearrangements in the gene encoding the *NTRK1* receptor tyrosine kinase have been identified in 5–15% of papillary thyroid carcinomas (Figure 1). These are discussed further in Chapter 12. In essence, *NTRK1* rearrangements bear strong resemblance to *RET* rearrangements in several respects. First, both NTRK1 and RET are receptors for neurotrophic ligands [103] and are not normally expressed in follicular epithelial cells. Second, both *NTRK1* and *RET* rearrangements were identified by transfection of papillary carcinoma DNA into NIH3T3 cells [11, 14, 85]. Third, both *NTRK1* and *RET* rearrangements arise frequently from subtle intra-chromosomal inversions. Fourth, both *NTRK1* and *RET* rearrangements lead to expression of fusion proteins with constitutive tyrosine kinase activation. For example, rearrangements at 1q21 often fuse the NTRK1 tyrosine kinase domain to other proteins such as *TPM* and *TPR* [104–106]. Fifth, both *NTRK1* and *RET* rearrangements may be more frequent in younger patients and in patients with lymph node metastases [95, 96, 107]. Last, the NTRK1 and RET fusion proteins activate related signal transduction pathways in thyroid follicular cells [66, 108–111] (Figure 3). Expression of the NTRK1 fusion proteins in the thyroid of transgenic mice leads to follicular hyperplasia- and papillary carcinoma-like proliferations [112].

RAS mutations

Somatic point mutations in *RAS* genes have been detected frequently in both non-thyroid [113] and thyroid (Figure 1) cancers. This contrasts the thyroid-specific gene rearrangements involving *PPARγ*, *RET,* and *NTRK1. RAS* mutations are most common in follicular versus papillary and Hurthle cell tumors [33, 91, 114–120] and have been detected in 20–50% of follicular adenomas and carcinomas [33, 91, 119–122]. The presence of *RAS* mutations in both follicular adenomas and carcinomas is consistent with a model in which many *RAS*-initiated follicular carcinomas develop from adenoma (morphologic) precursors. Experimental evidence supports this contention in that mutated RAS is insufficient to induce a fully transformed phenotype *in vitro* [66, 123–126] or follicular carcinoma *in vivo* [127, 128]. *N-RAS* mutations appear to predominate over *K-RAS* and *H-RAS* mutations in follicular thyroid tumors and mutations in codon 61 of *N-RAS* may be the most prevalent [33, 119, 120, 129]. The possibilities that *K-RAS* mutations are more frequent in papillary compared to follicular thyroid tumors [114, 115, 130], radiation-associated carcinomas [114], and/or aggressive thyroid cancers [130] require further investigation, particularly in view of the primary role of *K-RAS* mutations in pancreatic ductal carcinomas [10, 131] that are highly aggressive.

Recent studies have correlated the clinical and pathologic features with *RAS* mutation status. Thyroid carcinoma patients with *RAS* mutations may present at older age and with larger tumors [33] and may more frequently have less differentiated, high stage cancers [130, 132–134]. Careful pathologic evaluation of classic from follicular variant papillary carcinomas has noted another potentially interesting pattern. Follicular variants seem to contain more *N-RAS* (75%) and *H-RAS* (25%) mutations and

few if any *RET* rearrangements, whereas classic papillary carcinomas seem to contain more *RET* rearrangements (30–35%) and few if any *RAS* mutations [98]. Follicular variants papillary carcinomas also had statistically lower rates of lymph node metastases and higher rates of tumor encapsulation and vascular invasion (follicular carcinoma-like features) compared to classic papillary carcinomas [98]. Thus, the existence of a morphologic and molecular "hybrid" thyroid cancer with some features of papillary and follicular carcinoma needs to be further explored.

Mouse modeling experiments have documented that *RAS* mutations are important role in tumorigenesis and tumor maintenance[128, 131, 135, 136] and RAS proteins transduce multiple stimuli from the thyroid follicular cell surface (Figure 3) as discussed further in Chapter 7.

BRAF mutations

Somatic point mutations in the *BRAF* gene have been identified recently in thyroid and other cancers [137]. *BRAF* encodes a serine/threonine kinase downstream of RAS and it transduces signals from multiple stimuli (Figure 3). A mutation that alters valine 599 to glutamic acid (V599E) in the BRAF kinase domain has been identified in 35–45% of papillary thyroid carcinomas [70, 90, 91, 120, 138–140] and in some undifferentiated/anaplastic thyroid carcinomas [90, 138]. *BRAF* mutations have been detected in few other benign or malignant thyroid tumors [70, 90, 91] and seem not to co-exist with *RAS* point mutations or *RET* rearrangements [70, 91, 138], thereby defining an additional sub-pathway in papillary carcinoma (Figure 1).

Papillary thyroid carcinoma patients with *BRAF* mutations tend to present at older age [90], at higher stage [90, 138], and with more frequent distant metastases compared to papillary carcinoma patients without *BRAF* mutation. Thus, mutated *BRAF* may define an aggressive papillary carcinoma form. In agreement with this possibility, mutated BRAF exhibits enhanced kinase activity and increased transformation efficiency compared to wild-type BRAF *in vitro* [137].

Thyroid stimulating hormone receptor and G protein mutations

Iodide uptake and thyroid hormone biosynthesis and metabolism are coordinately regulated with proliferation in thyroid follicular epithelial cells. These differentiated thyroid functions are controlled by the thyroid stimulating hormone receptor (TSHR) and its downstream signaling molecules (Figure 3) such as cyclic AMP and phospholipase C [141–143]. Somatic mutations in molecular components of the TSHR pathway have been detected in 60% or more of benign TSH-independent (autonomous/hyperfunctioning) thyroid nodules. The remaining 40% of autonomous nodules are postulated to contain undefined alterations in the same TSHR system [144]. Approximately 90% of mutations involve TSHR, often in the third intracellular loop or transmembrane regions of this seven-spanning membrane receptor [145, 146]. 5–10% of the mutations involve the G protein subunit Gsα/gsp activated by TSHR ligands [147]. Thus, constitutive stimulation of the TSHR pathway underlies most autonomous thyroid tumors [148].

Autonomous thyroid tumors usually exhibit hyperplastic morphology and transgenic mice and other animal models with an activated TSHR-Gsα/gsp-cAMP axis [149, 150] develop follicular hyperplasia and hyper-functioning thyroid tumors, supporting a fundamental role of the TSHR system. Furthermore, nodular hyperthyroidism in non-autoimmune autosomal dominant hyperthyroidism [151] and the McCune-Albright Syndrome [152] have been associated with germ-line mutations in TSHR-Gsα/gsp axis. Although chronic stimulation of the TSHR pathway promotes formation of benign thyroid nodules, this seems to provide little increased risk of thyroid cancer. Additional cellular alterations [153], potentially including the down-regulation of PPARγ[154], are apparently required.

B-catenin and *p53* mutations

Stage at presentation is a key prognostic factor in thyroid carcinoma. Mutations in the genes encoding B-*catenin,* a component of the Wnt signaling pathway [155], and *p53,* an important tumor suppressor and a sensor of genome stability, have been identified most often in advanced stage thyroid cancer. Mutations in exon 3 of B-*catenin* have been detected in 25–60% of poorly differentiated and anaplastic thyroid carcinomas [156, 157], and the expression of *B-catenin* protein is often reduced or re-localized from the plasma membrane to the cytoplasm and nucleus in these [156–158] and some follicular and papillary [157–160] thyroid carcinomas. p53 mutations have been identified mostly in poorly differentiated and anaplastic thyroid carcinomas [161–164] and they appear to interfere with differentiated functions in thyroid cells [165, 166] and promote thyroid cancer invasion and metastases in transgenic mouse models [83, 167]. The p53 pathways are discussed in detail in Chapter 8.

Aneuploidy and other chromosomal aberrations

A low level of chromosomal instability is observed in benign thyroid tumors and well-differentiated thyroid cancers such as papillary carcinoma, a moderate level of chromosomal instability is observed in follicular carcinoma, and higher levels of chromosomal instability are observed in Hurthle cell, poorly differentiated/anaplastic, and metastatic carcinomas. Thus, increased chromosomal instability and aneuploidy correlate generally with increased thyroid cancer aggressiveness. On the other hand, microsatellite instability is relatively infrequent in thyroid cancer [168–174]. Exposure to ionizing radiation increases genetic instability and thyroid carcinoma prevalence as discussed in Chapter 11.

Analyses of human thyroid tumors with conventional cytogenetics and fluorescence in situ hybridization have identified additional recurrent chromosomal abnormalities. Hyperplastic nodules from thyroid goiters often contain one or two clonal numerical changes, including trisomies of chromosomes 7, 10, 12, 17, and/or 22, whereas follicular adenomas more frequently contain three or more numerical chromosomal alterations and/or balanced chromosomal rearrangements [25, 175–178]. However, it should be kept in mind that karyotypes frequently present an incomplete picture of chromosomal content because the cultures may frequently appear diploid as the result

of contaminating normal cells. All suspected genetic alterations must be verified in primary thyroid tumor tissues.

The chromosomal regions 2p21 and 19q13 are rearranged in approximately 10% and 20%, respectively, of thyroid follicular adenomas with clonal cytogenetic aberrations. Both the 2p21 [26, 175, 179] and 19q13 [24, 175, 180] loci fuse with multiple different partner chromosomes in different follicular adenomas. The 2p21 and 19ql3 breakpoints have been mapped using follicular adenoma cell lines that contain t(2;7)(p21;p15), t(2;20;3)(p21;q11;p25), t(5;19)(q13;13), or t(1;19)(p35;q13).The 2p21 breakpoint appears to involve a novel candidate gene termed *THADA* [181, 182] and the 19q13 breakpoint a novel transcription factor gene termed *ZNF331/RITA* [183–185]. It will be informative to define the cell biologic and biochemical mechanisms of these new thyroid rearrangements.

Additional genetic imbalances have been defined in follicular thyroid tumors using loss of heterozygosity studies and comparative genomic hybridization techniques. Genetic gains predominate over losses in follicular adenomas, whereas genetic losses predominate over gains in follicular, Hurthle, and anaplastic thyroid carcinomas. The most consistent losses in follicular cancers involve chromosomes 2p [186–189], 2q [186–188], 3p [169, 174, 187–191], 7q [188, 192, 193], 9 [174, 187, 188, 194, 195], 10q [196–198], 11q [187, 189, 195, 197, 199, 200], 13q [187, 188, 196, 197], 17q [201], 18q [174, 187, 197], and 22q [187, 188, 195, 202, 203] regions. In addition to these losses, Hurthle cell carcinomas harbor deletions at 1q, 8q, 9q, 14q, and 17p [174, 194, 201]. The possibility that at least some of these genomic loci contain genes important in thyroid tumor pathogenesis is reinforced by the fact that three regions (2q13, 3p25 and 7q31) have been shown to be involve follicular carcinoma rearrangements [12, 30]. Thus, functionally important loci may be targeted by multiple genetic mechanisms.

Summary

Knowledge of the molecular events that govern human thyroid tumorigenesis has grown considerably in the past ten years. Key genetic alterations and new oncogenic pathways have been identified. Molecular genetic aberrations in thyroid carcinomas bear noteworthy resemblance to those in acute myelogenous leukemias. Thyroid carcinomas and myeloid leukemias both possess transcription factor gene rearrangements—*PPARγ*-related translocations in thyroid carcinoma and *RARα*-related and *CBF*-related translocations (amongst others) in myeloid leukemia [204]. PPARγ and RARα are closely related members of the same nuclear receptor subfamily, and the PML-RARα and PAX8-PPARγ fusion proteins both function as dominant negative inhibitors of their wild-type parent proteins [12, 205, 206]. Thyroid carcinomas and myeloid leukemias [207–210] also both harbor *NRAS* mutations (15–25% of both cancers) and receptor tyrosine kinase mutations – *RET* mutations in thyroid carcinomas and *FLT3* mutations in myeloid leukemias [211, 212]. The *NRAS* and tyrosine receptor kinase mutations are not observed in the same thyroid carcinoma or leukemia patients [209, 213], suggesting that multiple initiating pathways exist in

both. Lastly, thyroid carcinomas [214] and myeloid leukemias [209, 215] possess p53 mutations at relatively low frequency (10–15%) in patients who tend to be older and have more aggressive, therapy resistant disease. Such parallels are unlikely to occur by chance alone and argue that common mechanisms underlie these diverse epithelial and hematologic cancers.

The comparison of thyroid carcinomas and myeloid leukemias may highlight areas of thyroid cancer investigation worthy of further focus. For example, few collaborating mutations have been defined in thyroid carcinomas even though they play a clear role in myeloid leukemias [212, 216], as exemplified by *RARα* rearrangements [217, 218] and *FLT3* mutations [219] that together dictate the promyleocytic leukemia phenotype. Functional interactions between collaborating mutations are possible at multiple levels, and it is tempting to speculate that some thyroid carcinomas might develop through an unique combination or co-activation of RET and RAS and/or RET and PPARγ (and/or other) signaling systems. In fact, the ELE1-RET (PTC3) fusion protein contains the ELE1 nuclear receptor co-activator domain [220, 221] and it appears to physically associate with and inhibit wild-type PPARγ in some papillary carcinomas [222].

The similarities of the fusion proteins in thyroid carcinoma and myeloid leukemia suggest that a more directed search for fusion genes in non-thyroid carcinomas is warranted. In fact, novel fusion genes have been identified recently in aggressive midline [223, 224], secretory breast [225], and renal cell [226–232] carcinomas, although the epithelial nature of the latter is not well-documented. Interestingly, these cancers all tend to present more frequently in adolescence and young adulthood in a manner similar to thyroid and myeloid [233] malignancies that have fusion genes. The analyses of cancers that present earlier in life may enhance fusion gene recognition in other carcinoma types.

Definition and biologic characterization of the precursor cells that give rise to thyroid carcinoma will also be important. Myeloid leukemias are thought to arise from stem/progenitor cells that acquire disturbed self-renewal and differentiation capacities but retain characteristics of the myeloid lineages. Although the presence of comparable stem/progenitor cells in the thyroid are not defined, distinct thyroid cancer lineages and patterns of differentiation exist and candidate stem/progenitor cells such as the p63-immunoreactive solid cell nests [234] are apparent.

A last important area is development of molecular-based therapies for thyroid carcinoma patients resistant to standard radio-iodine treatment. Treatments for such cancers are limited and pathways defined by thyroid cancer mutations are prime targets for pharmacologic interventions with molecular inhibitors. Tyrosine kinase inhibitors [235–239] and nuclear receptor ligands [240–242] have proven dramatically effective in some myeloid leukemia patients. Various molecular inhibitors are being investigated now in thyroid cancer models [45, 243–249]. Such developments predict that the thyroid cancer model will continue to provide biologic insights into human carcinoma biology and that improved pathologic diagnosis and treatment for thyroid cancer patients sit on the not too distant horizon.

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