

Perspective: Lessons Learned from Molecular Genetic Studies of Thyroid Cancer—Insights into Pathogenesis and Tumor-Specific Therapeutic Targets

Thyroid cancer derived from follicular cells is the most common endocrine malignancy. The past decade has seen progress in our understanding of the pathogenesis of the various forms of this condition, and translation of these discoveries to clinical practice now seems within reach. In this review, I will discuss some recent salient observations on the molecular genetics of these tumors, and how they impact on our understanding of their pathogenesis.

Rearrangements of the RET tyrosine kinase receptor gene in papillary thyroid carcinomas

RET encodes the membrane tyrosine kinase receptor for a family of ligands, the prototype of which is glial cell-derived neurotrophic growth factor (1). The *RET* protooncogene is involved in the regulation of growth, survival, differentiation, and migration of cells of neural crest origin. Germline mutations of *RET* confer predisposition to multiple endocrine neoplasia type 2, familial medullary thyroid carcinoma and Hirschsprung's disease. The *RET* gene is not normally expressed in thyroid follicular cells. Aberrant expression of various chimeric forms of *RET* in papillary thyroid cancers result from chromosomal rearrangements in which the promoter/s of unrelated gene/s are linked to the C-terminal fragment of *RET*. There are several types of *RET* rearrangements found in human thyroid papillary carcinomas, formed by the fusion of the intracellular tyrosine kinase domain of the gene with different 5' gene fragments. *RET/PTC1* is formed by a paracentric inversion of the long arm of chromosome 10 leading to fusion with a gene named *H4/D10S170* (2). *RET/PTC2* is formed by a reciprocal translocation between chromosomes 10 and 17, resulting in the juxtaposition of the TK domain of *c-RET* with a portion of the *R α* regulatory subunit of cAMP-dependent PKA (3). *RET/PTC3* is also a result of an intrachromosomal rearrangement and is formed by fusion with the *RFG/ELE1* gene (4, 5). Although *RET/PTC1* and *RET/PTC3* are the most common recombined forms of *RET*, recently several new variants of *RET/PTC* have been identified in which the oncoprotein is rearranged with other upstream partners in papillary carcinomas from children exposed to radiation after Chernobyl (6–8). In all cases examined so far, the truncated fragment of *RET* lacks the extracellular and transmembrane domains, and the aberrant protein is located within the cytosol. The respective promoters of the 5' partners in the *RET/PTC* rearrangements drive expression of the chimeric gene products. Signaling is dependent on dimerization, and the domain conferring this property is derived from the N-terminal fragment donated

by the respective upstream gene. Indeed, all N termini contain coiled-coil or leucine zipper motifs that cause unregulated oligomerization and *RET* kinase activation.

RET/PTC rearrangements are involved in tumor initiation

RET rearrangements are found in 2.6–34% of papillary carcinomas in the adult population. By contrast, *RET* rearrangements are particularly common in pediatric papillary thyroid carcinomas (9, 10), and in cancers from children exposed to radiation after the Chernobyl nuclear accident (11, 12), or to external irradiation for treatment of benign diseases of the head and neck (13). There are several lines of evidence indicating that *RET/PTC* rearrangements are one of the very first genetic changes leading to development of papillary thyroid carcinomas: 1) A high proportion of occult microscopic foci of papillary thyroid cancers, thought to be precursors of fully manifest forms of the disease, display *RET* immunoreactivity, and express *RET/PTC* mRNA as determined by RT-PCR (14). 2) Overexpression of *RET/PTC1* or *RET/PTC3* in thyroid cells of transgenic mice results in tumors with a typical papillary histotype (15–17). 3) Irradiation of human fetal thyroid explants, undifferentiated thyroid carcinoma or fibrosarcoma cells *in vitro* induces *RET/PTC1* rearrangements in a dose-dependent fashion within a short time frame (18, 19). 4) Chromosomal loci participating in the *RET/PTC1* rearrangement (*i.e.* *RET* and *H4*) are juxtaposed during interphase in normal human thyroid cells, providing a target for radiation to induce simultaneous double-stranded DNA breaks leading to illegitimate nonhomologous recombination via end-joining (20, 21).

Thus, chromosomal architecture during interphase may be an important prerequisite for *RET* recombination in thyroid cells. Despite their linear distance (30 megabases in the case of *H4* and *RET*), physical contiguity of the genes involved in the rearrangement during interphase may increase the likelihood of illegitimate recombination after exposure to genotoxic agents. By contrast, lack of association between these chromosomal loci may explain in part why *RET/PTC* rearrangements do not occur in other cell types in which radiation may serve as a tumor initiator, such as mammary epithelial cells (21), despite the fact that *RET* activation in experimental models has been shown to lead to their transformation (22).

Reappraisal of radiation susceptibility studies for thyroid tumorigenesis in animals

The relative impact of different forms of ionizing radiation on thyroid tumor formation has been the subject of considerable interest, primarily because of the common use of ¹³¹I

Abbreviations: PTC, Papillary thyroid carcinoma; RET, rearranged during transfection.

in diagnosis and therapy of benign thyroid diseases. Based on evidence that therapeutic doses of ^{131}I are not associated with induction of thyroid cancer in humans, or do so with a low probability (23), the relative risk of cancer following internal radiation exposure has long been thought to be less than that of external radiation (reviewed in Ref. 24). This contention has been buttressed by animal studies, which in general support the notion that ^{131}I is less tumorigenic than shorter-lived radioiodines and external radiation (25). Based on the present information on the molecular genetics of radiation-induced human thyroid cancer, the value of animal studies must now be questioned. Thus, as opposed to humans, radiation does not induce papillary thyroid cancers in either rodents or dogs. Moreover, *RET/PTC* rearrangements are almost certainly unique to human cells, presumably because of a requirement for a particular three-dimensional configuration of human chromosome 10 to favor these recombination events. Because of this, data on thyroid cancer formation after radiation in animals is probably not relevant to humans.

The same may not be true for benign neoplasms. Thus, radiation induces benign follicular adenomas in both rodents and humans. Although the precise genetic target/s of radiation leading to thyroid adenoma formation in humans is not known, there is evidence that ionizing radiation may favor point mutations of *K-RAS* in thyroid adenomas in rats (26). As oncogenic *RAS* has also been implicated in adenoma initiation in humans, rodents may represent appropriate models for radiation-induced benign thyroid neoplasia.

Papillary thyroid cancer multicentricity: multiple initiation events or micrometastases from a single primary?

Papillary thyroid carcinomas are frequently multicentric at the time of presentation. Until recently, it was not clear whether multiple foci of papillary thyroid cancer within the same gland represented micrometastases originating from the same primary lesion, or multiple distinct primary tumors. A study by Sugg *et al.* (27) has gone a considerable way toward resolving this quandary. They found that 17 of 21 patients with multifocal disease were positive for *RET/PTC* rearrangements, and 15 of these had diverse types of *RET/PTC* mRNA in the individual tumors from the same patients, indicating that they had arisen through distinct initiating events. This would predict that the thyroid glands of these patients might have been exposed to a significant genotoxic insult that resulted in widespread DNA damage. Accordingly, thyroid cancers from patients exposed to radiation during childhood are commonly multicentric (28). In addition, certain patients with multiple independent primary lesions may have an underlying predisposition to thyroid cancer conferred by germline mutations of genes such as *APC* (29). It is worth speculating that others may have germline mutations of genes coding for components of double-stranded DNA break repair complexes, and that this may favor development of multicentric, *RET/PTC*-positive, papillary thyroid cancers.

The puzzling derivation of Hurthle cell tumors

Hurthle or oxyphilic cell tumors have traditionally been considered variants of follicular neoplasms. Recently, several independent groups have challenged this notion (30). Based on nuclear morphological features, a subset of Hurthle cell tumors appears to resemble papillary thyroid carcinomas. More compelling support for this comes from the observation that most, but not all, Hurthle cell adenomas and carcinomas harbor *RET/PTC* rearrangements, and immunostain for *RET* (30). It remains to be seen whether *RET/PTC* rearrangements occur as an early event in the development of this subset of Hurthle cell tumors, or whether they develop later in the course of the disease. The reclassification of Hurthle cell tumors as variants of papillary carcinoma is not yet universally accepted but may have significant practical implications. It appears that “Hurthle cell papillary carcinomas” may have a distinct biological behavior and tend to spread to regional lymph nodes, as opposed to Hurthle cell carcinomas without *RET* rearrangements, which metastasize at a distance (30). Moreover, this adds to the list of neoplastic diseases in which *RET* may play a pathogenetic role, which as discussed below, may provide opportunities for new treatment modalities.

RET tyrosine kinase as a target for drug development

The amino sequence and three-dimensional structure of protein kinases have a high degree of homology to each other. The number of protein kinases for which there is x-ray crystal structure has increased remarkably, with over 70 protein kinases reported in the Protein Data Bank. All members of the kinase family bind the same nucleotide cofactor, ATP. The availability of structural information from multiple protein kinase family members in complex with selective site-directed inhibitors of ATP binding reveal that these compounds make contacts with both conserved and non-conserved residues within the ATP-binding site. This enables design of relatively specific small cell-permeable kinase inhibitors (31). A notable success has been the treatment of chronic myelogenous leukemia with STI 571 (imatinib mesylate), an inhibitor of constitutively activated *abl* kinase (32, 33). This compound is also effective against other kinases, such as the platelet-derived growth factor receptor and *c-kit*, which offers opportunities for additional therapeutic applications (34). Additional trials are planned to investigate the efficacy of imatinib mesylate to treat a variety of solid tumors whose pathogenesis is driven by the other tyrosine kinase targets. The *RET* kinase has a 99.2% homology with the eukaryotic protein kinase domain. Recently, compounds have been identified that exhibit significant inhibitory activity on *RET* kinase (35, 36). As *RET* oncoproteins are involved in tumor initiation of both medullary and papillary thyroid carcinomas, this new class of drugs may prove to be clinically beneficial for patients with advanced forms of these diseases.

A novel rearrangement in follicular carcinomas

We have known for several years that follicular adenomas and carcinomas arise through an oncogenic pathway distinct from that of papillary carcinomas, probably from the point

of clonal initiation. Key molecular genetic differences include a higher prevalence of activating mutations of all three *RAS* genes, and a much greater predisposition to develop DNA copy abnormalities. Until recently, however, there were no genetic abnormalities that could distinguish follicular adenomas from carcinomas. Kroll *et al.* (37) reported the molecular basis for a chromosomal translocation t(2:3)(q13;p25) that apparently is unique to follicular carcinomas and that may offer insights into the mechanisms of malignant transformation of follicular neoplasms. The chromosome 2q13 breakpoint lies within the coding region of the thyroid transcription factor PAX8, and the 3p25 break within the coding region of the PPAR γ 1. This rearrangement was found in 5/8 follicular carcinomas, but in none of 20 follicular adenomas. The fusion protein consists of the paired and homeobox binding domains of PAX8, and the DNA binding, ligand binding, dimerization, and transactivation domains of PPAR γ 1. The functional impact of this rearrangement remains unclear. When the PAX8/PPAR γ 1 fusion gene was transfected to heterologous cells, it did not transactivate promoter constructs containing PPAR response elements, either alone or in the presence of the PPAR ligand agonist troglitazone. The fusion construct did, however, prevent wild-type PPAR γ 1-mediated transactivation, indicating that it may have a dominant negative effect. This is of interest because PPAR γ agonists induce terminal differentiation and growth suppression of normal preadipocytes and human liposarcoma cells *in vitro* and *in vivo* (38). At this point, there are no data on whether thyroid follicular carcinoma cells will respond similarly, or conversely, whether loss of PPAR γ 1-mediated responses may adversely affect thyroid cell differentiation.

Clues to pathogenesis of follicular carcinomas from genetic studies of familial cancer syndromes

The past few years have seen the discovery of genes that when mutated confer predisposition to two familial syndromes of follicular neoplasia: Cowden disease and the Carney complex. Inactivating germline mutations of *PTEN*, which encodes a dual-specificity phosphatase, have been found in up to 80% of patients with Cowden disease, a syndrome of multiple hamartomas, tumors and hyperplastic lesions that may develop in almost any organ (39). Because follicular adenomas and carcinomas occur in this syndrome, a role for the tumor suppressor gene *PTEN* has also been proposed in the pathogenesis of sporadic follicular thyroid tumors. However, and despite the fact that LOH of markers within the *PTEN* locus at 10q22-24 occurs in about 25% of follicular carcinomas, somatic intragenic mutations in *PTEN* are rare. Decreased *PTEN* expression is seen in a significant proportion of follicular neoplasms, suggesting that haplo-type insufficiency and/or epigenetic factors may play a role in disease pathogenesis (40). This is also suggested by the fact that activation of the PI3K substrate Akt appears to be increased in a subset of follicular carcinomas, consistent with a relaxation of the inhibitory effects of the tumor suppressor *PTEN* on this signaling pathway (41).

Carney complex, an autosomal dominant disorder associated with cardiac myxomas and endocrine tumors, has recently been reported to be caused in part by inactivating

mutations of the gene encoding the regulatory subunit 1A of the cAMP-dependent PKA (*PKAR1A*), resulting in its inappropriate activation (42, 43). Although the Carney complex is primarily associated with benign endocrine neoplasms, thyroid follicular carcinomas may also be observed (44).

As is the case for RET in papillary and medullary thyroid carcinomas, new information on molecular genetic changes associated with progression toward follicular thyroid carcinomas may offer a new set of promising therapeutic targets. This may be either in the form of small molecule cell permeable kinase inhibitors or nuclear hormone receptor agonists, the latter if PPAR γ 1 were to play an important role in cell cycle control or differentiated gene expression in advanced thyroid cancers.

James A. Fagin
Division of Endocrinology and Metabolism
University of Cincinnati College of Medicine
Cincinnati, Ohio 45267-0547

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Address all correspondence and requests for reprints to: James A. Fagin, M.D., Division of Endocrinology and Metabolism, University of Cincinnati College of Medicine, Vontz Center for Molecular Sciences, 3125 Eden Avenue, Cincinnati, Ohio 45267-0547. E-mail: James.Fagin@uc.edu.

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