

## 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 $\alpha$*  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 <sup>131</sup>I

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

in diagnosis and therapy of benign thyroid diseases. Based on evidence that therapeutic doses of  $^{131}\text{I}$  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  $^{131}\text{I}$  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  $\gamma$ 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  $\gamma$ 1. The functional impact of this rearrangement remains unclear. When the PAX8/PPAR  $\gamma$ 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  $\gamma$ 1-mediated transactivation, indicating that it may have a dominant negative effect. This is of interest because PPAR  $\gamma$  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  $\gamma$ 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  $\gamma$ 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

#### Acknowledgments

I am grateful to present and former postdoctoral fellows and research associates in my laboratory for their many intellectual and experimental contributions. In particular, I am grateful to Yuri Nikiforov, Jianwei Wang, Jeffrey Knaut, Efsio Puxeddu, Mickey Croyle, Marina Nikiforova, and Bin Ouyang.

Received January 31, 2002. Accepted February 14, 2002.

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.

This work was supported in part by NIH Grants CA-50706 and M01-RR-08084.

#### References

- Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R, Louis JC, Hu S, Altrock BW, Fox GM 1996 GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR- $\alpha$ , a novel receptor for GDNF. *Cell* 85:1113–1124
- Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della PG, Fusco A, Vecchio G 1990 PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell* 60:557–563
- Bongarzone I, Monzini N, Borrello MG, Carcano C, Ferraresi G, Arighi E, Mondellini P, Della Porta G, Pierotti MA 1993 Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI  $\alpha$  of cyclic AMP-dependent protein kinase A. *Mol Cell Biol* 13:358–366
- Minoletti F, Butti MG, Coronelli S, Miozzo M, Sozzi G, Pilotti S, Tunnaciff A, Pierotti MA, Bongarzone I 1994 The two genes generating RET/PTC3 are localized in chromosomal band 10q11.2. *Genes Chromosomes Cancer* 11:51–57
- Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, Fusco A 1994 Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. *Oncogene* 9:509–516
- Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM 1996 A new form of RET rearrangement in thyroid carcinomas of children after the Chernobyl reactor accident. *Oncogene* 13:1099–1102
- Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM 1998 Detection of a novel type of RET rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved RET-fused gene RFG5. *Cancer Res* 58:198–203
- Klugbauer S, Rabes HM 1999 The transcription coactivator HTIF1 and a related protein are fused to the RET receptor tyrosine kinase in childhood papillary thyroid carcinomas. *Oncogene* 18:4388–4393
- Bongarzone I, Fugazzola L, Vigneri P, Mariani L, Mondellini P, Pacini F, Basolo F, Pinchera A, Pilotti S, Pierotti MA 1996 Age-related activation of the tyrosine kinase receptor protooncogenes RET and NTRK1 in papillary thyroid carcinoma. *J Clin Endocrinol Metab* 81:2006–2009
- Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA 1997

- Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Res* 57:1690–1694
11. Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM 1995 High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. *Oncogene* 11:2459–2467
  12. Fugazzola L, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, Bongarzoni I, Greco A, Astakhova L, Butti MG, Demidchik EP 1995 Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. *Cancer Res* 55:5617–5620
  13. Bounacer A, Wicker R, Caillou B, Cailleux AF, Sarasin A, Schlumberger M, Suarez HG 1997 High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. *Oncogene* 15:1263–1273
  14. Viglietto G, Chiappetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, Visconti R, Mastro A, Santoro M, Fusco A 1995 RET/PTC oncogene activation is an early event in thyroid carcinogenesis. *Oncogene* 11:1207–1210
  15. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, Picone A, Portella G, Santelli G, Vecchio G, Fusco A 1996 Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. *Oncogene* 12:1821–1826
  16. Jhiang SM, Sagartz JE, Tong Q, Parker-Thornburg J, Capen CC, Cho JY, Xing S, Ledent C 1996 Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. *Endocrinology* 137:375–378
  17. Powell DJJ, Russell J, Nibu K, Li G, Rhee E, Liao M, Goldstein M, Keane WM, Santoro M, Fusco A, Rothstein JL 1998 The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. *Cancer Res* 58:5523–5528
  18. Ito T, Seyama T, Iwamoto KS, Hayashi T, Mizuno T, Tsuyama N, Dohi K, Nakamura N, Akiyama M 1993 *In vitro* irradiation is able to cause RET oncogene rearrangement. *Cancer Res* 53:2940–2943
  19. Mizuno T, Kyoizumi S, Suzuki T, Iwamoto KS, Seyama T 1997 Continued expression of a tissue specific activated oncogene in the early steps of radiation-induced human thyroid carcinogenesis. *Oncogene* 15:1455–1460
  20. Nikiforov YE, Koshoffer A, Nikiforova M, Stringer J, Fagin JA 1999 Chromosomal breakpoint positions suggest a direct role for radiation in inducing illegitimate recombination between the ELE1 and RET genes in radiation-induced thyroid carcinomas. *Oncogene* 18:6330–6334
  21. Nikiforova MN, Stringer JR, Blough R, Medvedovic M, Fagin JA, Nikiforov YE 2000 Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. *Science* 290:138–141
  22. Portella G, Salvatore D, Botti G, Cerrato A, Zhang L, Mineo A, Chiappetta G, Santelli G, Pozzi L, Vecchio G, Fusco A, Santoro M 1996 Development of mammary and cutaneous gland tumors in transgenic mice carrying the RET/PTC1 oncogene. *Oncogene* 13:2021–2026
  23. Ron E, Doody MM, Becker DV, Brill AB, Curtis RE, Goldman MB, Harris BS, III, Hoffman DA, McConahey WM, Maxon HR, Preston-Martin S, Warshauer ME, Wong FL, Boice Jr JD 1998 Cancer mortality following treatment for adult hyperthyroidism. Cooperative Thyrotoxicosis Therapy Follow-up Study Group. *JAMA* 280:347–355
  24. Robbins J, Schneider AB 2000 Thyroid cancer following exposure to radioactive iodine. *Rev Endocr Metab Disord* 1:197–203
  25. Robbins J, Schneider AB 1998 Radioiodine-induced thyroid cancer: studies in the aftermath of Chernobyl. *Trends Endocrinol Metab* 9:87–92
  26. Lemoine NR, Mayall ES, Williams ED, Thurston V, Wynford-Thomas D 1988 Agent-specific ras oncogene activation in rat thyroid tumours. *Oncogene* 3:541–544
  27. Sugg SL, Ezzat S, Rosen IB, Freeman JL, Asa SL 1998 Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. *J Clin Endocrinol Metab* 83:4116–4122
  28. Roudebush CP, Asteris GT, DeGroot LJ 1978 Natural history of radiation-associated thyroid cancer. *Arch Intern Med* 138:1631–1634
  29. Harach HR, Williams GT, Williams ED 1994 Familial adenomatous polyposis associated thyroid carcinoma: a distinct type of follicular cell neoplasm. *Histopathology* 25:549–561
  30. Cheung CC, Ezzat S, Ramyar L, Freeman JL, Asa SL 2000 Molecular basis of Hurthle cell papillary thyroid carcinoma. *J Clin Endocrinol Metab* 85:878–882
  31. Traxler P, Bold G, Buchdunger E, Caravatti G, Furet P, Manley P, O'Reilly T, Wood J, Zimmermann J 2001 Tyrosine kinase inhibitors: from rational design to clinical trials. *Med Res Rev* 21:499–512
  32. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M 2001 Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344:1038–1042
  33. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL 2001 Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 344:1031–1037
  34. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD 2001 Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 344:1052–1056
  35. Lanzi C, Cassinelli G, Pensa T, Cassinis M, Gambetta RA, Borrello MG, Menta E, Pierotti MA, Zunino F 2000 Inhibition of transforming activity of the ret/ptc1 oncoprotein by a 2-indolinone derivative. *Int J Cancer* 85:384–390
  36. Croyle ML, Knauf JA, Traxler P, Fagin JA, Specific inhibition of kinase activity of Ret oncoproteins and of Ret-induced cell growth by PKI166. Program of the 84<sup>th</sup> Annual Meeting of The Endocrine Society, San Francisco, CA, in press
  37. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA 2000 PAX8-PPAR $\gamma$ 1 fusion oncogene in human thyroid carcinoma. *Science* 289:1357–1360
  38. Demetri GD, Fletcher CD, Mueller E, Sarraf P, Naujoks R, Campbell N, Spiegelman BM, Singer S 1999 Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor- $\gamma$  ligand troglitazone in patients with liposarcoma. *Proc Natl Acad Sci USA* 96:3951–3956
  39. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R 1997 Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 16:64–67
  40. Bruni P, Boccia A, Baldassarre G, Trapasso F, Santoro M, Chiappetta G, Fusco A, Viglietto G 2000 PTEN expression is reduced in a subset of sporadic thyroid carcinomas: evidence that PTEN-growth suppressing activity in thyroid cancer cells mediated by p27kip1. *Oncogene* 19:3146–3155
  41. Ringel MD, Hayre N, Saito J, Saunier B, Schuppert F, Burch H, Bernet V, Burman KD, Kohn LD, Saji M 2001 Overexpression and overactivation of Akt in thyroid carcinoma. *Cancer Res* 61:6105–6111
  42. Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA, Stratakis CA 2000 Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. *Hum Mol Genet* 9:3037–3046
  43. Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA 2000 Mutations of the gene encoding the protein kinase A type I- $\alpha$  regulatory subunit in patients with the Carney complex. *Nat Genet* 26:89–92
  44. Nwokoro NA, Korytkowski MT, Rose S, Gorin MB, Penles SM, Witchel SF, Mulvihill JJ 1997 Spectrum of malignancy and premalignancy in Carney syndrome. *Am J Med Genet* 73:369–377