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Translational Research in Endocrine Surgery

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Synopsis

This article reviews translational research in endocrine surgery, with a focus on disorders of the thyroid, parathyroids, adrenals, and endocrine pancreas. Discovery of genes responsible for heritable endocrine cancer syndromes, including MEN1, MEN2, von Hippel-Lindau, Neurofibromatosis, and Familial Pheochromocytoma/Paraganglioma syndromes, has increased knowledge of the causes and mechanisms of endocrine cancer and refined surgical treatment options. Knowledge of mutations in sporadic cancer, such as *BRAF* in thyroid cancer, has led to rapid progress in small-molecule kinase inhibitor strategies. These breakthroughs and their influence on current endocrine therapy are discussed to provide endocrine surgeons with an overview of the basic science research currently creating new clinical treatments and improving patient care.

Keywords

Endocrine cancer; heritable cancer syndromes; pancreatic neuroendocrine tumors; thyroid surgery; MEN2

Introduction

The past thirty years have seen incredible advances in the science of endocrine surgery. From early successes in mapping and cloning genes responsible for heritable endocrine cancer syndromes, to sequencing the human genome, to adoption of next-generation sequencing techniques, a broad understanding of genes responsible for familial and sporadic endocrine cancers now exists. This has enabled genetic testing of at-risk family members and even prophylactic surgery for some carriers of mutant genes. Parallel efforts to determine the function of these altered genes have defined cell-signaling pathways susceptible to treatment. High-throughput gene expression methodologies now give insight

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into entire networks of cellular processes perturbed in endocrine malignancy. The knowledge gained has led to development of small-molecule kinase inhibitors and other therapies able to specifically target the genes, pathways, and cells responsible for disease. New treatments based on rational drug development and targeted therapies continue to be the focus of aggressive investigation and ongoing clinical trials. Diagnosis and prognostication in endocrine cancer has likewise been improved using the results of mutation and gene expression data. The aim of this work is to review advances in the basic science of endocrine cancer, and highlight how these discoveries are being translated into real-world tests and therapies that will impact the practice of endocrine surgery today and in the near future. Heritable and sporadic tumors of the thyroid, parathyroids, adrenals, and pancreas will be emphasized. We expect that familiarity with these breakthroughs and with the ongoing challenges in endocrine cancer surgery will enhance clinicians' abilities to apply the latest scientific developments to the optimal care of their patients.

Thyroid

Overview of MAPK

The mitogen-activated protein kinase (MAPK) cascade is a cellular signaling pathway now established as central to thyroid cancer. In this cascade, extracellular signals such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and many others activate membrane-bound receptor tyrosine kinases, including RET, which cause RAS activation and induction of the RAS-RAF-MEK-ERK-MAP signaling cascade^{1,2} (Fig. 1). Activation of the MAPK pathway influences diverse cellular processes including cell cycle control, proliferation, differentiation, motility, and apoptosis^{3,4}. The pathway is highly regulated through expression of multiple isoforms of component proteins and cross-talk with related pathways, such as phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR), Janus-kinase, PKC/NF- κ B, and Wnt/ β -catenin, each contributing to different functional roles in different tissues and contexts^{2,5,6}. Apart from its physiologic role in thyroid differentiation, growth, and function, the MAPK pathway can also contribute to development of thyroid cancer by aberrant activation at several points. In sporadic and hereditary medullary thyroid cancer (MTC), mutant RET activates RAS, causing constitutive MAPK signaling⁷, while mutations in both *RET* and *RAS* are common in follicular thyroid cancer⁸. Downstream in the pathway, mutation in the BRAF serine/threonine kinase has emerged as the most common genetic abnormality in papillary thyroid cancer (PTC), and is also present in anaplastic thyroid cancer (ATC)^{9,10}. In total, mutation in some element of the MAPK pathway is present in over 70% of thyroid cancers, marking this as the central cellular control element in thyroid oncogenesis¹¹. Over the past twenty-five years, basic and translational research has defined the role of the MAPK pathway in thyroid cancer and produced promising new diagnostic and therapeutic strategies for this heterogeneous disease.

RET proto-oncogene

While the phenotype and autosomal dominant inheritance pattern of multiple endocrine neoplasia type 2 had been recognized for some time, it was not until 1987 that genetic linkage analysis mapped the causative locus for MEN2A to near the centromere of chromosome 10^{12,13}. Other heritable MTC phenotypes, such as MEN2B and familial medullary thyroid cancer (FMTC), were subsequently linked to the same region¹⁴. A gene known to map to this part of chromosome 10 was the *RET* proto-oncogene. *RET* (REarranged during Transfection) was first identified as a human lymphoma oncogene capable of transforming cells *in vitro*¹⁵. A gene isolated from papillary thyroid cancer specimens (*PTC*) had the same effect and also mapped to chromosome 10^{16,17}. Further investigation showed that this *PTC* oncogene was actually chimeric *RET* rearranged and

fused with another gene to form the *RET/PTC* proto-oncogene^{18,19}. In 1993, linkage narrowed the MEN2A locus to a small area near the *RET* proto-oncogene²⁰, and *RET* was identified as the causative gene in MEN2A and FMTC, with two groups reporting heterozygous germline mutations in affected patients, but not in normal controls and unaffected family members^{21,22}. Description of *RET* mutations in MEN2B kindreds followed soon thereafter^{23–25}.

Genetic tests to presymptomatically identify affected individuals in families with MEN2 and FMTC became more directed after identification of these mutations²⁶. Wells *et al.* became the first to use a genetic test to recommend prophylactic surgery, performing total thyroidectomy and parathyroidectomy in asymptomatic patients within MEN2A families found to carry *RET* mutations²⁷. Interestingly, even in patients with normal calcitonin levels, all of these patients' thyroidectomy specimens contained evidence of pre-cancerous C-cell hyperplasia or overt MTC.

With *RET* identified as the mutation responsible for MEN2A, 2B, and FMTC, it became clear that disease features varied according to the specific mutations present. In 1994, Mulligan *et al.* reported that MEN2A families with parathyroid hyperplasia and pheochromocytoma carried the C634R *RET* mutation much more frequently than families lacking these disease features²⁸. In 1996, the International *RET* Consortium pooled sequencing and clinical data from 477 MEN2 and FMTC families to catalogue the various mutations' phenotypic associations (Table 1)²⁹. This revealed that codon 634 mutations accounted for 85% of MEN2A cases, and that the C634R mutation was significantly more likely to lead to hyperparathyroidism and pheochromocytoma. Families with the less aggressive FMTC had mutations in several codons including 634, but none carried the C634R mutation. Finally, 75 of 79 MEN2B families had the same M918T mutation. These results and others demonstrated the robustness of the genetic diagnoses, provided valuable information on which patients were at highest risk for additional disease features beyond MTC, and provided a strong rationale for very early surgery in the highest-risk patients to preempt development of metastatic MTC^{29,30}.

Consensus guidelines grouping all known mutations into risk categories with recommendations for screening and age of thyroidectomy were developed in 2001³¹, and updated by the American Thyroid Association in 2009³². For the highest risk MEN2B mutations, thyroidectomy is recommended as soon as possible and ideally before 1 month of age, with central node dissection recommended if surgery is delayed beyond 1 year of age³² (Table 2). For the next highest risk group, thyroidectomy is recommended before 5 years, and for some less high risk mutations, patients and surgeons may consider delaying thyroidectomy to before age 10 in the setting of normal calcitonin and ultrasound, less aggressive family history, and strict compliance with screening^{31,32}. Certain MEN2A mutations, such as C609Y, lead to MTC usually only after age 20–40³³, and thyroidectomy between ages 5 and 10 for these patients can be acceptable under the ATA guidelines³². Still, rare examples of MTC in very young patients with mutations thought to confer lower risk exist^{31,32,34}, and therefore early thyroidectomy (before age 5) remains a reasonable option for these groups^{31, 32}. With the high sensitivity and specificity of genetic testing, and the fact that 98% of MEN2 patients have a known *RET* mutation³¹, genetic testing is now the standard to determine prophylactic surgical therapy in MEN2.

RET in sporadic MTC

RET mutations are found with high frequency in sporadic MTC. A study of 100 sporadic MTC patients with 10 year median follow-up identified somatic *RET* mutations in 43%, and found that their presence strongly correlated with lymph node and distant metastases (70 and 30% vs. 26 and 12% in *RET*+ and - patients, $p < 0.0001$)³⁵. Of patients with mutations, 79%

had the same M918T mutation found in most MEN2B cases. Sporadic *RET* mutations also independently predicted worse outcomes, with significantly reduced disease-free, and overall survival³⁵. In a separate study of 36 patients with sporadic and 21 with familial MTC, 75% of tumors had *RET* mutations and 16% had *RAS* mutations³⁶. Knowledge of mutational status in sporadic MTC will likely become increasingly important for stratification in clinical trials, or to direct therapy towards specific pathways.

Overview of BRAF

RAF kinase is normally activated by RAS and in turn activates MEK in the MAPK pathway (Fig. 1). Three genes encode different isoforms of RAF (A-RAF, B-RAF, and C-RAF), and of these, *BRAF* has proven important in several human cancers⁶. In 2002, a genome-level screen for cancer-related mutations found activating somatic *BRAF* mutations in many human tumor cell types, including 59% of melanoma lines studied³⁷. Although over 30 *BRAF* mutations have been described, a single base-pair substitution (T1799A) replacing valine with glutamate at residue 600 (V600E) is both the most common and most potent mutation, leading to 700-fold increased kinase activity, constitutive activation of the MAPK pathway, transformation of cells *in vitro*, and growth of tumors in mice *in vivo*^{4,37,38}.

Soon after the initial report of *BRAF* mutations in human cancer, the V600E mutation was reported in a high percentage of human papillary thyroid cancers. Kimura *et al.* found the *BRAF* mutation in 28/78 (36%) of adult, non-radiation exposed PTC specimens, while *RET* and *RAS* mutations accounted for only 16% of cases each³⁹. Concurrently, another group reported the V600E mutation in 24/35 (69%) of PTC specimens in a screen of 476 samples from diverse types of primary tumors⁴⁰. *BRAF* mutation was determined to occur more frequently in tall cell and less commonly in follicular variants of PTC⁴, and a recent meta-analysis established a 45% overall prevalence of the V600E mutation in papillary thyroid cancer (1,118 of 2,470 published cases)¹⁰. While medullary thyroid cancers do not carry *BRAF* mutations, 24–40% of anaplastic thyroid cancers do, and *BRAF* mutations are common in poorly differentiated, recurrent, and radioiodine-resistant thyroid cancers^{4,9}.

Intense research in the past decade revealed much about the molecular, cellular, and clinical effects of *BRAF* mutation. Determination of the crystal structure of mutant BRAF protein showed how the V600E mutation, nestled between activating phosphorylation sites at T599 and S602, disrupts the hydrophobic interactions that stabilize the inactive form of the protein, and mimics the conformation of a phosphorylated state³⁸. This change creates a pseudo-phosphorylated conformation and leads to constitutive pathway activation and tumorigenic behavior. Consistent with this model, invasiveness of thyroid cancer cell cultures expressing BRAF V600E was found to require signaling via the MAP kinase pathway⁴¹. Thyroid cells with the V600E mutation also show reduced markers of differentiation. Using inducible *BRAF*V600E mutant rat thyroid cell lines, Mitsutake and colleagues showed suppressed expression of genes necessary for iodine handling and thyroid differentiation, such as thyrotropin receptor (*TSHR*), sodium-iodine symporter (*NIS*), and thyroglobulin (*Tg*), as well as increased chromosomal instability⁴². In both rat and human thyroid cell lines, these phenotypes could be corrected by treatment with the MAP kinase inhibitor U0126⁴¹.

Despite the clear importance of the MAPK pathway, there is a growing understanding that *BRAF* mutations also influence related pathways. In thyroid cell culture with an inducible V600E mutation, Palona *et al.* showed upregulation of matrix-metalloproteinases and inhibitors of apoptosis. The upregulation was not blocked by inhibitors of the MAPK pathway, but instead depended on NF- κ B⁶. Similarly, Liu showed that while a MEK kinase inhibitor led to reduced invasiveness and cell cycle arrest in thyroid lines harboring *BRAF* mutations, this effect was potentiated by concurrent blockade of the NF- κ B pathway⁵. Wild-

type BRAF must form homodimers or heterodimers with CRAF to signal, and it forms these dimers only in response to RAS signaling. In contrast, BRAF V600E is always active, and forms homo or heterodimers in the absence of RAS signaling, and can additionally activate MEK as a monomer⁴³. Monomers and homodimers of mutant BRAF as well as heterodimers with wild-type RAF proteins may participate in MEK-independent signaling with the PI3K/AKT/mTOR, NF- κ B, and other pathways⁴⁴. Through both direct activity of mutant BRAF and through feedback from the constitutively active MAPK pathway⁴⁵, mutant BRAF may contribute to crosstalk effects in other pathways. These complex interactions belie the standard linear depiction of the MAPK pathway and have frustrated development of some pathway inhibitor-based therapeutics.

BRAF in Clinical Risk

As data accumulated to elucidate the molecular effects of the *BRAF*V600E mutation in thyroid cancer, clinical data has similarly accrued to support a picture of uncontrolled pro-malignant signaling. Tumors with the *BRAF*V600E mutation show increased risk for recurrence, lymphatic and distant metastases, and unfavorable pathologic characteristics predictive of more aggressive disease. In one study, 76% (24/34) of *BRAF*V600E positive (*BRAF*⁺) PTC primary tumors had positive sentinel lymph nodes, while only 17% (12/69) of *BRAF*V600E negative (*BRAF*⁻) primary tumors did⁴⁶. In another series, correlation of outcomes with retrospective *BRAF* testing of 190 PTC fine-needle aspiration (FNA) specimens showed that patients with *BRAF*⁺ FNAs had higher rates of unfavorable pathologic characteristics, with more frequent recurrence (36 vs. 12%), more lymph node metastases (39 vs. 19%), and more capsular invasion (29 vs. 16%) than *BRAF*⁻ FNAs⁴⁷. A long-term study of 203 PTC patients with median follow-up of over seven years found significant association between *BRAF*⁺ status and recurrence (21 vs. 7%, $p=.04$), but *BRAF* status itself was not an independent predictor of recurrence on multivariate analysis⁴⁸. Pooling 2470 patients from 452 studies regarding *BRAF* mutational status and clinical outcomes in PTC, Tufano *et al.* determined that *BRAF* mutation correlates with a higher risk of recurrence and metastasis. Overall, *BRAF* positivity was associated with significantly higher relative risk (RR) of tumor recurrence (24.9 vs. 12.6%, RR 1.93), lymph node metastasis (54.1% vs. 36.8%, RR 1.32), stage III or IV disease (35.4% vs. 19.6%, RR 1.70), and extrathyroidal extension (46.2% vs. 23.6%, RR 1.71) compared to *BRAF*⁻ PTCs. There was no difference in distant metastasis (8.0% vs. 7.9%)¹⁰. Testing for *BRAF* mutation informs risk estimates for resected thyroid cancer, and inclusion of *BRAF* status modestly improves performance of the AMES, MACIS, TNM, and ATA risk scores⁴⁹. Likewise for thyroid papillary microcarcinoma (TPMC), a risk score incorporating *BRAF* status allowed identification of high risk cancers that would not be identified by histology alone in a small study of 29 aggressive TPMCs matched to 30 nonaggressive cancers and validated in 40 others⁵⁰. Although papillary thyroid cancer has a favorable prognosis with over 90% survival at 10 years¹⁰, the *BRAF*V600E mutation puts patients at higher risk for recurrence, subjecting them to additional treatments and interventions, and likely causing worse quality of life, even if it does not cause decreased survival.

While knowledge of mutations in resected cancers improves prognostication, some have sought to apply molecular testing to prospective surgical decision-making. Based on their results correlating *BRAF*⁺ FNAs with higher recurrence risk even after correcting for unfavorable tumor characteristics, Xing *et al.* suggested that preoperative knowledge of *BRAF* status by FNA could help surgeons choose whether to perform prophylactic central neck dissection⁴⁷. The place for lymphadenectomy in well-differentiated thyroid cancer remains controversial. Some retrospective studies have suggested incremental improvements in recurrence and survival, but these are balanced against higher rates of hypocalcemia and nerve injury⁵¹. Prospective studies to determine whether preoperative *BRAF* testing of FNA

specimens is useful for predicting occult lymph node metastases have yielded conflicting results. One study (n=51) found that *BRAF* positivity in excised tumors did not correlate with nodal metastases, but did not test for *BRAF* preoperatively and had a low rate of *BRAF* mutation (29%)⁵². A larger study (n=148) found that *BRAF*⁺ FNA specimens were significantly associated with occult lymph node metastases after prophylactic central neck dissection, and concluded that *BRAF* status may be helpful in the decision whether to perform nodal dissection in a clinically node-negative neck⁵³. Unless a prospective randomized trial with long-term follow-up is conducted, the optimal extent of nodal dissection will likely remain uncertain.

Chemotherapeutics targeting BRAF and MAPK

Multiple proteins in the BRAF-MAPK pathway have been targeted for thyroid cancer treatment, and these pathways remain among the most active areas of pharmaceutical research. In 2008, a phase I trial showed promise for selumetinib/AZD6244, a selective MEK1/2 inhibitor, which included a patient with metastatic MTC with a prolonged period of stable disease⁵⁴. In *BRAF*-mutated metastatic melanoma, a phase-III trial of the MEK inhibitor trametinib demonstrated significantly prolonged progression-free and overall survival in the treatment group⁵⁵. More recently, researchers completed an early phase trial of selumetinib in metastatic, radioiodine-refractory thyroid cancer⁵⁶. Applying the preclinical observation that treatment with MEK inhibitors seemed to restore thyroid differentiation and iodine uptake in thyroid cells, they treated 20 patients with well-differentiated, metastatic, radioiodine-refractory, follicular-origin thyroid cancer with selumetinib. After four weeks, iodine uptake was reassessed with ¹²⁴I PET scans and 12/20 patients had increased uptake. Of these patients, 8 had enough uptake to justify treatment with ¹³¹I, and of these, 5 had partial responses and 3 had stable disease 6 months after treatment. Cancers with *RAS* mutations responded best to selumetinib (5/5), while only 1/9 cancers with *BRAF*V600E had enough uptake to warrant treatment with ¹³¹I. Although limited in size, this rapid translation of cell culture and mouse data to human clinical trials may herald a new approach to kinase-inhibitor treatment in thyroid cancer.

Sorafenib, a multi-kinase inhibitor, has some activity in thyroid cancer, but early human use has shown low response rates. In 2008, sorafenib was shown to more potently inhibit thyroid cancer cells harboring the *RET/PTC1* gene rearrangement than those with *BRAF*V600E⁵⁷. In a phase II trial of sorafenib for anaplastic thyroid cancer, only 2/20 patients showed a partial response, and another 5 had stable disease; median progression free survival was only 1.9 months⁵⁸. Despite these modest results, there is growing evidence that response to treatment might be improved by prospective tumor genotyping, allowing improved selection of patients most likely to respond. In a trial of another multi-kinase inhibitor, Pisceazzi *et al.* analyzed results by gene expression, finding no relationship between response to sunitinib treatment and tumor expression of traditional sunitinib targets VEGFR, cKIT, and PDGFR. Response did correspond to expression of mutant *RET*, where mutation positive patients enjoyed higher response rates than those with *BRAF* or *RAS* mutations, providing further enthusiasm for prospective genetic profiling of thyroid cancers in future trials¹. Still another multiple tyrosine kinase inhibitor, cabozantinib/XL184, showed improved progression-free survival of 11.2 months versus 4.0 months with placebo in a phase III trial of the drug in progressive MTC⁵⁹. Cabozantinib was approved by the FDA for advanced MTC in November, 2012.

Kinase inhibitors specifically targeting BRAF have also been developed. One such is PLX4720, which inserts into the ATP-binding site of mutant BRAF, stabilizing its inactive state. *In vitro*, the inhibitor blocks phosphorylation of downstream BRAF targets and leads to upregulation of suppressed markers of thyroid differentiation⁶⁰. In a mouse model, Nehs and colleagues injected V600E anaplastic thyroid cancer cells into mouse thyroids, and then

treated with PLX4720. Treated mice had resectable tumors after 7 days and lived to the endpoint of 50 days, while untreated mice were found to have unresectable tumors at 7 days and were sacrificed by day 35 due to tumor burden⁶¹. To more closely mimic the advanced stage at which ATC usually presents, this group next started inhibitor treatment at 28 days after administration of tumor cells to allow metastatic disease to become established, and still demonstrated tumor regression⁶². Based on these studies and data in melanoma with the similar but orally available BRAF inhibitor vemurafenib, Rosove and colleagues reported a complete response to vemurafenib in a critically ill patient with metastatic *BRAF*^{V600E} ATC⁶³, providing clinical evidence of the promise of this approach in human ATC.

In one of the most encouraging inhibitor trials to date, a phase III, manufacturer-sponsored trial of the RET-inhibitor vandetanib recently showed efficacy in patients with advanced MTC⁶⁴. Treatment with vandetanib was associated with median progression-free survival time of 30.5 months versus 19.3 months for placebo (HR 0.46, CI 0.31 to 0.69), and objective response rates by RECIST criteria of 45 versus 13% ($p < 0.001$, with high placebo response rate due to 93% crossover to open-label vandetanib treatment among patients who progressed on placebo). Mild toxicities were common and serious toxicities occurred, including grade 3 or 4 diarrhea, hypertension, and QTc prolongation in 11%, 9%, and 8%, respectively, of treated patients. These results suggest that this drug will play a role in treatment of MTC in the future.

Translational research in thyroid nodules

An area of persistent clinical interest is the thyroid nodule with indeterminate cytology by FNA. Around 30% of all nodules have indeterminate FNA results, and of these, around 30% harbor malignancy⁶⁵. Due to the risk of malignancy, most patients with indeterminate nodules undergo surgery. Several novel methods have recently emerged to derive additional information from FNA specimens to select patients at low risk of malignancy who do not need surgery, and to identify those likely to have cancer, who will benefit from total thyroidectomy. McCoy and colleagues studied 670 patients at a single institution in cohorts before and after introduction of routine molecular testing of all non-benign thyroid FNA specimens⁶⁶. They reported that testing for mutations in *RET*, *RAS*, *BRAF*, and *PAX8/PPAR γ* (a fusion of the thyroid transcription factor *PAX8* promoter with the peroxisome proliferator-activated receptor-1 gene, which is found in some follicular thyroid cancers⁶⁷) identified mutations in 15% of indeterminate or nondiagnostic FNAs. Of 25 patients with positive molecular testing and no other indication for thyroidectomy, all were ultimately found to have some malignant changes, and 22/25 (88%) required total thyroidectomy based on current guidelines. With knowledge of the molecular testing results, 18 of these patients elected to undergo total thyroidectomy at their initial surgery. Concurrently, due to the increased preoperative knowledge, the sensitivity of intraoperative frozen section dropped to only 1.7% and was abandoned at their institution. The authors concluded that routine molecular testing (at an additional \$104 per specimen) adds valuable knowledge, helps to select the correct initial surgery more often, and is cost-effective by limiting the need for later reoperation for completion thyroidectomy⁶⁶. Overall, detecting a known malignant mutation on thyroid FNA has a positive predictive value for malignancy of nearly 100% for *BRAF*, *RET*, or *PAX8/PPAR γ* and 74–87% for *RAS*, and these patients should undergo total thyroidectomy⁶⁵.

While testing for mutations of known malignant potential can identify patients who should undergo total thyroidectomy, with its low negative predictive value, it cannot rule out malignancy. An emerging approach to this challenge uses differences in microRNA expression (miRNA) to separate benign and malignant thyroid nodules. MicroRNA can distinguish benign from malignant thyroid FNA specimens with high accuracy, however, when applied to indeterminate nodules, negative predictive values remain below 90%, and

are insufficient to recommend against surgery⁶⁸. Nevertheless, potential discovery of additional informative miRNAs or improvements in classification algorithms make this a promising avenue of research.

Another strategy assesses differential mRNA expression by quantitative PCR. In 2012, a multicenter validation study of a commercially available gene expression assay for indeterminate thyroid FNA specimens reported results from 265 patients who underwent thyroidectomy⁶⁹. The assay measures expression of 142 genes and uses a proprietary algorithm to classify specimens as benign or malignant. In this study, 32% of patients were ultimately found to have malignancy, and the gene expression classifier correctly identified 78 of these 85 (91.7%) as suspicious for malignancy. The negative predictive value of the test was 85–95%, depending on the Bethesda classification assigned to the specimen. These results were reported as evidence that the test could help avoid unnecessary thyroidectomies. At least one study funded by the company reported that the rate of surgery in indeterminate nodules classified as benign by the gene expression test fell to 7% among patients of participating endocrinologists, compared to 74% overall for indeterminate nodules prior to adoption of the test⁷⁰. Despite the promise of these reports, we believe it is premature to decide against surgery based on their results. All data collection and analysis in both studies was performed by the manufacturer, and the validation study employed a relatively small sample size of 265 patients. As such, these results could represent a best-case scenario of the test's performance. Furthermore, in the utilization study, the proportion of total tests classified as benign was not reported, making determination of the total rate of surgery impossible⁷⁰. An industry-sponsored study claiming cost-effectiveness of this \$3,200 test used a cost model which assumed very high 30 and 44% complication rates for hemi and total thyroidectomy and did not account for the costs and quality-of-life impact of missed cancer diagnoses⁷¹. Finally, while this test clearly adds information over cytology results alone, the unanswered question is whether negative predictive values of as low as 85% in the highest risk category (implying a 15% rate of malignancy) would be sufficient for most surgeons to recommend against surgery. A larger, independent validation of the test should be done⁷², and studies of reduced thyroidectomy rates after application of the test must include appropriate follow-up to determine how many patients not having surgery based on a negative test result ultimately develop cancer or undergo thyroidectomy at a later time.

Until completion of such studies, we agree with the management algorithm of Nikiforov *et al.* for solitary thyroid nodules with indeterminate cytology, which was based on analysis of 1,056 FNA specimens⁷³. Indeterminate FNAs have a cancer risk of 14–54% and should undergo molecular testing with a limited and less-expensive panel (*BRAF*, *RAS*, *RET*, and *PAX8/PPAR-γ*). Patients with positive results should undergo total thyroidectomy due to the high risk of malignancy (87–95%). Patients with follicular lesions or atypia of undetermined significance and negative testing have a risk of malignancy of 6% and may consider observation, repeat FNA, or diagnostic lobectomy, while those with follicular neoplasm/suspicion for follicular neoplasm or suspicion for malignancy have a risk of malignancy of 14–28% and should undergo diagnostic lobectomy⁷³.

Parathyroid/MEN1

Parathyroid abnormalities encompass both benign and malignant disease, and hyperparathyroidism is a feature of MEN1 and MEN2A syndromes. Parathyroid carcinoma remains exceedingly rare, but is strongly associated with the tumor suppressor *HRPT2/CDC73*. Germline *HRPT2* mutations cause familial hyperparathyroidism/jaw-tumor syndrome and somatic mutation of *HRPT2* is found in over 75% of sporadic parathyroid carcinomas^{74–76}. Some patients with seemingly sporadic parathyroid carcinoma will be found to have germline *HRPT2* mutations, making genetic testing of parathyroid carcinoma

patients potentially helpful by identifying related gene carriers who will benefit from serum calcium screening⁷⁵. Parafibromin, the protein product of *HRPT2*, acts as a cell-cycle regulator in the Wnt pathway through interaction with β -catenin and is involved in histone modification during transcription⁷⁷. Loss of immunohistochemical staining for parafibromin is highly suggestive of parathyroid carcinoma, and in the future may aid in diagnosis of malignant parathyroid tumors^{78,79}.

Hyperparathyroidism (HPT) occurs in a minority of MEN2A patients, with the risk strongly influenced by the specific *RET* mutation present²⁹ (Table 1). The incidence of HPT in MEN2A is known to be 20–35%⁸⁰, and is much more common with mutations of codon 634, although HPT also occurs in 1–5% of patients with codon 609, 611, 618, and 620 mutations⁸¹. By contrast, HPT is the most common manifestation of MEN1, with nearly 100% of carriers affected by the age of 50³¹ (Table 3). The *MEN1* gene was first mapped to chromosome 11 by linkage in 1988⁸², and cloned in 1997^{83,84}. The menin tumor suppressor protein encoded by *MEN1* interacts with many different proteins, including transcription factors and cell-cycle regulatory proteins, can bind DNA directly, and while it seems to play a role in chromatin remodeling and genomic stability, its exact function remains unclear^{85–87}. Unlike MEN2 mutations, which cluster at particular codons, *MEN1* mutations are highly variable, with over 1,100 distinct germline mutations described and the most common occurring in only 4.5% of families (as opposed to 85% of MEN2A families having *RET* codon 634 mutations)^{29,86}. Also unlike MEN2, in MEN1 individuals show similar manifestations of the disease within affected families, but have considerable variation between families, even those sharing the same mutation⁸⁶. Thus, while genetic testing for *MEN1* mutations is available, identifying carriers does not suggest a specific prophylactic surgical therapy, as in MEN2, but rather identifies those who require screening for development of different manifestations of the disease. Lairmore *et al.* found that genetic testing for MEN1 helped identify biochemical changes 5–10 years before the development of clinically apparent tumors, allowing for early surgical intervention in some cases⁸⁸. Those at risk for MEN1 by family history or confirmed through genetic testing should begin serum calcium screening before the age of 10 years to detect development of HPT⁸⁹. In sporadic HPT, keen attention to family history suggestive of MEN1 (pituitary adenoma, pancreatic neuroendocrine tumor, hyperparathyroidism, thymic carcinoid, cutaneous angiofibroma, ependymoma, nodular adrenocortical hyperplasia, or multiple lipomas) helps identify patients who may benefit from genetic testing and four-gland exploration^{88,90}.

The importance of the *MEN1* gene to parathyroid disease is highlighted by the frequency of somatic *MEN1* mutation in sporadic parathyroid adenomas. Cromer and colleagues performed whole exome sequencing on 8 sporadic parathyroid adenomas and corresponding genomic DNA, identifying 29 somatic mutations in the adenomas⁹¹. Screening 185 additional sporadic parathyroid adenomas for these by direct sequencing revealed *MEN1* mutations in 35.2% of the validation cohort, while only 1 of the other mutations (occurring in 1/185 patients) was found. Thus, while the exact mechanisms by which *RET* and *MEN1* lead to parathyroid disease remain unknown, somatic *MEN1* mutation is a frequent event in sporadic and heritable parathyroid disease.

Pheochromocytoma/Paraganglioma

In addition to MEN2A, other autosomal dominant heritable disorders predispose patients to developing pheochromocytomas or paragangliomas (PCC/PGL), including Neurofibromatosis Type 1 (NF1), von Hippel-Lindau syndrome (vHL), and Familial Paraganglioma syndromes⁸⁵. Genetic testing for these conditions is available, but as with MEN1, no specific prophylactic surgical procedure exists. Genetic testing therefore allows screening and early tumor discovery, as well as prevention of anesthesia complications by

preoperative β -blockade in patients who could have PCCs. Overall, approximately 25% of PCCs display malignant characteristics, and approximately one third of all PCCs are associated with a known germline mutation⁹².

Patients with NF1 are at increased risk for PCC. Although the *NF1* gene has been known for some time^{93,94}, due to its large size (300kb, 57 exons), complexity (36 different pseudogenes and multiple splice variants), and the diversity of mutations causing the disorder, NF1 continues to be diagnosed based on clinical phenotype rather than genetic testing⁹⁵. Around 6% of NF1 patients will develop PCC⁹⁵. Among NF1 patients developing PCC, 67% show loss of the non-mutated *NF1* region in tumor specimens, suggesting that PCCs in NF1 require a “second hit,” for tumor development⁹⁵. *NF1* is also the most common somatically mutated gene in sporadic PCC (26% of sporadic tumors), with loss of heterozygosity of the wild-type allele in 91% of these⁹⁶. Neoplasia in NF1 follows pathways familiar from other endocrine cancers. One function of the *NF1* protein product neurofibromin is in RAS/MAPK signaling, where it interacts with RAS to stimulate the RAS GTPase, which terminates its signal⁹⁷ (Fig. 1). Lack of functional neurofibromin thereby allows the RAS signal to persist, activating the MAPK and PI3K/AKT/mTOR pathways. Activation of mTOR in *NF1*-mutated tumors is influenced by inactivation of tuberlin, the product of the Tuberous Sclerosis Complex gene *TSC2*⁹⁸. As such, development of tumors in neurofibromatosis is intimately related to the mechanisms underlying tumors in MEN2 and Tuberous Sclerosis⁸⁵.

After identification of the gene causing vHL in 1993⁹⁹, it became possible to screen at-risk patients based on genetic testing for PCCs, cerebellar hemangioblastomas, retinal angiomas, renal cell carcinomas, pancreatic neuroendocrine tumors, endolymphatic sac tumors, and cystadenomas of the pancreas, epididymis, and broad ligament, which characterize the disease^{85,100}. Although less common than the hallmark CNS and retinal hemangioblastomas (60–80% of patients) and renal clear cell carcinomas (70% lifetime risk)¹⁰¹, PCCs occur in 10–20% of vHL patients⁹². In 573 vHL patients, Ong *et al.* found PCCs were significantly more common (penetrance ~60% by 50 years versus 5–20% in other groups) and occurred earlier (mean 21.7 vs. 27.8 years, $p=0.012$) in patients with missense mutations of surface residues of the VHL protein, compared to those with deletions, truncations, or missense mutations of core residues¹⁰². This genotypic distinction between mutations with low and high risk of PCC correlates closely with the earlier clinical designation of vHL type 1 and 2¹⁰². VHL protein interacts with elongin as part of an E3 ubiquitin ligase complex, and also directly with hypoxia-inducible factor (HIF) 1 and 2 alpha subunits to target them for degradation¹⁰² (Fig. 1). Mutant proteins may fail to cause degradation of HIF proteins, leading to pro-malignant signaling, but as some patients with mutations that do not disrupt HIF interactions still develop PCC, additional mechanisms, such as failure of normal apoptosis of adrenal progenitor cells, may be responsible for PCC features of vHL syndrome^{101,102}.

Germline mutations in each of the four succinate dehydrogenase complex subunits and of a gene required for flavination and function of the complex (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, collectively called SDHx) predispose to autosomal dominant Familial Paraganglioma syndrome, with distinct phenotypes and inheritance. The SDH genes form mitochondrial complex II, which is involved in both the TCA cycle and electron transport chain⁹². *SDHD* was first identified as a cause of Familial Paraganglioma syndrome¹⁰³, and *SDHD* mutations were soon reported in sporadic PGLs and hereditary and sporadic PCCs^{104,105}. Inheritance of *SDHD* is autosomal dominant, but with a maternal-imprinting effect, such that patients inheriting the maternal copy display no symptoms, while those receiving a paternal allele have partial penetrance¹⁰³. In affected patients, somatic loss of the maternal chromosome 11, containing the wild-type allele, is required for tumor

development¹⁰⁶. After identification of the *SDHD* mutation, reports of mutations causing the syndrome in the A¹⁰⁷, B¹⁰⁸, and C¹⁰⁹ SDH subunits, as well as in the SDH assembly-factor 2 (*SDHAF2*) gene followed¹¹⁰. No imprinting effect is observed in *SDHB* and *C*, and inheritance is autosomal dominant, while *SDHAF2* shows paternal inheritance similar to *SDHD*^{108,109,111}. As with HIF-stabilization in vHL syndrome, the SDHx mutations inactivate the SDH complex, leading to a defect in electron transport, accumulation of succinate, and induction of a pseudo-hypoxic state with consequent persistence of HIF factors. This in turn induces angiogenesis and other proliferative and pre-neoplastic signaling¹⁰⁷ (Fig. 1).

Features of PCC/PGL syndromes depend on the mutation present and the tumor's location. PGL tumors in the head and neck rarely produce active catecholamines, while up to 50% of their abdominal PCCs/PGLs do^{85, 112}. Malignant PCCs occur more commonly in families with *SDHB* mutations, while *SDHD* PCCs are more often benign¹¹³. In malignant PCC/PGL, *SDHB* mutations also portend a worse prognosis, and are associated with lower 5-year survival when compared to tumors without *SDHB* mutations¹¹⁴. Mutations of the other SDHx genes rarely lead to PCCs, and these syndromes are marked mostly by head and neck paragangliomas¹¹³.

Two additional PCC-related tumor suppressor genes, *TMEM127* and *MAX*, were identified in hereditary PCC kindreds without known mutations (Fig. 1). Qin *et al.* reported autosomal dominant inheritance of 6 distinct germline *TMEM127* mutations in 7 families, representing 30% of familial and 3% of apparently sporadic PCCs out of 102 screened¹¹⁵. While the function of *TMEM127* remains unknown, it seems to behave as a tumor suppressor and negative regulator of mTORC1. It co-localizes with mTORC1, and siRNA knockdown of *TMEM127* increases mTORC1 signaling, which is likewise observed with *NF1* mutations¹¹⁵. Screening of 642 sporadic PCCs found *TMEM127* mutations in only six patients (0.9%), making *TMEM127* the least common PCC susceptibility gene¹¹⁶. *MAX* mutations causing loss of protein expression were discovered in three kindreds by exome sequencing¹¹⁷, and then confirmed to affect 1.12% of 1,694 sporadic and hereditary PCC patients without a known mutation¹¹⁸. *MAX* functions in dimerization of MYC/*MAX*/MXD1 transcription factors where it helps to repress cell growth, and may influence the mTOR pathway¹¹⁷. Like *SDHAF2* and *SDHD*, transmission of familial *MAX* mutation is preferentially paternal, with tumors of affected patients expressing only the paternal allele either through uniparental disomy or loss of heterozygosity for chromosome 14q¹¹⁷.

Despite the host of mutations predisposing to PCCs and PGLs, there is an increasing understanding that the affected genes cluster into only a few pathways and relate to other forms of neuroendocrine cancer. Burnichon *et al.* analyzed PCC/PGL specimens from 190 patients, performing microarray gene expression testing and analysis for germline and somatic mutations by comparative genomic hybridization and direct sequencing. They found that differences in gene expression allowed unsupervised hierarchical cluster analysis to correctly assign 67/69 hereditary tumors to one of three clusters concordant with their known mutations (*SDHx*, *VHL*, and *NF1/RET/TMEM127*). Analysis of 78 sporadic tumors which grouped to these same clusters uncovered somatic mutations of *RET* and *VHL* in 17, loss of *SDH* heterozygosity in 2/2 sporadic tumors that clustered with familial *SDHx* tumors, and loss of heterozygosity of the *VHL* locus in 16/16 tumors clustering with familial vHL tumors. Genes differentially expressed in the different clusters corresponded to mTOR and MAPK-pathway targets in the *NF1/RET/TMEM127* cluster, and to hypoxia-induced factors in the *SDHx* and *VHL* clusters¹¹⁹. This distinction between tumors with induction of genes for pseudo-hypoxia response versus genes for proliferative signaling likely represents a fundamental biologic difference in these tumors that will influence targeting of personalized tumor therapies in the future.

Adrenocortical Carcinoma

Adrenocortical carcinoma (ACC) remains a rare and deadly cancer, and has seen only limited improvements in therapy over time. The FIRM-ACT trial, which randomized patients with advanced ACC to treatment with mitotane plus either streptozocin (Sz+M) or etoposide, doxorubicin, and cisplatin (EDP+M), established EDP+M as the standard of care due to its higher objective response rate (23.2 vs. 9.2%, $p < 0.001$) and similar toxicity¹²⁰. Despite higher response rates with EDP+M, overall survival remained disappointing, with no significant difference between the two treatment groups (median 14.8 vs. 12.0 months, $p = 0.07$).

In light of the poor performance of current treatments, several groups have analyzed ACC gene expression to find new therapeutic targets. Gene expression arrays identified 2–6 fold overexpression of fibroblast growth factor receptor 1 (*FGFR1*) and 14–100 fold overexpression of insulin-like growth factor II (*IGF2*) as key components of ACC relative to benign adenomas and normal adrenal cortex¹²¹. Prior to this, *IGF2* was recognized to play a role in ACC. Patients with Beckwith-Widemann syndrome (BWS) are prone to ACC¹²² and the chromosomal region 11p15.5 that is altered in BWS contains *IGF2*, and is also altered in sporadic ACC¹²³. At this parentally-imprinted locus, duplications, deletions, gene methylation, chromosomal loss, and uniparental disomy cause variations in the effective copy number of *IGF2*, with increased gene dosage driving proliferation and malignancy in neural-crest derived tissues^{122,124}. High expression of *IGF2* also correlates with earlier recurrence in ACC¹²⁵. Based on these observations, small-molecule inhibitors of FGFR1 and IGF2's receptor, IGFR1, are currently in clinical trials, with results for the IGFR1-inhibitor OSI-906 expected in 2013¹²⁴.

Trials of other small-molecule kinase inhibitors in ACC are ongoing. After failures of gefitinib, imatinib, and sorafenib to produce responses in advanced ACC, a phase II trial of the multi-kinase inhibitor sunitinib reported stable disease at 12 weeks in 5 of 35 treated patients (14%), with improved overall survival in responders¹²⁶. A major problem with these kinase inhibitors is that similar to findings in other cancers, inhibition of one kinase protein may be overcome by compensatory upregulation of others^{45,124,127}. Lin *et al.* provided an example of this in ACC cell culture, finding that monotherapy with sunitinib effectively blocked phosphorylation of its targets VEGFR, PDGFR, and RET, but their downstream MAPK targets MEK and ERK actually showed increased phosphorylation¹²⁸. Combination therapy with sunitinib and the ERK inhibitor PD98059 resulted in greater proliferation inhibition (68% with both vs. 23% and 19% inhibition with sunitinib or PD98059 alone). These results suggest that personalized therapy with gene expression analysis leading to specific targeting of upregulated pathways could one day improve therapeutic response rates, but the additive toxicities of combination therapy remain an important practical obstacle to *in vivo* application.

Functional Adrenal Adenomas

As in other areas of endocrine dysfunction, exome sequencing has proven a powerful tool for identifying specific mutations in both familial and sporadic functional adrenal adenomas. Three genes, *KCNJ5*, *ATP1A1*, and *ATP2B3* were recently found to cause aldosterone-producing adenomas (APAs). Somatic mutation of *KCNJ5* occurs in 30–40% of APAs and germline mutations have been reported in a dominantly inherited syndrome of hyperaldosteronism and bilateral adrenal hyperplasia^{129–131}. The *KCNJ5* gene encodes a potassium channel that causes depolarization and excessive aldosterone release when mutated¹²⁹. Mutations in the Na⁺/K⁺ ATPase *ATP1A1* and the Ca²⁺ ATPase *ATP2B3* likewise cause depolarization with aldosterone release, and somatic mutations in these genes occur in 7% of APAs¹³⁰. Notably, a screen of 380 APAs found *KCNJ5* mutations in 49% of

females but only 19% of males ($p < .001$)¹³¹, while 17/21 (81%) of ATPase mutations occurred in males¹³⁰. Knowledge of these APA-causing mutations has not yet altered surgical management, but medical therapies targeting these genes could someday reduce the need for adrenalectomy in these patients.

Pancreatic Neuroendocrine Tumors

Pancreatic neuroendocrine tumors (PNETs) present with distant disease in more than 60% of cases and, for unknown reasons, their incidence has nearly doubled over the past 30 years¹³². In addition to sporadic cases, PNETs occur in von Hippel-Lindau, NF1, MEN1, and Tuberous Sclerosis syndromes (Table 3). Translational research in neuroendocrine cancer has shown progress in defining cell-surface receptor targets for imaging and treatment and in unraveling the genetic alterations present in this disease.

For pancreatic tumors associated with MEN1 and other heritable syndromes, optimal timing of surgery remains problematic. Functional (gastrinomas, insulinomas, glucagonomas, somatostatinomas, etc.) and nonfunctional PNETs have a penetrance of 30–50% by age 40 in MEN1 patients and are a major cause of mortality^{31,133}. Since surgical elimination of all at-risk tissue is not possible, intervention focuses on controlling symptoms and preempting metastatic malignant disease. Symptomatic functional tumors should be resected to prevent complications of their secreted hormones³¹. In non-functional and asymptomatic tumors, size has emerged as the best indication for intervention. Early reports indicated no association between tumor size and outcomes¹³⁴, but more recently, larger, non-functional pancreatic tumors were found to be significantly associated with poorer survival and higher rates of metastasis in a long-term study of more than 500 MEN1 patients¹³³. While only 10% of patients with tumors under 10mm had synchronous metastases, 18% did with tumors 20–30mm, and patients with tumors over 30mm had a 43% rate of synchronous metastasis. The authors therefore recommended resection when tumors become larger than 20mm or are rapidly growing, as the risks from metastases outweigh the risks of pancreatic surgery at that point.

Receptor-directed treatment strategies

Prognosis in sporadic PNETs has improved in recent years, with a Dutch registry showing all-stage five-year survival for low-grade PNETs increasing from 51% in 1990–2000 to 65% in 2000–2010¹³⁵. Recognition of the benefits of surgery for even metastatic disease may account for some of this improvement¹³⁶, but treatments targeting somatostatin receptors have also played an important role¹³⁵. Somatostatin analogues (SSAs) have been investigated since the 1970s to control hormonal symptoms of functional neuroendocrine tumors¹³⁷. While SSAs were initially studied for their relief of symptoms of carcinoid and other hormone overproduction syndromes, it is now understood that neuroendocrine tumor tissues actually overexpress somatostatin receptors^{138,139}. Somatostatin receptors were first cloned in 1992¹⁴⁰. Of the five human subtypes, most SSAs bind principally to type 2 (SSTR2) and type 5 (SSTR5) receptors¹⁴¹. SSTR signal transduction is complex, involving many pathways with abundant crosstalk, and is also dependent on cellular context¹⁴². Of interest in PNETs, SSTR2-mediated inhibition of the MAPK pathway and of cAMP accumulation has been reported in the PNET-derived BON-1 cell line, and causes reduced release of chromogranin A¹⁴³.

In 1998 a long-acting somatostatin analogue (octreotide LAR) was introduced, allowing more stable drug delivery and treatment compliance. Octreotide benefits patients with functional and non-functional tumors, and treatment achieves disease stabilization in 50–60% of patients, with occasional partial or complete responses¹⁴⁴. The PROMID study provided validation of SSAs' benefits in a randomized trial, allocating 90 patients with

metastatic functional and non-functional midgut NETs to treatment with octreotide LAR or placebo¹⁴⁵. Octreotide LAR demonstrated improved median progression free survival of 14.3 vs. 6.0 months ($p < 0.0001$), with a low death rate which prevented evaluation for overall survival¹⁴⁵.

SSTR overexpression in neuroendocrine tissues makes these receptors useful not only for their signaling effects, but also as specific markers of tumor cells. This property has been exploited by coupling SSA molecules like octreotide to radioactive isotopes, which permit tumor imaging and tumor-directed peptide receptor radionuclide therapy (PRRT). SSTR-based imaging is now recommended along with CT for staging of all tumors¹⁴⁴. Variations in the specific SSTR ligand, the radio-chelator, the imaging isotope used, and the combination of emission data with CT imaging have all improved the performance of SSTR-based imaging¹⁴⁶. Somatostatin receptor scintigraphy (SRS) with ¹¹¹In-octreotide was the first to show widespread utility, but is being supplanted by positron-emitting isotopes, such as ⁶⁸Ga¹⁴⁶. While imaging with ¹¹¹In may miss clinically important primary or metastatic tumors in around 25–40% of cases, Gabriel *et al.* reported sensitivity of 97% in 84 NET patients imaged with ⁶⁸Ga-DOTA-TOC, versus 52% with SRS and 61% with CT^{147,148}.

Lesions positive by SSTR-directed imaging may be treatable by PRRT. In this modality, the low-energy imaging radioligand is exchanged for β -emitting ⁹⁰Y or ¹⁷⁷Lu, with a somatostatin analogue directing the isotope to tumor cells¹⁴⁹. Using ¹⁷⁷Lu-DOTA-TATE in a series of 310 patients with advanced GEPNETs, complete or partial responses were reported in 30% of patients overall and in 42% of nonfunctional PNETs¹⁵⁰. Median overall survival was 46 months from treatment and 128 months from diagnosis. While relative benefits of PRRT compared to chemotherapy and small-molecule therapies require continuing investigation, PRRT stands out by its ability to shrink tumors in a significant proportion of patients.

The success of somatostatin analogues raises the question of whether additional receptors might be helpful for combination treatment, for tumors that do not respond to SSA therapy, or for tumors that do not express high levels of SSTRs. Imaging with radiolabeled GIP has successfully visualized GIPR-expressing adrenal tumors¹⁵¹, and recent work by our group has found that receptors for gastric inhibitory polypeptide (GIPR) and oxytocin (OXTR) are overexpressed in neuroendocrine tumors relative to normal tissue^{139,152}. GIPR has expression similar to SSTR2 in PNETs¹³⁹, suggesting that these and other new receptor targets could provide expanded treatment options for PNETs.

Genetic alterations in sporadic PNETs

While SSTR-directed therapies have shown success, the reasons for SSTR overexpression remain unclear. Unlike in MTC where mutations in RET lead to constitutive activation, providing a mechanism for tumorigenesis, the overexpressed receptors in PNETs are not mutated, suggesting that their overexpression does not drive malignancy, but rather represents an upregulatory reaction to primary mutations in other genes¹⁵³. Missiaglia *et al.* advanced understanding of these primary genetic defects, performing gene expression profiling on 72 PNET tumor specimens with clinical and immunohistochemistry correlation¹⁵⁴. This revealed that 85% of primary tumors had reduced staining for TSC2 or PTEN protein, with low expression correlating with decreased survival and increased metastasis. High expression of *FGF13*, which assists in p38 MAP kinase recruitment, also correlated with worse outcomes. *TSC2* and *PTEN* function to negatively regulate the PI3K/Akt/mTOR pathway, with mutations in either leading to increased proliferation and loss of hypoxia-induced growth inhibition (Fig. 2).

Jiao *et al.* performed exome sequencing on 10 sporadic PNETs to discover somatically mutated genes, with verification in 58 additional tumors⁸⁷. This showed that PNETs have a different mutational profile than pancreatic adenocarcinomas, with a low rate of mutation in *KRAS*, *TGF- β* , *CDKN2A*, and *TP53* (which are common in adenocarcinoma). Instead, the most commonly mutated genes in PNETs were *MEN1*, *DAXX/ATRX*, *TSC2*, and *PTEN* in 44, 25, 9, and 7% of tumors, respectively. Death-associated domain protein and alpha thalassemia/mental retardation syndrome X-linked (*DAXX/ATRX*) function in telomeric histone incorporation and chromatin remodeling, and had not been previously implicated in cancer. In Jiao's study, mutations in these genes were associated with significantly improved survival compared to PNETs without *DAXX/ATRX* mutations. Subsequent studies found a perfect correlation between PNET *DAXX/ATRX* mutation and the telomerase-independent telomere maintenance (ALT) phenotype¹⁵⁵, which can arise in cells with impairment of normal telomere maintenance and correlates with cell immortalization¹⁵⁶. This, along with the finding of *ATRX* mutations in additional cancer types with the ALT phenotype implies that *DAXX* and *ATRX* play important roles in telomere maintenance¹⁵⁵. Testing for these mutations promises to provide important prognostic information and further investigation of *DAXX/ATRX* function may uncover new insights into the pathogenesis of PNETs and other cancers.

Small-molecule Therapies in Pancreatic Neuroendocrine Cancer

Recognition that genes in the mTOR pathway are mutated in at least 16% of PNETs and that the pathway's function may be impaired in up to 85% of tumors has spurred the use of mTOR inhibitors in PNETs^{87,154}. The RADIANT-3 trial, a manufacturer-sponsored phase III trial, randomized 410 patients with advanced and progressing neuroendocrine tumors of multiple primary sites to the mTOR inhibitor everolimus or best supportive therapy, and reported significantly improved progression-free survival in the everolimus arm (median 11.4 vs. 5.4 months, $p < 0.001$)¹⁵⁷. Everolimus was effective in delaying progression, but tumor shrinkage occurred in only 5% of treated patients. No difference in overall survival was detected, possibly due to 73% crossover to everolimus among patients initially randomized to placebo.

Pancreatic neuroendocrine tumors express VEGF and PDGF receptors, which contribute to angiogenesis, and provided a rationale for use of the multi-kinase inhibitor sunitinib in PNETs¹⁵⁸. A manufacturer-sponsored, phase III trial randomized 154 patients with advanced, progressive PNETs to sunitinib or placebo. The trial was halted when interim analyses detected a significant advantage in progression-free survival with sunitinib treatment (median 11.4 vs. 5.5 months, $p < 0.001$). The effect on overall survival could not be estimated. Grade 1 and 2 side effects occurred in the majority of patients during treatment with either everolimus or sunitinib^{157, 158}. Grade 3 or 4 neutropenia and hypertension occurred in >10% of patients receiving sunitinib¹⁵⁸, while a 7% rate of grade 3 or 4 stomatitis was the most frequent serious complication in patients taking everolimus¹⁵⁷.

Conclusion/Future Directions

From genetic testing to targeted therapies, translational research in endocrine cancer has improved care and outcomes in endocrine surgery, but much work remains. Genetic markers will continue to help distinguish benign and malignant processes in difficult-to-diagnose cancers, such as indeterminate thyroid nodules, and malignant parathyroid and adrenal tumors. However, adequate validation of new tests is essential to ensure that surgery is not delayed in those who will benefit from it, and avoided in those who will not. Further research in basic and clinical aspects of small-molecule kinase inhibitors will clarify the optimal uses for these agents, but the challenges of inhibitor resistance and paradoxical induction of related pathways remains a vexing problem. The plummeting cost of genetic

sequencing promises dramatic advances in personalized, rational cancer therapy based on knowledge of specific pathways affected. As individualized combination therapies proliferate, managing or avoiding toxicities of potent new drugs will assume even greater importance. Investigators and clinicians should feel encouraged by the strides made in translational endocrine cancer care and continue to integrate these findings into practice to deliver superior patient outcomes.

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Key Points

1. Basic science research has identified the genes responsible for a number of hereditary endocrine tumor syndromes and elucidated the cell-signaling pathways critical to development of endocrine cancer.
2. Genetic testing for these mutations allows identification of at-risk individuals for screening prior to the onset of symptoms and in some cases permits prophylactic surgery..
3. Mutations in genes of the MAP-kinase signaling pathway (most commonly *RET* or *BRAF*) are found in most familial and sporadic thyroid cancers and cause constitutive proliferative signaling, leading to malignancy..
4. Small-molecule kinase inhibitors block aberrant pro-malignant signaling in several endocrine cancers and represent an active area of research with great potential. Improvements in progression-free survival have been reported with these drugs for thyroid, adrenal, and endocrine pancreatic cancer, but responses are usually not durable, and efforts to understand and overcome inhibitor resistance are ongoing..
5. In pancreatic neuroendocrine tumors, drugs targeting somatostatin receptors alleviatesymptoms, are useful for imaging, and can prolong life. Targeted radiotherapy directed towards these receptors and development of additional receptor targets promise to improve treatment of these tumors in the future..

Figure 1. Simplified overview of commonly mutated oncogenes, tumor suppressors, and pathways in endocrine cancer. In the mitogen-activated protein kinase (MAPK) pathway (right center), extracellular ligands activate receptor kinases such as RET, VEGFR, PDGFR, and others, initiating the RAS/RAF/MEK/ERK signaling cascade, resulting in gene transcription and proliferation. Activating mutations in these proteins lead to constitutive signaling. In the phosphatidylinositol 3-kinase (PI3K) cascade (right side), receptor activation causes activation of Akt, also affecting cellular survival and regulating apoptosis. Mammalian target of rapamycin (mTOR) signaling promotes survival and is stimulated by Akt and repressed by TMEM127. In neurofibromatosis type 1, loss of NF1 function prevents termination of RAS signaling. In von Hippel-Lindau syndrome, VHL (left top) targets hypoxia-inducible factor proteins for ubiquitination (Ub) and proteasomal degradation. When HIF persists due to mutated VHL, continuous activation of hypoxia genes leads to angiogenesis and tumor development. In the mitochondrion (left side), defects in subunits of complex II, SDHA, B, C, D, and assembly factor 2 (AF2), impair electron transport and induce a pseudo-hypoxic state. MAX (in nucleus) interacts with MYC and other transcription factors to repress cell growth and loss of function allows proliferation. S: succinate, F: fumarate, OH-P: hydroxy-proline residue, PIP2,3: Phosphatidylinositol bis- or tris-phosphate. Adapted from Fishbein L, Nathanson KL. Pheochromocytoma and

paraganglioma: understanding the complexities of the genetic background. *Cancer Genet.* 2012;205:1; with permission.

Figure 2.

mTOR and MAPK pathways. Receptor tyrosine kinases (RTKs) activate Ras or PI3K. Ras activates the MAPK cascade of Raf (including BRAF), MEK, and ERK kinases, leading to cell survival, gene transcription, and proliferation. Activated PI3K phosphorylates PIP₂ to PIP₃ (Phosphatidylinositol bis- and tris- phosphate). PTEN dephosphorylates PIP₃ to PIP₂, inhibiting its signal. PIP₃ activates Akt, which causes dissociation of TSC2 from TSC1, releasing inhibition of RHEB (Ras Homolog Enriched in Brain), which then activates mTORC1. Rapamycin analogues such as everolimus inhibit mTOR proteins. Crosstalk between MAPK and PI3K pathways is indicated (Ras/PI3K, ERK/mTORC1, and Akt/Raf). Adapted from Gadgeel SM, Wozniak A. Preclinical Rationale for PI3K/Akt/mTOR Pathway Inhibitors as Therapy for Epidermal Growth Factor Receptor Inhibitor-Resistant Non-Small-Cell Lung Cancer. Clin Lung Cancer. 2013; with permission (see also¹⁶⁴).

Table 1

Frequency of common *RET* mutations in 477 families with MEN2A, 2B, and FMTC by codon and presence of medullary thyroid carcinoma (MTC), hyperparathyroidism (HPT), and pheochromocytoma (PCC). From Eng C, Clayton D, Schuffenecker I, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA. 1996;276:1575; with permission.

MEN Type	Codon, No. (%) of Families With Mutations							
	609	611	618	620	634	768	804	918
2B	0	0	0	0	0	0	0	75 (100)
2A(1)	0	0	5 (6)	2 (2)	84 (92)	0	0	0
2A(2)	0	3 (3)	4 (4)	12 (13)	76 (80)	0	0	0
2A(3)	1 (8)	2 (15)	1 (8)	0	9 (69)	0	0	0
FMTC	2 (7)	1 (3)	10 (33)	5 (17)	9 (30)	3 (10)	0	0
Other	2 (1.5)	7 (5)	18 (13)	13 (10)	93 (68)	1* (<1)	2* (1.5)	0

* Families with medullary thyroid carcinoma (FMTC) but too few cases (3 or less) per family to exclude other MEN2A disease features.
MEN2A(1): MTC+HPT+PCC; MEN2A(2): MTC+PCC; MEN2A(3): MTC+HPT

Table 2

Recommended age for prophylactic thyroidectomy by codon for selected *RET* mutations based on risk of medullary thyroid cancer (MTC). Adapted from Calva D, O'Dorisio TM, Sue O'Dorisio M, et al. When is prophylactic thyroidectomy indicated for patients with the RET codon 609 mutation? *Ann Surg Oncol*. 2009;16:2237; with permission and updated to reflect current American Thyroid Association guidelines. For additional mutations and details, see^{31,32}

Recommended age for thyroidectomy	MTC Risk Category ³²	Codons
Before 1 year (preferably before 1 month)	D	883, 918, 922, compound 804 *
Before 5 years	C	634
Consider surgery before 5 years	B	609, 611, 618, 620, 630
Consider delaying to before 5–10 years **	A	768, 790, 791, 804 *, 891

* Compound 804+(778, 805, 806, or 904) mutations have higher risk than isolated codon 804 mutations³²

** With less-aggressive family history and appropriate negative screening³²

Table 3
Selected autosomal dominant endocrine cancer syndromes and penetrance of phenotypic features.

		Overall penetrance of disease feature ^{***}				
Syndrome	Gene(s)	MTC	HPT	PCC/PGL	PNET	
MEN1	<i>MEN1</i>	NA	100% at age 50 ³¹	<1% ³¹	53% at age 50 ¹³³	
MEN2A	<i>RET</i>	90–100% ^{31,80}	20–35% ⁸⁰	40% ⁸⁰	NA	
MEN2B	<i>RET</i>	100% ^{14,31}	Rare ¹⁴	19–53% ^{159–161}	NA	
FMTc	<i>RET</i>	100% by definition	0% by definition	0% by definition	NA	
vHL	<i>VHL</i>	NA	NA	10–20% ^{82,102}	10–17% ¹⁶²	
TSC	<i>TSC1, TSC2</i>	NA	NA	NA	1% ¹⁵³	
NF1	<i>NF1</i>	NA	NA	6% ⁹⁵	<10% ¹⁵³	

* Individual risk highly dependent on particular mutation present

** Reported penetrance tends to increase with age/length of follow-up

Abbreviations: MEN: Multiple Endocrine Neoplasia, FMTc: Familial Medullary Thyroid Cancer, vHL: von Hippel-Lindau syndrome, TSC: Tuberous Sclerosis Complex, NF1: Neurofibromatosis type 1, MTC: Medullary thyroid cancer, HPT: Hyperparathyroidism, PCC/PGL: Pheochromocytoma/paraganglioma, PNET: Pancreatic neuroendocrine tumor, NA: Not applicable