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Next-Generation Sequencing Has Identified New Oncogenic Mutations in Thyroid Nodules

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Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab 2013;98:E1852-60. Epub August 26, 2013.

SUMMARY

Background

Detection of oncogenic mutations is becoming very important for the diagnosis of thyroid cancer in nodules. Ideally the oncogenes are found in thyroid fine-needle aspiration (FNA) biopsy material, especially when the cytologic diagnosis is in the indeterminate category, because the oncogenes will confirm the diagnosis of cancer. The current report shows the application of a new method called "next-generation sequencing" (NGS), with the aim of detecting most point mutations and small insertions or deletions known to occur in thyroid cancer.

Methods

NGS provides simultaneous analysis of large regions of the genome with a high sensitivity for detection of mutations and quantitative assessment of mutant alleles. Using this method, the authors studied 228 thyroid neoplastic and nonneoplastic specimens, including 57 papillary thyroid carcinomas (PTCs) (27 classical PTCs and 30 follicular variant of papillary carcinomas [FVPTCs]), 36 follicular carcinomas (18 conventional and 18 oncocytic), 10 poorly differentiated carcinomas, 27 anaplastic carcinomas, 15 medullary carcinomas, 83 histologically benign hyperplastic nodules, and 51 FNA samples from nodules that were subsequently resected .

Results

BRAF, TP53, or NRAS mutations were detected with a sensitivity that corresponds to 6% of cells with heterozygous mutation. Altogether, 115 mutations were detected in 228 thyroid specimens tested, including 110 mutations in 145 cancer samples and 5 mutations in 83 benign nodules. Most cancers contained a single mutation, but several contained multiple mutations.

In 19 classical PTCs, BRAF mutation was found in 16, PIK3CA in 3, TP53 in 2, and NRAS in 1. In FVPTC, RAS mutations were the most common. In follicular cancers, mutations included RAS, TSHR, TP53, and PTEN. A total of 74% of anaplastic thyroid cancers were found to have mutations, including TP53, BRAF, RAS, PI3KCA, PTEN, and CTNNB1, and 73% of 15 sporadic medullary thyroid cancers contained either RET or RAS mutations. Most mutations in the tumor samples were heterozygous with allele frequencies that corresponded to 40% to 96% of cells with a heterozygous mutation.

Only 5 of 83 benign hyperplastic nodules contained mutations, of which 2 were TSHR. DNA sufficient for the sequencing method was found in 50 of 51 FNA samples.

Conclusions

NGS allows simultaneous testing for multiple mutations with high accuracy and sensitivity and requires only a small amount of DNA. Point mutations were detected in 30% to 83% of specific types of thyroid cancer and in only 6% of benign thyroid nodules.

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ANALYSIS AND COMMENTARY

This new method for genotyping thyroid tissue, including FNA samples, expands the repertoire of oncogenic mutations that have been used up to this time (1). It has led to the discovery of uncommon mutations, such as PTEN, TP53, PI3KCA, and TSHR. Activating mutations of the TSH receptor (TSHR) were reported previously in hot nodules (2). With the addition of these mutations to those of BRAF, RAS, and the PAX8/PPARg and RET/PTC rearrangements (now called "gene fusions"), 80% of thyroid cancers are now believed to have detectable mutations. When these mutations are added to the commercial panel of oncogenes, it is likely that a very high proportion of thyroid cancers will be detectable by NGS.

It is pertinent that the authors of this paper in the Nikiforov laboratory continue to be the leaders in this field. This was especially evident at the recent meeting of the American Thyroid Association.

Because of cost considerations, the main use of NGS may be restricted to indeterminate biopsies that now constitute the two categories of follicular lesion of undetermined significance and follicular neoplasm, according to the Bethesda classification of FNA cytology. However, reduction of cost by competition and by further improvements in sequencing may eventually permit extensive use of NGS on virtually all FNA biopsies.

References

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2. Davies TF, Ando T, Lin RY, Tomer Y, Latif R. Thyrotropin receptor-associated diseases: from adenomata to Graves disease. J Clin Invest 2005;115:1972-83.