Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer

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Marina N. Nikiforova, Abigail I. Wald, Somak Roy, Mary Beth Durso, and Yuri E. Nikiforov

Division of Molecular and Genomic Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA

Objectives: Next-generation sequencing (NGS) allows for high throughput sequencing analysis of large regions of the human genome. We explored the use of targeted NGS for simultaneous testing for multiple mutations in thyroid cancer.

Design: A custom panel (ThyroSeq) was designed to target 12 cancer genes with 284 mutational hotspots. Sequencing was performed on Ion Torrent PGM to analyze DNA from 228 thyroid neo- plastic and non-neoplastic samples including 105 frozen, 72 formalin-fixed, and 51 FNA samples representing all major types of thyroid cancer.

Results: Only 5–10 ng of input DNA was sufficient for successful analysis of 99.6% of samples. The analytical accuracy for mutation detection was 100% with the sensitivity of 3–5% of mutant allele. ThyroSeq DNA assay identified mutations in 19/27 (70%) of classic papillary thyroid carcinomas (PTC), 25/30 (83%) of follicular variant PTC, 14/18 (78%) of conventional and 7/18 (39%) of oncocytic follicular carcinomas, 3/10 (30%) of poorly differentiated carcinomas, 20/27 (74%) of anaplastic (ATC), and 11/15 (73%) medullary carcinomas. In contrast, 5/83 (6%) of benign nodules were positive for mutations. Most tumors had a single mutation whereas several ATC and PTC demon- strated two or three mutations. The most common mutations detected were *BRAF* and *RAS* fol- lowed by *PIK3CA, TP53, TSHR, PTEN, GNAS, CTNNB1* and *RET. BRAF* mutant allele frequency was 18–48% in PTC and was lower in ATC.

Conclusions: ThyroSeq NGS panel allows simultaneous testing for multiple mutations with high accuracy and sensitivity, requires a small amount of DNA and can be performed in a variety of thyroid tissue and FNA samples, and provides quantitative assessment of mutant alleles. Using this approach, point mutations were detected in 30 – 83% of specific types of thyroid cancer and in only 6% of benign thyroid nodules, and were shown to be present in the majority of cells within the cancer nodule.#