OR17-4 - The Mutational Landscape of 774 Variants and 132 Fusions in 513 Genes Measured in Thyroid FNAs from a Multi-Center, Blinded Cohort

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Abstract

Introduction

Few DNA variants have high positive predictive value (PPV) for thyroid cancer, with BRAF V600E and RET-PTC fusions being exceptions. Understanding the mutational landscape in a diagnostic setting can help guide patients with thyroid nodules to appropriate surgical intervention. As cytological interpretation of fine-needle aspiration (FNA) can yield indeterminate results, it may be advantageous to identify mutations and fusions (i.e. variants) that might provide preoperative guidance. We measured a large panel of DNA mutations and gene fusions in thyroid FNAs with surgical histopathology truth to determine their occurrence in benign and malignant lesions.

Methods

The patient cohort we tested was from VERA001, a prospectively collected, blinded, multicenter clinical trial used to independently validate the GEC and the next generation test, the Genomic Sequencing Classifier (GSC). We developed a custom DNA AmpliSeq panel measuring 774 single- and di-nucleotide variants in 346 genes reported in the literature, including the TCGA study. Sufficient DNA was available for 151 of 191 Bethesda III/IV FNAs reported in the primary GSC validation cohort. An additional 29 Bethesda V/VI samples from the secondary GSC validation test set were also tested. We measured 132 gene fusions in all samples using RNA sequencing data analyzed by the STAR-fusion pipeline.

Results

Most samples (96/151) harbored no detectable variant. Examination of samples with malignant histopathology showed 23/43 had a variant, resulting in a sensitivity of 53.5% [CI: 37.7-68.8%]. Samples with benign histopathology showed 76/108 had no variant, resulting in a specificity of 70.4% [CI: 60.8-78.8%]. Mutations in the RAS family were the most commonly detected, observed in 34/55 (62%) variant positive samples. The PPV of RAS variants was 44.1% [CI: 27.2-62.1%]. BRAF V600E was the only variant observed more than once that was specific to malignant histopathology in Bethesda III/IV FNAs, present in 3/43 (7%) cytologically indeterminate malignant samples. Fusions were observed in 4/151 (2.6%) Bethesda III/IV samples. TERT promoter mutations were observed in 3/151 (2%) indeterminate FNAs, with two in benign lesions and one in a papillary thyroid carcinoma (PTC). All three TERT mutations co-occurred with RAS mutations. BRAF V600E was the most common variant in Bethesda V/VI

FNAs, present in 14/29 (48%) samples. Fusions were observed in 3/29 (10%) Bethesda V/VI samples. The variant-only sensitivity for Bethesda V/VI samples was 79.2% [CI: 57.8-92.9%] and specificity was 40% [CI: 5.27-85.3%]. TERT mutations were observed in 2/29 (6.9%) Bethesda V/VI, both in PTCs and both co-occurring with a BRAF V600E mutation.

Conclusion

A large portion (46.5%) of thyroid cancers with Bethesda III/IV cytology do not contain a nucleotide or fusion variant, resulting in inadequate sensitivity or specificity to rule in or rule out cancer.

Disclosures

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