



Poster #1192

Detection of Malignancy in Thyroid Carcinoma Samples Through Targeted DNA Sequencing

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Detection of Malignancy in Thyroid Carcinoma Samples Through Targeted DNA Sequencing



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OBJECTIVE:

Thyroid carcinomas frequently contain genetic variants associated with transformation. Recent studies from a number of groups, including The Cancer Genome Atlas (TCGA) have elucidated several of these mutations. However, it is unclear how well a comprehensive panel of mutations performs in the detection of malignancy across a broad range of thyroid malignancy subtypes. We sought to measure this performance in the context of both fine needle aspirate (FNA) and tissue samples.

METHODS:

FNA samples (n=82) were collected pre-operatively from patients with thyroid nodules, and both RNA and DNA was extracted from each FNA. All patients underwent surgical resection of the thyroid and nodules were diagnosed by histopathology as malignant or benign. A separate set of 38 snap-frozen thyroid surgical tissue samples with histopathology truth were also analyzed. Three different targeted DNA sequencing strategies were applied to identify variants within these samples and RNA sequencing was used to identify fusions. The first approach assessed 229 nucleotide variants in 14 genes using the AmpliSeq Cancer Hotspot Panel v2 (HotSpot Panel; Figure 1A). Secondly we analyzed a custom panel targeting 854 variants in 357 genes derived from the literature, including TCGA (Custom Panel; Figure 2A). Lastly, we analyzed tissue samples with The Jackson Laboratory Cancer Treatment Profile (JAX-CTP; Figure 3A), which targets 358 genes associated with cancer diagnosis and therapeutic treatment selection.

RESULTS:

The sensitivity for detection of malignancy in the 82 FNA samples with the HotSpot Panel was 55% (95% CI 38-71%) and the specificity was 90% (77-97%) (Figure 1B). The sensitivity for detection of malignancy in 38 thyroid tissue samples using variants detected with the Custom Panel was 67% (41-87%), similar to that observed in FNA samples with the smaller Cancer Hotspot panel, while the specificity was 65% (41-85%) (Figure 2B).

The JAX-CTP assay was analyzed in 19 tissue samples that contained sufficient DNA. The sensitivity obtained with a smaller set of commonly studied thyroid cancer specific genes (12 genes, as the JAX-CTP assay does not include EIF1AX or the TERT promoter variants) was 50% (19-81%) and the specificity was 67% (30-93%) (Figure 3B). Using the entire set of genes in the JAX-CTP assay, the sensitivity increased to 90% (55-100%), but was accompanied by a drastic decrease in specificity to 11% (0-48%).

CONCLUSIONS:

Three methods detecting DNA variants and fusions lacked sensitivity when assessing common thyroid cancer associated variants. This finding is consistent with previous analyses using an RNA-based sequencing assay. Increasing variants from 14 genes to 357 did not improve performance. These results demonstrate that these variant panels applied to thyroid FNA samples have limited sensitivity for detection of malignancy. With a 24% prevalence of malignancy in indeterminate FNAs, the resulting NPV of 81% (Figure 4) is too low to rule out cancer.

FIGURE 1A. Comparison of Genes Identified Using the HotSpot Panel to ThyroSeq v2

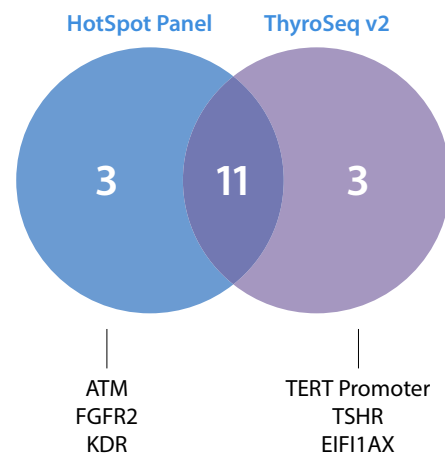


FIGURE 1B. HotSpot Panel Performance on FNA Samples

| | Histo M | Histo B |
|----------|---------|---------|
| Positive | 22 | 4 |
| Negative | 18 | 38 |

| Sensitivity | Specificity | PPV | NPV |
|-------------|-------------|-------------|-------------|
| 55% (38-71) | 90% (77-97) | 85% (68-94) | 68% (60-75) |

| Variant/Fusion | Histo B | Histo M |
|----------------|---------|---------|
| BRAFV600E | | 15 |
| HRASQ61R | 1 | 1 |
| KRASG12D | | 1 |
| NRASQ61K | 2 | |
| NRASQ61R | | 2 |
| TP53R248Q | | 1 |
| BRAF:SND1 | 1 | |
| PAX8:PPARG | | 2 |
| RET:SPECC1L | | 1 |

FIGURE 2A. Comparison of Genes Identified Using the Custom Panel to ThyroSeq v2

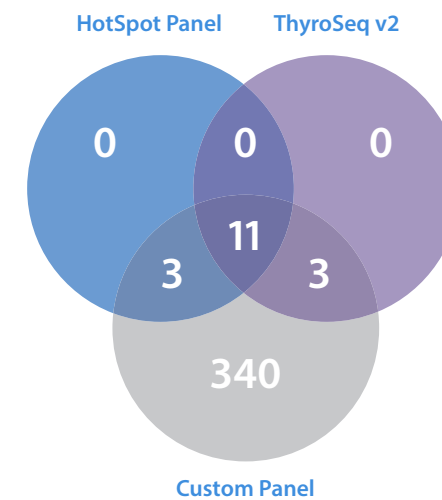


FIGURE 2B. Custom Panel Performance on Tissue Samples

| | Histo M | Histo B |
|----------|---------|---------|
| Positive | 12 | 7 |
| Negative | 6 | 13 |

| Sensitivity | Specificity | PPV | NPV |
|-------------|-------------|-------------|-------------|
| 67% (41-87) | 65% (41-85) | 63% (46-77) | 68% (51-82) |

| Variant | Histo B | Histo M |
|---------------|---------|---------|
| BRAF V600E | | 6 |
| EVPL V1416M | 1 | |
| FAT1 V912I | 1 | |
| HRAS Q61K | | 2 |
| KRAS G12V | 1 | |
| NRAS Q61K | 1 | |
| NRAS Q61R | 1 | 2 |
| PIK3CA T1025T | 1 | |
| TERT | 1 | 6 |
| TSHR D633Y | 1 | |
| TSHR I486F | 1 | |

FIGURE 3A. Jackson Labs Cancer Treatment Profile Panel Compared to Custom Panel

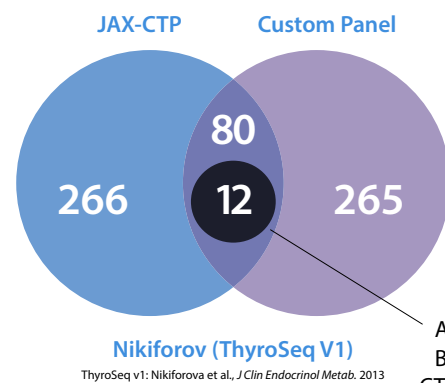


FIGURE 3B. Jackson Labs Cancer Treatment Profile Panel Performance on Tissue Samples

12 THYROID-SPECIFIC GENES

| | Histo M | Histo B |
|----------|---------|---------|
| Positive | 5 | 3 |
| Negative | 5 | 6 |

| Sensitivity | Specificity | PPV | NPV |
|-------------|-------------|-------------|-------------|
| 50% (19-81) | 67% (30-93) | 62% (35-84) | 55% (36-72) |

| Variant/Fusion | Histo B | Histo M |
|----------------|---------|---------|
| BRAF V600E | | 3 |
| HRAS Q61K | | 1 |
| NRAS Q61K | 1 | |
| NRAS Q61R | | 1 |
| RET G691S | 3 | 2 |
| TP53 R249S | | 1 |
| CCDC6:RET | 1 | |

358 GENES (JAX-CTP PANEL)

| | Histo M | Histo B |
|----------|---------|---------|
| Positive | 9 | 8 |
| Negative | 1 | 1 |

| Sensitivity | Specificity | PPV | NPV |
|--------------|-------------|-------------|------------|
| 90% (55-100) | 11% (0-48) | 53% (45-61) | 50% (7-93) |

| Variant/Fusion | Histo B | Histo M |
|----------------|---------|---------|
| AURKA F31I | 4 | 7 |
| BRAF V600E | | 3 |
| FGFR4 G388R | 6 | 4 |
| HRAS Q61K | | 1 |
| JAK3 V722I | 1 | 1 |
| KDR Q472H | 5 | 7 |
| KIT V530I | | 1 |
| NRAS Q61K | 1 | |
| NRAS Q61R | | 1 |
| RET G691S | 3 | 2 |
| TP53 R249S | | 1 |
| CCDC6:RET | 1 | |

FIGURE 4. Predictive Value of Variant Test on Indeterminate FNA

