Clinical Oral 30

Thyroid Nodules & Goiter Saturday Oral Clinical

XPRESSION ATLAS FINDINGS IN THE GENOMIC SEQUENCING CLASSIFIER (GSC) CLINICAL VALIDATION COHORT

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Afirma GSC utilizes RNA sequencing and machine-learning algorithms to classify cytologically indeterminate thyroid nodules into benign (B) and suspicious (S) categories. Detection of genomic variants and fusions was recently expanded beyond *BRAF* V600E and *RET/PTC1*&*3* by the Xpression Atlas (XA), which identifies 761 nucleotide variants and 130 fusion gene pairs in 511 genes. Here we used XA to analyze the mutational spectrum of 190 Bethesda III/IV nodules with gold standard histologic diagnoses.190 nodules previously collected in a prospective multicenter blinded trial design were analyzed with the XA. Among the 145 histologically benign nodules, 35 (24%) contained a variant or fusion (XA+). In the 99 benign nodules with GSC-B results there were 15 (15%) with XA variants and none with a fusion. These variants were 7 *TSHR*, 3 *SPOP*, 2 *EIF1AX*, 1 *PTEN*, 1 *TSHR* + *EZH1*, and 1 *GNAS*. In the 46 benign nodules with GSC-S results, 18 (39%) harbored a variant and 2 (4%) a fusion. There were 9 *NRAS*, 6 *HRAS*, 2 *TSHR*, and 1 *SPOP*. Two had a *PAX8/PPRARG* fusion.

Among the 45 histologically malignant nodules (41 GSC-S; 91% sensitivity), 22 were XA+ (49% sensitivity). In the 41 malignant nodules with GSC-S results, there were 19 variants (46%) and 2 fusions (5%). The variants were 9 *NRAS*, 3 *HRAS*, 3 *BRAF* V600E, 1 *SPOP*, 1 *KRAS* + *EIF1AX*, 1 *EIF1AX*, and 1 *BRAF* K601E. Fusions were *BRAF/MKRN1* and 1 *ETV6/NTRK3*. In the 4 GSC-B false negative nodules (2 PTC, 1 fvPTC, 1 HCC), only the HCC contained a variant (*TSHR)*. In 190 thyroid nodules with definitive histology, malignant nodules were twice as likely to be XA+ than

benign nodules (49% vs 24%, $p = 0.003$ [χ^2]). Although GSC-S nodules were nearly 3 times more likely than GSC-B nodules to be XA+ (47% vs 16%, $p < 0.0001$), the PPV for malignancy did not differ among all GSC-S, GSC-S XA+, and GSC-S XA- nodules (47%, 51%, and 43%, respectively; $p = 0.77$). When XA+, GSC S nodules expressed mainly *RAS* variants, and GSC B nodules predominantly *TSHR* variants. Conversely, the NPV for XA was 83%. These findings support GSC as better than XA to rule-out cancer while the addition of XA to GSC-S nodules may provide additional insights into pathway activation and potential cancer treatment targets.