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Vitamin D Signaling Expression Markers in Thyroid Tumors

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Potential conflict of interest may exist. Refer to the meeting app.

INTRODUCTION

- 25(OH)D deficiency has been associated with higher thyroid cancer risk; however, it is unclear if it mechanistically affects biologic behavior and aggressiveness.[1,2]
- Studies have demonstrated an association between vitamin D receptor (*VDR*) polymorphisms and thyroid cancer.[3]
- *BRAF*V600E is the most common mutation seen in PTC.[4]
- *CYP27B1* encodes 1α-hydroxylase which converts 25(OH)D to $1,25(OH)_2D$.
- *CYP24A1* encodes for 25-hydroxyvitamin D3-24-hydroxylase which converts 25(OH)D and $1,25(OH)_2D$ to inactive metabolites.
- Increased expression of *VDR* (CYP24A1) is seen in PTC as well as tumors that have *BRAF*V600E mutations.[5]
- It is unclear how Vitamin D Activity relates to tumor aggressiveness and disease progression.

STUDY AIM

The aim of this study was to characterize *VDR*, VD regulatory enzymes, and VD pathway target gene expression in thyroid nodules sent for Afirma Genomic Sequencing Classifier (GSC) molecular testing.

METHODS

- The Afirma thyroid nodule molecular database (DB) was analyzed to:
  - Characterize vitamin D expression and genes involved in its synthesis in Afirma GSC-(B)enign and GSC-(S)uspicious (B)ethesda III/IV nodules and BV/VI malignant nodules.
  - Further characterize expression according to *BRAF*V600E+ vs *BRAF*wt and other molecular variants.
- The mRNA expression levels of four vitamin D target genes were evaluated.
  - *CAMP, CD14, ORM1, CLMN*
- A Vitamin D Activity Score was developed as a measure of VD signaling and correlated with Afirma result, expressed variant and fusion status, and ERK signaling.
  - This score is the average expression level of the 4 vitamin D target genes.

RESULTS

- 47,695 thyroid nodules from the Afirma DB were analyzed.
  - 30,259 BIII/IV (GSC-B)
  - 15,815 BIII/IV (GSC-S)
  - 1,621 BV/VI
- *VDR* expression is lower in the presence of *BRAF*V600E relative to *BRAF*wt (figure 1).
- *CYP27A1* (sterol 27-hydroxylase – conversion of VD to 25(OH)D) expression was not significantly different from GSC-B levels in any other category.
- *CYP27B1* (1α-hydroxylase – generation of active $1,25(OH)_2D$) expression was lower in GSC-S and BV/VI nodules compared to GSC-B nodules and even lower in *BRAF*V600E mutant GSC-S and BV/VI nodules compared to those with *BRAF*wt (figure 2a).
- *CYP24A1* (24-hydroxylase – catabolism of $1,25(OH)_2D$) expression was higher in GSC-S compared to GSC-B, and in BV/VI nodules, and higher still in *BRAF*V600E compared to *BRAF*wt subgroups (figure 2b).
- The four VD signaling target genes (*CAMP, CD14, ORM1, CLMN*) showed expression levels that were lower in GSC-S and BV/VI nodules compared to GSC-B nodules and even lower in *BRAF*V600E mutant GSC-S and BV/VI nodules compared to those with *BRAF*wt.
- The novel VD activity score followed the same pattern, with the lowest levels in samples with *BRAF*V600E (figure 3).
- The VD activity score in nodules with *RAS* like mutations (*N/H/KRAS* and *PAX8::PPARγ*) was lower than the median level with GSC-B results and higher than *BRAF*V600E (figure 4).
- The VD activity score was strongly positively correlated with *VDR* expression (r=0.45) and strongly negatively correlated with ERK activity (r= -0.48).

CONCLUSION

- Bethesda III/IV-GSC-S and Bethesda V/VI malignant nodules have reduced VD signaling compared with GSC-benign nodules.
- *BRAF*-like alterations have lower VD signaling than *RAS*-like alterations.
- Novel VD Activity score was lowest in *BRAF*V600E+ GSC-S and malignant nodules when compared to those negative for *BRAF*V600E.
  - VD Activity score is inversely correlated with ERK signaling.
  - VD Activity score may be a novel marker predicting tumor behavior, especially in nodules that do not express canonical mutations.

FIGURE 1.
***VDR* expression by Afirma GSC, Bethesda category, and *BRAF* mutational status**

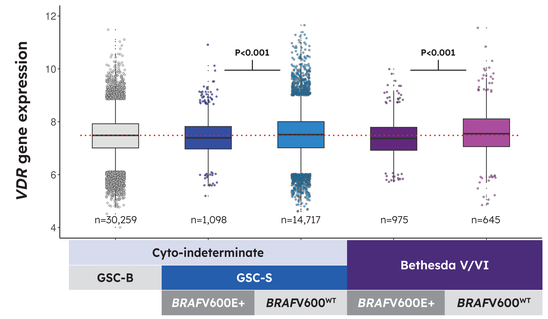


FIGURE 2.
***CYP27B1* (1α-hydroxylase – generation of active 1,25 VD)**
(A) and *CYP24A1* (24-hydroxylase – catabolism of 1,25 VD)
(B) expression by Afirma GSC, Bethesda category, and *BRAF* mutational status

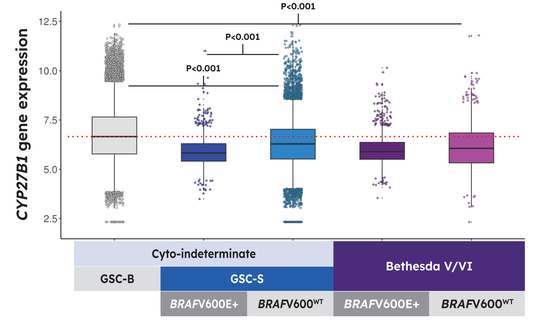


FIGURE 3.
VD activity score by Afirma GSC, Bethesda category, and *BRAF* mutational status

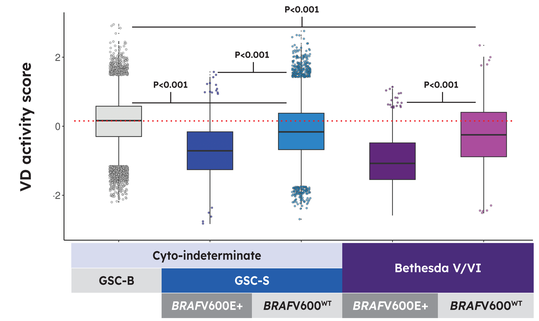
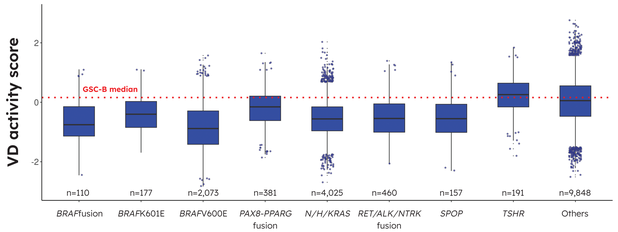


FIGURE 4.
VD activity score in GSC-S and BV/VI nodules by expressed variants and fusions



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