Differential Interactions of Vitamin D Binding Protein and Vitamin D Receptor in Different Ethnic Groups with Aggressive Thyroid Cancer

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ABSTRACT

ABSTRACT: Thyroid cancer affects ethnic groups at different rates and severity. Despite this, thyroid cancer health disparities are still understudied area. Our laboratory has shown a differential expression of vitamin D binding protein (DBP) in Filipino Americans (FA) versus European Americans (EA) [1]. Higher DBP levels correlated to a better prognosis. Another study showed a DBP-dependent (VDR) promoter activation. In this study, we investigated whether VDR and DBP are expressed differentially in different ethnicities, including African Americans (AA), Hispanic Americans (HA), EA, and FA. We were also able to determine the differential DBP polymorphisms, VDR variant expressions, and DBP-VDR interactions in the different ethnicities. By PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), we showed a higher frequency of DBP gene polymorphism in FA versus EA. Analyzing the Cancer Genome Atlas (TCGA) thyroid cancer datasets with the UALCAN assay, we found the differential expressions of DBP and VDR genes based on cancer stages, sample types, race, and histological subtypes. By immunohistochemistry, we detected strong nuclear VDR (nVDR) and very low membranous VDR (mVDR) expression that correlated with low DBP in FA thyroid cancer tissues. In contrast, there was a higher expression of both mVDR and DBP in HA versus the other ethnicities. Co-immunoprecipitation analysis revealed a stronger DBP interaction with mVDR in FA compared to other ethnicities. Our data suggest that low DBP correlates with low mVDR in FA, whereas high DBP correlates with high mVDR in the other ethnicities. In conclusion, the strong interaction of DBP with mVDR in FA may implicate the potential role of DBP-mVDR crosstalk in aggressive thyroid cancer. In the future, we will determine the pathways involved in BDP-mVDR crosstalk in thyroid cancer health disparities

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INTRODUCTION

Despite being one of the most common forms of endocrine cancer, the variation in the biology of thyroid cancers amongst ethnic groups is not fully understood. Amongst various ethnic groups, Filipino Americans have a distinctly high rate of thyroid cancer, even more so than other Asian Americans or European Americans (Figure 1) [2,3]. Vitamin D bind protein (DBP) has numerous functions that include both vitamin D-dependent and vitamin D-independent functions. Recent evidence has shown that DBP has a wide array of essential physiologic roles, including vitamin D transportation, globular actin, fatty acid-binding, and influence on immune response and inflammation. The vitamin D-dependent roles have been evaluated extensively in the setting of cancer with mixed results [4]. However, DBP is implicated in many cancer-related mechanisms, such as angiogenesis and apoptosis. Higher levels of DBP have been associated with better prognosis but the impact of DBP in thyroid cancer is not very well studied [5]. Prior research from our lab has shown that DBP loss may promote intracellular signaling within TC oncogenesis in Filipino Americans [1]. Some studies have shown that DBP acts in a dependent manner upon the vitamin-D receptor (VDR) promoter to enhance activation. In this study, multiple ethnicities were investigated for potential varying expression patterns for both DBP and VDR.

METHODS AND MATERIALS

We characterized differential expressions of vitamin D binding protein and vitamin D receptor antibodies by immunohistochemistry in matched samples of Filipino Americans, and European Americans malignant thyroid tissues.

RESULTS

Filipino Americans were shown to have reduced expression of DBP protein when compared to European Americans (EA) (Figure 2 & 3). The level of DBP expression amongst thyroid cancers (TC) was correlated to the cancer stage in FA and EA tumors. This demonstrated higher rate of T3/4 stage amongst FA with lower levels of DBP correlating to higher stage (Figure 4). FA were shown to have a higher occurrence of DBP gene polymorphisms when compared to EA.

Differential expression of DBP in FA versus EA

Fig. 1. Thyroid cancer incidence and death rates across ethnic groups. **A**. Age-adjusted percent incidence rates in males vs. females; **B**. Age-adjusted percent mortality rates in males vs. females across ethnicities (95% confidence interval).

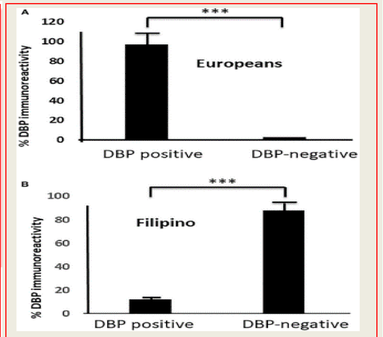

Figure 2. DBP positive tissues in EA versus FA

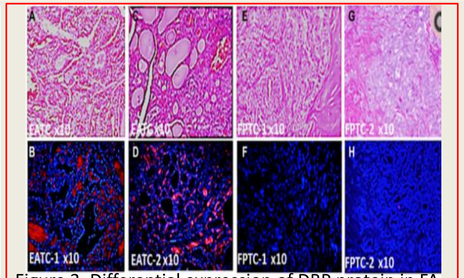

Figure 3. Differential expression of DBP protein in FA vs. EA. A ,C ,E & G. H&E sections for EATC-1, EATC-2, FATC-1, and FATC-2, respectively. EATC/FATC= European American/Filipino American thyroid cancer.

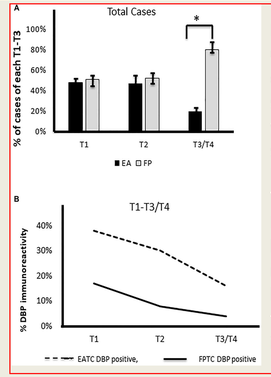

Figure 4. Correlation of DBP staining with staging. A. Higher T3/T4 in FA. B. The lower DBP reactivity, higher the tumor stage in FA

Differential expression of VDR in FA versus EA

RESULTS

Furthermore, FA thyroid cancer tissue showed a high level of nuclear VDR (nVDR) within the nucleus but a very low level of membranous VDR (mVDR) by immunohistochemistry (IHC) (Figure 5). In Hispanic Americans (HA), there was a significantly higher level of DBP and mVDR by IHC in comparison to other ethnic groups. FA were also found to have a very strong interaction between DBP and mVDR by co-immunoprecipitation. This interaction was much stronger than other ethnicities. A representative schematic of the DBP-mVDR & nVDR interaction demonstrated in figure 6.

DISCUSSION

These results indicate that the interaction between DBP and mVDR is stronger in FA compared to other ethnicities. Furthermore, the level of expression for mVDR by IHC was significantly lower in FA but significantly higher for HA. These results indicate that the nature of thyroid cancer within FA may be partially influenced by a DBP-mVDR crosstalk.

CONCLUSIONS

DBP levels have been shown to correlate with a better prognosis. In thyroid cancer, FA have lower levels of DBP and mVDR compared to other ethnicities. Future studies will further investigate the BDP-mVDR interaction to determine the pathways involved.

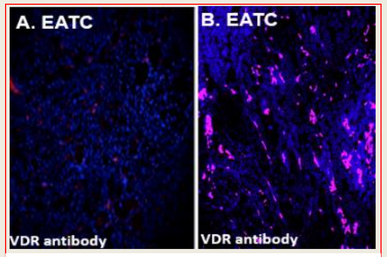

Figure 5. nuclear VDR expression in EATC versus FATC. A. EATC, European American thyroid cancer; B. FATC, Filipino American thyroid cancer tissues

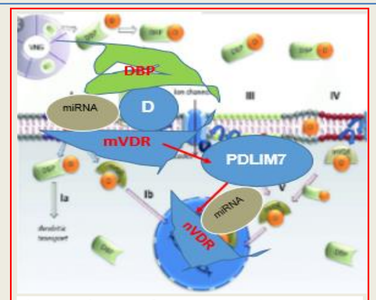

Fig. 6. Schema of DBP-mVDR & nVDR interaction at the cell membrane. mVDR, membrane VDR; nVDR, nuclear VDR

ACKNOWLEDGEMENTS

Special thanks to Dr. Salma Khan, Dr. Alfred Simental, and the LLU Center for Health Disparities and Molecular Medicine.
Dr. Mia Perez, Pathology and Human Anatomy.

Funding statement: Partial support by grant 5P20MD006988/P20MD006988 from the NIH-National Institute on Minority and Health Disparities, Award Number 2R25GM060507, LLUMC Department of Otolaryngology research fund, and GCAT.

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