Increased DNA methylation of genes regulating intracellular calcium levels alter androgen receptor expression and activity in prostate cancer from African American men

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Background: African American (AA) men are 1.7 times more likely to develop and 2.4 times more likely to die of prostate cancer (PrCa) than their European American (EA) counterparts; however, the underlying biology causing this disparity is unknown. The purpose of this study was to determine how DNA methylation mediated transcriptomic changes drive PrCa health disparity in AA men.

Methods: Illumina arrays and RNA-sequencing were used to identify transcriptomic alterations potentially mediated by DNA methylation in PrCa from 32 EA and 30 AA men. MDA PCa 2a (2a)/MDA PCa 2b (2b) cells derived from an AA man and DU145/LaPC-4 cells derived from EA men were stimulated with ionomycin, a calcium ionophore, followed by intracellular calcium measurement using fluorescent-based live imaging. Androgen receptor (AR) protein expression was measured in cells treated with a calcium chelator (BAPTA-AM) and T and in PrCa tissue microarrays from 95 EA and 92 AA men.

Results: Unsupervised hierarchical clustering revealed a DNA methylation cluster (Cluster A) enriched in loci regulating intracellular calcium, including RYR2, TRPC6, and TRPA1, which was associated with reduced disease-free time (DFT) (21.65 vs 46.71 months, $p<0.05$) only in AA men with PrCa. RYR2 (-0.122 vs -0.004, $p=0.69$), TRPC6 (0.006 vs -0.639, $p=0.06$), and TRPA1 (-0.070 vs -0.269, $p<0.05$) transcripts were lower in Cluster A. These data suggest DNA methylation potentially reduces calcium regulatory gene and thus protein expression which can lower their activity. We found rapid increase in intracellular calcium in ionomycin stimulated 2a/2b cells (within 60 seconds) compared to DU145/LAPC-4 cells (120-300 seconds). This suggests that calcium regulatory genes in PrCa cells from an AA man have reduced intracellular calcium buffering capacity. Additionally, we found that depleting calcium in the presence of T increased AR expression only in PrCa cells derived from an AA man. Furthermore, AR protein expression in a subset of tumors and adjacent non-tumor was lower in Cluster A. AR low PrCa with basal-like features respond poorly to androgen deprivation therapy. We found that AR target and PAM50 basal-luminal genes were differentially expressed in AA and EA PrCa and between 2b and DU145 cells.

Conclusion: Our study shows that AA patients with worse DFT have epigenetically dysregulated calcium signaling that can alter AR expression and activity. Our ongoing work seeks to dissect the mechanisms of DNA methylation mediated changes in PrCa models from AA men. We will investigate how these molecular lesions can serve as novel subtypes and guide the design of rationale therapies for AA men with PrCa.