Androgen Receptor-Mediated Regulation of the Prostate Tumor Microenvironment in Black Men

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Background: We previously reported that innate immune cell infiltration varies between races, and others report differences in androgen receptor (AR) expression by race or ancestry. Herein, we evaluated AR status and immune cell infiltration in prostate cancer (PCa) tissues from Black American, White American and Kenyan men**.**
Methods: RNA *in situ* hybridization (RISH) AR analysis was done on a tissue microarray (TMA) constructed from radical prostatectomy tissues from Black American men (n=120) matched to White American men (n=60) on age, grade and stage. RISH or immunohistochemistry (IHC) analysis of AR, NKX3.1, prostate specific antigen (PSA), mast cells (tryptase), neutrophils (CD66), and macrophages (CD206, CD68, CD80) was done on a TMA containing rapid autopsy tissues from metastatic sites (n=11) collected from Black (n=5) and White (n=16) American men. Multiplexing IHC (mIHC) was used to assess AR, neutrophils, mast cells, T-cells (CD4, CD8), and M2 macrophages (CD163) on radical prostatectomy whole-tissue sections from American men and biopsy tissues from Kenyan men. Tumor regions on TMAs were annotated by a pathologist and TMAJ and FrIDA software image analysis done. HALO Image Analysis was used for mIHC tissues. Statistical analysis and graphing were done using GraphPad Prism.
Results: There was increased AR mRNA expression in primary PCa from Black compared to White men ($p<0.0001$) after negative binomial regression with the adjustment of TMA set, age, grade and stage. There was a negative correlation between AR and $CD66^+$ neutrophils in tissues from Black men ($R = -0.318$; $p = 0.002$). Interestingly, we measured a positive correlation between AR mRNA expression and $tryptase^+$ mast cells among White men ($R= 0.320$; $p=0.016$), but a negative relationship trended among Black men ($R= -0.252$; $p = 0.075$). There was a positive correlation between AR and $CD163^+$ ($R= 0.324$; $p<0.001$), $CD68^+$ ($R= 0.514$; $p<0.001$), and $CD80^+$ ($R= 0.386$; $p<0.001$) macrophages in primary tumor tissues from all men.

There was a higher frequency of AR loss (AR^-) in metastatic tissues from Black (33%) compared to White (n=8%) American men. Further, NKX3.1 and PSA proteins were significantly lower in metastatic tissues from Black compared to White men. Conversely, M1 macrophages were higher in Black compared to White men. $CD66^+$ neutrophils and M2 macrophages were increased in AR^- compared to AR intact metastatic tissues.

AR protein was not apparent on immune cells in primary cancer tissues. However, we observed increased T cell infiltration in AR^- regions in primary cancer tissues in both American and Kenyan men.
Conclusions: Immune cell infiltration varies by AR presence in both primary and metastatic PCa tissues. We observed differences in AR mRNA and AR-related proteins between Black and White patients which may contribute to the differences observed in immune cell infiltration between races.

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