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Racial Disparities in Expression of GDF15 and NFκB in Prostate Cancer and Benign Prostatic Epithelium

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ABSTRACT: Prostate cancer (PC) outcomes are more adverse for African-American (AA) than white/European American (EA) men. Growth differentiation factor 15 (GDF15, PDF, NAG-1) is a stress-induced anti-inflammatory cytokine with immunosuppressive and tumor growth-promoting functions. GDF15 inversely regulates NFκB, a transcription factor enabling pro-inflammatory gene expression and becomes constitutively activated in androgen-independent PC. Tissue microarrays (TMAs), prepared from prostatectomy tissue at three institutions, comprised 688 cases (364 EA and 324 AA). Each case included ≥3 tumor punches plus ≥3 non-neoplastic punches. TMAs were stained separately for GDF15 and NFκB and evaluated by two pathologists, using the 0-3+ scale. PC, compared to benign epithelium, had elevated median GDF15 expression (1.93 vs. 0.99) and also, NFκB (1.18 vs. 0.96, both $P<0.0001$). Only in AA men did PC show gradewise or stagewise altered expression of these markers. In AA men, GDF15 expression fell as stage rose in PC ($P=0.007$) and also in benign epithelium ($P=0.003$). In EA men, GDF15 expression in *benign* epithelium fell as stage ($P=0.01$) and grade ($P=0.01$) rose. NFκB expression was higher in AA than EA men only in high-grade PC ($P=0.01$). NFκB expression rose with increasing tumor grade only in AA men ($P=0.027$) and in the benign prostate component only in EA men ($P=0.007$). Benign and tumor NFκB expression did not vary with stage. PC showed significant alterations in GDF15 and NFκB expression in accord with cancer aggressiveness in AA men only: stagewise decrease in GDF15, and gradewide increase in NFκB. Findings suggest a racial disparity in cell growth, immune response, stress response, or other functions relevant to prostate carcinogenesis.

KEYWORDS: Racial disparity, prostate cancer, GDF15, NFκB.

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Introduction

African-American (AA) men generally have worse prostate cancer (PC) outcomes than white/European American (EA) men or other races. The role of chronic inflammation in development of prostate cancer is well documented (Puhr et al., 2016). Lymphocytic infiltration is normal in the prostate (Bostwick et al., 2003), but whether there are racial differences in the level of prostatic inflammation is unclear. Immune response-associated gene expression differs between AA and EA prostate cancers (Wallace et al., 2008); and some studies have found chronic inflammation to be more frequent in AA men (Eastham et al., 1998) while others have noted no difference (Bostwick et al., 2003; Vidal et al., 2016) although the studies reporting no difference did not distinguish types of inflammatory cells and digital quantification was not used. Observed differences were not accounted for by race-related differences in patients' age, serum testosterone level, or prostate volume (Eastham et al., 1998). Although many studies have shown that interaction of inflammatory cytokines with inflammatory pathways greatly influence prostate cancer risk, the role of some cytokines such as growth differentiation factor 15 (GDF15) in prostate cancer is ambiguous (Vaňhara et al., 2012). GDF15, also called prostate-derived factor or PDF, NAG-1, or MIC-1, is a stress-induced anti-inflammatory cytokine possessing immunomodulatory functions. It is known to interact with Nuclear Factor of kappa B (NFκB) which regulates genes involved in cellular proliferation, apoptosis, migration and angiogenesis (Bennett et al., 2018). GDF15 is known to have both pro-tumorigenic and tumor-suppressing functions. GDF15 is a divergent member of the TGF-β and bone morphogenic protein family. It directly induces p53, and its high expression is associated with progression of several cancers, including PC (Iczkowski and Pantazis, 2003). GDF15 was found to be up-regulated *in situ* and in primary cultures of cancer-associated fibroblasts from prostate cancer. Ectopic expression of GDF15 in fibroblasts produced prominent paracrine effects on PC cell migration, invasion, and tumor growth (Bruzzese et al., 2014) and the same effects were noted in cervical cancer (Li et al., 2018). Consistent with an anti-inflammatory role, inflammatory lesions in the prostate correlated with decreased prostatic GDF15 expression (Lambert et al., 2015). Moreover, an inverse correlation was demonstrated between GDF15 and CD3+, CD4+, CD8+, CD68+, and iNOS (NO Synthase)+ leukocytes (Bennett et al., 2018); and GDF15 exerts immunosuppressive effects (Zhang et al., 2018).

Nuclear factor kappa-light-chain-enhancer of activated B cells, NFκB is a transcription factor (also called p65(RelA)) that regulates pro-inflammatory gene expression and is constitutively activated in androgen-independent prostate cancer, increasing anti-apoptotic bcl-2 and angiogenesis (Jin et al., 2008). GDF15 expression was shown to correlate inversely with inflammatory lesions in the prostate and acts through the PI3K pathway to suppress NFκB activity (Lambert et al., 2015); cervical cancer demonstrated this same inverse relationship (Li et al., 2018). Expression of the NFκB target, interleukin 8 (IL-8), was downregulated by GDF15 in PC3 cells (Lambert et al., 2015).

Whether these pro-tumorigenic factors show a racial disparity in PC is uncertain. In this study we examined immunoexpression of GDF15 and NFκB in tissue from African-American and white cancerous and benign prostate.

Materials and Methods

Retrospective study in prostatectomy tissue

Tissue microarrays (TMAs), prepared at three different institutions, comprised prostatectomy tissue from 697 cases. Each TMA contained at least 3 individual 0.6 mm punches of the dominant tumor nodule plus at least 3 punches of non-neoplastic epithelium. Sources were Medical College of Wisconsin (57 cases), Prostate Cancer Biorepository Network (PCBN, via Johns Hopkins) (153 cases), PCBN High-Grade Racial Disparity (120 cases), and Henry Ford Hospital (up to 12 evaluable cores each of tumor and benign, 367 cases). Each study set contained an approximate 1:1 match of AA to white cases, based on grade and stage, for a total of 364 EA and 324 AA men. Gleason score according to current consensus (Epstein et al., 2016) and tumor pathologic stage (pT) were available for all groups; for analysis, grade was expressed according to the 5-tier International Society of Urological Pathology (ISUP) Grade Group system (Epstein et al., 2016). Patient ages were available only for the Henry Ford and Medical College of Wisconsin cases. The results from two Hopkins study sets were combined in all analyses going forward.

Separate slides were stained with polyclonal antibody to GDF15 or monoclonal antibody to NFκB. Slides were dried 30 min at 60°C, then deparaffinized down to deionized water. Antigen retrieval was performed on a PT Link (Dako) by preheating Target Retrieval Solution to 65°C and heating for 20 min at 97°C in pH=6 (Dako). Slides were washed with buffer for 5 minutes. All IHC staining was performed on the Dako Autostainer Plus (Agilent) using the Dako EnVision™ FLEX High pH Detection Kit (catalog K8010) with 3 drop zones at 100 µl each and Dako Protein Block (Agilent). Antibodies used were goat polyclonal antibody to GDF15 (1:150, 20 min incubation, catalog AF957, R&D Systems, Minneapolis) or rabbit monoclonal antibody to NFκB (1:2000, 10 min incubation, clone D14E12, Cell Signaling Technologies, Beverly, MA). Background was stained with hematoxylin Dako FLEX. Slides were rinsed with deionized water and oven dried 15 min.

Evaluation of Immunostaining

TMA cores of tumor and benign prostatic tissue for each case were evaluated by two pathologists. (Digital evaluation of the TMAs was not feasible because the frequent admixture of tumor glands and benign glands in many spots required assessment by pathologists. Moreover, occasional spots that were sampled as benign were actually cancer and vice versa, and again pathologists' interpretations were needed to visually dissect out the admixture and evaluate the tumor or benign epithelium separately.) Immunoreactivity (Figures 1-2) was scored on a scale of 0 (negative) to 3+ (strong and diffuse), including half-steps (0.5). Disagreements ≥1 were resolved by consensus.

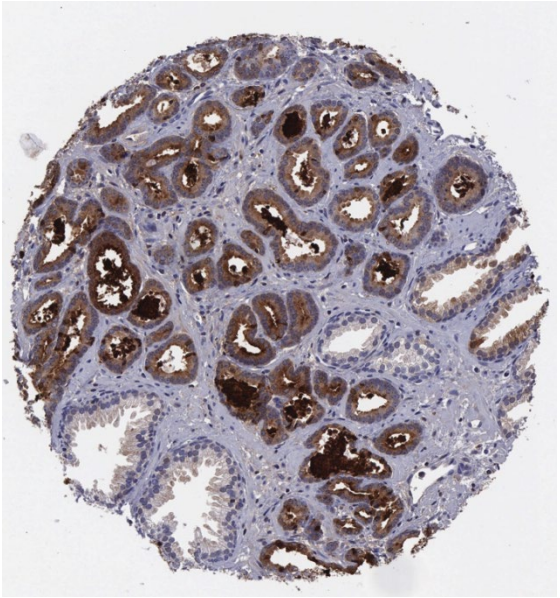

Figure 1. Example of strong GDF reactivity in cancer. Non-neoplastic glands at lower left are negative. 10x objective.

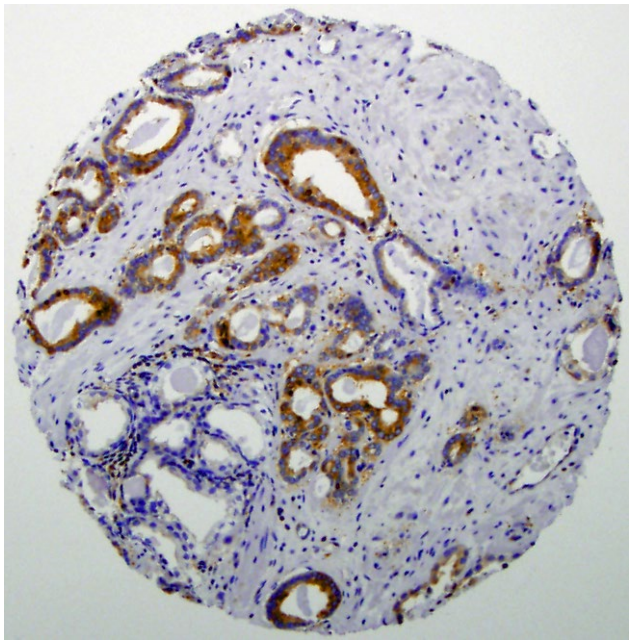

Figure 2. Example of strong NFκB reactivity in cancer. Non-neoplastic glands at lower left are negative. 10x objective.

Statistical analysis

To adjust for potential batch effects from TMAs from 3 sites, we first digitally measured the expression of both markers in the *benign* tissue spots corresponding to Gleason grade group 2 tumors from all sites using the QuPath software (Bankhead et al., 2017). In QuPath, the stained color for a marker (brown) is separated from counter stain (blue) using stain vectors auto-estimated by the software. Percentage of positive expression area (PPEA) is determined as the ratio of the number of positive brown stained pixels (brown optical density (OD) > 0.2) over total number of tissue pixels (overall OD > 0.05). The average expression intensity (AEI) is calculated as the mean brown OD of positively stained pixels. The log transformed product of PPEA and AEI, log(PPEA*AEI), is used as the marker expression level of a subject. Under the assumption that the marker expression in benign regions of Gleason grade group 2 tumors should be the same across the 3 study sites, the batch correction factor for a study site with respect to the reference Henry Ford study site was defined as the ratio of median marker expression level for the benign prostate spots evaluated for the study site over median marker expression level of benign prostate spots of the Henry Ford study site. Subsequently, all pathologist-assessed data for each study site were adjusted by dividing original measures over the batch correction factor (the reference Henry Ford site had a batch correction factor of 1).

Mean age difference between races was tested by Wilcoxon signed rank test. Comparisons of expression in PC vs. benign prostate were done by Wilcoxon signed rank test.

The non-parametric Mann-Whitney test was used for testing expression differences between two races. Kruskal-Wallis test was applied for testing expression differences across tumor stages (pT2, pT3a, or pT3b) and Gleason grade groups (ISUP groups 1-5). Correlation of the two markers with each other (in benign or tumor) was examined by Pearson and Spearman correlation tests. Statistical significance was set at $P < 0.05$.

Results

The racial distribution of cases did not differ by grade (P=0.9) or pT stage (2, 3a, or 3b) (P=0.9). The mean age of AA men was 60.7; this was less than for EA men at 62.2 (P=0.03). GDF15 reactivity was cytoplasmic, while NFκB reactivity was both cytoplasmic and nuclear, as expected (Domingo-Domenech et al., 2005).

Between mean reactivities of the TMAs from 3 study sites—in both cancer and benign spots, some batch effect was noted in both normal and

tumor regions. This was attributed to differences in tissue processing across institutions, different lots of antibodies used, and other technical procedures. Therefore, the above-described normalization was applied to all benign and tumor results. The degrees of deviation in expression before and after batch correction are shown in normal and tumor spots of prostate tissue from men from all sites for GDF15 expression and NFκB expression (Supplementary Figure 1).

Representative marker reactivity is shown (Figure 3). Median GDF15 (1.93 vs. 0.19, $P<0.0001$) and NFκB (1.18 vs. 0.96, $P<0.0001$) expression was elevated in PC compared to benign prostate (Table 1). Median GDF15 expression for EA men was 2.06 in tumor versus 0.87 in benign ($P<0.0001$); comparable values for AA men were 2.06 vs. 0.83 ($P<0.0001$). Median NFκB expression for EA men was 1.04 in tumor versus 0.88 in benign ($P<0.0001$); comparable values for AA men were 1.04 vs. 0.96 ($P<0.0001$). Tumor-tumor and benign-benign comparisons of expression levels for both proteins by race were not significant except that NFκB was borderline-higher in the prostate benign epithelium of AA men ($P=0.06$). (NFκB expression in prostate benign epithelium was also significantly higher in AA than EA men when we separately analyzed the PCBN High-Grade TMA set ($P=0.01$) but not in the other cases; namely 41.4% of AA men had reactivity >1 in benign glands but 30.2% of EA did ($P=0.03$); for further analysis, however, both PCBN cohorts were combined.)

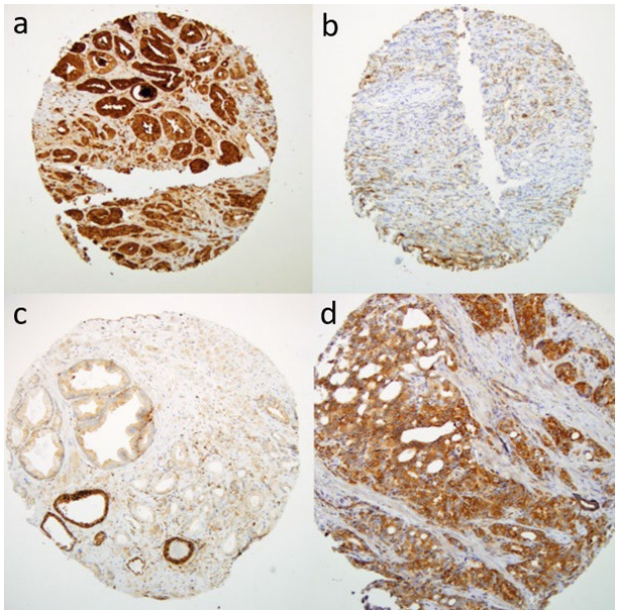

Figure 3. Representative TMA spots illustrating trends in AA patients. (a) GDF15 in stage 2 tumor, (b) GDF15 in stage 3b tumor, (c) NFκB in low grade (group 1) tumor with a few stronger-staining benign glands at bottom, (d) NFκB in high grade (group 5) tumor.

Gradewise GDF15 in tumor showed changes of indeterminate direction ($P=0.010$), while NFκB expression was increased in Gleason grade groups 3, 4, and 5 in tumor ($P=0.009$) and benign ($P=0.003$) (Table 2). By pathologic stage (Table 3), GDF15 expression markedly decreased in tumor ($P=0.001$) and benign epithelium ($P<0.001$) with increasing stage. NFκB expression rose in tumor ($P=0.02$) with increasing stage. Results for each site-specific sampling generally followed similar trends as the combined sample (Supplementary Tables 1-5).

Racial disparities of GDF15 and NFκB expression emerged, according to tumor grade and stage, in both tumor and non-neoplastic prostate. The only expression trend by grade in tumor was for increasing NFκB expression with grade in AA ($P=0.03$) (Table 4). GDF15 significantly increased with grade only in EA men ($P=0.01$). By stage (Table 5),

GDF15 significantly decreased in AA men in prostate tumor (P=0.007), compared to a non-significant trend in the same direction observed in EA men (P=0.07); thus the significant decrease in the overall study group was driven by AA men. GDF15 expression in benign epithelium decreased in EA men with increasing stage (P=0.01), as well as in AA men (P=0.003). NFκB showed no race-specific trends according to stage in either tumor or benign epithelium. Finally, the two markers did not correlate with each other in normal (Supplementary Figure 1A-1C) or tumor samples (Supplementary Figure 1D-1F), either overall or in EA or AA cohorts, shown as scatter plots. Trends are summarized (Table 6).

Discussion

The current study shows a newly-described, significant stagewise decline in GDF15 expression in prostate tumors of African-American (AA) men not observed in European American (EA) men, and a grade-proportional rise of NFκB expression in AA tumors. Also, NFκB expression was higher in benign epithelium of AA men than EA men in the PCBN High-Grade Disparity cohort. Although AA men have shown higher Gleason scores in other series (P = 0.037) (Powell et al., 2013) the lack of significant differences between our AA and EA men groups rules out differing average grades as a cause of the differences observed. Since GDF15 is considered to repress NFκB, this may explain the increase in NFκB expression in tumor (although the latter was gradewise, not stagewise) that was noted in AA men but not EA, a finding possibly related to androgen independence (Jin et al., 2008). These findings suggest racially differing effects of these molecules on biologic functions including immune response.

GDF15 is widely associated with inflammation, regulating apoptosis, cell repair, growth, and tumorigenesis. GDF15 expression is normally relatively high in the prostate, and GDF15 immunoreactivity in human prostatectomy specimens had shown an inverse relationship to inflammatory cells (Bruzzese et al., 2014). The lowering of GDF15 expression in AA tumor progression may be consistent with the known effect of vitamin D to upregulate GDF15 (Lambert et al., 2015). That is, higher prevalence of vitamin D deficiency in AA men (Hollis et al., 2013) could explain our finding that AA men have lower tumor GDF15 expression as the stage progresses. Stated differently, whereas white men do not show a lowering of GDF15 expression as tumor spreads outside the prostate, such a reduction occurs in AA men. Whether this highly significant (P=0.003) lowering of GDF15 expression in benign epithelium in AA men predicts future cancer detection will require a prospective trial, based on repeated biopsies. Notably, increased circulating GDF15 was significantly correlated with AA race, smoking, and hypertension (Powell et al., 2013), suggesting that some GDF15 originates from extraprostatic sources. This finding supports a role for GDF15 in tumor development; for example, increased GDF15 targets p53 and acts through the PI3K/AKT and MAPK/ERK signaling pathways, with upregulation of cyclins D1 and E1, to promote proliferation in cervical carcinogenesis (Li et al., 2018).

Recent evidence supports the concept that the greater modulation of GDF15 in AA men is related to higher risk of PC progression. A racial disparity was found in the link between PSA velocity and eventual PC diagnosis such that NSAID use was associated with increased PC risk only in AA men but not EA men (Wallace et al., 2008; Kryvenko et al., 2019). Since NSAID use induces GDF15 expression (Wang et al., 2013), this aligns with astronger role of immune-related genes in tumor development in AA men than

in EA men (Wallace et al., 2008), and may relate to the current study where a greater stagewise modulation of GDF15 was found in AA men than in EA men (Table 5).

NFκB is the most important transcription factor for oxidative susceptibility in the body. After activation, NFκB can activate and regulate the expression of many inflammatory factors, which makes it the key promoter of the inflammatory response. Infection or hypoxia activates NFκB, which is inactive in cells, and activates inflammatory genes, induces the up-regulation of cytokines, adhesion molecules, and vasoactive regulators and increases the concentration of further downstream cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8) and others (Moresco et al., 2011). Our finding of more pronounced rises in NFκB in cancer glands in AA tumor development may correlate with the more frequent prostatic inflammation reported by some (Eastham et al., 1998) (but not all) studies in AA men (Bostwick et al., 2003).

Previous studies suggested NFκB to have a reciprocal interaction with GDF15 (Lambert et al., 2015; Zhang et al., 2018). Thus, a firefly luciferase construct was used to show that expression of the NFκB target, interleukin 8 (IL-8), was downregulated by GDF15 in PC3 cells (Lambert et al., 2015). GDF15 also inactivates NFκB signaling in dendritic cells, enabling induction of immune tolerance after heart transplantation (Zhang et al., 2018). On this basis, upregulation of NFκB with tumor stage might be expected, but this was noted in the tumor only in AA men (with stagewise upregulation in benign glands in EA men). This could be explained by lower GDF15 in progression of AA men's tumors, allowing a greater rise in NFκB expression in response to tumor. The altered NFκB in AA men could correlate with inflammatory response to the cancer tissue, but the non-neoplastic cells also have a greater rise in EA men which may cause localization of inflammatory cells to the tumor.

In cancer, NFκB becomes constitutively activated in a high proportion of androgen-independent prostate cancers (Jin et al., 2008; Nadiminty et al., 2008). Apparently, the ability of NFκB to promote transcription of the prominent anti-apoptotic protein Bcl-2 and cyclin D1, cyclooxygenase-2, matrix metalloproteinase 9, nitric oxide synthase-2 (NOS-2), and vascular endothelial growth factor aids the survival of cells that would otherwise die owing to loss of androgen activity (Shukla et al., 2004). We observed a gradewise increase of tumor expression of NFκB in AA men but not EA men, a finding possibly related to greater attainment of androgen independence in AA men. Signaling linked to NFκB and inflammatory cytokine factors was preferentially upregulated in PC from AA race (Powell et al., 2013). In the face of lower NFκB expression by the tumor, this suggests a greater sensitivity of the post-NFκB cascade in AA men.

Aside from these two molecules, other signaling pathways involved in the immune response have also been noted to show a racial disparity in prostate cancer. A germline variant called interferon lambda 4 was twice as common in prostate tumors of AA men than white men (42-67% versus 18-33%); this relied on pro-tumorigenic JAK-STAT signaling, and was associated with decreased survival (Tang et al., 2018).

It is uncertain whether our results are consistent with the current understanding of the prostatic inflammatory environment. The normal prostate contains lymphocytes, of which >90% are T cells; and those in the epithelium are predominantly cytotoxic/suppressor (CD8+) (Bostwick et al., 2003; Eastham et al., 1998). Studying just tumor-infiltrating T-cell density with three immunohistochemical

markers and image analysis, Kaur et al. found no association with EA or AA racial ancestry (Kaur et al., 2018) although increased T-cell density was associated with ERG positivity and PTEN loss in both races. The REDUCE study, a 4-year, multicenter, placebo-controlled study in which a negative prostate biopsy was criterion for enrollment, involved 7,982 men: 7,271 white and 180 AA men. No differences were noted in chronic inflammation, but AA men were less likely (OR = 0.65, 95%CI: 0.41-1.03, *P*= 0.07) and Asian men (OR = 1.74, 95%CI: 1.14-2.65, *P*= 0.001) more likely, to have acute inflammation (Vidal et al., 2016). Inflammation in biopsies was associated with decreased cancer risk in other case-control studies (Kryvenko et al., 2012; Yli-Hemminki et al., 2013). Our topographic/spatial study of atrophy had found only a weak association of inflammation with cancer provided the inflammation was accompanied by atrophy (Iczkowski et al., 2014). Acute inflammation has also been linked with lower future PC risk (Allott et al., 2018; Moreira et al, 2014). Inflammation was not significantly predictive of PC in another study (Khani et al., 2014). High BMI was a greater risk factor for PC in AA men than in white men (Barrington et al., 2015), and adiposity causes generalized inflammation.

One limitation of the study was its exclusion of stromal changes. There is increased reactive stroma associated with chronic inflammation in prostate cancer of AA men, and fibroblasts isolated from AA prostate cancer tissues showed increased growth response to androgens, fibroblast growth factor 2, and platelet-derived growth factor. Conditioned media from AA-derived fibroblasts enhanced the proliferation, motility, and in vivo tumorigenicity of prostate cancer cells more than European-American-derived fibroblasts did, and they had elevated markers of myofibroblast activation such as expression of SMA, vimentin, and tenascin-C. Also, proinflammatory paracrine mediators BDNF, C HI3L1, DPPIV, FGF7, ILI8BP, IL6, and VEGF were comparatively enriched in AA-derived fibroblasts (Gillard et al., 2018). It is possible that the high, and rather constant level of GDF15 we observed in the benign and cancer epithelia is counteracting these stromal proinflammatory mediators. A second limitation is not knowing the anatomic sites within the prostate from which TMA cancer cores were derived. Dominant tumor nodules in AA men are larger and more often in the anterior peripheral zone, making them less amenable to rectal palpation than posterior tumors, and this location correlates with an adverse outcome (Sundi et al., 2014). A third limitation was that serum PSA measurements were not available for comparison to marker expression; this would have given further insight into their roles in tumor development, although inflammation had been shown to be unrelated to the racial disparity in serum PSA (Zhang et al., 2000).

In summary, we report that tumor immunoreactivity showed significant alterations with progression in AA men only: a stagewise decrease in GDF15 expression, and a gradewide increase in NFκB expression. These findings have ramifications for tumor development and androgen independence, and further work is needed to determine whether the racial disparities observed in non-neoplastic prostate are influenced by the presence of tumor or independent of it.

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Conflict of interest

Dr. Iczkowski has no consultancies, stock ownership, equity interest, patent-licensing agreements, research support, or honoraria from companies whose product figures prominently in this manuscript.

Authors' contributions

Study design: KAI, MSL, BAR. Immunostain protocol and science background: JRL. Data acquisition: KAI, OK, SS, WP, BAR. Statistical analysis: YC, KCT.

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Table 1. Expression of GDF15 (top) and NFκB (bottom) according to race in benign and tumor, tissue microarray.									
	African-American, median (range)			European American, median (range)			Overall population, median		
	Tumor	Benign	*P*	Tumor	Benign	*P*	Tumor	Benign	*P*
GDF15	2.06 (0, 3)	0.83 (0, 3)	<0.0001	2.06 (0, 3)	0.87 (0, 3)	<0.0001	1.93	0.99	<0.0001
P (AA-EA)				0.96	0.08				
NFκB	1.04 (0, 3)	0.96 (0, 3)	<0.0001	1.04 (0, 3)	0.88 (0, 2.83)	<0.0001	1.18	0.96	<0.0001
P (AA-EA)				0.82	0.06				

AA= African-American; EA = European-American White

Table 2. Expression of GDF15 and NFκB according to Gleason Grade Group.						
Grade group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	2.06 (0, 3)	0.010	221	0.83 (0, 3)	0.9475	250
2	2.06 (0, 3)		198	0.83 (0, 3)		210
3	2.06 (0, 3)		87	0.93 (0, 3)		94
4	2.42 (0.4 ,3)		52	0.95 (0, 3)		59
5	1.70 (0, 3)		70	0.83 (0, 3)		72
Grade group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1.00 (0, 3)	0.009	221	0.88(0, 2.7)	0.003	248
2	0.97 (0, 3)		195	0.85 (0, 2.5)		210
3	1.17 (0, 3)		87	1.08 (0, 2.3)		94
4	1.50 (0, 2.9)		57	1.06 (0, 3)		59
5	1.06 (0, 3)		72	0.96 (0, 2.8)		71

n= number of informative cases

Table 3. Expression of GDF15 and NFκB according to pathologic stage.						
Pathologic stage	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
2	2.20 (0, 3)	0.001	462	1.43 (0.188, 2.56)	<0.001	514
3a	1.91 (0, 3)		106	1.82 (0.5, 2.81)		109
3b	1.65 (0, 3)		56	1.16 (0.25, 2.56)		58
4	1.33 (0.6, 2.8)		4	1.16 (0.625, 1.69)		4
Pathologic stage	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
2	1.04 (0, 3)	0.02	466	0.9 (0, 3)	0.10	511
3a	0.97 (0, 3)		105	0.9 (0, 2.5)		109
3b	1.20 (0, 3)		57	1.08 (0, 2.1)		58
4	0.54 (0, 0.7)		4	0.86 (0.7, 1.2)		4

n= number of informative cases

Table 4. Racial disparity of expression of GDF15 and NFκB according to Gleason Grade Group.

	African-American						European American					
Grade Group	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
1	2.15 (0, 3)	0.08	107	0.83 (0, 3)	0.80	118	2.06 (0, 3)	0.19	114	0.97 (0, 2.9)	0.01	132
2	2.06 (0.4, 3)		93	0.83 (0, 3)		97	2.00 (0, 3)		105	0.83 (0, 3)		113
3	2.17 (0.2, 3)		42	0.83 (0, 3)		44	2.01 (0, 3)		45	1.1 (0, 3)		50
4	2.71 (0.4, 3)		22	0.88 (0, 3)		27	2.34 (0.5, 3)		30	1.15 (0, 3)		32
5	1.63 (0, 3)		36	0.96 (0 ,3)		37	2.03 (0.3, 3)		34	0.69 (0, 2.5)		35
Grade Group	**NFκB.** Tumor, median (range)	*P*	N=	**NFκB.** Benign, median (range)	*P*	n=	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=
1	0.96 (0 ,3)	0.03	107	0.96 (0, 2.7)	0.37	117	1.04(0, 3)	0.23	114	0.82 (0, 2.5)	0.007	131
2	1.04 (0 ,3)		89	0.91 (0, 2.3)		97	0.87 (0, 3)		106	0.75 (0, 2.5)		113
3	1.15 (0, 3)		42	1.08 (0, 2.3)		44	1.25 (0, 3)		45	1.04 (0, 2.1)		50
4	1.50 (0, 2.9)		25	1.00 (0, 3)		27	1.46 (0, 2.9)		32	1.07 (0, 2.1)		32
5	1.12 (0, 3)		37	0.96 (0, 1. 8)		36	1.04 (0.2, 3)		35	0.96 (0, 2.8)		35

n= number of informative cases

Table 5. Racial disparity of expression of GDF15 and NFκB according to pathologic stage.

	African-American						European American					
Stage	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
2	2.20 (0, 3)	0.007	221	0.83 (0, 3)	0.003	242	2.08 (0, 3)	0.07	241	1.00 (0, 3)	0.01	272
3a	1.61 (0.4, 3)		49	0.42 (0, 2)		50	2.06 (0, 3)		57	0.80 (0, 3)		59

3b	1.63 (0, 3)		28	1.10 (0, 2.5)		29	1.65 (0.3, 3)		28	0.75 (0, 2.5)		29
4	2.01 (0, 2.8)		2	1.38 (0.8, 1.9)		2	1.02 (0.6, 1.4)		2	0.92 (0.2, 1.7)		2
Stage	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=
2	1.04 (0, 3)	0.21	222	0.96 (0, 3)	0.96	240	1.08 (0, 3)	0.15	244	0.86 (0, 2.8)	0.18	271
3a	1.06 (0, 3)		48	0.96 (0, 1.9)		48	0.89 (0, 3)		57	0.88 (0, 2.5)		59
3b	1.23 (0, 2.8)		28	0.96 (0, 1.8)		28	1.12 (0.2, 3)		29	1.12 (0.1, 2.1)		29
4	0.54 (0.4, 0.7)		2	0.93 (0.7, 1.2)		2	0.36 (0, 0.7)		2	0.86 (0.8, 1.0)		2

n= number of informative cases

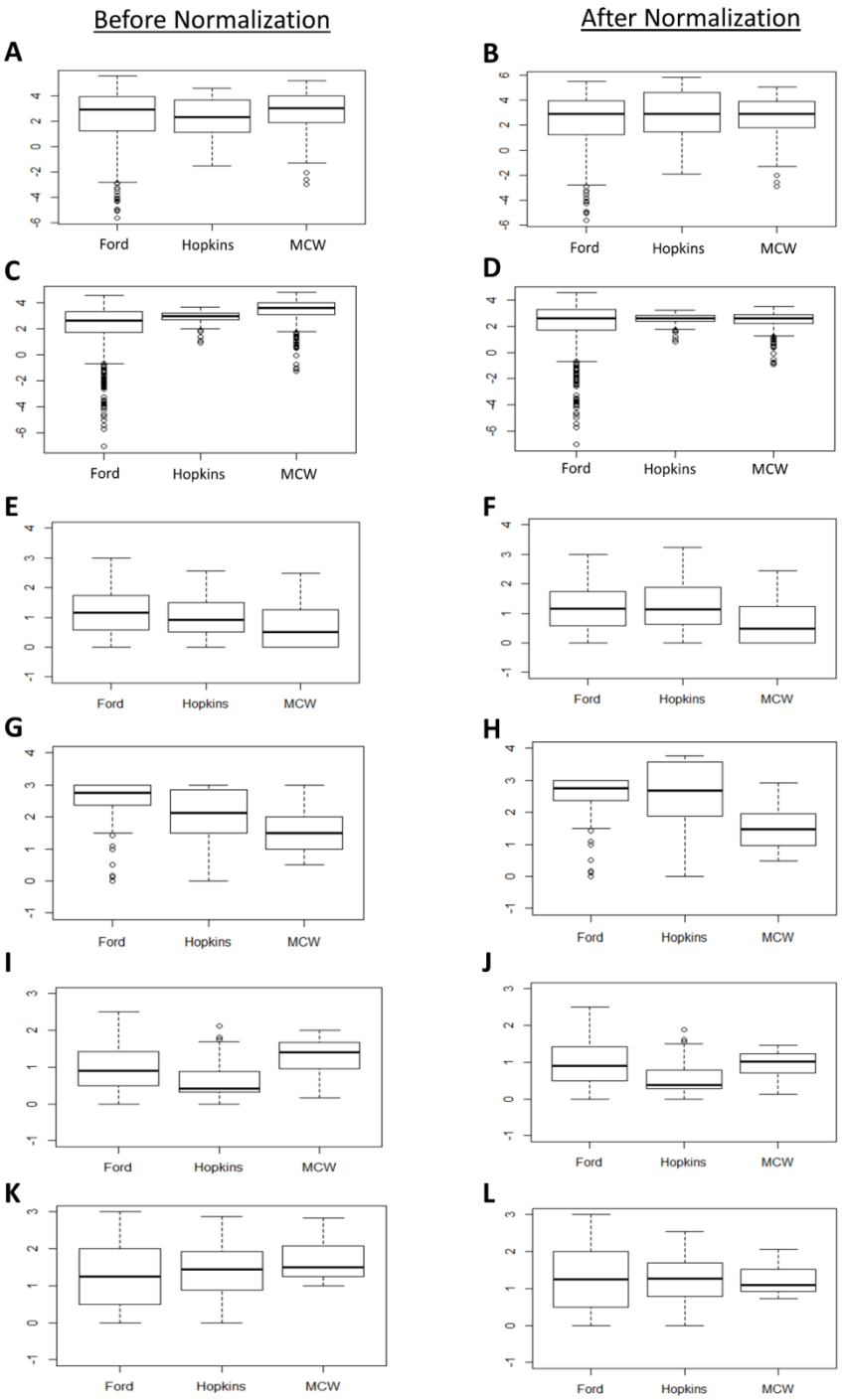
Note: With only 2 cases for Stage 4, values are probably not representative.

Table 6. Summary of inflammatory markers in prostate.

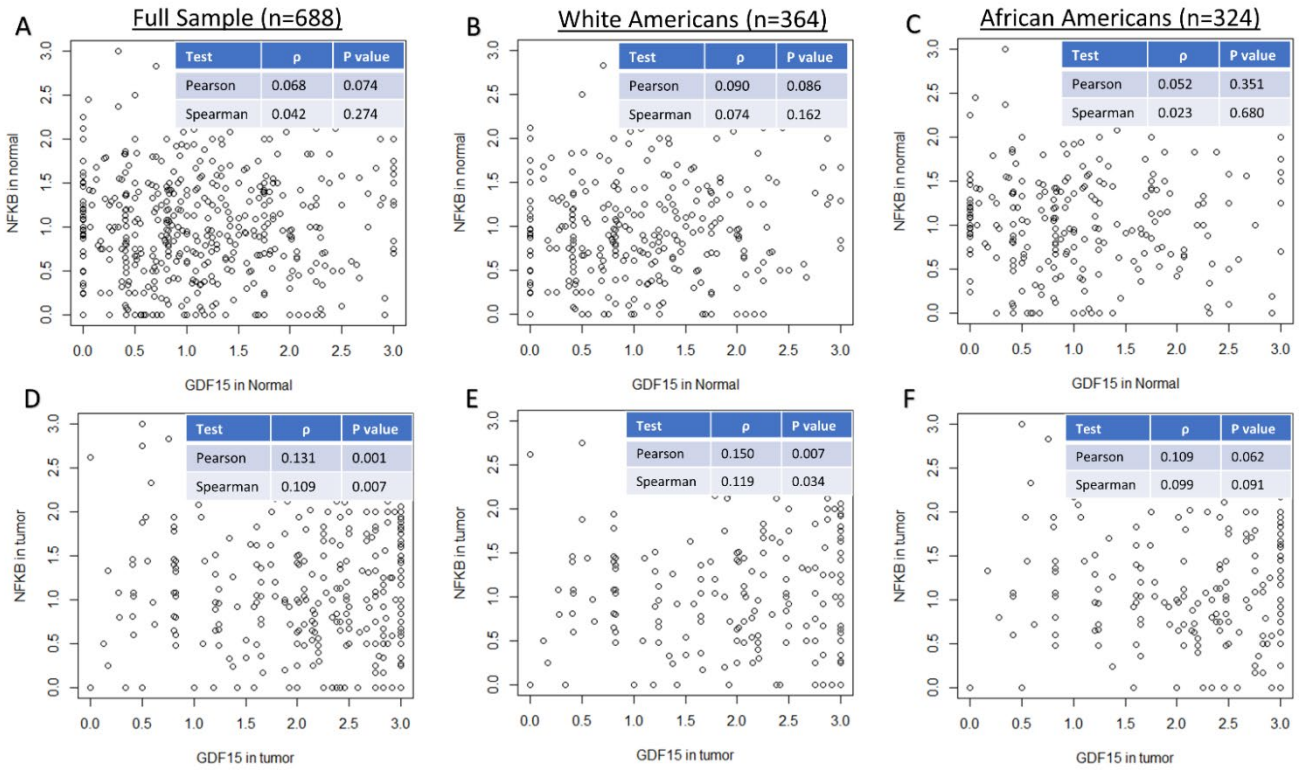
Marker	By increasing stage[K]	By increasing grade[K]
GDF15 in EA men	Decreases in benign (*P*=0.01), not in tumor	Decreases in benign only (*P*=0.01)
GDF15 in AA men	Decreases in tumor (*P*=0.007) as well as benign (*P*=0.003)	No change in benign or tumor
NFκB in EA men	No change in benign or tumor	Increases in benign (*P*=0.007) but not tumor (*P*=0.23)
NFκB in AA men	No change in benign or tumor	Increases in tumor (*P*=0.027) but not benign (*P*=0.37)

AA= African-American; [K]Kruskal-Wallis; [W]Wilcoxon test; EA= European American

Supplementary Figure 1. Normalization of GDF15 and NFκB expression in normal and tumor regions of prostate of men with Gleason Grade group 2 prostate cancer from the three study sites. Box plots showing digitally assessed GDF15 (A,B) and NFκB (C,D) expression in normal prostate before (A,C) and after (B,D) normalization. Additional Box plots show effect of normalization on pathologically assessed GDF15 (E-H) and NFκB (I-L) expression in normal (E,F & I,J) and malignant (G,H & K,L) prostate before (E,G,I,K) and after (F,H,J,L) normalization. Ford=Henry Ford, Hopkins=Johns Hopkins, MCW=Medical College of Wisconsin.



Supplementary Figure 2. Correlation of GDF15 and NFκB expression in normal and tumor regions of prostate in European/White American (EA) and African American (AA) men with prostate cancer. Scatter plots and associated correlation coefficients are shown for all normal samples (A), normal prostate regions in EA (B), Normal prostate regions in AA (C), all tumor samples (D), tumor regions in EA (E), tumor regions in AA (F).



Supplemental Table 1. Expression of GDF15 (top) and NFκB (bottom) according to race in benign and tumor, tissue microarray stratified by Study Site.

Henry Ford									
	African-American, median (range)			White American, median (range)			Overall population, median (range)		
	Tumor	Benign	*P*	Tumor	Benign	*P*	Tumor	Benign	*P*
GDF15	2.83 (0, 3)	1.14 (0, 3)	<0.0001	2.75 (0, 3)	1.18 (0, 3)	<0.0001	2.75 (0, 3)	1.17 (0, 3)	<0.0001
P (AA-W)				0.251	0.931				
NFκB	1.50 (0, 3)	1.15 (0, 3)	<0.0001	1.42 (0, 3)	0.90 (0, 2.83)	<0.0001	1.45 (0, 3)	1.00 (0, 3)	<0.0001
P (AA-W)				0.992	0.039				

Johns Hopkins									
	African-American, median (range)			White American, median (range)			Overall population, median		
	Tumor	Benign	*P*	Tumor	Benign	*P*	Tumor	Benign	*P*
GDF15	2.50 (0.5, 3)	0.83 (0, 3)	<0.0001	2.00 (0, 3)	1.00 (0, 3)	<0.0001	2.00 (0, 3)	1.00 (0, 2.6)	<0.0001
P (AA-W)				<0.0001	0.148				
NFκB	1.25 (0.2, 2.94)	0.60 (0, 2.52)	<0.0001	1.56 (0.38, 3)	0.67 (0, 2.5)	<0.0001	1.47 (0, 3)	0.67 (0, 2.5)	<0.0001
P (AA-W)				<0.0001	0.73				
Medical College of Wisconsin (MCW)									
	African-American, median (range)			White American, median (range)			Overall population, median		
	Tumor	Benign	*P*	Tumor	Benign	*P*	Tumor	Benign	*P*
GDF15	2.00 (0.67, 3)	0.88 (0, 2.33)	<0.0001	1.00 (0, 3)	0.67 (0, 2.5)	0.082	1.58 (0, 3)	0.77 (0, 2.5)	<0.0001
P (AA-W)				0.007	0.454				
NFκB	2.00 (0.75, 3)	1.83 (0.17, 3)	0.161	2.00 (0, 3)	1.67 (0.5, 3)	0.943	2.00 (0, 3)	1.75 (0.17, 3)	0.297
P (AA-W)				0.283	0.384				

AA= African-American; W= White

Supplemental Table 2. Expression of GDF15 and NFκB according to Gleason Grade Group Stratified by Study Site.						
Henry Ford						
Grade group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	2.81 (0, 3)	0.668	103	1.17 (0, 3)	0.268	132
2	2.75 (0, 3)		101	1.17 (0, 3)		113
3	2.83 (0.167, 3)		32	1.30 (0.056, 3)		39
4	2.75 (0.5 ,3)		43	1.11 (0, 3)		50
5	2.50 (0.75, 3)		20	0.92 (0, 3)		33
Grade group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1.25 (0, 3)	0.128	106	0.95 (0, 2.67)	0.544	130
2	1.25 (0, 3)		101	0.90 (0, 2.5)		113
3	1.47 (0, 3)		32	1.19 (0, 2.33)		39
4	1.71 (0, 2.92)		48	1.25 (0, 3)		50
5	1.83 (0.167, 3)		22	1.23 (0, 2.82)		21
Johns Hopkins						
Grade group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	2 (0,3)	0.458	112	1 (0,2.56)	0.040	112

2	2.12 (0,3)		80	0.91 (0,2.56)		81
3	2.41 (0.5,3)		26	1.47 (0,2.56)		26
4	2.44 (2.06,2.83)		9	1.4 (0.67,2.56)		9
5	2.12 (0.5,3)		45	1.44 (0.25,2.6)		45
Grade group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1.35 (0,3)	0.001	110	0.40 (0,2.38)	<0.0001	112
2	1.44 (0,2.88)		80	0.42 (0,2.12)		81
3	1.5 (0.25,2.69)		26	0.86 (0.1,2.5)		26
4	1.62 (0.69,2.94)		9	1.06 (0.58,1.69)		9
5	2 (0.25,2.81)		45	1.31 (0.38,2.52)		45
Medical College of Wisconsin (MCW)						
Grade group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	1.25 (0.5,2)	0.085	6	0.75 (0,1)	0.573	6
2	1.5 (0.5,3)		16	0.5 (0,2.5)		16
3	2.33 (0,3)		29	1 (0,2.5)		29
4	---		0	---		0
5	1.67 (0.5,2.17)		5	0.5 (0,2.33)		5
Grade group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1.5 (0.75,3)	0.049	5	1.5 (0.8,1.8)	0.0003	6
2	1.5 (1,2.83)		16	1.4 (0.17,2)		16
3	2.08 (0,3)		29	2 (0.5,3)		29
4	---		0	---		0
5	2.5 (2.25,2.62)		5	2 (1.4,2.83)		5

n= number of informative case

Supplemental Table 3. Expression of GDF15 and NFκB according to pathologic stage stratified by Study Site.						
Henry Ford						
Pathologic stage	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
2	2.83 (0,3)	.015	243	1.25 (0,3)	0.01	295
3a	2.71 (0.167,3)		34	0.88 (0,3)		37
3b	2.2 (0.75,3)		20	1.19 (0.25,2.5)		22
4	2.1 (1.42,2.79)		2	1.06 (0.19,1.94)		2
Pathologic stage	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
2	1.42 (0,3)	0.203	253	1.00 (0,3)	0.589	292
3a	1.42 (0,3)		33	0.88 (0,2.5)		37
3b	1.62 (0,3)		21	1.25 (0,2.08)		22
4	0.18 (0,0.36)		2	0.71 (0.67,0.75)		2

Johns Hopkins						
Pathologic stage	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
2	2.25 (0,3)	0.081	183	1 (0,2.56)	0.900	183
3a	2 (0.5,3)		57	1 (0,2.6)		58
3b	2 (0.5,3)		30	1.04 (0.25,2.5)		30
4	1.41 (1.31,1.5)		2	1.16 (0.63,1.69)		2
Pathologic stage	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=
2	1.38 (0,3)	0.005	181	0.58 (0,2.5)	<0.0001	183
3a	1.38 (0,2.88)		57	0.5 (0,1.98)		58
3b	2.25 (0.25,2.81)		30	1.11 (0.42,2.52)		30
4	1.68 (1.55,1.81)		2	1.38 (1.25,1.5)		2
Medical College of Wisconsin (MCW)						
Pathologic stage	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
2	1.5 (0.33,3)	0.589	36	1 (0,2.5)	0.101	36
3a	1.83 (0,3)		14	0.5 (0,2.5)		14
3b	1.75 (0.5,2.67)		6	0.88 (0,2.33)		6
4	---		0	---		0
Pathologic stage	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=
2	2 (0.75,3)	0.206	35	1.73 (0.75,3)	0.860	36
3a	1.71 (0,2.83)		14	1.77 (0.17,2.88)		14
3b	2.42 (1,3)		6	1.92 (1.4,2.83)		6
4	---		0	---		0

n= number of informative cases

Supplemental Table 4. Racial disparity of expression of GDF15 and NFκB according to Gleason Grade Group stratified by Study Site.												
Henry Ford												
	African-American						White American					
Grade Group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	2.69 (0,3)	0.557	48	1.17 (0,3)	0.591	59	2.83 (0,3)	0.585	55	1.18 (0,2.88)	0.104	73
2	2.75 (0.5,3)		44	1.2 (0,3)		48	2.75 (0,3)		57	1.17 (0,3)		65
3	2.83 (0.17,3)		15	1.42 (0.06,3)		17	2.75 (1,3)		17	1.27 (0.13,3)		22
4	2.96 (1,3)		18	0.92 (0,3)		23	2.75 (0.5,3)		25	1.33 (0,3)		27
5	2.79 (0.75,3)		9	1.17 (0.25,3)		10	2.25 (1.15,3)		11	0.73 (0,2.5)		12
Grade Group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1.17 (0,3)	0.754	48	1.17 (0,2.67)	0.912	58	1.33 (0,3)	0.122	58	0.81 (0,2.5)	0.511	72
2	1.33 (0,3)		41	1 (0,2.25)		48	1.21 (0,3)		60	0.75 (0,2.5)		65
3	1.6 (0,3)		15	1.42 (0,2.33)		17	1.25 (0,3)		17	0.98 (0,2.12)		22
4	1.75 (0,2.88)		21	1.25 (0,3)		23	1.5 (0,2.92)		27	1.25 (0,2.08)		27
5	1.25 (0.36,3)		10	1.23 (0,1.8)		9	2.31 (0.17,3)		12	1.12 (0,2.83)		12
Johns Hopkins												
	African-American						White American					
Grade Group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	2.38 (0.5,3)	0.867	56	1 (0,2.56)	0.872	56	1.81 (0,3)	0.174	56	1 (0,2.5)	0.007	56
2	2.5 (0.5,3)		41	1.21 (0,2.56)		41	1.5 (0,3)		39	0.63 (0,2.5)		40

3	2.5 (0.5,3)		13	1.06 (0,2.56)		13	2.25 (0.5,3)		13	1.5 (0,2.5)		13
4	2.53 (2.12,2.83)		4	0.74 (0.67,2.35)		4	2.44(2.06,2.81)		5	1.81 (1,2.56)		5
5	2.38 (0.88,2.94)		23	1.44 (0.38,2.38)		23	1.88 (0.5,3)		22	1.34 (0.25,2.6)		22
Grade Group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1 (0,2.42)	0.019	55	0.33 (0,2.38)	<0.0001	56	1.5 (0.38,3)	0.034	55	0.5 (0,1.75)	<0.0001	56
2	1.19 (0,2.81)		41	0.42 (0.08,1.81)		41	1.5 (0.44,2.88)		39	0.44 (0,2.12)		40
3	1.62 (0.25,2.69)		13	0.75 (0.1,2.08)		13	1.38(0.75,2.69)		13	0.88 (0.25,2.5)		13
4	1.81 (0.83,2.94)		4	1.24 (0.75,1.62)		4	1.62 (0.69,2)		5	1.06 (0.58,1.69)		5
5	1.81 (0.25,2.69)		23	1.28 (0.38,2.52)		23	2.12(0.81,2.81)		22	1.31 (0.58,2.25)		22
Medical College of Wisconsin (MCW)												
	African-American						White American					
Grade Group	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
1	1.5 (1.5,2)	0.512	3	0.5 (0,1)	0.867	3	1 (0.5,1)	0.127	3	1 (0,1)	0.757	3
2	2 (1,3)		8	0.75 (0,1.5)		8	1 (0.5,1.5)		8	0.5 (0,2.5)		8
3	2.42 (0.67,3)		14	0.94 (0,1.5)		14	2.17 (0,3)		15	1 (0,2.5)		15
4	---		0	---		0	---		0	---		0
5	1.83 (1.5,2.17)		4	0.56 (0,2.33)		4	0.5 (0.5,0.5)		1	0.5 (0.5,0.5)		1
Grade Group	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
1	1 (0.75,1.5)	0.005	3	1.25 (0.8,1.8)	0.010	3	2.33 (1.67,3)	0.559	2	1.5 (1.5,1.5)	0.078	3
2	1.5 (1,2.83)		8	1.5 (0.17,2)		8	1.69 (1,2.75)		8	1.23 (0.75,2)		8

3	2.17 (1,3)		14	2 (1.4,3)		14	2 (0,2.5)		15	2 (0.5,3)		15
4	---		0	---		0	---		0	---		0
5	2.5 (2.33,2.62)		4	2.17 (1.4,2.83)		4	2.25 (2.25,2.25)		1	1.83 (1.83,1.83)		1

n= number of informative cases

Supplemental Table 5. Racial disparity of expression of GDF15 and NFκB according to pathologic stage stratified by Study Site.

Henry Ford

	African-American						White American					
Stage	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
2	2.83 (0,3)	0.528	113	1.21 (0,3)	0.080	134	2.82 (0,3)	0.009	130	1.25 (0,3)	0.042	161
3a	2.49 (1.04,3)		12	0.9 (0.06,1.7)		13	2.79 (0.167,3)		22	0.787 (0,3)		24
3b	2.88 (0.75,3)		8	1.25 (0.25,2.5)		9	2.08 (1.15,3)		12	1.12 (0.35,2.5)		13
4	2.79 (2.79,2.79)		1	1.94 (1.94,1.94)		1	1.42(1.42,1.42)		1	0.19 (0.19,0.19)		1
Stage	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=	**NFκB.** Tumor, median (range)	*P*	n=	**NFκB.** Benign, median (range)	*P*	n=
2	1.45 (0,3)	0.545	115	1.16 (0,3)	0.773	114	1.38 (0,3)	0.244	138	0.9 (0,2.83)	0.432	160
3a	0.92 (0,3)		11	1.32 (0,1.92)		11	1.49 (0,3)		22	0.71 (0,2.5)		24
3b	1.75 (0,2.83)		8	0.91 (0,1.75)		8	1.62 (0.17,3)		13	1.25 (0.13,2.08)		13
4	0.36 (0.36,0.36)		1	0.67 (0.67,0.67)		1	0 (0,0)		1	0.75 (0.75,0.75)		1

Johns Hopkins

	African-American						White American					
Stage	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=	**GDF15.** Tumor, median (range)	*P*	n=	**GDF15.** Benign, median (range)	*P*	n=
2	2.5 (0.5,3)	0.496	92	1 (0,2.56)	0.813	92	2 (0,3)	0.162	91	1 (0,2.56)	0.974	91
3a	2.5 (0.5,3)		29	1 (0,2.56)		29	1.5 (0.5,2.69)		28	1 (0,2.6)		29
3b	2.31 (0.88,3)		15	1.06 (0.38,2.5)		15	1.81 (0.5,2.94)		15	1.02 (0.25,2.38)		15
4	1.31 (1.31,1.31)		1	1.69 (1.69,1.69)		1	1.5 (1.5,1.5)		1	0.63 (0.63,0.63)		1

Stage	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
2	1.25 (0,2.94)	0.250	91	0.46 (0,2.38)	0.008	92	1.5 (0.38,3)	0.010	90	0.6 (0,2.5)	0.002	91
3a	1.12 (0,2.75)		29	0.5 (0,1.98)		29	1.5 (0.5,2.88)		28	0.5 (0,1.94)		29
3b	1.75 (0.25,2.69)		15	0.94 (0.42,2.52)		15	2.31 (0.83,2.81)		15	1.25 (0.58,2.25)		15
4	1.81 (1.81,1.81)		1	1.5 (1.5,1.5)		1	1.55 (1.55,1.55)		1	1.25 (1.25,1.25)		1
Medical College of Wisconsin (MCW)												
	African-American						White American					
Stage	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=	GDF15. Tumor, median (range)	*P*	n=	GDF15. Benign, median (range)	*P*	n=
2	2 (1,3)	0.696	16	1 (0,1.5)	0.104	16	1 (0.33,3)	0.435	20	1 (0,2.5)	0.485	20
3a	2.25 (1,3)		8	0.5 (0,1)		8	1.58 (0,2.67)		6	0.42 (0,2.5)		6
3b	2 (0.67,2.67)		5	1.12 (0,2.33)		5	0.5 (0.5,0.5)		1	0.5 (0.5,0.5)		1
4	---		0	---		0	---		0	---		0
Stage	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=	NFκB. Tumor, median (range)	*P*	n=	NFκB. Benign, median (range)	*P*	n=
2	1.56 (0.75,3)	0.589	16	1.68 (0.8,3)	0.546	16	2 (0.83,3)	0.101	19	1.75 (0.75,3)	0.281	20
3a	1.96 (1.5,2.83)		8	2.12 (0.17,2.88)		8	1.19 (0,2.17)		6	1.45 (0.5,2)		6
3b	2.5 (1,3)		5	2 (1.4,2.83)		5	2.25 (2.25,2.25)		1	1.83 (1.83,1.83)		1
4	---		0	---		0	---		0	---		0

n= number of informative cases

Note: With only 1 cases for Stage 4, values are probably not representative.