Chapter 9
Epigenetics and Racial Disparities in Prostate Cancer

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Abstract Epigenetic changes are drivers of prostate carcinogenesis and may be one biological explanation for racial disparities in prostate cancer incidence and mortality. Surprisingly, despite the growing body of knowledge concerning the role of epigenetics in prostate carcinogenesis, few studies have analyzed methylation differences in prostate cancer by race. To date, the evidence suggests that racial differences in gene methylation patterns in prostate do exist; in benign prostate tissue, African Americans appear to have higher methylation levels of several key genes, such as *APC*, *RARB* and *NKX2.5*, that are known to be involved in prostate carcinogenesis. Whether higher methylation levels in benign prostate translate into a higher prostate cancer risk is unclear, but if one assumes higher methylation levels presage prostate malignancy, then the pre-cancer "field" in prostate defined by methylation may be more primed in African Americans. Only one study to date has examined gene methylation in benign prostate as it relates to cancer risk—however, this study found greater risk in African Americans. In summary, the limited evidence to date suggests that epigenetic changes plays some role in the observed racial disparities in prostate cancer, but more studies are needed to define a broader spectrum of epigenetic profiles in prostate cancer by race—particularly if methylation markers are to have utility as biomarkers and tools for clinical decision making in prostate cancer.

Keywords Racial disparities • Gene promoter hypermethylation • Prostate cancer • Biomarkers • Field cancerization

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9.1 Introduction—Is Prostate Cancer Biologically Different in African Americans?

Rates of early prostate cancer detection have increased and survival outcomes have improved in the US over the last 20 years [1], but racial disparities still persist in both incidence and clinical course of the disease. African Americans experience more aggressive disease presentation [2, 3], are more likely to die of prostate cancer [1], and may have higher incidence of prostate cancer after a negative biopsy than Whites [4]. While screening behaviors and access to medical care vary by race [5, 6], a study of prostate cancer mortality among Medicare-eligible men found that social factors explained only 25 % of these disparities [7].

A number of biological markers suggest that androgen biosynthesis and function may vary by race. Mean serum testosterone is higher in African American men under 40 than in White men [8, 9], although this difference diminishes as men age [10]. Androgen receptor expression may also be higher in African American compared with White men [11]. The distribution of prostate specific antigen (PSA) levels also varies across race [3, 12]; among men who do receive prostate cancer screening, established diagnostic protocols may have less efficacy in African Americans, as there is evidence that appropriate interpretation of PSA levels [13] and pathology findings [14] varies by race. Such differences are observed both for men with [3] and without [12] prostate cancer, suggesting that racial differences in underlying prostate biology exist not only for cancer but for other prostate-related conditions.

A recent study found that prostate cancer volume after radical prostatectomy was greater in African American than in White men, and that metastatic disease was four times more common [2], suggesting that differences in the biological presentation of prostate cancer—such as growth rate or transformation to more aggressive phenotype—likely influence racial disparities in diagnosis and mortality rates. Several studies have demonstrated differences in gene expression in prostate tumors from African American and White men, particularly in pathways associated with immune response [15, 16]. Other studies have found racial differences in copy number variation in tumors. Rose et al. [17] found significant enrichment of copy number alterations in genes related to immune response. Our own research group recently analyzed DNA copy number changes in prostate tumors and found no significant differences between prostate tumors of White and African Americans in overall mean allelic balance, combined loss, or copy-neutral events [18]. Further analyses of these data using a copy number biomarker validated in Whites [19] found that 80 % of African American patients positive for this biomarker went on to develop biochemical recurrence; however, the majority of African American patients in our study population (82 %)—including many patients that experienced recurrent disease—were biomarker-negative, resulting in a low negative predictive value of 61 %. Such findings emphasize that, while a biomarker optimized in a White population may have predictive utility in some African Americans, additional discovery is often needed to identify biomarkers with wider applicability across all ethnicities.

If the biology of prostate cancer differs by race, then it is reasonable to consider whether patterns of DNA methylation in prostate also vary between African American and White men. Unfortunately, little is known about the prostate cancer methylome in African Americans, but the existing studies that have examined racial differences suggest that gene methylation patterns in prostate may indeed differ by race [20–25] emphasizing the importance of studying race-specific methylation patterns; in fact, our own work suggests that the relationship between methylation of the *APC* and *RARB* genes and risk of prostate cancer varies by race [23].

Characterization of tumor suppressor genes inactivated by hypermethylation—and the timeline when such changes occur—may improve early prostate cancer detection and disease management, which could have a significant impact upon racial disparities. In the chapter that follows, we discuss what is known about racial differences in gene methylation on a population level, and in the female counterpart of prostate cancer, breast cancer. Next, we discuss how methylation plays a role in all stages of prostate carcinogenesis. We then conduct a comprehensive review of the existing literature regarding racial differences in DNA methylation in prostate cancer, highlighting our recent work regarding racial differences in prostate cancer risk associated with methylation in the benign prostate. We discuss differences in methylation patterns in a key gene, *RARB*, that has been studied extensively in prostate cancer risk methylation studies, and how CpG regions interrogated across studies influence results. We finish with some closing thoughts on deficiencies in our current knowledge of prostate methylation as it relates to racial disparities and key areas that should be areas of focus for future studies.

9.2 Racial Differences in Methylation—Leukocytes and Breast Cancer Tissue

Much research has emphasized how DNA methylation patterns vary among cell types and across time; however, methylation patterns may also be inherited, and remarkably only two studies have investigated how inherited epigenetic patterns vary by race, and whether methylation differences present at birth are associated with later differences in the incidence and mortality of cancers. One of these investigated CpG sites associated with cancer-related genes [26] whereas the other investigated methylation more broadly [27]. Adkins et al. [26] studied whole blood acquired at birth, finding that methylation patterns in 13.7 % of autosomal CpG islands differed between African American and Whites. In regions where methylation was significantly associated with race, they found a 1.8-fold ($p = 0.00139$) enrichment of 31 genes involved in the prostate carcinogenesis. In contrast, Zhang et al. studied methylation levels in leukocytes from 161 cancer-free adult subjects; they observed a 2.2 % lower level of leukocyte *LINE-1* methylation in non-Hispanic African Americans versus Whites [27]. The authors did not offer any explanations for the lower levels of methylation in African Americans and noted that since African Americans carry the lowest frequency of the methylation disrupting

MTHFR C677T polymorphism, the opposite result would be expected. The main methodological difference between the Adkins et al. and Zhang et al. studies in terms of methylation and cancer is that the former measured methylation at CpG sites biased towards cancer-related genes whereas the latter was a more general measure of global methylation.

Like prostate cancer, breast cancer presentation varies by race, and shares pathogenic pathways (such as sex hormone sensitivity) with prostate cancer [28, 29]. Although overall breast cancer incidence is higher in White women, incidence prior to age 50 is higher and the disease is more deadly in African American women. A study of methylation in invasive ductal carcinomas found similar levels of methylation across groups—except in (estrogen/progesterone-receptor negative) tumors from African American women younger than 50. These tumors displayed significantly higher methylation levels in four genes (*HIN-1*, *Twist*, *Cyclin D2*, and *RASSF1A*) involved in apoptosis and tumor suppression [30]. Another study, using cluster analysis of methylation levels in 773 cancer-related genes, found unique methylation profiles across races [31]; the majority of tumors from African American women were in a cluster associated with greater tumor size and younger age at presentation.

A study of candidate-gene CpG island methylation in breast cancer found differences between African American and White patients in the *CDH13* gene; these differences were even more pronounced among younger patients and those with estrogen receptor-negative disease [32]. The *CDH13* tumor suppressor gene produces a protein that mediates cell-cell interaction and cancer cell invasion and metastasis [33]; the authors hypothesized that hypermethylation of this gene may contribute to racially-distinct molecular alterations contributing to early onset of breast cancer. The study also found three loci (*RASSF1A*, *RARB,* and *CDH13*) that were methylated more often in breast tumors from African American women; methylation of these genes was associated with poor prognoses, suggesting that differences in methylation patterns may contribute to more aggressive disease among African Americans. Methylation of certain genes has also been associated with more aggressive disease and poor outcomes in prostate cancer, but no studies exist that have directly tested gene methylation as a potential confounding factor for racial differences in prostate cancer outcomes.

9.3 Preneoplastic Methylation Changes in Prostate Carcinogenesis

Prostate cancer is characterized by its marked multi-focal nature—67–96 % of prostatectomy specimens display more than one tumor focus [34, 35]. Hanson et al. [36] first showed that gene promoter methylation occurs in the non-neoplastic cells of the prostate tumor microenvironment. Troyer et al. [37] found that 84 % of men with a methylated *RARB* gene had a subsequent diagnosis of prostate cancer upon repeat biopsy. Steiner et al. found that the same *RARB* gene studied by Troyer et al.

was often found methylated in normal prostate cells located as far as 10–20 mm from the primary tumor [38]. We recently found that men with *RARB* methylation in benign prostate had an increased risk for subsequent prostate cancer that persisted almost 10 years after initial benign biopsy, but this increased risk while nominal in Whites was consistently greater than two in African Americans.

The multifocal nature of prostate cancer and methylation changes in histologically appearing benign prostate tissue that either becomes malignant after a short time or is adjacent to cancer suggests the presence of a 'field effect,' whereby factors underlying carcinogenesis result in molecular changes in areas beyond the tumor foci. Epigenetic change is a potential measure of a generalized field effect in the prostate [39]. As described above, such changes are measurable in histologically normal cells and often precede overt carcinogenesis. Another example is gene expression patterns of histologically benign tissue adjacent to tumor that more closely resembles a malignant gene expression phenotype than that of normal prostate cells [40]. In a sample of prostate cancer cases that was 60 % African American [41], expression of the let-7 family of miRNAs in histologically normal prostate tissues from Gleason grade 7 or higher tumor was decreased compared to histologically normal tissue from Gleason grade 6 tumors. Further analysis of expression data comparing the two adjacent normal specimens from grade 6 and 7 tumors with normal tissue controls found only the normal adjacent tissue from grade 6 tumors was similar to the normal tissue controls suggesting normal prostate tissue adjacent to grade 7 tumors has already undergone a molecular malignant transformation. Investigation into whether this field cancerization effect is more pronounced in African American cases may provide insights about why African Americans have more aggressive prostate cancer.

9.4 Methylation Changes in Prostate as Markers for Progression

Methylation of specific genes at different stages of prostate carcinogenesis provides insight into potential carcinogenic pathways. DNA methylation status may also serve as a biomarker for prostate cancer detection and disease progression. As described above, methylation changes in prostate tissue often occur before histological changes are evident; methylation changes are also more common and consistent than many somatic genetic changes. Thus, identification of methylation markers that occur throughout the course of prostate carcinogenesis may also provide insight into pathogenic pathways that are targets for intervention.

For instance, prostatic intraepithelial neoplasia (PIN) [42] is the precursor lesion of prostate cancer; in these lesions, methylation in the gene *GSTP1* is common [43, 44]. Other genes—including *RARB*, *APC*, *MGMT*, and *RASSF1A*—display increasing methylation as carcinogenesis progresses [44–47]. In benign prostate tissue, *RARB* is more often methylated than *APC* [23, 44], but *APC* methylation may serve as a pre-malignant marker with high negative predictive value for subsequent

prostate cancer [48]. *APC* methylation is also strongly associated with high-grade tumors [23] and shown to be an independent predictor of poor prognosis [49, 50]. In the most comprehensive study to date of whole genome methylation changes associated with different prostate cancer phenotypes, Mahapatra et al. [47] measured methylation status in 14,495 genes. They found methylation of different genes associated with presence of disease and disease recurrence—genes such as *GSTP1* and *RARB* were associated with prostate cancer incidence, while genes such as *BCL11B* and *RASGRF2* were associated with systemic recurrence.

Aberrant methylation changes may arise from any number of insults to the prostate, incidental or cumulative [51]. Such changes appear consistently in prostate cancer; for example, methylation of the *GSTP1* gene is the most reliable biomarker of the disease [52] and researchers have identified over 30 genes hypermethylated in prostate cancer [53]. These include tumor-suppressor genes, genes involved in hormone responses, tumor-cell invasion, cell cycle control, and DNA damage repair. Changes occurring in the early stages of tumor development are homogeneous and persist through the progression of the disease—these may serve as biomarkers for early detection. However, methylation patterns in recurrent tumors are heterogeneous—suggesting that prostate tumor cells acquire distinct epigenetic changes as they progress. A key question with regard to prostate cancer risk and racial disparities is whether early and/or late epigenetic changes associated with disease outcomes follow a race-specific pattern.

9.5 Genes Displaying Racial Variation in Methylation in Prostate

While publications regarding methylation and prostate cancer now number in the hundreds [53], those including race as a factor are far less common (Table 9.1). The first reported study that examined racial differences in prostate cancer methylation studied hypermethylation of the *GSTP1*, *CD44*, and *E-Cadherin* genes in tumor tissues of African American and White prostate cancer patients [25]. Although *GSTP1* methylation was found in most tumor tissues (~84 %), there were no differences across groups. In contrast, *CD44* was methylated 70 % more frequently ($p = 0.05$) in African American than White cases; methylation frequency also correlated with tumor grade. A later study by this same group examined racial differences in methylation for eight genes (*GSTP1, RASSF1A, RARB, CD44, EDNRB, E-cadherin, Annexin-2*, and *Caveolin-1*) [20]. Methylation of *CD44* actually showed a stronger association with race (OR (odds ratio) = 2.0 versus 1.7), but none of the eight genes showed a statistically significant difference in terms of methylation percentages by race. It should be noted, however, that the sample size decreased from 111 to 90, reducing statistical power compared to the previous study.

Methylation in the gene *GSTP1* is highly specific [52] for prostate cancer. A study of *GSTP1* by Enokida et al. investigated whether promoter hypermethylation

Table 9.1 Summary of studies that have examined racial differences in prostate cancer-related gene methylation

Study	Sample			Tissue type	Genes analyzed	Genes with significant results	Notes
Woodson et al. [25]		**AA**	**W**	FFPE from RP, TURP, or needle biopsy	*GSTP1; CD44; E-cadherin*	*CD44*	1.7-fold higher frequency of *CD44* methylation in AA (43 vs. 25 % in AA vs. W; P = 0.05)
	Cases	47	64				
	Controls	NA	NA				
Woodson et al. [20]		**AA**	**W**	FFPE from RP, TURP, or needle biopsy + cell lines	*GSTP1; RASSF1A; RARB2; CD44; EDNRB; E-cadherin; Caveolin-1; ANXA-2*	*CD44*	Methylated in 41.5 % (AA) vs 26.5 % (W), P = 0.10
	Cases	41	49				
	Controls	NA	NA				
Enokida et al. [22]		**AA**	**W**	FFPE from RP (cases); TURP (BPH, controls)	*GSTP1*	*GSTP1*	When complete *GSTP1* methylation present, HR = 13.4 (AA) vs 3.8 (W)
	Cases	44	77				
	Controls	38	38				
Das et al. [21]		**AA**	**W**	RP (cases); TURP (BPH, controls) + cell lines	*PYCARD*	*PYCARD*	*PYCARD* methylated more in AA cases, but only a risk factor in W cases (OR = 7.6)
	Cases	21	45				
	Controls	12	22				
Kwabi-Addo et al. [24]		**AA**	**W**	RP (paired tumor/normal) (cases); needle biopsy (controls)	*GSTP1; AR; RARB; SPARC; TIMP3; NKX2.5;*	all except *GSTP1*	Methylation of *RARB* and *NKX2.5* increased more rapidly with age in African Americans
	Cases	39	113				
	Controls	18	13				
Tang et al. [23]		**AA**	**W**	Benign biopsy & TURPs (case-control pairs), RP (cases)	*RARB, APC, MGMT, RASSF1, CCND2*	*RARB, APC*	*RARB* methylation a risk factor only for AA (OR = 2.09); *APC* methylation a risk factor for high grade disease
	Cases	211	300				
	Controls	211	300				

Abbreviations: *AA* African Americans, *W* Whites, *FFPE* fixed formalin paraffin embedded, *RP* radical prostatectomy, *TURP* transurethral resection of the prostate, *BPH* benign prostatic hypertrophy

of this gene correlated with clinico-pathological findings in a mixed-race (Asian, African American, and White) sample of 291 prostate cancer cases. The researchers also compared methylation percentages from these cases with 172 benign prostate hypertrophy specimens [22], using assays specific to each of the two sites necessary for functional *GSTP1* promoter activation [54]. In Whites, the ratio of positive methylation results from at least one assay for prostate cancer versus BPH was 2:1; in African Americans, it was almost 6:1. This difference increased further when limited to positive results from both assays (i.e., more complete methylation across the *GSTP1* promoter region); the ratio was 4:1 in Whites, but 13:1 in African Americans. These results clearly demonstrate how gene promoter methylation in prostate cancer may vary by race.

A similar study of 66 prostate cancer and 34 BPH tissue samples investigated methylation patterns in the *TMS1/ASC* (aka *PYCARD*) gene [21], known to play a role in apoptosis [55]; methylation of this gene is associated with breast cancer [56]. Interestingly, the authors found that *TMS1/ASC* methylation was more prevalent in prostate cancer cases than controls in White patients (OR = 7.6; p 0.002) while no difference between the cases and controls was seen in African American patients. A subsequent analysis of five additional genes known to be methylated in prostate cancer—*GSTP1, CD44, ECAD, RASSF1A* and *EBR*—did not reveal any statistically significant differences in methylation by race. As in previous studies, however, the modest number of specimens limited the statistical power; although the risk associated with *RASSF1A* methylation was much higher for African Americans than Whites (OR = 8.6 vs. 3.2) none of the risk estimates reached statistical significance.

Perhaps the most comprehensive study of racial differences in prostate cancer methylation by race was performed by Kwabi-Addo et al. [24]. These authors used pyrosequencing to quantitatively measure the methylation status of *GSTP1, AR, RARB, SPARC, TIMP3*, and *NKX2-5* in prostate tumor and normal tissue specimens from African American and White patients to assess differences in methylation by age and race. Overall, they observed significant methylation differences by race after adjusting for cancer status. Tumor specimens from White patients displayed slightly higher Gleason score and similar pathologic staging when compared with the African American samples. Thus, the higher prevalence of methylation seen in the African American cancer samples was not simply an artifact of differences in disease aggressiveness or stage between the two groups. In addition, regression analysis revealed significantly higher age-adjusted methylation levels for *NKX2-5* and *TIMP3* genes in the normal prostate tissue samples of African American cases. Of the six genes that were analyzed in the normal prostate tissue samples, methylation of *NKX2-5* also showed modest evidence for a race-by-age interaction, suggesting that this gene may also be a more sensitive methylation biomarker in African Americans.

While this study provided evidence that methylation levels of key genes in the prostate vary by race, the authors were unable to demonstrate that these methylation differences translated into prostate cancer risk differences [24]. Although receiver operator characteristics analyses showed suggestive racial differences in

the predictive potential of DNA methylation for the *GSTP1, RARB, SPARC, TIMP3*, and *NKX2-5* genes, the study was underpowered to demonstrate statistically significant differences by race. Despite this shortcoming, the results of Kwabi-Addo et al. raised the possibility of designing "ethnic-sensitive" biomarkers for prostate cancer detection.

Notably, the four studies reviewed above all compared the methylation status of genes in prostate tumor tissue of cases with that of benign prostate tissue of controls; such cross-sectional study designs do not provide insight into temporal associations between methylation events and prostate carcinogenesis. Our own research was designed to address this limitation by nesting a case-control study within a longitudinal cohort of men with benign prostate specimens; we tested. the association of methylation of five tumor suppressor genes, *MGMT, RASSF1, RARB, APC and CCND2* in benign prostate tissue with risk of subsequent prostate cancer [23]. We also measured methylation in a subset of paired benign-tumor specimens to validate that methylation of *RARB* and *APC* in prostate are stable events in the prostate carcinogenesis pathway.

In our sample of 211 African American and 300 White case-control pairs, the methylation-associated prostate cancer risk varied by race; a positive association between *RARB* methylation and prostate cancer risk was found only in African Americans (OR = 2.09; p = 0.002) while a negative association for methylation of the *MGMT* gene was observed only in Whites (OR = 0.50; p = 0.03). When cases were stratified by tumor grade (low vs. high), the highest risk estimates were observed in African Americans; the association of methylation of *APC* with high grade tumors was greater in African Americans than Whites (OR = 3.21 vs. 2.04). In African Americans, *APC* and *RARB* methylation appeared to act in concert to increase risk, particularly after adjusting for PSA level and presence of high-grade PIN (OR = 3.04; p = 0.003). The same was not true for whites, with the joint OR for *RARB* and *APC* methylation only slightly elevated (OR = 1.14; p = 0.7) and significantly different from that observed in African Americans (p = 0.01). Our results showed that in African Americans, methylation of the *RARB* and *APC* genes that occurs before histological evidence of disease are biomarkers of subsequent disease risk.

Across the studies that have investigated racial differences in prostate cancer methylation, methylation results in 15 genes have been reported (Table 9.2). Most have been investigated in only one study, and therefore any significant findings—such as variation in aged-related *NKX2.5* methylation by race [24] or a positive association between *RARB* and *PYCARD* methylation and prostate cancer risk limited to African Americans [21, 23]—require replication. Although most of the observed racial differences show higher levels of methylation in African Americans, some exceptions exist; for example, two studies have both shown higher levels of *RASSF1A* methylation in benign prostate of Whites compared with African Americans [21, 23]. Results are also not consistent across all studies: while Enokida et al. found significantly higher risk associated with *GSTP1* methylation in African Americans [22], this finding was not replicated by two other studies [21, 24]. Likewise, the higher methylation levels of *CD44* in prostate tumors of African Americans reported by Woodson et al. [20, 25] were not replicated by Das et al.

[21]. Of the associations listed in Table 9.2, the most consistent are for *RARB*. This is likely due to both the targeted region of methylation that has been studied as well as the critical role this gene plays in prostate carcinogenesis.

9.6 An Exploration of *RARB* Methylation Differences by Race

Retinoic acid receptor-beta (*RARB*) is a tumor suppressor gene on chromosome 3p24, where a high incidence of loss of heterozygosity is detected in many types of tumors. Retinoic acid suppresses cancer cell growth through binding to retinoic acid receptors (RAR), especially RAR-beta. Selective loss or down-regulation of RAR-beta mRNA and protein has been reported in prostate cancers [57], and the distinct cellular distributions of RAR subtypes in benign, pre-neoplastic, and malignant prostate tissues suggest links between altered RAR signaling and deregulated cell growth and carcinogenic processes [58]. *RARB* methylation is a sensitive and specific marker for prostate cancer [37], and appears to increase in the course of prostate malignancy [59]. As described above, levels of *RARB* methylation in normal prostate tissue are higher in African American than White men [23, 24].

The study by Adkins et al. of CpG methylation in leukocytes collected at birth examined four *RARB* CpG loci; one of the four—cg26124016—exhibited significant methylation differences between African Americans and Whites [26], with an average methylation percentage of 3.5 % in African Americans and 5.7 % in Whites ($p = 10^{-12}$). This locus resides within the *RARB* promoter region, about 300 base-pairs upstream of the transcription initiation site.

This same *RARB* promoter region (Fig. 9.1) has been highly interrogated by three studies that have reported racial differences in *RARB* methylation [20, 23, 24]. There are 21 CpG loci spanning 200 base-pairs within the *RARB* gene promoter region and exon 1; for the 13 of the 21 CpG loci where methylation was measured in one or more of the three studies, the results have been remarkably consistent. In overlapping CpG loci residing in *RARB* exon 1, Tang et al. [23]and Kwabi-Addo et al. [24]both reported 34–37 % methylation in benign prostate tissue and 63–67 % methylation in tumor tissue of African Americans, versus 23–27 % methylation in benign and 59–68 % methylation in tumor tissue of Whites. Both studies found lower methylation levels in benign prostate tissue specimens from Whites than African Americans (Fig. 9.2), although Kwabi-Addo et al. found slightly higher *RARB* methylation in prostate tumors of African Americans (67 % vs. 59 %) while Tang et al. found the opposite (63 % vs. 68 %). Neither study could report statistically significant racial differences in *RARB* methylation in prostate tumors. Woodson et al. [20] reported similar results in their investigation of methylation levels in prostate tumor tissue for four CpG loci upstream of the transcription initiation site (Fig. 9.1).

If racial differences in *RARB* methylation occur in benign prostate tissues, these differences apparently disappear when the prostate becomes malignant. While the clinical profile of the prostate cases among the above studies varied (Kwabi-Addo et al.

Table 9.2 Summary of genes tested for methylation differences by race in prostate

Gene	Gene product	Cancer pathway	Tumor	Racial variations		CpG Island	References
				Normal	Risk		
APC	Adenomatous polyposis	Tumor suppressor		√	√	Promoter/exon 1	[23]
AR	Androgen receptor	Hormone receptor gene; metabolism of androgens and testosterone				Promoter/exon 1	[24]
CCND2	Cyclin D2	Cell cycle regulation				Promoter	[23]
CD44	CD44 antigen	Lymphocyte activation; recirculation and homing; tumor metastasis	√			Promoter/exon 1	[20, 21, 25]
ERRFI1	ERBB receptor feedback inhibitor 1	Cell growth and signaling				Exon 2	[21]
CDH1	E-cadherin protein	Adhesion molecule—loss of function increases cell proliferation, invasion and/or metastasis				Exon 1	[20, 21, 25]
EDNRB	Endothelin receptor type B	Cell growth and signaling				Promoter	[20]
GSTP1	Glutathione S-transferase P1	DNA repair			√	Promoter	[21, 22, 24]
MGMT	O(6)-methylguanine-DNA methyltransferase	DNA repair				Exon 1/intron 1	[23]
NKX2.5	Homeobox protein	Gene transcription	√	√		Exon 1	[24]
PYCARD	PYD and CARD domain containing	Inflammatory and apoptotic signaling pathways		√	√	Promoter	[21]
RARB	Retinoic acid receptor beta	Gene transcription		√	√	Promoter/exon 1	[20, 23, 24]
RASSF1	Ras-association domain family 1A	Tumor suppressor		√		Promoter/exon 1	[21, 23]
SPARC	Secreted protein, acidic and rich cysteine	Cell-matrix interactions; migration and angiogenesis	√			Exon 1	[24]
TIMP3	Tissue inhibitors of metalloproteinase inhibitors 3	Degradation of extracellular matrix	√	√		Exon 1	[24]

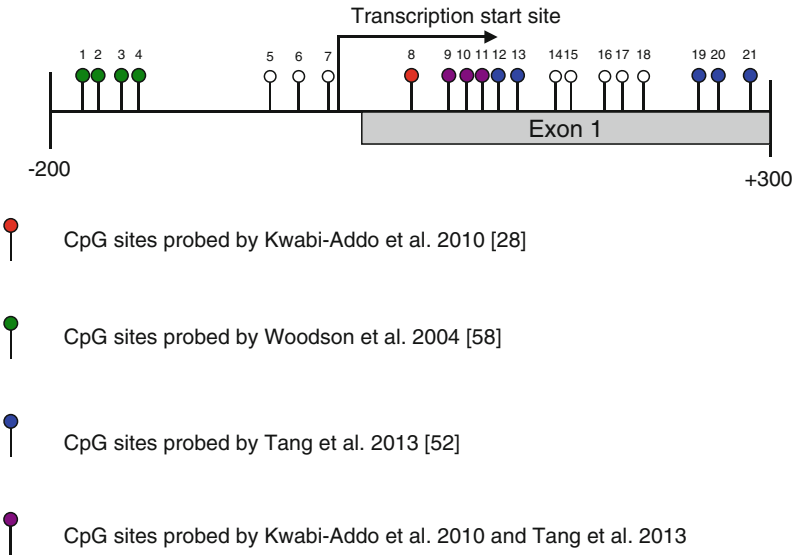

Fig. 9.1 CpG loci spanning a 500 base pair region across the *RARB* promoter and exon 1 where racial variation of methylation status in prostate has been previously studied. The region in question is located on chr3: 25469522-25470022 and contains 21 CpG loci

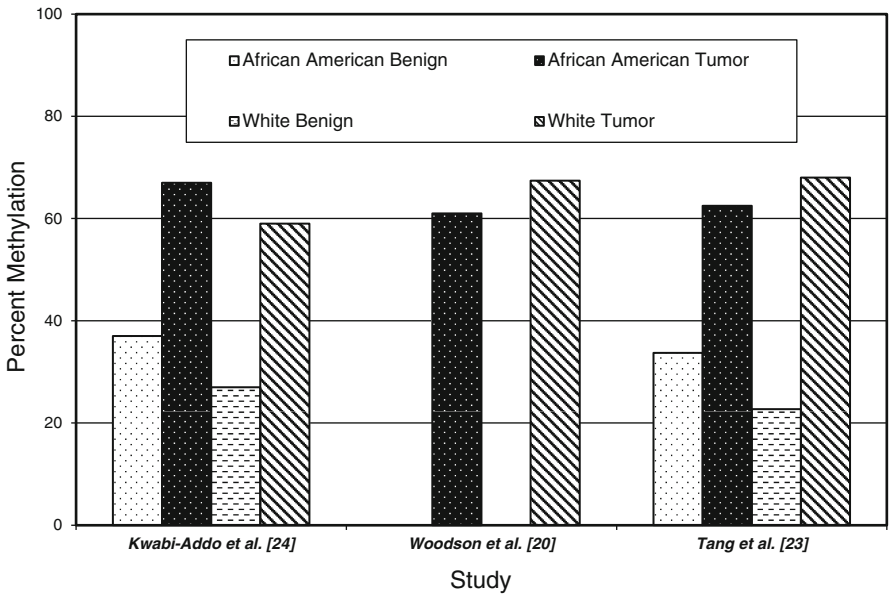

Fig. 9.2 Racial variation in *RARB* methylation levels in benign prostate and prostate cancer across three studies

included more advanced stage cases, but tumors of lower Gleason grade than Woodson et al. or Tang et al.), these clinical differences do not appear to be associated with *RARB* methylation. Nor did the choice of which CpG loci were queried—suggesting that methylation changes occur over a broad span of the *RARB* promoter region and likely occur in the earliest stages of carcinogenesis. If high levels of *RARB* methylation are a precursor for prostate cancer, then the elevated *RARB* methylation observed in histologically benign prostate tissue from African American men [23, 24] may suggest a higher baseline risk for this racial group; this is consistent with epidemiologic observations. As we noted previously from our work [23], *RARB* methylation in benign prostate is associated with prostate cancer in African Americans but not in Whites—this further underscores the importance of considering race in studies of epigenetic change and cancer.

9.7 Conclusions

Can gene methylation and its effect on prostate carcinogenesis explain some of the racial disparities observed in prostate cancer? In prostate tumors, it does not appear that methylation levels vary significantly by race for most genes. However, given that methylation across a field of cells happens gradually and the current thinking that certain epigenetic events might help define the "cancer field" [39], it is conceivable that racial differences in both baseline methylation levels of key cancer-related genes within benign prostate as well as the rate at which these genes become methylated with age might affect overall prostate cancer risk. Our understanding of racial differences in gene methylation in prostate is based on only a few studies, and more work is clearly needed to define race-specific epigenetic profiles of prostate cancer—particularly if methylation markers are to have utility as biomarkers of disease presence and as tools for clinical decision making. Ideally, future studies will be racially diverse and will include whole genome methylation surveys [47] to explore the full range of methylation marks in prostate tissue that may vary by race. While the concept of race is complex and involves both social and biologic constructs [60], as the field of epigenetics becomes richer and its role in molecular medicine grows, we can expect to learn more about the impact of aberrantly-methylated genes upon prostate cancer risk and how this risk is manifest across the full spectrum of men affected by this disease.

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