A CD24-p53 Axis Contributes to African-American Prostate Cancer Disparities

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Abstract

Background: Using a functional analysis of prostate cancer cells, we found a CD24-dependent inactivation of mutant p53, but the clinical significance of this observation remained uncertain. Here, we validated these results with samples of human prostate cancer and explored the role of a CD24-p53 axis in racial disparities of prostate cancer.

Methods: Samples of formalin-fixed, paraffin-embedded prostate cancer from 141 European-Americans (EAs) and 147 African-Americans (AAs) in two independent sample cohorts were assessed for protein expression of CD24, mutant p53, MDM2, and ARF using immunohistochemical analyses. All samples were analyzed for TP53R175H and TP53R273H.

Results: CD24, mutant p53, MDM2, and ARF proteins were expressed in 55%, 24%, 39%, and 68% of prostate cancer samples, respectively. CD24 and mutant p53 were present more frequently...
in late-stage and metastatic prostate cancer. The presence of CD24 was associated with a > 4-fold risk of metastasis, which included lymph node and distant metastases. H-score analysis showed positive correlations of CD24 expression with mutant p53 (r = 0.308, p<0.001) and MDM2 (r=0.227, p=0.004). There was a negative correlation for CD24 with ARF (r=-0.280, p<0.001). A racial disparity was evident for CD24 (AAs/EAs: 64% vs. 47%; p=0.004) but not for mutant p53 (AA/EA: 28% vs. 21%; p=0.152). In 32 CD24+/mutant p53+ cases, a TP53\textsuperscript{R273H} mutation was found in five cases, but no TP53\textsuperscript{R175H} mutation was found.

**Conclusion:** The CD24-p53 axis may contribute to aggressive and metastatic prostate cancers, especially those of AAs. This observation enhances understanding of the pathogenesis of prostate cancer and its associated racial disparities.

**Keywords**

CD24; TP53; prostate cancer; racial disparity; metastasis

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### 1 | INTRODUCTION

CD24, a cell-surface protein anchored by glycosyl-phosphatidyl-inositol (GPI), often is over-expressed in cancers and serves as a marker for poor prognosis of human cancer, including prostate cancer\textsuperscript{2,3}. Analyses involving ectopic and/or inducible expression\textsuperscript{4–6}, targeted mutations\textsuperscript{6,7}, gene silencing\textsuperscript{4,6}, and antibody blocking\textsuperscript{8,9} in cancer cells demonstrate the oncogenic function of CD24. The effects of CD24 on tumor progression and metastasis were validated using xenogenic\textsuperscript{4–6,9} and transgenic tumor models\textsuperscript{6,7}, which suggests that CD24 promotes tumor progression and metastasis. A recent study characterizes CD24 as a “don’t eat me” signal through macrophage receptors, a process that promotes tumor immune escape\textsuperscript{10} and perhaps tumor metastasis. Tumor-expressed CD24 promotes immune evasion through its interaction with Siglec-10, which is expressed by tumor-associated macrophages\textsuperscript{10}. Genetic ablation of either CD24 or Siglec-10 or a blockade of the CD24-Siglec-10 interaction by monoclonal antibodies augmented the phagocytosis of CD24-expressing tumor cells. A CD24-p53 axis regulated hepatocellular carcinogenesis by sustaining intrahepatic macrophages\textsuperscript{11}. Thus, CD24 has roles in cancer and its immune microenvironment.

CD24 is expressed in 50% of prostate cancers but not in normal prostates\textsuperscript{2,3,12}. CD24 expression is associated with aggressive and metastatic prostate cancers\textsuperscript{12}. Mutant Kras-induced upregulation of CD24 enhances prostate cancer stemness and bone metastasis\textsuperscript{13}. Recently, we identified a CD24-p53 axis, which functions in prostate cancer cells most likely through a CD24/NPM-ARF-MEM2-p53 signaling pathway\textsuperscript{6,12}. Silencing of CD24 prevented functional inactivation of mutant p53\textsuperscript{6,12}, a gene mutated in 30% of prostate cancers\textsuperscript{14}. Mutation or loss of function of TP53, which encodes the p53 protein, promoted the invasion and metastasis of prostate cancer cells\textsuperscript{15,16}. Mutant p53 expression was associated with an increased risk of disease-specific death and the development of distant metastases\textsuperscript{16,17}. CD24 amplification correlated with CD24 mRNA overexpression and metastases\textsuperscript{18}, and higher CD24 mRNA levels correlated with higher rates of TP53 mutations\textsuperscript{6}. Thus, CD24-dependent inactivation of mutant p53 may contribute to progression
and metastasis of prostate cancer. However, the biological association of CD24 protein with mutant p53 protein in human prostate cancer has remained elusive.

African-American (AA) men have the highest rate of prostate cancer among racial or ethnic groups. They also have a higher rate of death due to more frequent development of metastatic prostate cancer. The causes of these higher rates among AA men remain largely unknown. Although several genetic and/or biologic factors correlate with aggressive prostate cancer, these factors need to be validated as predictors of aggressive disease for AA patients. Such validation would have the potential to determine which AA men are expected to have unfavorable outcomes or to benefit from earlier treatment. Recent studies report the relevance of biomarkers to the aggressiveness and recurrence of disease among AA men. Various genes (ERG, LSAMP, AMACR, SPINK1, NKX3–1, GOLM1, and AR) show differential expression in prostate cancers of AA men compared to European-American (EA) men. AA men have triple-negative (ERG-negative/ETS-negative/SPINK1-negative) disease more often than EA men. In the present study, we addressed the association of CD24 with mutant p53 and their differential expression between AA and EA prostate cancers and assessed whether cells with CD24 and mutant p53 contribute to health disparities in prostate cancer.

2 | Materials and Methods

2.1 | Human tissue specimens

We evaluated 288 formalin-fixed paraffin-embedded (FFPE) prostate cancer tissues. Of these tissues, 156 were collected at the University of Alabama at Birmingham (UAB) Hospital between 2015 and 2019; 132 FFPE tissue microarray (TMA) samples were obtained from the North Carolina-Louisiana Prostate Cancer Project (PCaP). Clinical and pathological characteristics of the subjects are presented in Table 1. The pathological stage of prostate cancer at the time of diagnosis was determined by the tumor-node-metastasis (TNM) system. Pathological grading was based on specimens corresponding to Gleason Grade groups of 2–6, 7, and 8–10. There were two races based cohorts (UAB: 78 EAs and 78 AAs; PCaP: 63 EAs and 69 AAs). This study was approved by the Institutional Review Boards of UAB, the University of North Carolina at Chapel Hill, and the Louisiana State University Health Sciences Center.

2.2 | Immunohistochemical (IHC) analysis

The ABC detection system (Vectastain Elite ABC) was used for immunostaining according to the manufacturerr’s protocol. Antibodies were human CD24 (ML5, BD Biosciences), p53 (DO-1, Santa Cruz Biotechnology), MDM2 (SMP14, BD Biosciences), and ARF (also p14-ARF, E3X6D, Cell Signaling). Specific primary antibodies were used to detect CD24 (1:100), p53 (1:200), MDM2 (1:100), and ARF (1:50). Protein expression of CD24 in the plasma membrane and cytoplasm, and p53 (a tumor suppressor), MDM2 (a tumor promoter), and ARF (that promotes degradation of MDM2) in the nuclei were classified as negative or positive. The overall result for each case was classified as negative if <10% of cells within tumor areas were stained or positive if 10%–100% were stained. Also, the percentage of positive tumor cells per slide (10% to 100%) was multiplied by the
dominant intensity pattern of staining (1, weak; 2, moderate; 3, intense); the overall scores ranged up to 300 H-scores. All slides were examined by pathologists in a blinded fashion.

### 2.3 Laser capture microdissection

FFPE tissue sections were used for laser capture micro-dissection to obtain prostate epithelial or cancer cells. Two thousand cells were micro-dissected from target tissues for analysis of TP53 mutations.

### 2.4 Mutation analysis of the TP53 gene

Genomic DNA was extracted from micro-dissected cells using PicoPure DNA Isolation kits (Thermo Fisher Scientific). TP53R175H and TP53R273H mutations were identified based on polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs). Briefly, a fragment encompassing the mutant sites in the TP53 gene was amplified using specific primers 5’- TGTGCAAGCTGTTGGGATT-3’ in sense and 5’- TATCTGAGCAGCGCTCATGT-3’ in antisense for the TP53R175H mutation, and specific primers 5’- TGGTAATCTACTGGGACGGAA-3’ in sense and 5’- TGGTGAGGCTCCCCTTTCTT-3’ in antisense for the TP53R273H mutation. PCR reactions were performed according to the manufacturer’s instructions (GoTaq Green Master Mix, Promega). After a 10-min initial denaturation step at 95°C, 35 cycles of PCR reaction consisting of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s were accomplished, followed by a 7-min final extension step at 72°C in a thermal cycler (SimpliAmp Thermal Cycler, Thermo Fisher Scientific). After confirmation of PCR amplification on 2.0% agarose gel electrophoresis, each PCR product was digested overnight with 5 units of enzymes (TspRI at 65°C for TP53R175H and MsiII at 37°C for TP53R273H, New England Biolabs) and electrophoresed on 3.0% agarose gels. For TP53R175H, a 128-bp PCR fragment was divided into 104- and 33-bp fragments when the TspRI site was present, and the mutation was designated as G to A when the TspRI restriction site was present. For TP53R273H, a 108-bp PCR fragment was divided into 69- and 39-bp fragments when the TspRI site was present, and the mutation was designated as G to A when the MsiII restriction site was present. The TP53 mutations were confirmed using direct sequencing of PCR samples with each mutation by comparing TP53 sequences of tumor tissue to those of benign tissues from the same patients.

### 2.5 Statistical analyses

Comparisons between groups were performed using Chi-square or Fisher exact tests for categorical data. Logistic regression analyses were performed, and odds ratios (OR) and confidence intervals (CI) were used for quantifying differences in IHC protein expression between groups. Similarities, dissimilarities, and relationships of protein expression levels between two groups were assessed using Pearson’s correlation coefficient. The relationship between expression and tumor stage was examined using the Cochran-Armitage trend test. All data were entered into an Access database using Excel 2016 and analyzed with SPSS (version 24, IBM).
3 | Results

3.1 | CD24 and mutant p53 expressions contribute to aggressive and metastatic prostate cancer

In a previous study, we determined, by IHC, the protein expression of CD24 in FFPE tissue samples, including 522 samples of prostate cancer, 34 samples of benign prostatic hyperplasia, and 141 samples of normal prostate\(^\text{12}\). CD24 was not expressed in benign prostatic hyperplasia or in normal prostate tissues but was expressed in 48\% of the prostate cancer tissues\(^\text{12}\). In the present study, we validated the previous results using two independent sample cohorts from UAB and PCaP (Table 1). CD24 was expressed more in the cytoplasm than in the plasma membrane (Fig. 1); these results are consistent with our previous finding that intracellular CD24 appears to be responsible for its oncogenic function\(^\text{6}\). Overall, CD24 protein was not expressed in normal prostate or benign prostatic hyperplasia tissues but was expressed in 58\% and 52\% of the prostate cancer tissues from the UAB and PCaP sample cohorts, respectively (Table 2 and Figs. 1A–D). Tumor-adjacent normal prostate epithelial cells were not stained (Fig. 1B). CD24 expression positively correlated with tumor stages in the UAB cohort (p=0.037) but not in the PCaP cohort (p=0.558) (Table 2). A Cochran-Armitage trend analysis of the UAB cohort showed that CD24\(^+\) was present more frequently in tumors of higher stages (for CD24, p=0.017; Table 2). In the UAB cohort, CD24 expression was associated with more than a 4-fold risk of tumor metastasis, which included lymph node and distant metastases (age-adjusted OR=4.73, 95% CI: 1.52–14.7, p=0.007) (Tables 3 and S1). Most of the PCaP samples were of T1–2 tumor stages; no metastatic cases were available (Table 1). We did not perform a Cochran-Armitage trend analysis for this cohort. For both cohorts, there were no significant associations of CD24 expression with tumor grades (Gleason scores) (Table 2).

Wild-type (WT) p53 is unstable, with half-life less than 20 min, mainly due to degradation by its E3 ubiquitin ligase, MDM2\(^\text{28}\). Within cells, WT p53 is maintained at low concentrations\(^\text{29}\), and IHC cannot detect expression of the WT p53 protein\(^\text{28}\). However, missense mutant p53 proteins are stable and detectable in tumor tissue\(^\text{30}\). Thus, we used IHC analysis to assess the protein expression of mutant p53 in prostate cancer tissues. In the nuclei of prostate cancer cells, mutant p53 protein accumulated; it was expressed in 30\% and 18\% of the prostate cancer samples from the UAB and PCaP cohorts, respectively (Table 2 and Figs. 1C and 1D). Mutant p53 expression was correlated with higher tumor stage (Chi-square test: p=0.035 and Cochran-Armitage trend test: p=0.010) in the UAB cohort, but not the PCaP cohort (Chi-square test: p=0.720) (Table 2). Mutant p53 expression was associated with a >2-fold risk of tumor metastasis, but the difference did not reach statistical difference (Table 3). There was no significant correlation between mutant p53 expression and tumor grade in either cohorts (Table 2).

We previously identified, in prostate cancer cells, a CD24-mediated inhibition of the ARF-nucleophosmin (NPM) interaction, that caused ARF degradation and resulted in elevated MDM2 levels and lower levels of p53 including mutant p53\(^\text{6}\). We assessed the protein expressions of MDM2 and ARF and their associations with tumor progression and metastasis in the UAB cohort. The nuclear MDM2 and ARF proteins were expressed in 39\%
and 68% of the prostate cancer samples, respectively (Table S2 and Fig. 1C). However, there was no significant association between MDM2 or ARF expression and tumor stage (Tables S2 and S3). There was a significant association of MDM2 expression with tumor grades (Gleason scores), but the expression was most often higher in cancers with Gleason 7 scores than in those either less or more than Gleason 7 (Table S2). For the UAB cohort, there was no significant correlation between ARF expression and tumor grade or Gleason score (Table S2).

3.2 | Association of CD24 and mutant p53 expressions with racial disparities of prostate cancer

In our previous study, we performed IHC analyses of 522 FFPE tissues or TMA samples of prostate cancer, consisted of EAs (27%), AAs (7%), and Asians (66%)\(^1\). Due to the few cases of AA men, we could not determine the role of CD24 and mutant p53 in prostate cancer racial disparity. Only 6 AA prostate cancer patients are available in The Cancer Genome Atlas (TCGA) dataset (Fig. S1). For the present study, we selected 156 specimens of prostate cancer FFPE tissues from 78 EA and 78 AA prostate cancer patients in the UAB cohort (Table 1). Of note, for the 78 EA and 78 AA patients, samples were matched by age (\(p=0.866\)) and Gleason score (\(p=0.559\)). We assessed an additional 132 TMA prostate cancer samples from 69 EA and 63 AA prostate cancer patients in the PCaP cohort (Table 1). For the UAB cohort, the frequency of CD24 expression showed a difference (\(p=0.023\)) between 66.7% of AA cases (52/78) and 48.7% of EA cases (38/78) (Table 4). The frequency of mutant p53 and MDM2 expression showed differences (\(p=0.014\) for mutant p53 and \(p=0.021\) for MDM2) between cases of AA and EA, but no significant differences for ARF expression (Table 4). For the PCaP cohort, the frequency of CD24 and mutant p53 expression appeared different between cases of AAs and EAs, but the differences were not significant (CD24, \(p=0.077\); mutant p53, \(p=0.390\); Table 4).

Since CD24 and mutant p53 expression in prostate cancer were associated with tumor progression and metastasis (Table 2)\(^1\), tumor stage may be a confounding factor in our analysis of racial disparities. For the two sample cohorts, we stratified by tumor stage. For the UAB cohort, the frequency of CD24 expression showed a difference between cases of AAs and EAs in the high-stage group (TNM T4, node-positive (N+), or metastasis-positive (M+), \(p=0.015\)) but not in the low/medium-stage group (TNM T2–3) (Table S4). However, the frequency of mutant p53 and MDM2 expression showed differences between cases of AAs and EAs in the low-stage group alone (\(p=0.050\) for mutant p53 and \(p=0.004\) for MDM2) but no significant difference for ARF expression (Table S5). For the PCaP cohort, the frequency of CD24 expression showed differences between cases of AAs and EAs in the T2-stage group alone (\(p=0.005\)), but no differences for CD24 in the other stage groups or mutant p53 in all stage groups (Table S4).

3.3 | Co-expressions of CD24 and mutant p53 in prostate cancer samples

Since our recent data showed that silencing CD24 restored at least part of the tumor suppressor activity of mutant p53 in prostate cancer cells\(^6\), CD24 may be a modulator of p53-driven tumor progression. To validate the CD24-dependent inactivation of mutant p53 in prostate cancer\(^1\), we assessed the expression of CD24 and p53 and their association in
prostate cancer tissues. The frequency of mutant p53 expression was less than CD24 expression in all samples, but mutant p53 accumulation was accompanied by CD24 expression in most cases (32/46 in the UAB cohort; 20/25 in the PCaP cohort) (Tables 2 and S6). H-score analysis of the UAB cohort showed positive correlations of mutant p53 with CD24 expression (r = 0.308, p<0.001) (Figs. 2A and Table S6). A positive correlation was evident between protein expression of CD24 and MDM2 (r = −0.280, p<0.001) (Figs. 2B and 2C). The use of TMA samples with a small core size in the PCaP cohort prevented the H-score. H-score analysis in the UAB cohort showed a difference in the expression levels of CD24 (p=0.001) in prostate cancer samples between AAs and EAs (Fig. 2D). The expression levels of mutant p53 and MDM2 appeared to be different (p=0.022 for mutant p53 and p=0.020 for MDM2) between AAs and EAs, but ARF expression levels were similar (Figs. 2E–G).

Most of the TP53 missense mutations are within the DNA binding domain, that disrupt WT p53 protein conformation (e.g., R175H, G245S, G245D, and R249S) or abolish DNA contact (e.g., R248Q, R248W, R273H, R273C, and R282W). As shown in our previous study, the DNA contact p53R273H mutant is as efficient as p53WT in suppressing colony formation after CD24 silencing. However, CD24 silencing does not confer tumor suppressor activity to the conformational p53R175H mutant. Thus, silencing of CD24 increases activities of WT TP53 and the DNA contact mutant p53R273H. In the present study, we tested whether p53R273H accumulation was accompanied by CD24 expression in primary prostate cancer. We obtained prostate cancer cells using laser capture microdissection from all UAB prostate cancer samples, which included 32 CD24+/mutant p53+ and 14 CD24−/mutant p53+ UAB samples (Table S6). A PCR-based analysis was used to detect mutant p53 (e.g., TP53R175H and TP53R273H) using various TP53WT, TP53R175H, and TP53R273H human cancer cells (Fig. 3A). Next, using this mutation analysis, we identified the TP53R273H mutation in 5 of 32 CD24+/mutant p53+ cases but no TP53R175H mutations were found in any of the 32 CD24+/mutant p53+ cases (Fig. 3B). In 14 CD24−/mutant p53+ tissue samples, we found the TP53R273H mutation in one case but no TP53R175H mutation in any case (Fig. 3B). Of note, all identified TP53R273H mutations were heterozygous (Fig. 3B) and were validated using Sanger sequencing (Fig. 3C).

4 | Discussion

Grasso et al. and Wallance et al. found that CD24 mRNA is overexpressed in human prostate cancer tissues, an observation validated by Yu et al. and Singh et al. Our bioinformatics analysis of public datasets including TCGA data confirmed higher levels of CD24 mRNA expression in late-stage and metastatic prostate cancer. In mouse models of prostate cancer, mutation of CD24 reduced tumor progression and metastasis, which suggests CD24 promotes tumor progression and metastasis. Furthermore, we found that protein expression of CD24 in human primary prostate cancer was associated with an aggressive tumor stage, especially distant metastasis. In the present study, we validated the previous results using an independent UAB sample cohort. However, the previous results were not validated in the independent PCaP cohort. Ninety-eight percent of the PCaP cases were diagnosed at low tumor stage (TNM T1–2), and the frequency of CD24+ samples was...
higher in T2 cases (59%) than in T1 cases (50%). In the UAB sample cohort, we validated our previous result that mutant p53 was associated with a higher tumor stage\textsuperscript{12}. However, for the independent PCaP cohort, there was no significant difference in the frequency of mutant p53 samples between T2 and T1 cases. These data suggest that CD24 and mutant p53 are implicated in late-stage and metastatic prostate cancers.

Prostate cancer has a strong disparity by race. In our previous study, we found that CD24 was expressed in 54% of EA cases and 66% of AA cases\textsuperscript{12}. Likewise, mutant p53 was expressed in 16% of EA cases and 23% of AA cases\textsuperscript{12}. Perhaps due to analysis of only 35 AA cases, these results were not significant. In the present study, we selected from the UAB cohort 78 AA and 78 EA cases that were age- and Gleason score-matched, and found differential expression of CD24 in prostate cancer tissues of AAs and EAs. A racial difference of CD24 expression was evident in the PCaP cohort, but did not reach statistical significance (p=0.077). Further studies with additional samples are required for validation. However, after combination of results for the prostate cancer samples from the two cohorts, there was a racial disparity for the expression of CD24 (AA/EA: 64% vs. 47%; p=0.004) but not for the expression of mutant p53 (AA/EA: 28% vs. 21%; p=0.152). These data indicate that CD24 contributes to AA racial disparities of prostate cancer and suggest that it is a biologic contributor to these disparities.

The mechanism underlying the AA racial disparities of CD24 remains elusive. Our previous study demonstrated that CD24 is a genetic modifier for the risk and progression of prostate cancer\textsuperscript{37}. Genetic variants of CD24 appear to be associated with CD24 expression that affects expression of CD24 in prostate cancer cells\textsuperscript{37}. The polymorphisms rs52812045\textsuperscript{7,38} and rs3838646\textsuperscript{7,39} in the CD24 transcript and a CGC haplotype (−534C/−492G/−442C) in the promoter region of CD24\textsuperscript{40} are functional genetic variants. First, rs52812045 C/T at exon 2 in the CD24 putative cleavage site for the GPI anchor resulted in replacement of the amino acid alanine with valine. In lymphocytes and hepatocellular cells, the T/T genotype expressed higher cell-surface CD24 than the C/T or C/C genotypes\textsuperscript{7,38}. Second, rs3838646 TG deletion in the 3′-untranslated region reduced levels of CD24 mRNA by >2-fold\textsuperscript{7,39}. Third, a haplotype (CGC) consisting of three single nucleotide polymorphisms (−534A/C, −492G/C, and −442C/T) bound to a transcription factor, SP1, which was required for promoter activity\textsuperscript{40}. Thus, these genetic variants may affect the levels of CD24 expression in prostate cancer cells. Since CD24 overexpression has been implicated in tumor progression and metastasis, CD24 variants may be associated with the genetic susceptibility of AA men to prostate cancer and contribute to the AA racial disparity. Thus, for future studies, it is appropriate to test the CD24 variants in tumors of AA versus EA patients.

TP53 mutations are more frequent in prostate cancers among American males, but it is uncertain if mutant p53 contributes to the AA racial disparity\textsuperscript{41}. The mutational landscape of AA prostate cancers revealed that there were fewer TP53 somatic aberrations in tumors from AAs relative to EAs\textsuperscript{23,42–45}. MDM2 protein expression is lower in AA prostate cancers than in EA prostate cancers\textsuperscript{46}. In the present study, we observed higher expression of mutant p53 and MDM2 in AA tumors relative EA tumors in the UAB cohort but not in the PCaP cohort. This discrepancy may result from confounding variables (e.g., age, tumor stage and grade, and/or socioeconomic factors). Age is a risk factor for being diagnosed with prostate cancer.
Older men tend to be diagnosed with more advanced disease\textsuperscript{47}, but AA men tend to be younger compared to men of other races at the time of diagnosis\textsuperscript{48}. Also, racial disparities are greatest for low-grade Gleason 6 disease compared with Gleason 7 to 10 disease, in which AA men are twice as likely to die of prostate cancer compared with non-AA men\textsuperscript{49}. In the present study, samples of the UAB cohort were matched by age and Gleason score between AAs and EAs. Stratified by tumor stage, there were racial differences only for mutant p53 and MDM2 expressions in the low-stage group. Co-expression of CD24 and mutant p53 was frequent in prostate tumors. Thus, mutant p53 may act as a passenger, with CD24 as the driver, to contribute to the AA racial disparity.

In previous studies, we showed that silencing of $CD24$ results in functional restoration of p53$^{R273H}$ but not p53$^{R175H}$, which demonstrated a CD24-dependent restoration of mutant p53 in prostate cancer cells\textsuperscript{6,12}. In the present study, CD24 expression and mutant p53 accumulation were higher in metastatic tumors compared to localized tumors. Of the mutations, > 80% are missense mutations that lead to less p53 transcriptional activity\textsuperscript{14–16}. Since mutant p53 most likely accompanies overexpressed CD24, we screened the $TP53^{R273H}$ and $TP53^{R175H}$ mutations in CD24+/mutant p53+ tumors. $TP53^{R273H}$ mutations were found in 5 of 32 (15.6%) CD24+/mutant p53+ cases, but no $TP53^{R175H}$ mutation was identified in any case. For all 954 $TP53$ mutations (18.4%) in 5192 human prostate cancer samples from 20 studies (cBioPortal), the top ranking of the TP53 missense mutant rates was $TP53^{R248Q}$ (5.1%), $TP53^{R273C}$ (4.6%), $TP53^{R175H}$ (2.6%), $TP53^{R245S}$ (2.5%), $TP53^{R220C}$ (2.3%), $TP53^{R282W}$ (2.2%), $TP53^{R248W}$ (1.7%), and $TP53^{R273H}$ (1.3%). These data in human prostate cancer suggest that DNA contact mutant $TP53^{R273H}$, but not conformational mutant $TP53^{R175H}$, is more likely co-expressed with CD24. However, other $TP53$ mutations, such as the more commonly occurring DNA contact $TP53$ mutations (e.g., R248Q, R273C, and R282W), may be accompanied by overexpressed CD24.

Limitations of the present study may include the inherent selection and observational bias in our sample cohorts. First, our results with a small sample size should be taken with caution because the cohort consisted of patients who had biased tumor stages. Also, our cohorts lacked follow-up data to analyze clinical outcomes. Second, socioeconomic factors likely contributed to the differences in clinical outcomes between AAs and EAs\textsuperscript{50}. However, two cohorts combined provided increased sample size that supported the strength and reproducibility of the results. In addition, CD24 is expressed in 68% of clinical samples across all major cancer types, especially late-stage cancers\textsuperscript{1}. Although CD24 is not expressed in stem cells of most types of cancer, it is a well-accepted negative cancer stem cell surface marker for various cancers, especially breast cancers\textsuperscript{51}. However, establishment of CD24 as a prostate cancer stem cell marker seems to be inadequate and requires confirmation\textsuperscript{51}.

5 | Conclusions

The CD24-p53 axis may contribute to aggressive and metastatic prostate cancers, especially those of AAs. This is the first characterization of a racial disparity for the CD24-p53 axis in prostate cancer, which furthers understanding of AA prostate cancer pathogenesis. Although the mechanism underlying a functional interaction of CD24 with mutant p53 in tumor
progression remains elusive, a combination of CD24 and mutant p53 may serve as a biomarker for aggressive and metastatic prostate cancers, especially AA prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES


Figure 1. Expression of CD24 and mutant p53 in human prostates

(A) Representative IHC data showed no CD24 staining in normal prostate (left panel) or benign prostatic hyperplasia (BPH, right panel). (B) Representative IHC data showed CD24 staining of prostate cancer cells with no staining of adjacent normal prostate epithelial cells. The red arrows indicate adjacent normal prostate epithelial cells. (C) CD24, mutp53, MDM2, and ARF staining of representative UAB prostate cancer samples. Representative IHC data showed co-expression of CD24 and mutant p53 from the same case. (D) CD24 and mutp53 IHC staining of two representative PCaP TMA prostate cancer samples. mutp53, mutant p53 protein. All experiments were repeated three times.
Figure 2. Protein expression of CD24, mutp53, MDM2, and ARF in human primary prostate cancers

Figure 3. Screening for $TP53^{R175H}$ and $TP53^{R273H}$ mutations

(A) PCR restriction fragment-length polymorphism analyses for $TP53^{R175H}$ and $TP53^{R273H}$ mutations. Representative $TP53^{R175H}$ genomic DNA samples for each of the genotypes (G/G, G/A, and A/A) were obtained from LNCaP, PCI-4B, and NCI-H196 cancer cells, respectively. Representative $TP53^{R273H}$ genomic DNA samples for each of the genotypes were obtained from the three types of cells. WT, wild-type; Mut: mutant. Numbers at the right represent sizes of each fragment (bp).

(B) Representative data identified $TP53^{R273H}$ mutations using PCR-based analysis of micro-dissected cells from UAB prostate cancer samples.

(C) Validation of $TP53^{R273H}$ mutations using Sanger sequencing. Down arrows indicate the $TP53^{R273H} G>A$ mutant site.
TABLE 1.

Human Subject Characteristics

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<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European-American (EA)</td>
<td>78</td>
<td>63</td>
</tr>
<tr>
<td>African-American (AA)</td>
<td>78</td>
<td>69</td>
</tr>
<tr>
<td>Tumor stage (TNM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0</td>
<td>82</td>
</tr>
<tr>
<td>T2</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>T3</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>T4 or N+ or M+</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Gleason grade (GG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG 2–6</td>
<td>16</td>
<td>72</td>
</tr>
<tr>
<td>GG 7</td>
<td>108</td>
<td>53</td>
</tr>
<tr>
<td>GG 8–10</td>
<td>32</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Formalin-fixed paraffin-embedded (FFPE) samples

UAB, University of Alabama at Birmingham

<sup>b</sup>FFPE TMA samples

PCaP, The North Carolina-Louisiana Prostate Cancer Project
### TABLE 2.
Association of CD24 and p53 expressions with tumor stage and grade

<table>
<thead>
<tr>
<th>Tumor stage or grade</th>
<th>CD24</th>
<th></th>
<th></th>
<th>p53</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>(p)-value(^a)</td>
<td>Negative</td>
<td>Positive</td>
<td>(p)-value(^a)</td>
</tr>
<tr>
<td><strong>Tumor stage (UAB)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>37 (49.3)</td>
<td>38 (50.7)</td>
<td>0.037</td>
<td>59 (78.7)</td>
<td>16 (21.3)</td>
<td>0.035</td>
</tr>
<tr>
<td>T3</td>
<td>24 (42.9)</td>
<td>32 (57.1)</td>
<td>0.558 (^b)</td>
<td>38 (67.9)</td>
<td>18 (32.1)</td>
<td>0.035</td>
</tr>
<tr>
<td>T4 or N+ or M+</td>
<td>5 (20.0)</td>
<td>20 (80.0)</td>
<td>0.356</td>
<td>13 (52.0)</td>
<td>12 (48.0)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Tumor stage (PCaP)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>41 (50.0)</td>
<td>41 (50.0)</td>
<td>0.382 (^b)</td>
<td>60 (82.9)</td>
<td>14 (17.1)</td>
<td>0.035</td>
</tr>
<tr>
<td>T2</td>
<td>18 (40.9)</td>
<td>26 (59.1)</td>
<td>0.382 (^b)</td>
<td>35 (79.5)</td>
<td>9 (20.5)</td>
<td>0.035</td>
</tr>
<tr>
<td>T3</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>0.382 (^b)</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Gleason grade (UAB)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG 2–6</td>
<td>9 (56.3)</td>
<td>7 (43.7)</td>
<td>0.356</td>
<td>12 (75.0)</td>
<td>4 (25.0)</td>
<td>0.903 (^b)</td>
</tr>
<tr>
<td>GG 7</td>
<td>42 (38.9)</td>
<td>66 (61.1)</td>
<td>0.356</td>
<td>76 (70.4)</td>
<td>32 (29.6)</td>
<td>0.903 (^b)</td>
</tr>
<tr>
<td>GG 8–10</td>
<td>15 (46.9)</td>
<td>17 (53.1)</td>
<td>0.356</td>
<td>22 (68.7)</td>
<td>10 (31.3)</td>
<td>0.903 (^b)</td>
</tr>
<tr>
<td><strong>Gleason grade (PCaP)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG 2–6</td>
<td>32 (44.4)</td>
<td>40 (55.6)</td>
<td>0.382 (^b)</td>
<td>60 (83.3)</td>
<td>12 (16.7)</td>
<td>0.677 (^b)</td>
</tr>
<tr>
<td>GG 7</td>
<td>26 (49.1)</td>
<td>27 (50.9)</td>
<td>0.382 (^b)</td>
<td>42 (79.2)</td>
<td>11 (20.8)</td>
<td>0.677 (^b)</td>
</tr>
<tr>
<td>GG 8–10</td>
<td>5 (71.4)</td>
<td>2 (28.6)</td>
<td>0.382 (^b)</td>
<td>5 (71.4)</td>
<td>2 (28.6)</td>
<td>0.677 (^b)</td>
</tr>
</tbody>
</table>

\(^a\)Pearson chi-squared test

\(^b\)Adjusted Pearson chi-squared test
<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Positive</th>
<th>Age-adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>OR&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>UAB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic (N+ or M1+)</td>
<td>5 (20.0)</td>
<td>20 (80.0)</td>
<td>3.49</td>
</tr>
<tr>
<td>Localized (pT1–4, N0, M0)</td>
<td>61 (46.6)</td>
<td>70 (53.4)</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic (N+ or M1+)</td>
<td>13 (52.0)</td>
<td>12 (48.0)</td>
<td>2.63</td>
</tr>
<tr>
<td>Localized (pT1–4, N0, M0)</td>
<td>97 (74.0)</td>
<td>34 (26.0)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Odds ratio (OR)

<sup>b</sup>Confidence interval (CI)
TABLE 4.

Ethnic/racial distribution comparisons in gene expressions

<table>
<thead>
<tr>
<th>Gene</th>
<th>UAB</th>
<th>PCaP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative n (%)</td>
<td>Positive n (%)</td>
</tr>
<tr>
<td>CD24</td>
<td>AA 26 (33.3)</td>
<td>52 (66.7)</td>
</tr>
<tr>
<td></td>
<td>EA 40 (51.3)</td>
<td>38 (48.7)</td>
</tr>
<tr>
<td>p53</td>
<td>AA 48 (61.5)</td>
<td>30 (38.5)</td>
</tr>
<tr>
<td></td>
<td>EA 62 (79.5)</td>
<td>16 (20.5)</td>
</tr>
<tr>
<td>MDM2</td>
<td>AA 41 (52.6)</td>
<td>37 (47.4)</td>
</tr>
<tr>
<td></td>
<td>EA 55 (70.5)</td>
<td>23 (29.5)</td>
</tr>
<tr>
<td>ARF</td>
<td>AA 24 (30.8)</td>
<td>54 (69.2)</td>
</tr>
<tr>
<td></td>
<td>EA 26 (33.3)</td>
<td>52 (66.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pearson chi-squared test