Basic and Translational Science

Regulation of the Antioxidant Response by MyoD Transcriptional Coactivator in Castration-resistant Prostate Cancer Cells

Shun Zhang*, Lin-hu Li*, Hong-mei Qiao, Xue Yang, Liang Chen, and Xiao-hui Luo

OBJECTIVE
To reveal the potential role of the basic helix-loop-helix myogenic transcription regulator MyoD in the regulation of castration-resistant prostate cancer.

METHODS
Expression level of MyoD was assessed in prostate cancer tissues using quantitative reverse transcription polymerase chain reaction and immunohistochemistry and in experimentally induced castration-resistant LNCaP/R cells using quantitative reverse transcription polymerase chain reaction and immunoblotting. Effect of MyoD knockdown on LNCaP/R cell progression was determined by assessing cell proliferation, apoptosis, and colony formation rate. The effect of MyoD knockdown on the oxidative stress state in PC3 cells was determined by assessing antioxidant response gene expression and glutathione synthetase-to-glutathione ratio. Finally, the functional link between the nuclear factor erythroid-derived 2-related factor 1 (NRF1) and the regulation of antioxidant response element-driven transcription by MyoD was studied at both molecular and functional levels.

RESULTS
MyoD expression was significantly upregulated in hormone-refractory prostate cancer tissues and in experimentally induced castration-resistant LNCaP/R cells, and MyoD knockdown effectively impaired LNCaP/R cell proliferation and promoted apoptosis under androgen-depleted condition. Moreover, MyoD enhanced the glutathione production and protected against oxidative stress by positively regulating a cluster of antioxidant genes known to be the downstream targets of NRF1. Mechanistically, MyoD could augment the antioxidant response element-driven transcription in an NRF1-dependent manner, and the stimulatory effect of MyoD on the antioxidant response was substantially compromised in the presence of NRF1 small interfering RNA treatment.

CONCLUSION
We have identified an unexpected collaboration between MyoD and NRF1 under androgen-depleted condition, which may serve as an important adaptive mechanism during the pathogenesis of castration-resistant prostate cancer. UROLOGY 123: 296.e9−296.e18. 2019. 2018 © 2018 Elsevier Inc.

Prostate cancer (PCa) is the most common type of noncutaneous cancer and the second leading cause of male cancer-related mortality in Western countries. In China, the incidence of PCa is increasing rapidly (~11 cases per 100,000 per year), and the mortality is especially high in rural areas.1 Because androgen receptor (AR) signaling plays an essential role in PCa development and progression, androgen-deprivation therapy (ADT), involving a reduction in androgen level via surgical castration or interference with AR function using antiandrogen agents, has exerted tremendous therapeutic effects on localized early-stage PCa (small tumor size [stage T2a or smaller], low prostate-specific antigen [PSA] [no more than 10 ng/mL], and low Gleason score [no more than 6]).2,3 Despite the initial effectiveness, most of patients with PCa receiving ADT eventually become resistant to the therapy and develop castration-resistant prostate cancer (CRPC) via unknown mechanisms.4 So castration resistance is often the natural history after a prolonged course of continuous ADT in many patients with PCa. Castration is known to generate excessive production of reactive oxygen species (ROS).5,6

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Elevated ROS could promote the conversion of androgen-dependent PCa into CRPC via regulation of AR expression. However, the mechanisms by which oxidative stress regulates PCa progression and CRPC are not fully understood and further investigation is required. MyoD, the basic helix-loop-helix myogenic transcription regulator, plays a decisive role in skeletal muscle development. Upon activation, MyoD induces a robust expression of several well-characterized myogenic genes such as myogenin, M-cadherin, myosin heavy and light chains, and muscle creatine kinase. These muscle-specific molecules work together to promote myogenesis. A growing body of evidence suggests that additional, as yet poorly characterized, nonmuscular actions of MyoD are likely to take place. MyoD has been shown to be a negative regulator of brown adipogenesis. Sarcoma metastasis is partially controlled through Pax7/MyoD-dependent activation of miR-182. Additionally, MyoD is responsible for the transcriptional repression of estrogen receptor α (ERα) expression in relapsed ERα-positive breast cancer cells. Clearly, the regulatory functions of MyoD exist on a broader scale and remain to be delineated.

An association between oxidative stress and deregulation of MyoD function has been recently revealed. Treatment with nitric oxide potentiates myoblast differentiation by regulating oxidative stress/MyoD cascade. Consistently, quercetin, a dietary supplement with powerful antioxidant properties, could reduce MyoD expression by attenuating oxidative stress in zebrafish embryos. On this basis, we hypothesize that MyoD may regulate the oxidative stress during the development or progression of CRPC and deregulated MyoD may play a potential role in CRPC. Thus, the current study was undertaken to study the expression and potential function of MyoD in PCa pathogenesis.

MATERIALS AND METHODS

Clinical Specimens

After obtaining institutional ethical board approval and signed informed consents from the participants, we enrolled 19 cases of fresh benign prostatic tissues from patients who underwent radical cystoprostatectomy due to bladder cancer, 13 cases of fresh non-CRPC tissues, and 21 cases of CRPC tissues (all the 34 patients underwent radical prostatectomy due to bladder cancer, 13 cases of fresh non-CRPC tissues, and 21 cases of CRPC tissues containing either 10% FBS or 10% FBS-CS at 37°C in 5% CO₂. Three days later, triplicate samples were counted using trypan blue exclusion with the assistance of a hemocytometer (Beckman Coulter, Fullerton, CA). In another experiment setting, transfected PC3 and LNCaP/R cells were incubated with different doses of H₂O₂ for 24 hours and assayed for cell viability afterward.

Viable Cell Counts

Forty-eight hours after siRNA treatment, LNCaP/R cells were plated onto a 96-well plate at 5 x 10⁵ cells/well in medium containing 10% FBS or 10% FBS-CS and incubated overnight at 37°C in 5% CO₂. Three days later, triplicate samples were counted using trypan blue exclusion with the assistance of a hemocytometer (Beckman Coulter, Fullerton, CA). In another experiment setting, transfected PC3 and LNCaP/R cells were incubated with different doses of H₂O₂ for 24 hours and assayed for cell viability afterward.

Cell Apoptosis

Forty-eight hours after siRNA treatment, LNCaP/R cells were cultured in medium containing 10% FBS or 10% FBS-CS for another 3 days, as described earlier. The extent of apoptosis was then quantitatively measured using apoptosis ELISA kit (Roche, Shanghai, China) in triplicate with a microplate reader at 405 nM. The optical density in the medium containing 10% FBS was arbitrarily set at 1, and values in the medium containing 10% FBS-CS were normalized and plotted accordingly.

Glutathione Synthetase-to-Glutathione Ratio Detection

The PC3 cells stably transfected with pEnter-His-MYOD1 or empty vector were cultured in fresh culture medium for 24 hours. Cells were then treated with different doses of H₂O₂ for another 24 hours, followed by measurement of glutathione synthetase-to-glutathione (GSH/GSSG) ratio by using a commercial GSH/GSSG Ratio Detection Kit (Abcam). Total RNA was extracted using MagMAX Total RNA Isolation Kit (Thermo Fisher Scientific), and cDNA was synthesized using SMARTer PCR cDNA Synthesis Kit (Takara, Beijing, China) according to protocols recommended by the manufacturer. PCR primers used for different targets were listed in Supplementary Table S1.
Subsequent RT-qPCR was performed using Quantifast one-step SYBR Green RT-PCR kit in Applied Biosystems 7300 Real-Time PCR System. β-Actin was used as the internal control.

**Western Blotting**

Total protein from PCa cells was isolated using Chemicon Total Protein Extraction Kit (Merck Millipore, Beijing, China), and protein concentrations were determined by a Protein assay kit (Bio-Rad, Hercules, CA). Western blotting was carried out as described previously, by employing the antibodies including rabbit-anti-MyoD (Novus Biologicals, Shanghai, China), rabbit-anti-GAPDH (Abcam), and rabbit-anti-NRF1 (Abcam).

**Immunohistochemistry**

Immunohistochemical staining was performed as previously described, with the aid of VECTASTAIN Elite ABC HRP Kit (Vector Laboratories, Burlingame, CA). The antibody used was rabbit-anti-MyoD polyclonal Ab (Novus Biologicals, Shanghai, China).

**Cotransfection Transactivation Assay**

The construction of the NQO1-ARE-Luc reporter, which contains the antioxidant response element (ARE) of NAD(P)H dehydrogenase quinone 1 (NQO1) and drives a minimal promoter upstream of the luciferase gene, has been described previously. pCMV6-NF-E2L1/NRF1 and pCMV6-NF-E2L2/NRF2 were purchased from OriGene Technologies. For reporter assay, subconfluent proliferating PC3 cells were transfected overnight with the indicated expression vector(s) (0.25 μg/well) and the pNQO1-ARE-Luc reporter (0.25 μg/well), along with 0.001 μg of the Renilla luciferase reporter, pRL-EF, using FuGENE 6 (Promega, Madison, WI). Forty-eight hours later, cells were harvested and subjected to luciferase activity measurements using a dual luciferase reporter assay kit (Promega). Firefly luciferase activity was normalized to cotransfected Renilla luciferase activity for transfection efficiency.

**Statistical Analysis**

Data were analyzed to determine normality, and significant differences were determined by either t test or one-way analysis of variance (Prism 5.00, GraphPad Software Inc.) followed by Tukey post hoc analyses. Data were presented as mean ± SEM, and significance was accepted at P <.05.

**RESULTS**

**Induction of MyoD Expression in CRPC Tissues and Cells**

To examine the expression of MyoD in human PCa tissues, RT-qPCR and immunohistochemical analyses were done on 19 cases of benign prostatic tissues, 13 cases of non-CRPC biopsies, and 21 cases of CRPC biopsies. As shown in Figure 1A,B, MYOD1 expression was remarkably induced in CRPC tissues compared with other tissues. Consistently, MyoD immunoreactivity, which was predominantly localized in the nuclear, was dramatically increased in CRPC tissues (Fig. 1C). Moreover, MyoD staining appeared to be more intensive in poorly differentiated PCa samples (Gleason scores >8) than that in well-differentiated PCa samples (Gleason scores <7) (Supplementary Fig. S1). We examined MYOD1 expression in a panel of human PCa cell lines: a significantly higher level of MYOD1 was detected in castration-resistant LNCaP/R cells and in androgen-independent 22Rv1, PC3, and DU145 cells (Fig. 1D). Because PC3 and DU145 cells are AR-negative (Supplementary Fig. S2), the results suggest that MyoD function may be AR-independent. Advanced PCa is initially treated with ADT (orchiectomy or treatment by analogues of luteinizing hormone-releasing hormone). These treatments are usually applied together with an AR antagonist to achieve a complete androgen blockade. However, the response to this combined therapy is time-limited and almost all tumors become refractory eventually. Bicalutamide (namely Casodex, Selleckchem, Shanghai, China) is a nonsteroidal AR blocker. Therefore, to mimic the natural course of CRPC development, many researchers have established castration-resistant LNCaP/R cells by culturing androgen-sensitive LNCaP cells in the androgen-depleted media in the presence of long-term stimulation by bicalutamide. As expected, LNCaP/R exhibited faster growth and higher PSA production in the androgen-depleted medium compared with LNCaP (Fig. 1E,F). Interestingly, along the pathogenesis of castration resistance, MyoD expression was gradually upregulated, with the highest levels observed at 3 and 4 months after LNCaP/R induction (Fig. 1G). This upregulation was also tightly associated with the high PSA production (Fig. 1H).

**Inhibition of Endogenous MyoD Expression Impairs LNCaP/R Cell Proliferation Under Androgen-depleted Condition**

Next, we transfected MYOD1 siRNA and Ctrl siRNA into LNCaP/R cells to reveal the loss-of-function effect on cancerous progression. Ablation of endogenous MyoD expression was verified using Western blotting (Fig. 2A). Attenuated MyoD expression significantly alleviated the viability of LNCaP/R cells under androgen-depleted condition (Fig. 2B). Consistently, androgen-depleted condition-induced apoptosis was significantly evoked in the cells deprived of MyoD expression (Fig. 2C). Moreover, MyoD knockdown suppressed colony formation efficiency in the presence of FBS-CS but exerted no effects on colony formation efficiency in the presence of FBS (Fig. 2D). Thus, MyoD potentiates CRPC survival and growth under androgen-depleted condition.

**MyoD Acts Against Oxidative Stress by Restoring Cellular Redox Balance**

The available data suggest that MyoD may exert a promoting effect on the antioxidant response. We therefore examined the expression levels of a range of antioxidant genes the LNCaP/R cells transfected with MyoD siRNA compared with the LNCaP/R cells transfected with Ctrl siRNA (Supplementary Fig. S3). Interestingly, we frequently observed that the mRNA expression levels of GCLC, GCLM, glutathione synthetase (GSS), and metallothionein-1 (MT1) were substantially repressed in the LNCaP/R cells transfected with MyoD siRNA (Fig. 3A). The GCLC gene encodes the catalytic
**Figure 1.** MyoD induction in castration-resistant prostate cancer (CRPC). (A) Relative expression of MYOD1 in prostate cancer (PCa) tissues was determined using quantitative reverse transcription polymerase chain reaction (RT-qPCR). Quantitative analysis was performed in which the level of MYOD1 expression value in different PCa samples was normalized against the MYOD1 value in the third hormone-naive (HN) tumor sample, which was randomly selected and arbitrarily set at 1. (B) Statistical comparison of MYOD1 expression levels between HN PCa tissues and hormone-refractory (HR) PCa tissues (**P < .01). (C) Immunohistochemical staining of MyoD in different PCa tissues. PA, preabsorption. (D) Relative MYOD1 level in different PCa cells. *P < .05 and **P < .01 compared with the value in prostate epithelial cell (PEC) cells. (E) PCa cells were incubated in media containing 10% charcoal-stripped fetal bovine serum (FBS-CS) for 3 days and then cultured in media containing 10% FBS-CS, followed by incubation with different concentrations of synthetic androgen R1881 for 9 days. Viable cell numbers were finally determined and relative cell growth was calculated. (F) Intracellular PSA levels in (E). (G) MyoD expression along the establishment of LNCaP/R cells. (H) Intracellular PSA levels in (F). *P < .05 and **P < .01.
enzyme subunit of glutamate-cysteine ligase (GCL) and the GCLM gene encodes the regulatory enzyme subunit of GCL. GCLC, GCLM, and GSS together regulate GSH biosynthesis and other oxidative defense enzymes. MT1 has a high content of cysteine residues that bind various heavy metals. GCLC, GCLM, GSS, and MT1 are very important endogenous antioxidants. To verify this, we transfected PC3 cells with pEnter-His-MYOD1, and the overexpression of the exogenous MyoD was confirmed by Western blotting (Fig. 3B). As expected, MyoD overexpression significantly stimulated the transcriptions of antioxidant response genes (Fig. 3C). To further determine whether the stimulation of antioxidant response genes by MyoD have any biological consequences, we evaluated the effects of alteration of MyoD expression on the cellular sensitivity to H$_2$O$_2$-elicited oxidization. In the presence of H$_2$O$_2$, MyoD overexpression effectively improved the cell viability in PC3 cells, whereas pretreatment of parental LNCaP/R or LNCaP cells with a MYOD1-siRNA caused significant sensitization to H$_2$O$_2$ (Fig. 3D,E, Supplementary Fig. S4). To ask whether the redox balance was affected by MyoD, we examined the effect of exogenous MyoD on the redox balance of PC3 cells after 24-hour treatment with H$_2$O$_2$. pEnter-His-MYOD1 transfected cells were able to maintain significantly higher ratios of GSH/GSSG than control cells, especially at high doses of H$_2$O$_2$ (Fig. 3F). Consistently, overexpression of the exogenous MyoD produced unanimous higher ratios of GSH/GSSG in LNCaP/R and LNCaP cells under androgen-depleted conditions (cultured in FBS-CS) (Fig. 3G). Unlike LNCaP/R cells that are AR-positive,
Figure 3. MyoD regulates antioxidant response in prostate cancer (PCa) cells. (A) Quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis of antioxidant gene expression in naïve and transfected LNCaP/R cells. Different superscript letters denote groups that are statistically different ($P < .05$). (B) MyoD overexpression in PC3 cells was confirmed using immunoblotting. (C) RT-qPCR analysis of antioxidant gene expression in PC3 cells overexpressing MyoD. (D-E) Transfected PCa cells were challenged with different concentrations of H$_2$O$_2$ for 24 hours, followed by cell viability analysis. (F) Glutathione synthetase-to-glutathione (GSH/GSSG) ratio in transfected PC3 cells was determined using a commercial kit. *$P < .05$ and **$P < .01$ compared with the value in control (Ctrl) vector-transfected cells. (G) GSH/GSSG ratio in transfected LNCaP/R and LNCaP cells under different culture conditions was determined using a commercial kit. *$P < .05$ and **$P < .01$ when comparing His-MYOD1 + FBS-CS group with the His vector + FBS-CS group.
castration-sensitive PCa cells, PC3 cells are androgen-independent cells that resemble castration-resistant PCa cells. In this regard, our observation that manipulation of MyoD expression produced the unanimous effects on antioxidant response in both cell types re-emphasizes that the regulation of redox balance by MyoD is independent of AR signaling in PCa cells.

**Regulation of ARE-driven Transcription by MyoD**

The above observations suggest that MyoD may regulate a cluster of antioxidant genes known to be the downstream targets of the nuclear factor erythroid-derived 2-related factor 1 (NRF1). Because the cellular antioxidant responses are mediated mainly by NRF1 and NRF2 via direct binding of ARE, we next evaluated the effects of MyoD on the ARE-driven transcription by transient transfection assays using an NRF1/-responsive reporter driven by the ARE of NQO1 (NQO1-ARE-Luc). Transfection alone with the pEnter-His-MYOD1 exerted no effects on NQO1-ARE-Luc activity but cotransfection with pEnter-His-MYOD1 and pCMV6-NRF1 caused a robust increase in NRF1-stimulated NQO1-ARE-Luc activity (Fig. 4A,E). This stimulatory effect was not observed in NRF2-transfected PCa cells (Fig. 4B,F). In contrast to pEnter-His-MYOD1, cotransfection with NRF1 siRNA significantly impaired NRF1-stimulated NQO1-ARE-Luc activity, even in the presence of MyoD overexpression (Fig. 4C,G). Accordingly, transient transfection with NRF1 siRNA sensitized PC3 cells to H$_2$O$_2$ and abrogated the ability of MyoD to protect PC3 cells against H$_2$O$_2$ insult (Fig. 4D,H, Supplementary Fig. S5). The specific regulation of NRF1-dependent ARE-driven transcription by MyoD was also confirmed in castration-sensitive LNCaP cells (Supplementary Fig. S6).

**DISCUSSION**

To date, the biological effects of MyoD have been mostly restricted to its ability to regulate myogenic determination via positively modulating the activity of myogenic transcription factors. Our results are in keeping with previous findings in nonmuscle tissues including lung, colonic mucosa, and brown adipose tissues, and to our knowledge, are the first to demonstrate a biological action of MyoD in PCa.

Previously, Mishra et al have found that the promoter of MYOD1 gene is significantly hypermethylated in PCa cells compared with that in normal prostate cells, suggesting MyoD expression would be much lower in PCa cells than that in normal prostate cells. In our study, however, MYOD1 expression was observed to be significantly higher in androgen-independent 22Rv1, PC3, and DU145 cells than that in normal PEC cells and androgen-sensitive LNCaP cells (Fig. 1C). This observation re-emphasizes the notion that promoter hypermethylation does not necessarily entail transcriptional repression. A good example is the thyroid-stimulating-hormone-alpha (TSH-$\alpha$), a very common subunit of the heterodimeric hormone TSH. Two CpG sites in the TSH-$\alpha$ promoter are remarkably hypermethylated in thyroid cancer tissues than those in normal thyroid tissues, but TSH-$\alpha$ expression remains inhibited in normal thyroid tissues while activated in cancer tissues. Moreover, both immunohistochemical analysis in clinical PCa tissues (Fig. 1A,B) and biochemical analysis in experimentally induced castration-resistant PCa cells (Fig. 1D-G) have revealed that MyoD expression is virtually stimulated in CRPC and its activation is closely associated with the pathogenesis of castration resistance. The molecular mechanisms responsible for MyoD upregulation in CRPC remain to be defined, but one factor has been so far reported to determine the MyoD activation. PCa cells are exposed to increased oxidative stress and especially, castration, the major approach for treatment of late-stage PCa, significantly increases the prostatic oxidative stress. Treatment with the exogenous nitric oxide increases oxidative stress and thereafter activates MyoD expression, thus potentiating myoblast differentiation in injured muscles. Thus, it is very likely that oxidative stress may act as a delicate brake on MyoD activation that is normally suppressed in hormone-naïve PCa.

Further evidence on the involvement of MyoD in the regulation of CRPC is provided by our loss-of-function assays in the castration-resistant LNCaP/R cells. Under androgen-depleted condition, MyoD knockdown significantly impaired LNCaP/R cell proliferation and colony formation efficiency but evoked a dramatic apoptosis (Fig. 2B-D). Notably, this therapeutic effect was only detectable in the androgen-depleted condition, suggesting that MyoD may function as a potential oncogene in promoting PCa progression, especially to the androgen-independent state. In favor of our hypothesis, a very recent study has shown that MyoD potentiates endocrine resistance in ER$\alpha$-positive breast cancer cells via negatively regulating ESRI transcription. Interestingly, MyoD has been shown to act as an essential tumor suppressor in medulloblastoma and gastric cancer. Thus, MyoD may be a bidirectional regulator in the regulation of cancerous progression and whether it acts as an oncogene or a tumor suppressor is largely dependent on the cellular context.

Accumulated data have evidenced a causal role of oxidative stress in the development and progression of CRPC. However, the expression of certain antioxidant genes may also be evoked in CRPC. NRF1 (NF-E2-related factor-1) is such a striking example. NRF1 expression is elevated in human-aggressive CRPC tissues and NRF1 is more highly expressed in the androgen-independent C4-2B cells, compared with the androgen-dependent LNCaP cells. NRF1 then stimulates the expressions of several antioxidant genes including Txn-1 and Prx-1. Both Txn-1 and Prx-1 work together to maintain compartmental ROS expression and enhance AR transactivation under androgen-deprivation condition, thus promoting CRPC. Our findings extend this knowledge by identifying MyoD as a potent protector against oxidative toxicity by augmenting the antioxidant defenses (eg, elevated expression of antioxidant genes and enhanced...
Figure 4. Regulation of antioxidant response element (ARE)-driven transcription by MyoD is nuclear factor erythroid-derived 2-related factor 1 (NRF1)-dependent. (A-B) NQO1-ARE-Luc reporter activity. PC3 cells were transfected with the indicated vectors for 24 hours, followed by luciferase reporter assay. The values are expressed relative to the control (Ctrl). (C) Effects of NRF1 knockdown on MyoD-stimulated NQO1-ARE-Luc reporter activity in PC3 cells. (D) Effects of NRF1 knockdown on MyoD-stimulated PC3 cells viability on H2O2 challenge. *P < .05 and **P < .01 compared with the value in Ctrl vector-transfected cells. (E-F) NQO1-ARE-Luc reporter activity. LNCaP/R cells were transfected with the indicated vectors for 24 hours, followed by luciferase reporter assay. The values are expressed relative to the control. (G) Effects of NRF1 knockdown on MyoD-stimulated NQO1-ARE-Luc reporter activity in LNCaP/R cells. (H) Effects of NRF1 knockdown on MyoD-stimulated LNCaP/R cells viability upon H2O2 challenge. *P < .05 and **P < .01 compared with the value in Ctrl vector-transfected cells.
production of GSH) (Fig. 3C,F). It is understandable that excessive production of ROS is detrimental to both normal and cancer cells. It damages lipids, DNA, and proteins, thus inevitably causing cell apoptosis. To get adapted to this ROS challenge, advanced PCa cells must develop mechanisms to utilize higher levels of ROS for mitogenic purposes by upregulating expression of antioxidant enzymes. Actually, numerous studies have shown that antioxidants play significant roles in regulation of cancer growth and survival. 27 To this end, the increased expression of MyoD may entitle the castration-resistant PCa cells more equipped for constitutive modification of antioxidant signaling, which could enhance the aggressive phenotype of these cells.

In contrast to NRF1, the expression of NRF2 (NF-E2-related factor-2), another oxidative stress-sensitive transcription factor, is suppressed in human-aggressive PCa tissues and in the androgen-independent C4-2B cells. 21 Consistently in the current study, regulation of ARE-driven transcription by MyoD in CRPC cells appears to be exclusively NRF1-dependent (Fig. 4A,B). It has been suggested that NRF2 responds to inducible oxidative stimuli, whereas NRF1 regulates more constitutive forms of oxidative stress. Therefore, MyoD-NRF1 cascade may serve as an essential adaptive mechanism, rather than an acute response mechanism, to maintain oxidative homeostasis and thereby promotes cancerous proliferation and survival through rebalancing the redox-sensitive pathways in CRPC cells.

**CONCLUSION**

The results presented here have identified a critical role of MyoD, both as a target and as a modifier of the oxidative stress signaling in conferring an optimal, sufficient antioxidant response during the pathogenesis of CRPC. As a mechanism of MyoD regulation of a subset of antioxidant genes, MyoD cooperates with NRF1, promotes antioxidant response, and acts as an indispensable adaptive mechanism under androgen-depleted condition. Overall, our study has pointed to an unexpected collaboration between MyoD and NRF1 to mobilize the antioxidant machinery of cells in CRPC.

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**References**


APPENDIX

SUPPLEMENTARY DATA
Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.urology.2018.04.028.