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The role of mitochondrial TCA cycle enzymes in determining prostate cancer chemosensitivity

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14. ABSTRACT
In the needle biopsy samples from high-risk prostate cancer patients, we further confirmed that TCA cycle enzymes MDH2 and CS are elevated in subsets of patients. In vitro, comparing three commonly used prostate cancer cell lines PC3, C42B and LNCaP to benign prostate cell line BPH1 showed that CS expressions are very robust in all cell lines, and MDH2 is overexpressed in the cancer cell lines only. We established stable MDH2 shRNA knockdown cell lines in PC3, C42B and LNCaP. The resulted cells have lower rates of proliferation, lower levels of ATP production, and higher sensitivity to docetaxel chemotherapy compared to the scramble control knockdowns. These confirm our original hypothesis that MDH2 plays a role in determining prostate cancer chemosensitivity.

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Docetaxel, chemosensitivity, MDH2, mitochondria.

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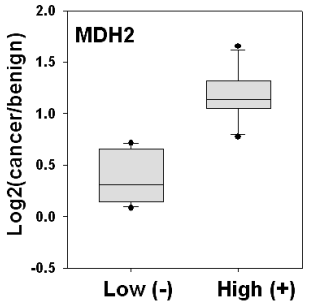
Introduction:

Genetic and epigenetic alterations in the metabolic pathways are associated with mitochondrial dysfunction and cancer development. In most cases, however, the mechanisms by which these alterations mediate cancer cell response or resistant to therapeutics are unknown. The goal of this proposal is to investigate one of such mechanisms that mediate prostate cancer docetaxel sensitivity. In matched-benign and cancer epithelium derived from pre-treatment needle biopsies of prostate cancer patients participating in a preoperative neoadjuvant chemotherapy trial (1), we observed significant upregulations in two mitochondria tricarboxylic acid (TCA) cycle genes, malate dehydrogenase 2 (MDH2) and citrate synthase (CS), which encode enzymes that catalyze the respective first and last step of TCA cycle that is central to cellular oxidative energy metabolism and reduction-oxidation (redox) balance. Subsequently, in prostate cancer cell line LNCaP treated with docetaxel and transient siRNA knockdown of either MDH2 or CS, we observed that CS enhanced the efficacy of docetaxel, whereas MDH2 conferred resistance.

Our hypothesis is: MDH2 and CS have opposite effects on cellular redox and docetaxel-chemosensitivity in prostate cancer cells. The overexpression of MDH2 generates antioxidant and cytoprotective intermediate OAA, which reduces the damage induced by oxidative stress (including stress by docetaxel-chemotherapy), and protects cancer cells. In contrast, the overexpression of CS removes this intermediate. Therefore, the unique combination of low MDH2 and high CS (as seen in a subset of patients) predispose prostate cancer cells to chemotherapy via a redox-dependent mechanism.

Body:

We used qRTPCR to confirm the gene expression results from cDNA microarray analyses of prostate cancer cells obtained from patient needle biopsy. Of the total of 31 patients, a significant subset had upregulation in either the CS gene or the MDH2 gene in cancer cells compared to the matched benign prostate cells (Figure 1). To investigate the biological significance of MDH2 and CS in prostate cancer growth and response to docetaxel chemotherapy, we measured the protein expression of both genes in prostate cancer cell lines LNCaP, C42B and PC3, and prostate benign cell line BPH1. All cell lines express robust and similar levels of CS, on the other hand, MDH2 is overexpressed only in prostate cancer cell lines compared to BPH1 (Figure 2). Based on the results in Figure 2, we used lentivirus containing MDH2 shRNA to establish stable PC3, LNCaP and C42B cell lines with MDH2 knockdown (Figure 3). In the MDH2 knockdown (shMdh2) and shRNA control (shScr) cell lines, we performed cell proliferation analysis. We observed that MDH2 inhibition significantly reduced the cancer cell proliferation (Figure 4). The reduction in proliferation is also accompanied by the reduction of cellular ATP biosynthesis (Figure 5). To test the effect of MDH2 knockdown on cellular response to chemotherapy, we treated the –shScr and –shMdh2 cells with increasing doses of docetaxel. 48 hours after the treatment, the viable cells were quantitated. Significantly, the MDH2 knockdown cells exhibited higher sensitivity to docetaxel, and the docetaxel efficacy is significantly increased (Figure 6).



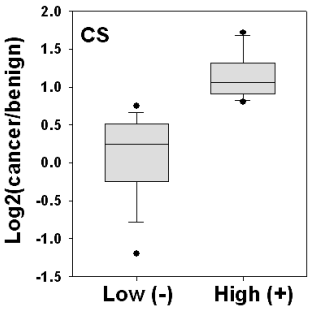
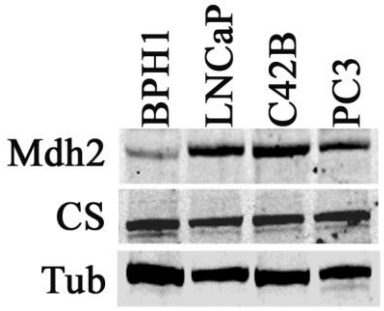


Figure 1: MDH2 and CS are upregulated in a subset of prostate cancer patients. Needle biopsy samples were processed by laser capture microdissection for prostate cancer and adjacent benign cells. The MDH2 and CS mRNA transcripts in cancer and benign cells were analyzed by qRT-PCR.

Figure 2: MDH2 and CS expressions in BPH1, LNCaP, C42B and PC3 cells were measured by western blots. Tubulin was sued as control.



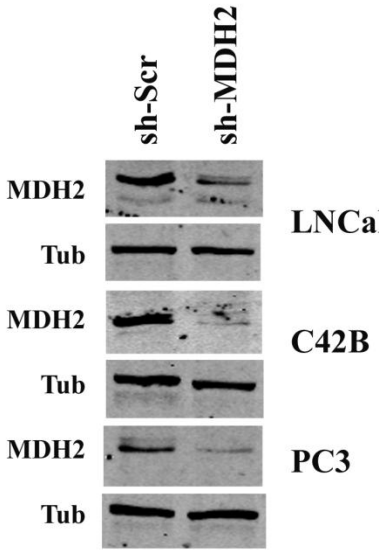
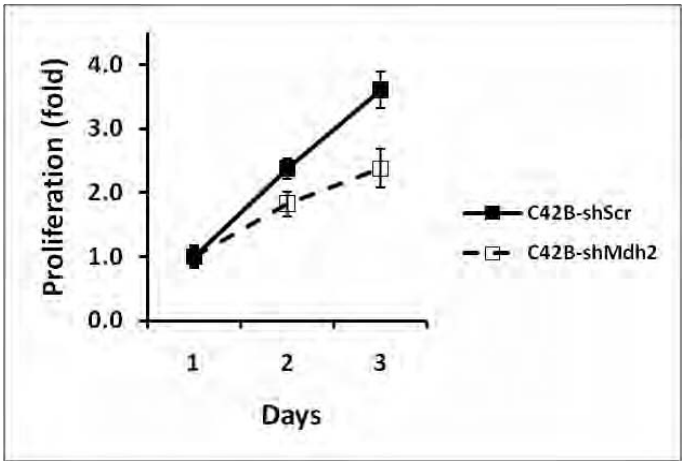


Figure 3: The MDH2 western blots showing MDH2 was effectively knocked down by lentivirus containing shRNA against MDH2. Tubulin (Tub) was used as control.

Figure 4: Cells were seeded and cultured in 6-well dish and counted daily for 3 days. The viable cell numbers are day 2 and day 3 were normalized to the day 1 values. N=4.



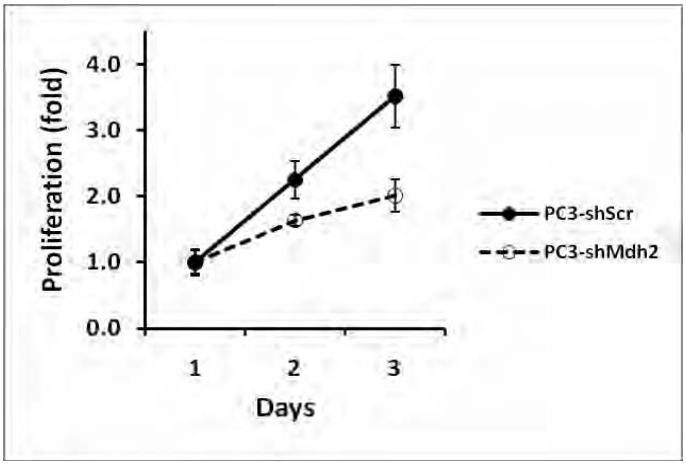


Figure 5: The PC3 and C42B cells were cultured in 96-well plates. The ATP and ADP levels were measured, n=4.

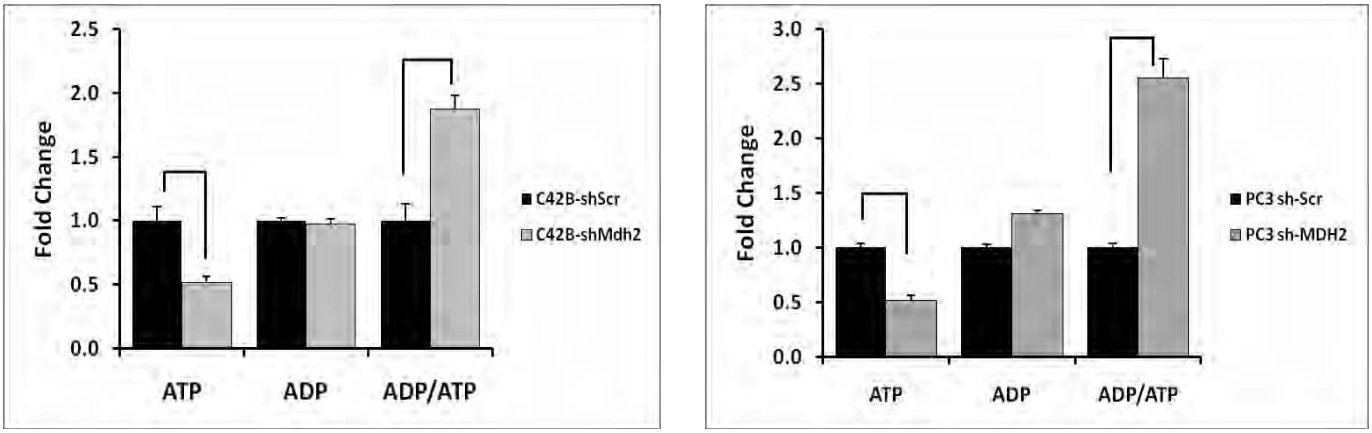
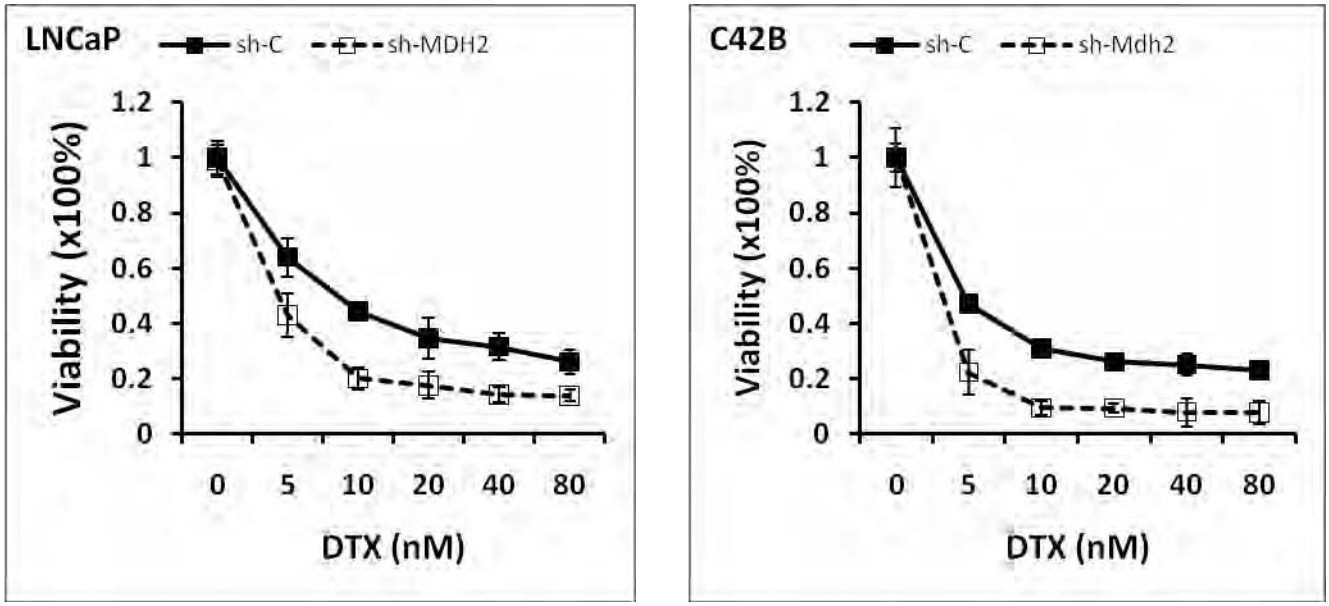
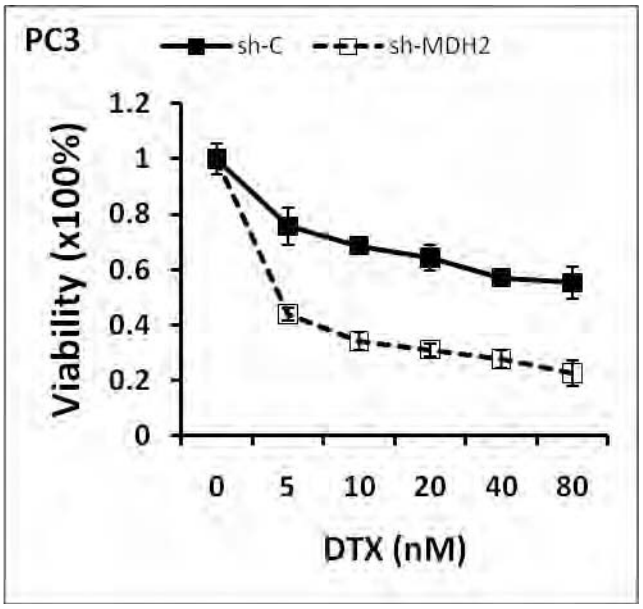


Figure 6: PC3, LNCaP and C42B cells were cultured in 24-well plate, treated with the indicated doses of docetaxel for 48 hours. The viable cells were counted and normalized to the solvent controls (0), n=4.





Key Research Accomplishments:

a. We validated the MDH2 and CS mRNA expression levels in patient needle biopsy samples.
b. We established stable prostate cancer cell lines (PC3, LNCaP and C42B) with MDH2 shRNA.
c. We observed that MDH2 plays an important role in regulating cellular energy production and proliferation.
d. We confirmed the original hypothesis that MDH2 is critical in regulating cellular resistance to docetaxel.

Reportable Outcomes:

a. MDH2 and CS are dysregulated (upregulation) in subset of clinical prostate cancers
b. MDH2 dysregulation contributes to the chemotherapy resistance

Conclusion:

These data confirms our hypothesis, and we will continue identifying the mechanism of MDH2-related chemoresistance. In addition, we will investigate the role of CS in prostate cancer growth and response to therapy.

Reference:

1. Qian DZ, Huang CY, O'Brien CA, Coleman IM, Garzotto M, True LD, Higano CS, Vessella R, Lange PH, Nelson PS, Beer TM. Prostate cancer-associated gene expression alterations determined from needle biopsies. Clin Cancer Res 15 (9): 3135-42, 2009.