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TITLE: The Role of Mitochondrial TCA Cycle Enzymes in Determining Prostate Cancer Chemosensitivity

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14. ABSTRACT Based on the in vitro results in preliminary data and in year #2 of the proposal (1), we conducted an in vivo xenograft mouse experiment using prostate cancer cell lines containing specific levels (endogenous, shRNA, and Flag-MDH2 compensation) of MDH2. We found that xenograft tumors are more sensitive to docetaxel chemotherapy when the endogenous MDH2 is inhibited by shRNA. In contrast, when the loss of MDH2 is compensated by Flag-MDH2 plasmid transfection, the drug sensitivity is lost. This suggests that MDH2 is important to in vivo chemotherapy sensitivity and resistance.					
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Introduction:

In previous funding cycles (year 1-2), we have determined that MDH2 overexpression in prostate cancer is associated with chemotherapy resistance in patients and in cancer cell lines. The results have been published (see reference 1). In the past funding cycle (year 3), we focused on the role of MDH2 in vivo. In vivo chemoresistance is driven by multiple mechanisms that both within the cancer cell and in the tumor microenvironment. A preclinical animal experiment enables us to more realistically evaluate the ability of MDH2 in contributing to docetaxel resistance in the context of these other mechanisms.

Body

In previous experiments (1), we have shown that prostate cancer cell line C42B contains high level of endogenous MDH2 level; we subsequently used shRNA to knockdown MDH2 expression (C42B-shMDH2) and found that the in vitro docetaxel sensitivity is significantly enhanced by shRNA. Then, we restored the MDH2 expression by transfecting a plasmid coding for the wild-type MDH2 (C42B-shMDH2 plus Flag-MDH2). We subcutaneously implanted these 3 types of C42B cell lines into nude mice, which represent MDH2 high (C42B), MDH2 low (C42B-shMDH2), and MDH2 high via molecular compensation (C42B-shMDH2-Flag-MDH2). When the tumors were palpable, we treated the mice with either vehicle (V) or docetaxel (D) (15 mg/kg, IP, weekly) for 4 weeks. Similar to the in vitro observation shown previously, the docetaxel efficacy is very limited in C42B tumors (25% inhibition docetaxel *versus* vehicle); MDH2 shRNA significantly increased the efficacy (70% inhibition), in contrast, Flag-MDH2 wild-type compensation restored the resistant phenotype (30% inhibition) (Figure 1). This result suggests that MDH2 plays a significant role in conferring docetaxel resistance in vivo.

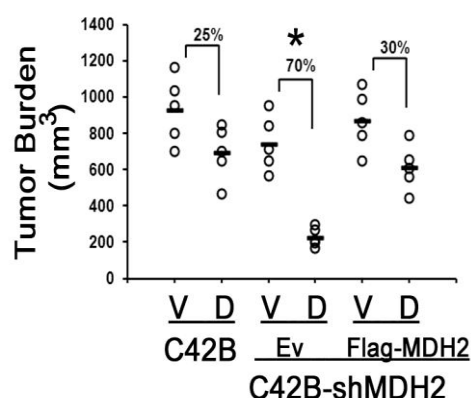


Figure 1. MDH2 confers docetaxel resistance in xenografts. C42B cells or C42B-shMDH2 that was transfected with Ev or Flag-MDH2 wild-type were injected into the flank of nude mice. After the tumors were palpable, mice were treated with vehicle (V) or docetaxel chemotherapy (D). Tumor volume was measured by a digital caliper three times a week. The tumor burden was calculated based on volume measurements. The tumor burden for each mouse at the end of 4 week treatment is shown. The antitumor efficacy of docetaxel in each cell line is shown as % by comparing the average tumor burdens between vehicle (V) and therapy (D). * $P < 0.05$, t-test, Ev vs. C42B or Flag-MDH2, $n=5$.

Key Research Accomplishments:

We have shown that MDH2 expression is critically important to docetaxel sensitivity and resistance in vivo.

Reportable Outcomes:

MDH2 mediates in vivo docetaxel resistance.

Conclusion:

Results in this cycle will provide the mechanistic rationale of targeting MDH2 to overcome in vivo docetaxel resistance in patients.

Reference

1. Prostate. 2013 Feb 6. doi: 10.1002/pros.22650. [Epub ahead of print]

Malate dehydrogenase 2 confers docetaxel resistance via regulations of JNK signaling and oxidative metabolism.

Liu Q, Harvey CT, Geng H, Xue C, Chen V, Beer TM, Qian DZ.

Oregon Health & Science University Knight Cancer Institute, Portland, Oregon.

BACKGROUND: Resistance to chemotherapy represents a significant obstacle in prostate cancer therapeutics. Novel mechanistic understandings in cancer cell chemotherapeutic sensitivity and resistance can optimize treatment and improve patient outcome. Molecular alterations in the metabolic pathways are associated with cancer development; however, the role of these alterations in chemotherapy efficacy is largely unknown. **METHODS:** In a bed-side to bench-side reverse translational approach, we used cDNA microarray and qRT-PCR to identify genes that are associated with biochemical relapse after chemotherapy. Further, we tested the function of these genes in cell proliferation, metabolism, and chemosensitivity in prostate cancer cell lines. **RESULTS:** We report that the gene encoding mitochondrial malate dehydrogenase 2 (MDH2) is overexpressed in clinical prostate cancer specimens. Patients with MDH2 overexpression had a significantly shorter period of relapse-free survival (RFS) after undergoing neoadjuvant chemotherapy. To understand the molecular mechanism underlying this clinical observation, we observed that MDH2 expression was elevated in prostate cancer cell lines compared to benign prostate epithelial cells. Stable knockdown of MDH2 via shRNA in prostate cancer cell lines decreased cell proliferation and increased docetaxel sensitivity. Further, MDH2 shRNA enhanced docetaxel-induced activations of JNK signaling and induced metabolic inefficiency. **CONCLUSION:** Taken together, these data suggest a novel function for MDH2 in prostate cancer development and chemotherapy resistance, in which MDH2 regulates chemotherapy-induced signal transduction and oxidative metabolism. Prostate © 2013 Wiley Periodicals, Inc.

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