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Ketone Body Metabolic Enzyme OXCT1 Regulates Prostate Cancer Chemoresistance

PRINCIPAL INVESTIGATOR: Dr. Qiong Liu

CONTRACTING ORGANIZATION: Oregon Health & Science University
Portland, Oregon, 97239

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6. AUTHOR(S)
Qiong Liu

E-Mail: liuqio@ohsu.edu, liuqiong082@gmail.com

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon Health & Science University
3303 SW Bond Ave
Portland, OR 97239

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14. ABSTRACT

OXCT1 is a key enzyme in ketone body metabolism and cellular energy homeostasis. Analysis of patient data indicated that higher OXCT1 levels are associated with docetaxel chemotherapy resistance. In order to investigate the role of OXCT1 in prostate cancer chemotherapy resistance and the underlying mechanisms, we established OXCT1 stable knock down prostate cancer cell lines. The results revealed that lowering OXCT1 level using shRNA knock down increased docetaxel chemosensitivity in prostate cancer cells. Lowering OXCT1 enhanced docetaxel induced JNK signaling pathway activation and downstream apoptotic signaling. OXCT1 knock down increased ADP/ATP, NAD^+/NADH and oxygen consumption in docetaxel treated cells compared to control knock down cells, therefore induced metabolic inefficiency. These results confirmed our hypothesis that OXCT1 plays important role in prostate cancer chemotherapy sensitivity and revealed the mechanisms.

15. SUBJECT TERMS
chemosensitivity, OXCT1, docetaxel, metabolism, JNK, ketone body

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1. Introduction

Ketone bodies are important alternative energy source other than glucose and fatty acids. The role of ketone body utilization pathway in regulating prostate cancer resistance to docetaxel-based chemotherapy has never been tested. OXCT1 encodes the rate limiting enzyme converting ketone bodies to acetyl-CoA. The goal of this project is to investigate the role of OXCT1 in prostate cancer chemotherapy sensitivity and the underlying molecular mechanisms.

2. Keywords

Chemosensitivity, OXCT1, docetaxel, metabolism, JNK, ketone body

3. Overall Project Summary

Current Objectives

To determine the role of OXCT1-mediated ketone body utilization in regulating prostate cancer cell response to docetaxel, cellular metabolism, and redox balance and investigate the molecular mechanisms.

Results

3.1 OXCT1 level is associated with chemoresistance in prostate cancer patients

Analysis of prostate cancer needle biopsy samples revealed that the OXCT1 gene was over-expressed in nearly 50% of patients (1). Kaplan-Meier survival analysis indicated that higher OXCT1 level was associated with lower relapse free survival rate (Fig. 1). Further analysis showed that in prostate cancer cells OXCT1 level was higher than benign cells (Fig. 2). This suggests that OXCT1 over-expression or ketone body utilization pathway may promote prostate cancer cells malignancy and mediate prostate cancer chemotherapy resistance.

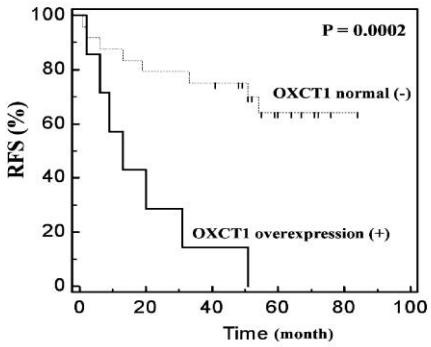


Figure 1. Relapse-free survival (RFS) based on pre-treatment OXCT1 mRNA in LCM-cancer compared to matched benign epithelium. n=31.

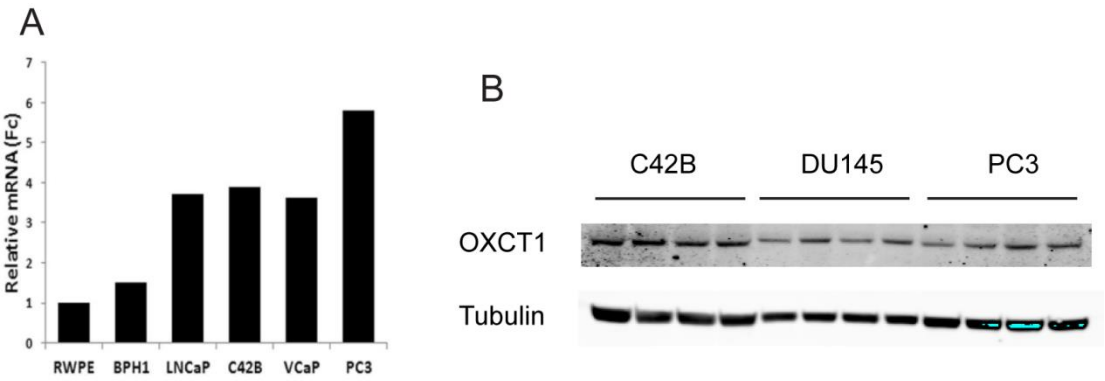


Figure 2. A. OXCT1 mRNA in prostate benign (RWPE and BPH1) and cancer cell lines. B. OXCT1 protein in prostate cancer cell lines.

3.2 OXCT1 knock down increased docetaxel chemosensitivity in prostate cancer cells

To further investigate the role of OXCT1 in docetaxel chemosensitivity, we established OXCT1 stable knockdown cell lines in prostate cancer cells lines C42B and DU145 (Fig. 3).

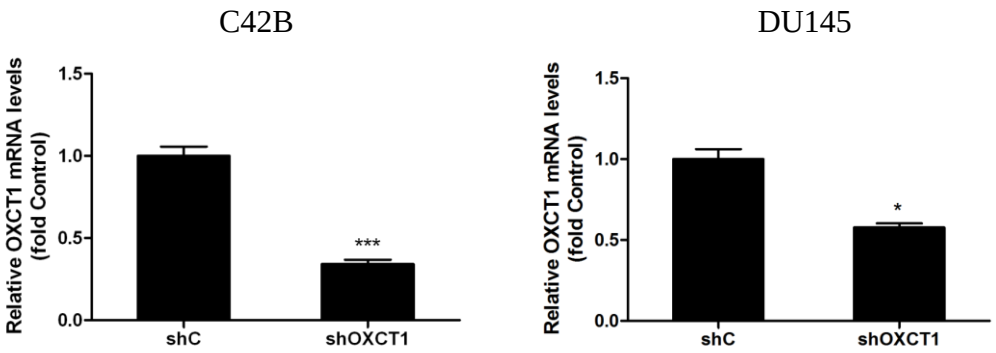


Figure 3. OXCT1 knock down stable cell lines. mRNA levels of OXCT1 with non-target control (shC) and OXCT1 (shOXCT1) stable knockdown in C42B and DU145 prostate cancer cells. *p<0.05, ***p<0.001.

Next, we measured the cell viability in OXCT1 knock down prostate cancer cells after 0-40 nM docetaxel treatment using the optimal docetaxel dose and time determined previously in annual report. The cell viability was significantly lower in OXCT1 knock down cells (shOXCT1) after 48 h docetaxel treatment than that in non-target control knockdown cells (shC, Fig. 4) in both C42B and DU145 cell lines. The results suggested that knock down of OXCT1 increased docetaxel sensitivity in prostate cancer cells.

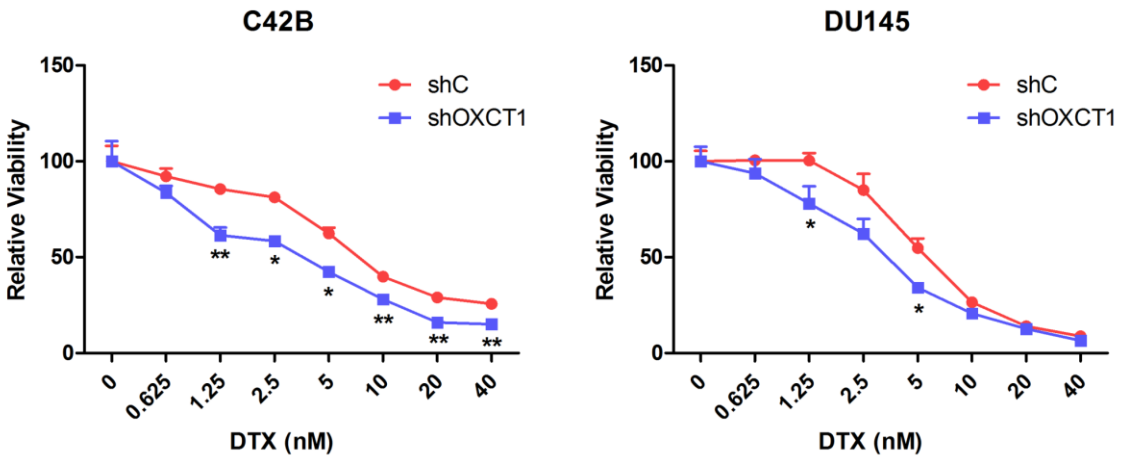


Figure 4. OXCT1 knock down increased docetaxel sensitivity in prostate cancer cells. Cell viability in shC and shOXCT1 prostate cancer cells were measured after 0 - 40 nM docetaxel (DTX) treatment for 48 h. shC or shOXCT1 cells without DTX treatment were set as 100, respectively. *p<0.05, **p<0.01.

3.3 OXCT1 knock down enhanced docetaxel induced JNK signaling pathway activation

Docetaxel treatment could lead to the activation of JNK signaling pathway which mediates cell apoptosis pathways [2, 3]. To investigate the mechanism of increased docetaxel sensitivity in OXCT1 knock down cells, we examined JNK signaling pathway activation in DU145 cells. The results showed that, as expected, docetaxel treatment activated the

JNK signaling cascade as seen in ATF2, JNK1/2, and c-Jun phosphorylation in the non-targeting control shRNA cells (shC). Importantly, in OXCT1 knockdown cells, the docetaxel-induced phosphorylation of ATF2, JNK1/2, and c-Jun were further increased (Fig. 5). Consistent with enhanced JNK signaling, OXCT1 knock down further increased PARP cleavage upon docetaxel treatment (Fig. 5). These results suggested that OXCT1 knock down enhance docetaxel induced JNK signaling pathway activation and the downstream apoptotic pathway.

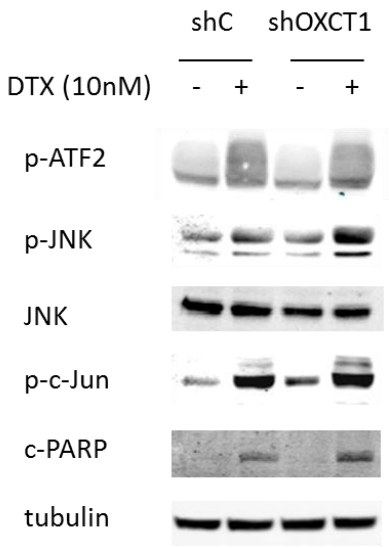


Figure 5. OXCT1 knock down enhanced docetaxel induced JNK signaling pathway activation and the downstream apoptotic pathway. DU145 cells were treated with 10 nM docetaxel for 24 h and cell lysates were analyzed by western blot using indicated antibodies.

3.4 OXCT1 knock down induced metabolic inefficiency upon docetaxel treatment

Since OXCT1 is a metabolic enzyme involved in energy homeostasis, next, to investigate the mechanism of increased docetaxel sensitivity in OXCT1 knock down cells, we measured ATP and ADP levels and determined ADP/ATP ratio in cells. The results showed that docetaxel treatment did not alter ADT/ATP ratio in control knock down cells, while in OXCT1 knock down cells, docetaxel treatment significantly increased ADP/ATP ratio (Fig. 6).

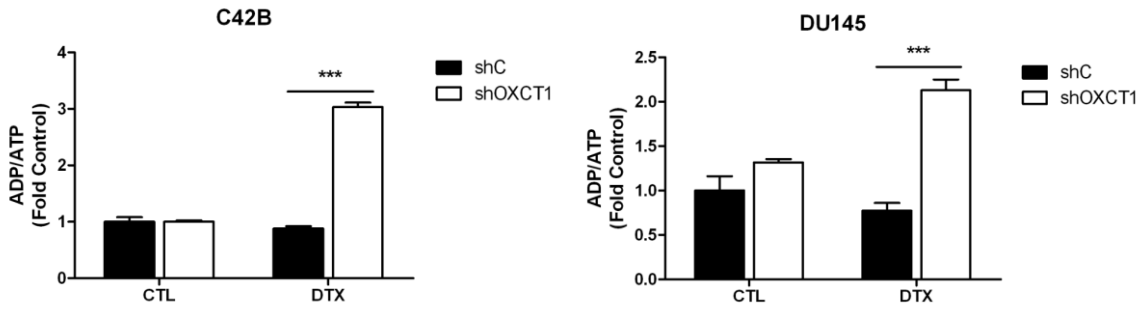
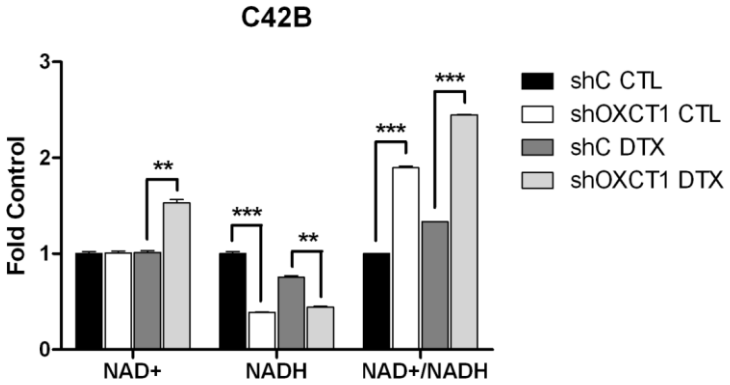


Figure 6. OXCT1 knock down increased ADP/ATP ratio after docetaxel treatment. ADP/ATP ratio was measured in shC and shOXCT1 C42B and DU145 stable cell lines after docetaxel treatment (10 nM, 24 h). ***p<0.001.

NADH is the metabolic substrate for mitochondrial oxidative phosphorylation and ATP biosynthesis. Next, we examined NAD^+/NADH levels in OXC1 knock down prostate cancer cells. The results showed that after docetaxel treatment, NAD^+ level was significantly increased in OXCT1 knock down cells compared to control knock down cells, while NADH levels was decreased in shOXCT1 cells compared to shC cells and NAD^+/NADH ratio was significantly increased in shOXCT1 cells compared to shC cells (Fig. 7).



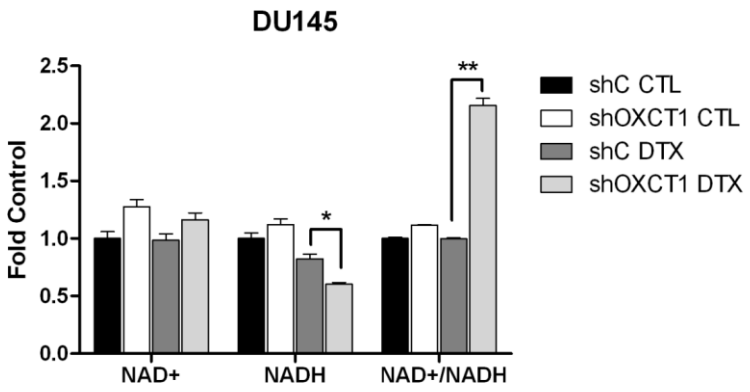


Figure 7. OXCT1 knock down increased NAD^+/NADH ratio after docetaxel treatment. NAD^+, NADH and NAD^+/NADH ratio were determined in C42B and DU145 cells with or without docetaxel treatment (5 nM, 24 h). *p<0.05, **p<0.01, p<0.001.

The above results suggested that OXCT1 knock down reduced mitochondrial oxidative phosphorylation upon docetaxel treatment. Next, to investigate whether this is associated with altered cellular respiration, we measured oxygen consumption in prostate cancer cells after docetaxel treatment. The results showed that upon docetaxel treatment oxygen consumption was significantly increased in OXCT1 knock down cells compared with control knock down cells in both C42B and DU145 cells (Fig. 8). Taken together, the increased oxygen consumption but decreased ATP and NADH production indicated that OXCT1 knock down induced metabolic inefficiency upon docetaxel treatment compared to control knock down cells.

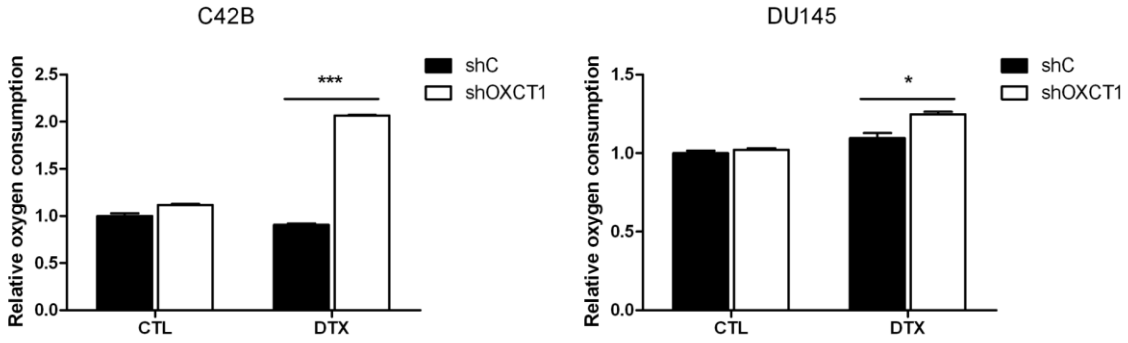


Figure 8. OXCT1 knock down increased oxygen consumption after docetaxel treatment. Oxygen consumption rate was measured in C42B and DU145 cells using a Clark-type electrode with or without docetaxel treatment (10 nM, 24 h). *p<0.05, **p<0.01, p<0.001.

3.5 The role of ketone body supplementation in docetaxel chemosensitivity

To further test the above hypothesis, we analyzed the effect of ketone body on cellular metabolism. The results showed siRNA knock down of OXCT1 induced similar growth inhibition in PC3 prostate cancer cells (Fig. 9A). Treatment of PC3 cells with acetoacetate increased cellular ATP levels (Fig. 9B) and decreased reactive oxygen species (ROS) levels (Fig. 9C), indicating that supplementation of ketone body improved cellular metabolism.

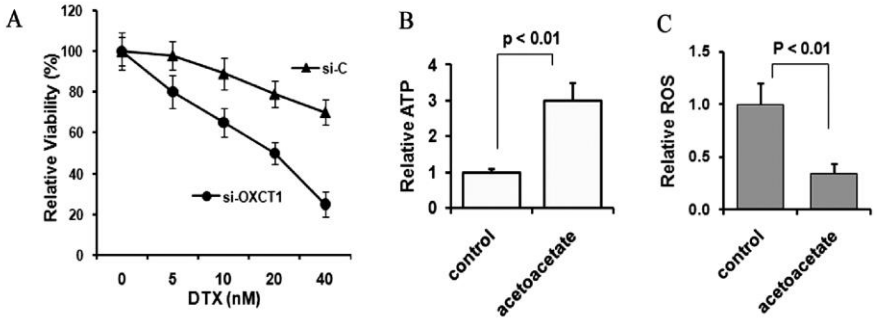


Figure 9 A. Cell viability in PC3 cells after 48h docetaxel treatment. Cells were treated with 1mM acetoacetate for 24h, then B. ATP and C. ROS levels were measured.

We next investigated whether supplementation of ketone body could abolish the enhanced docetaxel sensitivity in OXCT1 knock down cells. We find that the ketone body Lithium Acetoacetate did not affect cell viability in low concentrations, but decreased cell viability in high concentrations, however, this may be due to the toxic effects of the compound's acidity (Fig. 10 upper). Based on the above results, next, we chose 3 Lithium Acetoacetate concentrations (low, medium and high) and combined them with docetaxel to evaluate the effect of ketone body on chemosensitivity in shOXCT1 cells. The results showed that docetaxel decreased cell viability in both shC and shOXCT1 cell lines, while Lithium Acetoacetate did not alter the effect of docetaxel (Fig. 10 lower), suggesting that supplementation of ketone body alone may not alter docetaxel sensitivity in OXCT1 knock down cells.

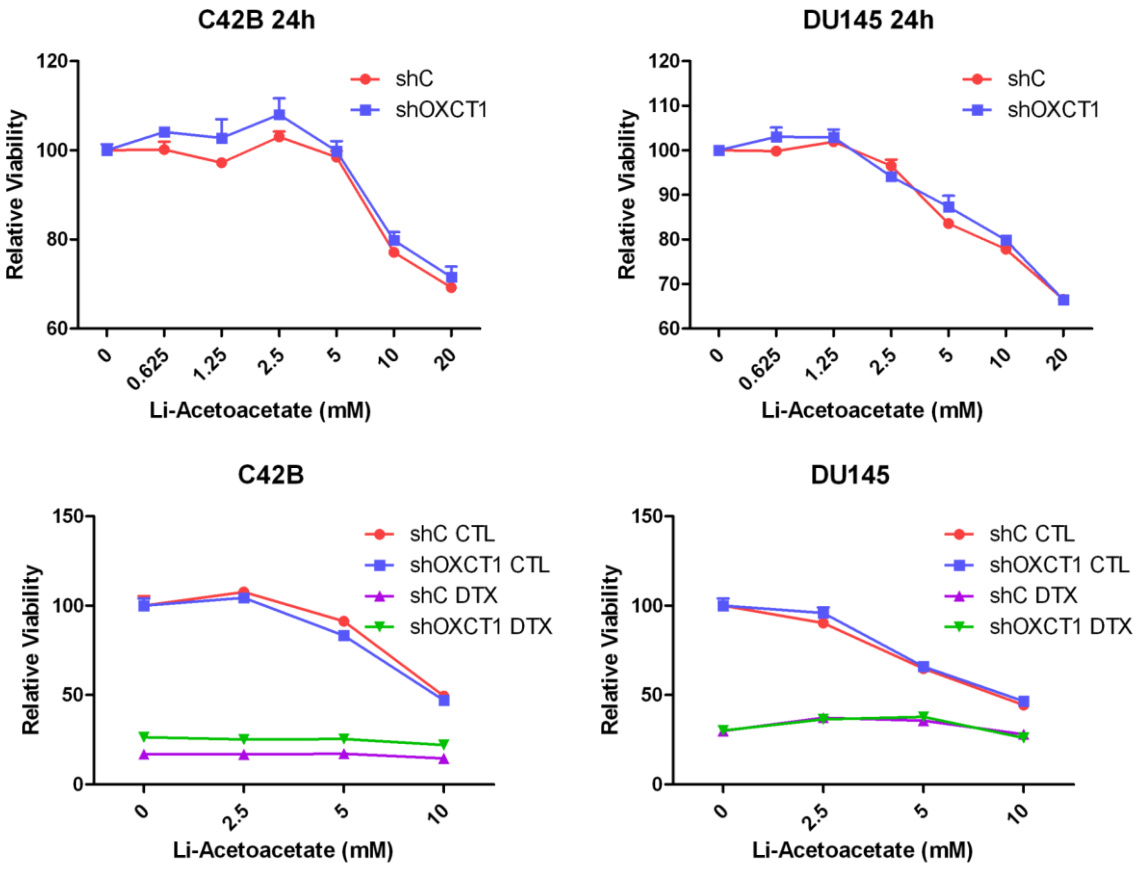


Figure 10. Supplementation of ketone body alone could not alter docetaxel sensitivity in OXCT1 knock down cells. Cells were treated with different concentrations of Lithium Acetoacetate (Li-Acetoacetate) without (upper) or with (lower) docetaxel (5 nM) for 24 h, and cell viability were measured using Cyto-60 staining method. shC or shOXCT1 cells without Li-Acetoacetate treatment were set as 100, respectively.

Progress and Accomplishments

We established OXCT1 stable knock down prostate cancer cell lines and evaluated docetaxel sensitivity in these cell lines. JNK signaling pathway and apoptosis pathway were evaluated. Cellular metabolic endpoints including ADT/ATP, NAD^+/NADH and oxygen consumption were measured. The role of ketone body supplementation in cellular metabolism and enhanced docetaxel chemosensitivity in OXCT1 knock down cells were evaluated. The results revealed that OXCT1 levels were associated with higher docetaxel sensitivity, lowering OXCT1 level using shRNA knock down increased docetaxel chemosensitivity in prostate cancer cells. Lowering OXCT1 enhanced docetaxel induced

JNK signaling pathway activation and downstream apoptotic signaling. OXCT1 knock down induced metabolic inefficiency in docetaxel treated prostate cancer cells. Supplementation of ketone bodies could improve metabolic efficiency but could not alter cell viability in OXCT1 knock down cell treated with docetaxel compared to control shRNA knock down cells.

Discussion

OXCT1 is a key enzyme in ketone body metabolism and cellular energy homeostasis. Analysis of our previous data from patient needle biopsy samples indicated that higher OXCT1 levels are associated with docetaxel chemotherapy resistance. In addition, we found that OXCT1 levels were higher in prostate cancer cells. In order to investigate the role of OXCT1 in prostate cancer chemotherapy resistance, we established OXCT1 stable knock down prostate cancer cell lines with distinct resistance mechanisms. For example, PC3 and DU145 cells without androgen receptor are highly resistant to docetaxel compared to C42B cells. The results showed that docetaxel chemosensitivity was increased in OXCT1 knock down cells compared to control knock down cells. We further investigated the mechanisms. JNK signaling pathway was reported to mediate docetaxel cytotoxicity, our results showed that OXCT1 knock down enhanced docetaxel induced JNK signaling pathway activation and the downstream apoptosis pathway. Since OXCT1 is a key enzyme involved in energy metabolism, we measured metabolic endpoints. The results showed that OXCT1 knock down increased ADP/ATP, NAD^+/NADH and oxygen consumption in docetaxel treated cells compared to control knock down cells, therefore induced metabolic inefficiency. Ketone body supplementation increased metabolic endpoints, however, ketone body did not alter cell viability in OXCT1 knock down cell compared to control knock down cells upon docetaxel treatment. This could be due to the interference of chemical substances in cell culture medium and needs further investigation. This could also be caused by cellular mechanisms involving other enzymes in ketone body metabolism which balance excessive ketone bodies. Taken together, our results suggested that lowering OXCT1 levels by shRNA in prostate cancer cells increased docetaxel chemosensitivity through increasing JNK signaling pathway and inducing metabolic inefficiency. Our work indicated that lowering OXCT1 could be developed as a new therapeutic strategy in prostate cancer chemotherapy resistance.

4. Key Research Accomplishments

a. OXCT1 was associated with prostate cancer chemotherapy resistance in patients, and higher OXCT1 levels were detected in prostate cancer cell lines.

b. Lowering OXCT1 increased prostate cancer cell chemosensitivity to docetaxel.

c. Lowering OXCT1 increased chemosensitivity through increasing JNK signaling pathway and inducing metabolic inefficiency.

5. Conclusion

The current work confirms the hypothesis that OXCT1 regulates prostate cancer cell chemotherapy resistance, and revealed the underlying mechanisms, suggesting a new potential therapeutic strategy to improve prostate cancer chemotherapy.

6. Publications, Abstracts, and Presentations

Nothing to report

7. Inventions, Patents and Licenses

Nothing to report

8. Reportable Outcomes

a. OXCT1 levels are upregulated in a subset of prostate cancer patients and prostate cancer cell lines.

b. OXCT1 upregulation was associated with prostate cancer chemotherapy resistance. Lowering OXCT1 increased docetaxel chemosensitivity in prostate cancer cells.

c. Knock down of OXCT1 increased JNK signaling pathway and induced metabolic inefficiency upon docetaxel treatment.

9. Other Achievements

OXCT1 stable cell lines were developed.

10. References

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3. Mhaidat NM, Zhang XD, Jiang CC, Hersey P. Docetaxel induced apoptosis of human melanoma is mediated by activation of c-Jun NH2-terminal kinase and inhibited by the mitogen-activated protein kinase extracellular signal-regulated kinase 1/2 pathway. Clin Cancer Res 2007;13(4):1308–1314.

11. Appendices

None.

12. Training and Development Opportunities

I have frequent one-on-one work with mentor, which greatly improved my scientific skills including experimental and analytical as well as scientific writing skills. I attended AACR (American Association for Cancer Research) 2014 annual meeting with the support of this grant, which helped to extend my professional networks and research resources. I also participated in various academic seminars in OHSU, which brought new insights from accomplished scientists. I finished manuscript writing and revision under supervision of the supervisor. I attended business writing classes offered by OHSU, which greatly improved my writing skills. I attended workshops for professional development such as applying for jobs and grant writing offered by OHSU, which greatly helped to develop my career.