Attenuating glucose metabolism by Fbxw7 promotes Taxol sensitivity of colon cancer cells through downregulating NADPH oxidase 1 (Nox1)

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Background: Colorectal cancer (CRC), one of the most common malignancies worldwide, is associated with poor survival and has a high mortality rate. Taxol is a chemotherapeutic agent that has been clinically applied as a first-line drug against diverse cancers. Yet, development of drug resistance has become the major challenge for anti-cancer treatments. F-box and WD40 domain protein 7 (Fbxw7) is a known tumor suppressor which is frequently downregulated in cancers. However, the biological roles and mechanisms of Fbxw7 in Taxol resistance are still under investigation.

Methods: We report that Fbxw7 is significantly inactivated in CRC tumors and cell lines compared with normal tissues and colon cells. Expressions of Fbxw7 and Nox1 were detected from human colon tumors and cells by qRT-PCR and Western blot. Glycolysis rate was assessed by glucose uptake and lactate product assay. Interactions between Fbxw7 and Nox1 were determined by co-immunoprecipitation (Co-IP). Chemosensitivity and resistance of colon cancer cells were determined by MTT assay and Annexin V-FITC assay.

Results: Overexpression of Fbxw7 sensitized colon cancer cells to Taxol. Moreover, we observed a negative correlation between Fbxw7 and glucose metabolism. From the established Taxol-resistant (TR) cell line from HCT-116, Fbxw7 was found to be markedly downregulated in HCT-116 TR cells. We detected that NADPH oxidase 1 (Nox1), a superoxide-generating NADPH oxidase, is negatively regulated by Fbxw7. The Co-IP assay showed that Fbxw7 interacted with Nox1, which was observed to be significantly upregulated in CRC tissues. Nox1 therefore promotes the Taxol resistance and glucose metabolism of colon cancer cells. Finally, rescue experiments demonstrated that the Fbxw7-promoted Taxol sensitivity was partially through the Nox1-glycolysis axis. Restoration of Nox1 in Fbxw7-overexpressed TR colon cancer cells significantly recovered the Taxol resistance, which could be further overridden by glycolysis inhibition.

Conclusions: Collectively, this study uncovered that targeting the Fbxw7-Nox1-glucose metabolism axis could be an effective strategy against chemoresistant colon cancer.

Keywords: Colorectal cancer (CRC); Taxol sensitivity; F-box and WD40 domain protein 7 (Fbxw7); NADPH oxidase 1 (Nox1)

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Introduction

Colorectal cancer (CRC), one of the leading causes of cancer-related death, is associated with poor survival and has a high mortality rate (1). Currently, surgery is an effective therapeutic approach for CRC at the early stages (2). In addition, radiotherapy and chemotherapy are the most common treatment methods for CRC, particularly at the advanced and metastatic stages (3). Paclitaxel (Taxol) is one of the most commonly used chemotherapeutic agents, and is the first-line treatment for multiple cancers (4). Taxol is known to directly target the microtubules of the mitotic spindle to impede chromosome processes, leading to the induction of apoptosis pathways (5). Moreover, Taxol is an inhibitor of cell replication and migration through arresting cells in the late G2/M phase of the cell cycle (6). Despite the fact that better outcomes have been achieved by chemotherapy over recent decades, a large fraction of CRC patients develop drug resistance, leading to disappointing survival rates of CRC (7). However, the molecular mechanisms for the development of Taxol resistance in CRC remain largely unknown. Thus, investigation of effective approaches against chemoresistance is an urgent task to improve the therapeutic outcomes of colon cancer patients.

F-box and WD40 domain protein 7 (Fbxw7), a conserved F-box WD40 protein, plays an important role in recognizing subunits of the SKP1/CUL1/F-box protein (SCF) E3 ubiquitin ligase (8). Accumulating evidence has revealed that Fbxw7 targets a network of oncoproteins which regulate tumorigenesis and progression for ubiquitination and proteasome degradation (9). Moreover, multiple cancer-associated mutations of Fbxw7 have been detected in cancers (10), leading to favoriting tumor progression. However, a precise understanding of the biological roles and molecular targets of Fbxw7 in Taxol sensitivity is still under investigation.

NOXs are a family of membrane-associated enzymes which catalyze the oxidation of NADPH or NADH to NADP+ or NAD+ (11). NADPH oxidase 1 (Nox1) belongs to the NOX family and is a multifunctional protein participating in diverse tumor processes, including tumorigenesis, invasion, metastasis, and drug resistance (12). Remarkably, accumulating studies have demonstrated that Nox1 functions as an oncogenic protein with frequent upregulation in diverse tumors (13), indicating that Nox1 could be a therapeutic target for anti-CRC treatments. This study aims to investigate the biological roles and molecular mechanisms of Fbxw7 in Taxol resistance of colon cancer. Further results demonstrated that Fbxw7 is a negative regulator of the Nox1 protein, which is positively associated with colon cancer and Taxol resistance by promoting glucose metabolism. Our observations uncovered that the Fbxw7-mediated Taxol sensitivity was through the Nox1-glucose metabolism axis. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-21-2076).

Methods

Patient sample collection

This study was approved by the Ethics Committee of The Fifth People’s Hospital of Shanghai, Fudan University. Forty human CRC specimens and the matched adjacent non-tumorous colon tissues were collected from CRC patients who underwent surgical removal in The Fifth People’s Hospital of Shanghai from March 2016 to October 2018. All patients were histopathologically examined as CRC and did not receive other surgery or chemo/radiotherapy. After dissection, specimens were immediately placed in liquid nitrogen and transferred to a –80 °C freezer for storage. All patients signed the informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cell culture and reagents

Human normal colon cell lines, CRL-1790 and CCD-18co, and CRC cell lines, HT29, HCT116, DLD-1, and LoVo, were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA). The cells were cultured in RPMI medium (HyClone, MA, USA) with 10% fetal bovine serum (FBS, Gibco, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, MA, USA). Cells were maintained in a humidified incubator at 37 °C in an atmosphere with 5% carbon dioxide. The HCT-116 Taxol-resistant (TR) colon cancer cell line was established by exposing cells to increased concentrations of Taxol for 3 months to select surviving cells. Rabbit Fbxw7 antibody was purchased from Sigma-Aldrich (#AB10620, Shanghai, China). Rabbit Nox1 antibody was purchased from Abcam (#ab78016, Shanghai, China). Rabbit β-actin antibody was purchased from Cell Signaling Technology (#4970, Danvers, MA, USA). Paclitaxel (Taxol) and 2-DG were purchased from Sigma-Aldrich (#ab78016, Shanghai, China). Rabbit Nox1 antibody was purchased from Cell Signaling Technology (#4970, Danvers, MA, USA). Paclitaxel (Taxol) and 2-DG were purchased from Sigma-Aldrich (Shanghai, China).
Transfection

Transfection of plasmid DNA or siRNA into colon cancer cells was performed using Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) according to the manufacturer’s instructions. Plasmid DNA was transfected at 1 μg/mL, and siRNA was transfected at 50 nM. After 48 h, cells were collected for downstream experiments. Transfection was performed in triplicate.

RNA isolation and qRT-PCR

Total RNA was isolated using the Trizol reagent (Invitrogen, USA) according to the manufacturer’s instructions. The quality and concentrations of RNA samples were examined using a NanoDrop™ 2000/2000c Spectrophotometer (Thermofisher, Waltham, MA, USA). The cDNA was synthesized from total RNA using the SuperScript II Reverse Transcriptase (Invitrogen, USA). The qRT-PCR reactions were performed using the SYBR Green PCR Master Mix (Thermofisher, Waltham, MA, USA). The reaction conditions were as follows: 95 ℃ for 3 min, followed by 40 cycles of 95 ℃ for 30 sec and 60 ℃ for 30 sec. Primer sequences were as follows: Fbxw7 forward: 5’-CGAACTCCAGTAGTATTGTGGACCT-3’ and reverse: 5’-TTCTTTTTCATTTTTGTGTTTTTGTATAGA-3’; Nox1 forward: 5’-GCCTGTGCCCGAGCGTCTGC-3’ and reverse: 5’-ACCAATGCCGTGAATCCCTAAGC-3’; GAPDH forward: 5’-GCCGCATCTTCTTTTGCGTCGC-3’ and reverse: 5’-TCCCGTTCTCAGCCTTGACGGT-3’. The relative gene expression was calculated by the 2–ΔΔCt method. Experiments were performed in triplicate and repeated three times.

Cell viability assay

After treatment with Taxol, cell viabilities were examined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer’s instructions (Sigma-Aldrich Co., St. Louis, MO, USA). Briefly, cells (8×10^3/well) were seeded in a 96-well plate for 24 h. After treatment, 0.025 mL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added and incubated for 4 h. Cells were then lysed with DMSO for 2 h at 37 ℃. The optical density (OD) value was detected at 570 nm on a 96-well multi-scanner plate reader (Biotek, USA). Experiments were performed in triplicate and repeated three times.

Clonogenic assay

Colon cancer cells (1×10^5/well) were seeded into six-well plates. After treatment with Taxol for 48 h, the medium was replaced, and cells were cultured for 7 days in drug-free medium. Cells were fixed by 4% paraformaldehyde (PFA) and stained with a 10% crystal violet solution for 10 min at room temperature. After washing by PBS, colonies were photographed under microscopy. Experiments were repeated three times.

Cell apoptosis analysis

Cell apoptosis/death was examined using an Annexin V apoptosis Detection Kit I from BD Biosciences (San Jose, CA, USA) following the manufacturer’s protocols. Colon cancer cells (2×10^5/well) were cultured in 6-well plates for 24 h. After treatment with indicated concentrations of Taxol or 2-DG, cells were collected and washed with PBS followed by adding the binding buffer (500 μL) to resuspend the cells. The Annexin-FITC (5 μL) and propidium iodide (PI) (5 μL) solutions were added to cells which were then incubated with the mixture for 15 min at room temperature. The assay was performed with FACS Calibur flow cytometry (Becton Dickinson, San Jose, CA, USA) and analyzed by Flowjo software. Experiments were performed in triplicate and repeated three times.

Measurements of glucose metabolism

The glucose metabolism of colon cancer cells was determined by the glucose uptake assay and the lactate production assay. The glucose uptake assay was performed using the Glucose Uptake Colorimetric Assay Kit (#MAK083, Sigma-Aldrich, Shanghai, China). The lactate production assay was performed according to previous reports (14). Experiments were performed in triplicate and repeated three times. Results were normalized by cell numbers of each reaction.

Western blotting

Proteins from colon cancer cells were extracted by adding 100 μL RIPA lysis buffer with 1x protease inhibitor cocktail (Beyotime, Shanghai, China) on ice for 15 min. Lysates were then centrifuged at 10,000 × g at 4 ℃ for 10 min. The protein concentration from each sample was determined by the Bradford method. Subsequently, 20 μg protein of each sample was resolved by electrophoresis with 10%
polyacrylamide gels. Proteins were then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, MO, USA), which were blocked by 4% non-fat milk in PBS with Tween 20 (PBST). The PVDF membranes were incubated with specific primary antibodies (1:1,000) at 4 °C for overnight. Membranes were washed by PBST for 3 times with 10 min each and incubated with HRP-conjugated secondary antibody at 1:3,000 at room temperature for 1 h. The enhanced chemiluminescent substrate (Millipore, MO, USA) was applied to visualize the protein bands. Experiments were repeated three times.

Data analysis
Statistical analysis was performed using the GraphPad Prism 6.0 software. Data were expressed as mean ± SD. The unpaired Student’s t-test and one-way analysis of variance (ANOVA) were applied to analyze the differences between the two groups and among three groups, respectively. A statistical difference of P<0.05 was considered significant.

Results
Fbxw7 is downregulated in CRC and correlates with Taxol sensitivity
Previous studies have revealed a positive association between Fbxw7 and improved prognosis and survival rates in diverse cancers (9,10), suggesting that Fbxw7 functions as a tumor suppressor in colon cancer. To evaluate the biological roles of Fbxw7 in colon cancer and chemosensitivity, we compared the expression of Fbxw7 in CRC tumors and their adjacent normal tissues. Expectedly, qRT-PCR results showed that Fbxw7 mRNA expression was significantly attenuated in colon tumor tissues (Figure 1A). Moreover, expression levels of Fbxw7 in four human colon cancer cell lines, HT-29, HT116, DLD-1, and LoVo, were significantly downregulated compared with the two normal colon cell lines, CRL-1790 and CCD-18co (Figure 1B). To assess the roles of Fbxw7 in regulating Taxol sensitivity of CRC cells, Fbxw7 was overexpressed in HCT-116 and HT-29 cells by transfection with a control or Fbxw7 overexpression plasmid (Figure 1C). Colon cancer cells were treated with gradually increased concentrations of Taxol. Results from Figure 1D,E consistently showed that HCT-116 cells with higher Fbxw7 expression were more sensitive to Taxol. The IC_{50} of HCT-116 and HT-29 cells were 9.2 and 8.7 nM, respectively. CRC cells with Fbxw7 overexpression had decreased IC_{50}s at 3.9 and 4.1 nM, respectively (Figure 1D,F). The Fbxw7-promoted Taxol sensitivity of HT-29 cells was further validated by the Annexin V/FITC cell apoptosis assay (Figure 1G).

Fbxw7 attenuates glucose metabolism of colon cancer cells
Metabolic reprogramming in response to cellular stresses as well as chemotherapeutic drugs is a known hallmark of cancer cells (15). Bioinformatics analysis and the glucose metabolism assay validated that compared with normal colon tissues and cells, CRC displayed significantly upregulated glucose metabolism enzyme expression and glucose metabolism rate (Figure S1A,B,C,D). We further characterized the roles of Fbxw7 in cellular glucose metabolism of CRC cells. As we expected, overexpression of Fbxw7 effectively inhibited glucose uptake and lactate production (Figure 1H,I), two key steps of glucose metabolism in colon cancer cells. Taken together, the above results demonstrated a tumor-suppressive role of Fbxw7 in colon cancer.

TR colon cancer cells display elevated glycolysis rates and suppressed Fbxw7 expression
We further revealed Fbxw7-mediated Taxol sensitization and glucose metabolism suppression. To investigate the molecular mechanisms of Fbxw7 in Taxol sensitivity, a TR colon cancer cell line was established from HCT-116 cells though treating cells with gradually increased concentrations of Taxol. Consequently, surviving cells were collected and characterized. As shown in Figure 2A,B, HCT-116 TR cells displayed higher tolerance under Taxol treatment compared with HCT-116 parental cells. The IC_{50} value of HCT-116 TR cells was 43.6 nM, which was approximately 5-fold higher than that of parental cells (Figure 2A,B). Consistently, results from the clonogenic assay demonstrated that the viability of HCT-116 TR cells was less inhibited by Taxol treatment than that of parental cells (Figure 2B). We then evaluated the correlation between Taxol resistance and glucose metabolism in colon cancer cells. HCT-116 TR cells exhibited markedly elevated glucose uptake (Figure 2C), lactate production (Figure 2D), and glycolysis key enzymes (GLUT1, HK2, and LDHA) expression (Figure 2E), suggesting that targeting dysregulated glucose metabolism could effectively overcome chemoresistance. Moreover,
Figure 1 Fbxw7 is negatively associated with CRC. (A) Expression of Fbxw7 in 40 CRC tissues and normal colon tissues was measured by qRT-PCR. (B) The mRNA expression of Fbxw7 was compared between two normal colon cell lines and four colon cancer cell lines. (C) HCT-116 cells were transfected with a control vector or Fbxw7 overexpression vector, and expression of Fbxw7 was examined by Western blot. (D) HCT-116 cells with or without Fbxw7 overexpression were treated with Taxol at the indicated concentrations. Cell viability was determined using the MTT assay and (E) flowcytometry assay. (F) HT-29 cells with or without Fbxw7 overexpression were treated with Taxol at the indicated concentrations for 48 h. Cell viability was determined by the MTT assay and (G) flowcytometry assay. (H) HCT-116 and (I) HT-29 cells were transfected with control vector or Fbxw7 for 48 h, and glucose uptake and lactate production were examined. *, P<0.05; **, P<0.01; ***, P<0.001. CRC, colorectal cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
with the observation that \textit{Fbxw7} was remarkably downregulated in TR colon cancer cells (\textit{Figure 2E}), we assessed whether overexpression of \textit{Fbxw7} could reverse the Taxol resistance. Expectedly, HCT-116 TR cells with \textit{Fbxw7} overexpression displayed significantly elevated Taxol sensitivity compared with control cells (\textit{Figure 2F}). Taken together, the above results clearly demonstrated that \textit{Fbxw7} contributes to anti-Taxol treatments.

\textbf{\textit{Fbxw7} interacts with \textit{Nox1} and downregulates \textit{Nox1} in colon cancer cells}

\textit{Fbxw7} is known to exert its tumor-suppressive roles mainly through mediating oncoprotein degradation (8,9). To identify potential substrates which are recognized by \textit{Fbxw7} and involved in cellular metabolism, we performed literature searches and noticed previous reports which uncovered that \textit{Nox1}, a member of the Nox enzyme family which is involved in the production of ROS (11), is critical for supporting the elevated glycolysis of cancer cells. We then tested whether \textit{Fbxw7} could regulate \textit{Nox1} expression. Interestingly, overexpression of \textit{WT-Fbxw7} significantly downregulated \textit{Nox1} expression in two colon cancer cell lines, HCT-116 and HT29 (\textit{Figure 3A}). However, overexpression of the inactive mutant \textit{Fbxw7} (\textit{Fbxw7 ΔF}) did not affect \textit{Nox1} expression (\textit{Figure 3A}). The qRT-PCR results showed that \textit{Nox1} mRNA was not regulated by neither \textit{WT-} nor mutant-\textit{Fbxw7} overexpression in colon cancer cells (\textit{Figure 3B}), suggesting that \textit{Fbxw7} regulates \textit{Nox1} at the protein level. We further performed co-immunoprecipitation (Co-IP) experiments on HCT-116 cells using the \textit{Fbxw7} specific antibody or IgG control antibody. IP results from \textit{Figure 3C} clearly illustrate that \textit{Fbxw7} could specifically bind with the \textit{Nox1} protein. Given the known functions of \textit{Fbxw7} in mediating protein ubiquitination and degradation, the above results uncovered that \textit{Fbxw7} interacts with \textit{Nox1} and downregulates \textit{Nox1} through the ubiquitin protein degradation pathway.

\textbf{\textit{Nox1} is positively associated with Taxol resistance and promotes glycolysis of colon cancer cells}

We then investigated the biological roles of \textit{Nox1} in the...
Taxol sensitivity of colon cancer. Bioinformatics analysis showed that Nox1 was significantly upregulated in CRC tissues (Figure S2). Consistent with this, Nox1 was apparently overexpressed in CRC tissues from our 40 CRC patient specimens (Figure 4A). To evaluate the roles of Nox1 in Taxol sensitivity, Nox1 was overexpressed in HCT-116 and HT29 cells (Figure 4B), followed by treatment with Taxol at increased concentrations. Expectedly, overexpression of Nox1 significantly decreased Taxol sensitivity of CRC cells as determined by the cell viability assay (Figure 4C,D) and clonogenic assay (Figure 4E,F). Nox1 was significantly upregulated in TR colon cancer cells compared with HCT-116 parental cells (Figure 5A). Meanwhile, silencing Nox1 effectively sensitized HCT-116 TR cells to Taxol (Figure 5B), suggesting that Nox1 could be a therapeutic target for overcoming Taxol resistance.

To assess the correlation between Nox1-promoted Taxol resistance and the upregulated cellular glucose metabolism in TR colon cancer cells, we evaluated the roles of Nox1 in glucose metabolism. Expected results in Figure S3A,B demonstrated that overexpression of Nox1 significantly promoted glucose uptake and lactate production in CRC cells. Moreover, glycolysis key enzyme expression was effectively downregulated by Fbxw7 (Figure S4A) and upregulated by Nox1 (Figure S4B). We then compared the glucose metabolism rates of HCT-116 TR cells with or without Nox1 silencing. Expected results showed that TR cells with lower Nox1 maintained a relatively lower glucose uptake (Figure 5C) and lactate production (Figure 5D).

In summary, these results revealed that Nox1 is positively associated with Taxol resistance and the glucose metabolism of colon cancer cells.

**The Fbxw7-promoted Taxol sensitivity is partially through the Nox1-glycolysis axis**

Finally, we summarized the above results and examined whether Fbxw7 sensitizes colon cancer cells to Taxol via the Nox1-glycolysis pathway. HCT-116 TR cells were transfected with a control vector, the Fbxw7 overexpression vector alone, or plus the Nox1 overexpression vector. Western blot results from Figure 6A showed successful rescue of Nox1 in Fbxw7-overexpressed cells. Glucose uptake (Figure 6B) and lactate production (Figure 6C) were consistently suppressed by Fbxw7 overexpression. Expectedly, this regulation by Fbxw7 was recovered by Nox1 restoration in HCT-116 TR cells (Figure 6B,C). Cells with the above transfections were treated with control, Taxol alone, or plus the glycolysis inhibitor 2-DG. As we expected, restoration of Nox1 in Fbxw7-overexpressed HCT-116 TR cells effectively recovered Taxol resistance (Figure 6D), which could be further overridden by glucose metabolism inhibition (Figure 6D). Taken together, our rescue experiments revealed that the Fbxw7-promoted Taxol sensitivity was partially through targeting the Nox1-glycolysis axis.

**Discussion**

CRC, the third most common cancer worldwide, is associated with poor survival and has a high mortality rate (1,2). Currently, despite the fact that improved outcomes have been achieved by chemotherapy, the acquired drug resistance is becoming the major challenge for cancer treatments (3). Therefore, investigation
into the molecular mechanisms of chemoresistance is essential to improve therapeutic effects. In this study, we report a Fbxw7-mediated Taxol sensitization in colon cancer. Based on an in vitro TR CRC cell model, we demonstrated that Fbxw7 was significantly downregulated in HCT-116 TR cells, suggesting that enhancing the Fbxw7 signaling pathway could contribute to overcoming Taxol resistance.

Fbxw7 functions as a tumor suppressor and is negatively associated with tumor progression through targeting a cluster of oncoproteins, such as c-Myc (16), cyclin E (16), mTOR (17), and Aurora A (18). Despite the fact that diverse Fbxw7 substrates have been validated, novel oncoproteins of potential Fbxw7 targets and the molecular mechanisms underlying the functions of Fbxw7 in chemosensitivity remain largely unknown. In this study, we detected Nox1, an enzyme which is capable of oxidizing NADPH or NADH to NADP+ or NAD+ to generate superoxide, as a substrate of
Fbxw7. We showed that Fbxw7 interacts with Nox1, leading to a significant downregulation of the Nox1 protein in colon cancer cells. Expectedly, overexpression of Fbxw7 did not affect the mRNA level of Nox1, indicating that Fbxw7 markedly promotes Nox1 protein degradation. Although a previous study described Fbxw7-mediated Nox1 degradation in smooth muscle cells (19), our results for the first time demonstrate that Nox1 is a Fbxw7-interacting protein and is downregulated by Fbxw7 in colon cancer cells. Importantly, this study integrated the Fbxw7-Nox1 interaction with Taxol sensitivity of colon cancer, potentiating the Fbxw7-Nox1 interaction as a novel therapeutic target against TR CRC.

In contrast to normal cells, tumor cells show increased glycolysis rate rather than oxidative phosphorylation for energy supply and fast proliferation, a phenomenon called the “Warburg effect” (15). Moreover, this dysregulated cellular metabolism reprogramming provides tumor cells with a survival advantage under a stressful microenvironment or chemotherapeutic agents (15). In particular, we observed that Nox1, which was shown to be positively associated with CRC progression, had the capacity to activate the glucose metabolism of CRC cells. As an NADPH oxidase, Nox1 has been implicated in the regulation of gene expression and ROS generation (11). In addition, a recent study uncovered that upregulation of Nox1 was critical for elevated glycolysis by providing additional NAD+ in cancer cells with mitochondrial dysfunction (12,13). Here, we showed that Nox1 was upregulated in TR CRC cells, which showed apparently elevated glucose metabolism. Silencing Nox1 effectively sensitized TR cells by blocking glucose metabolism. Thus, our data linked Nox1-mediated glucose metabolism and Taxol resistance in CRC cells, presenting a novel therapeutic target for overcoming Taxol resistance. The present study has limitations, in that an in vivo xenograft model is needed to validate the above in vitro cellular mechanisms. Moreover, the precise mechanisms of Nox1-promoted glucose metabolism are still under investigation.

In summary, this study unveils the Fbxw7-Nox1-glucose metabolism axis in regulating the Taxol sensitivity of CRC. Our conclusions will enlighten researchers to develop Fbxw7-Nox1-based anti-chemoresistant drugs which can effectively enhance the clinical applications of Taxol against colon cancers.
Figure 6 The Fbxw7-Nox1-glucose metabolism axis in TR CRC cells. (A) HCT-116 TR cells were transfected with a control, Fbxw7 alone, or plus Nox1 overexpression vector for 48 h, and protein expression levels of Fbxw7 and Nox1 were examined by Western blot. (B) The glucose uptake and (C) lactate production were examined from the above transfected cells. (D) The above transfected cells were treated with control or Taxol with or without 2-DG for 48 h, and the Taxol sensitivity was determined by the MTT assay. *, P<0.05; **, P<0.01. TR, Taxol-resistant; CRC, colorectal cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-21-2076). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by institutional ethics committee of The Fifth People’s Hospital of Shanghai, China. Written informed consent was taken from all individual participants included in the study.

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References


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Figure S1 Comparison of glucose metabolism rates in normal colon tissues and CRC tissues. (A) The mRNA expression levels of GLUT1 (SLC2A1) and (B) LDHA in normal colon tissues and CRC tissues. (C) Glucose uptake and (D) lactate production were assessed in normal colon cell lines and CRC cell lines. *, P<0.05; **, P<0.01. CRC, colorectal cancer.
Figure S2 Expression of Nox1 in cancer and normal tissues. The Nox1 expression profiles were analyzed by gepia.cancer-pku.cn from TCGA database.
**Figure S3** Modulation of glucose metabolism by *Fbxw7* and *Nox1* in colon cancer cells. (A) HCT-116 and (B) HT-29 cells were transfected with control, *Fbxw7*, or *Nox1* for 48 h, and glucose uptake and lactate production were then examined. *, P<0.05; **, P<0.01.

**Figure S4** Modulation of glucose metabolism enzymes by *Fbxw7* and *Nox1* in colon cancer cells. (A,B) HCT-116 cells were transfected with control, *Fbxw7*, or *Nox1* for 48 h, and protein expression levels of *GLUT1*, *HK2*, and *LDHA* were examined by Western blot. β-actin was an internal control.