Original Article

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

Hui-Peng Wang[#], Wen-Jie Chen[#], Jia-Men Shen, Tao Ye[*], Hong-Wei Xie[*]

Department of General Surgery, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai, China

Contributions: (I) Conception and design: HP Wang, WJ Chen, T Ye, HW Xie; (II) Administrative support: HW Xie; (III) Provision of study materials or patients: T Ye; (IV) Collection and assembly of data: HP Wang; (V) Data analysis and interpretation: T Ye; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

[*]These authors contributed equally to this work as co-corresponding authors.

Correspondence to: Tao Ye; Hong-Wei Xie. Department of General Surgery, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200240, China. Email: yetao105@sina.com; yetao104@126.com; 13816819761@163.com.

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*)

Submitted Apr 08, 2021. Accepted for publication May 17, 2021.
doi: 10.21037/atm-21-2076
View this article at: http://dx.doi.org/10.21037/atm-21-2076

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 ℃ 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 ℃ 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 (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'-TTCTTTTTCATTTTTGTTGTTTTTGTATAGA-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 $2^{-\Delta\Delta Ct}$ 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 (1×10^3/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 1× 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 ℃ 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}s 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,

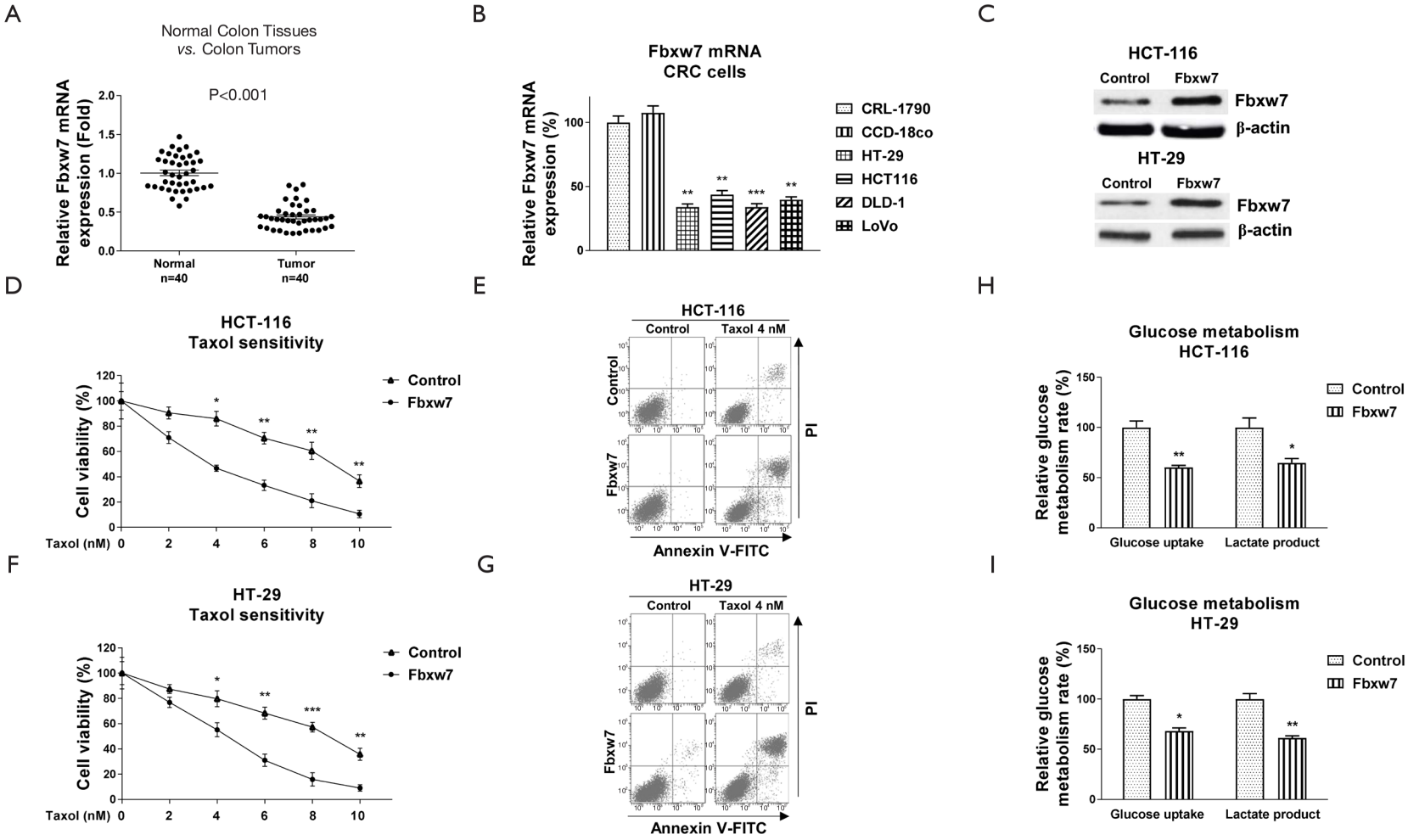

Figure 1 *Fbxw7* is negatively associated with CRC. (A) Expression of *Fbxw7* in 40 CRC tissues and normal colon tissues was measured by qRT-PCR. (B) The mRNA expression of *Fbxw7* was compared between two normal colon cell lines and four colon cancer cell lines. (C) HCT-116 cells were transfected with 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.

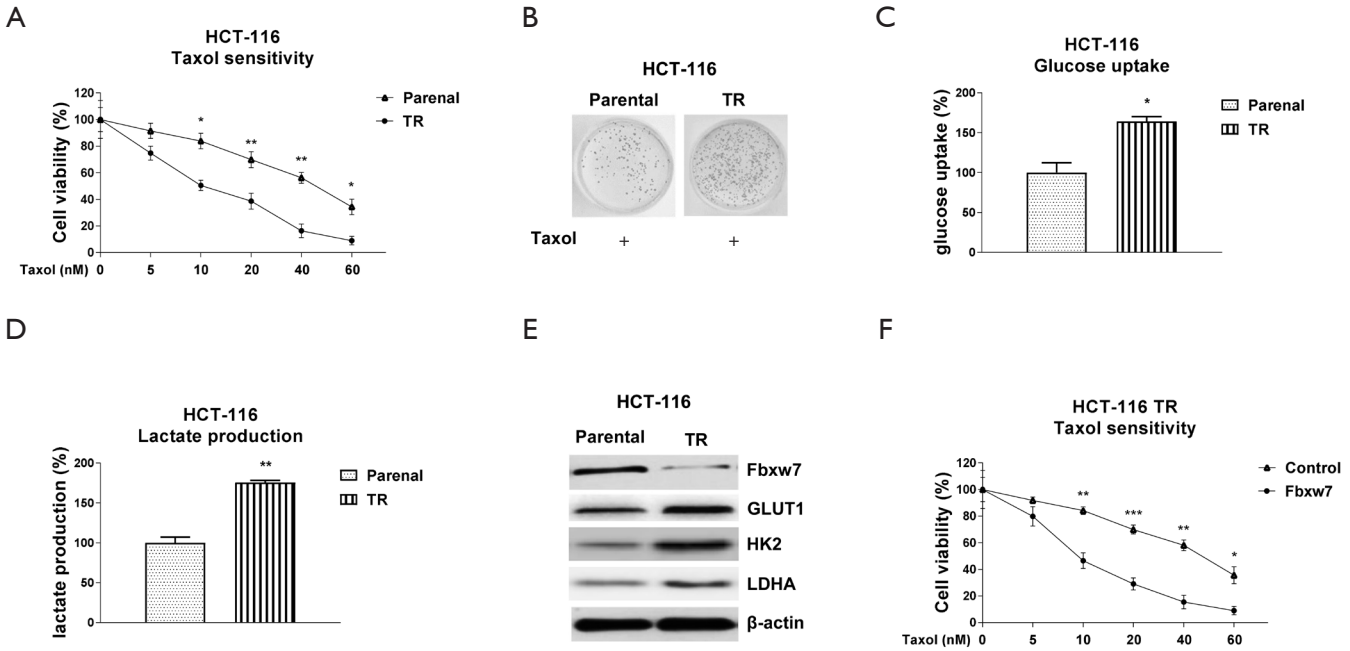

Figure 2 TR CRC cells display elevated glucose metabolism and downregulated *Fbxw7*. (A) Validation of Taxol resistance in HCT-116 TR cells by the MTT assay and (B) clonogenic assay. (C) Glucose uptake, (D) lactate production, and (E) protein expression of *Fbxw7*, *GLUT1*, *HK2*, and *LDHA* from HCT-116 parental and TR cells were examined. (F) HCT-116 TR cells were transfected with a control vector or *Fbxw7* overexpression vector for 48 h, and cells were treated with Taxol at the indicated concentrations. Cell viabilities were determined by the MTT assay. *, P<0.05; **, P<0.01; ***, P<0.001. TR, Taxol-resistant; CRC, colorectal cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

with the observation that *Fbxw7* was remarkedly downregulated in TR colon cancer cells (*Figure 2E*), we assessed whether overexpression of *Fbxw7* could reverse the Taxol resistance. Expectedly, HCT-116 TR cells with *Fbxw7* overexpression displayed significantly elevated Taxol sensitivity compared with control cells (*Figure 2F*). Taken together, the above results clearly demonstrated that *Fbxw7* contributes to anti-Taxol treatments.

Fbxw7 interacts with Nox1 and downregulates Nox1 in colon cancer cells

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

Nox1 is positively associated with Taxol resistance and promotes glycolysis of colon cancer cells

We then investigated the biological roles of *Nox1* in the

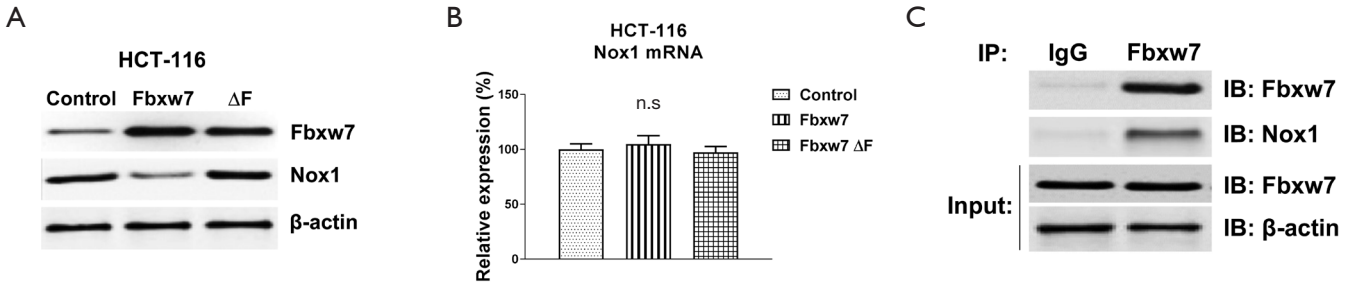

Figure 3 *Fbxw7* interacts with *Nox1* and downregulates *Nox1* in colon cancer cells. (A) HCT-116 cells were transfected with a control vector, *WT-Fbxw7*, or inactive mutant *Fbxw7* for 48 h, and expression levels of *Fbxw7* and *Nox1* were detected by Western blot. (B) The mRNA expression of *Nox1* from the above transfected cells was detected by qRT-PCR. (C) HCT-116 cells were subjected to the immunoprecipitation assay using a control antibody, IgG, or *Fbxw7* specific antibody, followed by Western blot analysis for detecting the *Nox1-Fbxw7* interaction.

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 aquired drug resistance is becoming the major challenge for cancer treatments (3). Therefore, investigation

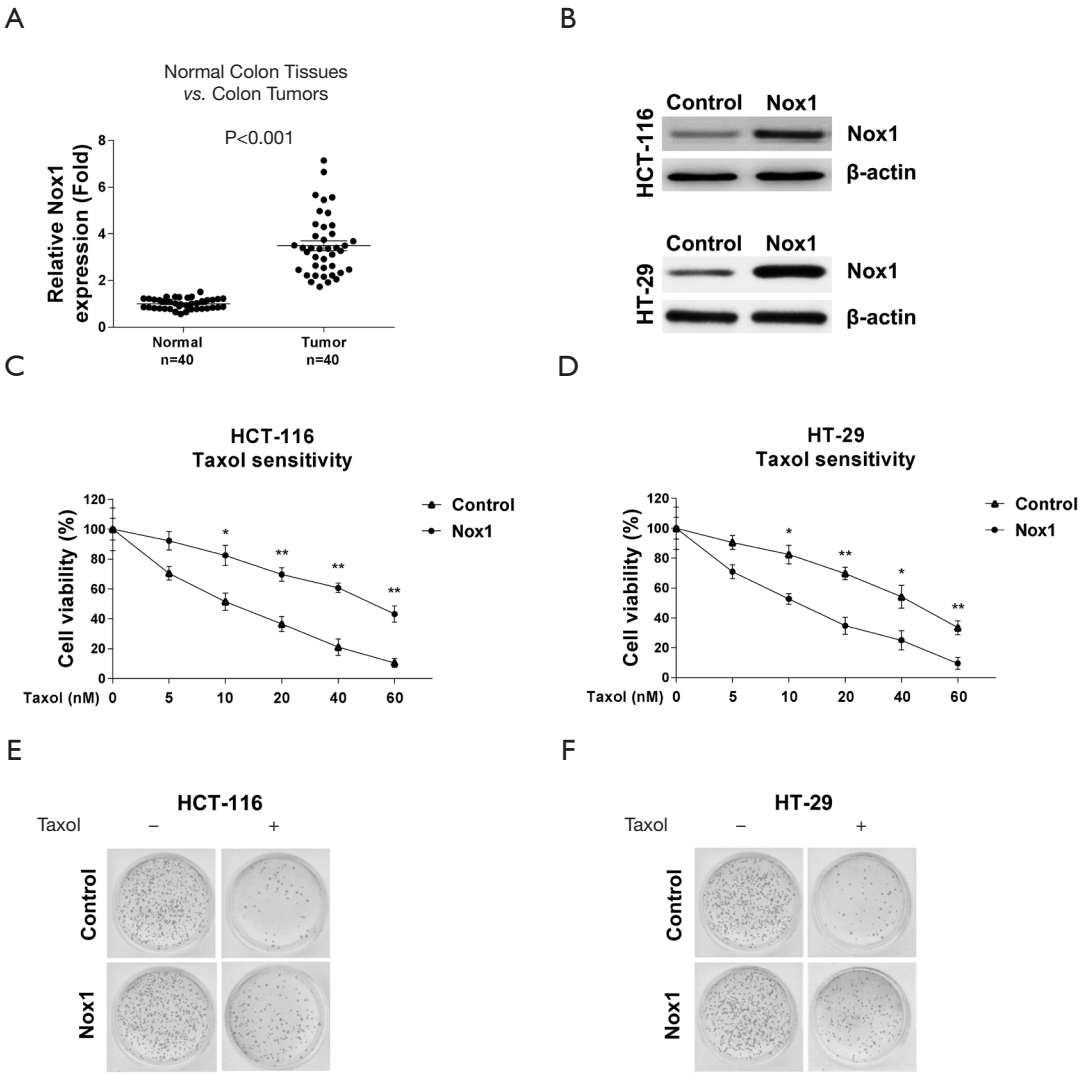

Figure 4 *Nox1* is upregulated and promotes Taxol resistance in colon cancer cells. (A) The mRNA expression of *Nox1* was compared in CRC tissues and their matched normal colon tissues. (B) HCT-116 cells were transfected with a control vector or *Nox1* overexpression vector, and expression of *Nox1* was detected by Western blot. (C) HCT-116 and (D) HT29 cells were transfected with a control vector or Nox1 overexpression vector for 48 h, and cells were treated with Taxol. Cell viability was determined by the MTT assay and (E,F) clonogenic assay. *, P<0.05; **, P<0.01. CRC, colorectal cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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

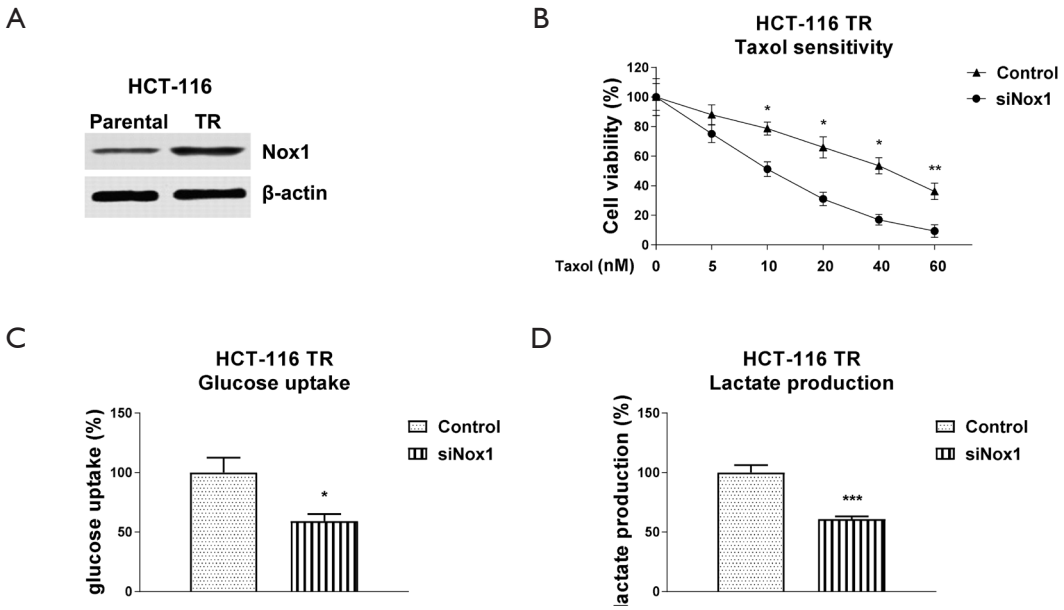

Figure 5 *Nox1* is upregulated in TR CRC cells and promotes glucose metabolism. (A) *Nox1* protein expression in HCT-116 parental cells and TR cells was examined by Western blot. (B) HCT-116 TR cells were transfected with control siRNA or si*Nox1* for 48 h, and cells were treated with Taxol. Cell viability was determined by the MTT assay. (C) HCT-116 TR cells were transfected with control siRNA or si*Nox1* for 48 h, and glucose uptake and (D) lactate production were measured. *, P<0.05; **, P<0.01; ***, P<0.001. TR, Taxol-resistant; CRC, colorectal cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

*Fbxw*7. We showed that *Fbxw*7 interacts with *Nox1*, leading to a significant downregulation of the *Nox1* protein in colon cancer cells. Expectedly, overexpression of *Fbxw*7 did not affect the mRNA level of *Nox1*, indicating that *Fbxw*7 markedly promotes *Nox1* protein degradation. Although a previous study described *Fbxw*7-mediated *Nox1* degradation in smooth muscle cells (19), our results for the first time demonstrate that *Nox1* is a *Fbxw*7-interacting protein and is downregulated by *Fbxw*7 in colon cancer cells. Importantly, this study integrated the *Fbxw*7-*Nox1* interaction with Taxol sensitivity of colon cancer, potentiating the *Fbxw*7-*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 reprograming 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 *Fbxw*7-*Nox1*-glucose metabolism axis in regulating the Taxol sensitivity of CRC. Our conclusions will enlighten researchers to develop *Fbxw*7-*Nox1*-based anti-chemoresistant drugs which can effectively enhance the clinical applications of Taxol against colon cancers.

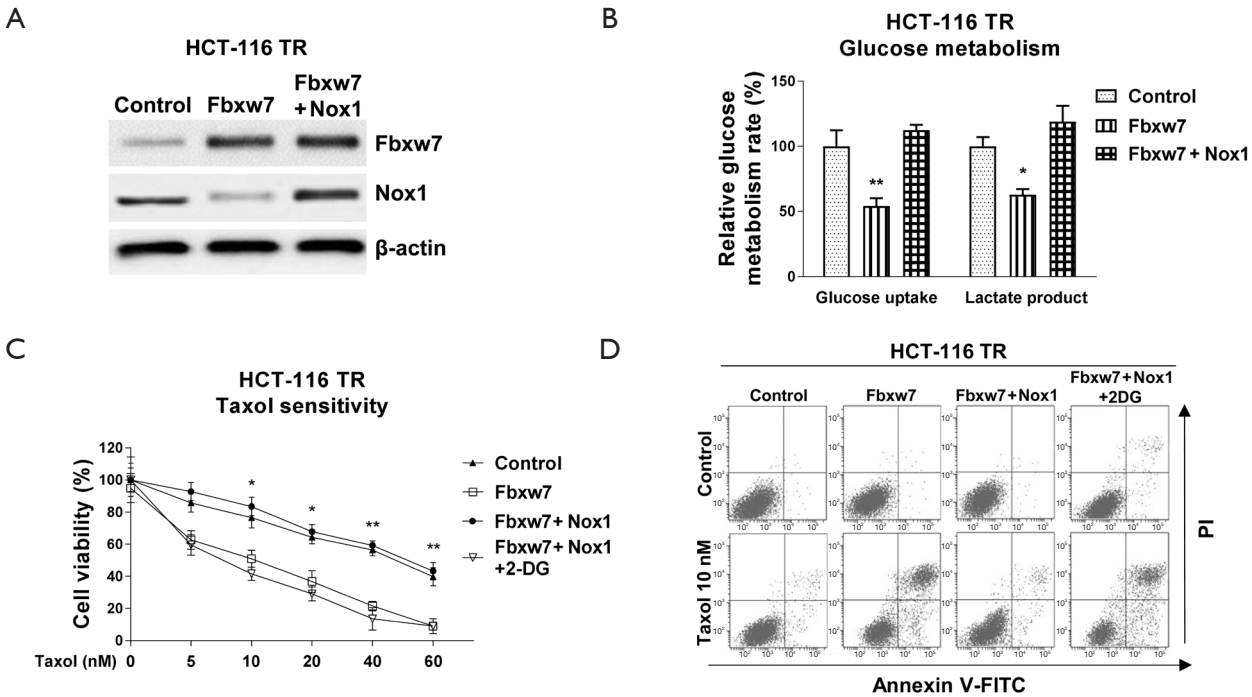

Figure 6 The *Fbxw7-Nox1*-glucose metabolism axis in TR CRC cells. (A) HCT-116 TR cells were transfected with a control, *Fbxw7* alone, or plus *Nox1* overexpression vector for 48 h, and protein expression levels of *Fbxw7* and *Nox1* were examined by Western blot. (B) The glucose uptake and (C) lactate 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.

Acknowledgments

Funding: The project was supported by the Medical System of Shanghai Minhang District (No. 2020MWDXK02), Natural Science Research Funds of Minhang District, Shanghai (No. 2018MHZ025, No. 2018MHZ037).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/atm-21-2076

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-21-2076

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.

References

1. Cappell MS. Pathophysiology, clinical presentation, and management of colon cancer. Gastroenterol Clin North Am 2008;37:1-24, v.
2. Hangaard Hansen C, Gögenur M, Tvilling Madsen M, et al. The effect of time from diagnosis to surgery on oncological outcomes in patients undergoing surgery for colon cancer: a systematic review. Eur J Surg Oncol 2018;44:1479-85.
3. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, et al. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. Int J Mol Sci 2017;18:197.
4. Yang CH, Horwitz SB. Taxol®: the first microtubule stabilizing agent. Int J Mol Sci 2017;18:1733.
5. Sofias AM, Dunne M, Storm G, et al. The battle of "nano" paclitaxel. Adv Drug Deliv Rev 2017;122:20-30.
6. Orr GA, Verdier-Pinard P, McDaid H, et al. Mechanisms of Taxol resistance related to microtubules. Oncogene 2003;22:7280-95.
7. Abal M, Andreu JM, Barasoain I. Taxanes: microtubule and centrosome targets, and cell cycle dependent mechanisms of action. Curr Cancer Drug Targets 2003;3:193-203.
8. Yeh CH, Bellon M, Nicot C. FBXW7: a critical tumor suppressor of human cancers. Mol Cancer 2018;17:115.
9. Sailo BL, Banik K, Girisa S, et al. FBXW7 in cancer: what has been unraveled thus far? Cancers (Basel) 2019;11:246.
10. Chang CC, Lin HH, Lin JK, et al. FBXW7 mutation analysis and its correlation with clinicopathological features and prognosis in colorectal cancer patients. Int J Biol Markers 2015;30:e88-95.
11. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86:494-501.
12. Faria CC, Fortunato RS. The role of dual oxidases in physiology and cancer. Genet Mol Biol 2020;43:e20190096.
13. Barton M, Meyer MR, Prossnitz ER. Nox1 downregulators: A new class of therapeutics. Steroids 2019;152:108494.
14. Wu Z, Han X, Tan G, et al. Dioscin inhibited glycolysis and induced cell apoptosis in colorectal cancer via promoting c-myc ubiquitination and subsequent hexokinase-2 suppression. Onco Targets Ther 2020;13:31-44.
15. Spencer NY, Stanton RC. The Warburg effect, lactate, and nearly a century of trying to cure cancer. Semin Nephrol 2019;39:380-93.
16. Tu K, Zheng X, Zan X, et al. Evaluation of Fbxw7 expression and its correlation with the expression of c-Myc, cyclin E and p53 in human hepatocellular carcinoma. Hepatol Res 2012;42:904-10.
17. Xu Y, Tian C, Sun J, et al. FBXW7-induced MTOR degradation forces autophagy to counteract persistent prion infection. Mol Neurobiol 2016;53:706-19.
18. Duhamel S, Girondel C, Dorn JF, et al. Deregulated ERK1/2 MAP kinase signaling promotes aneuploidy by a Fbxw7β-Aurora A pathway. Cell Cycle 2016;15:1631-42.
19. Shen Y, Chen X, Chi C, et al. Smooth muscle cell-specific knockout of FBW7 exacerbates intracranial atherosclerotic stenosis. Neurobiol Dis 2019;132:104584.

(English Language Editor: C. Betlazar-Maseh)

Cite this article as: Wang HP, Chen WJ, Shen JM, Ye T, Xie HW. Attenuating glucose metabolism by *Fbxw7* promotes Taxol sensitivity of colon cancer cells through downregulating *NADPH* oxidase 1 (*Nox1*). Ann Transl Med 2021;9(10):886. doi: 10.21037/atm-21-2076

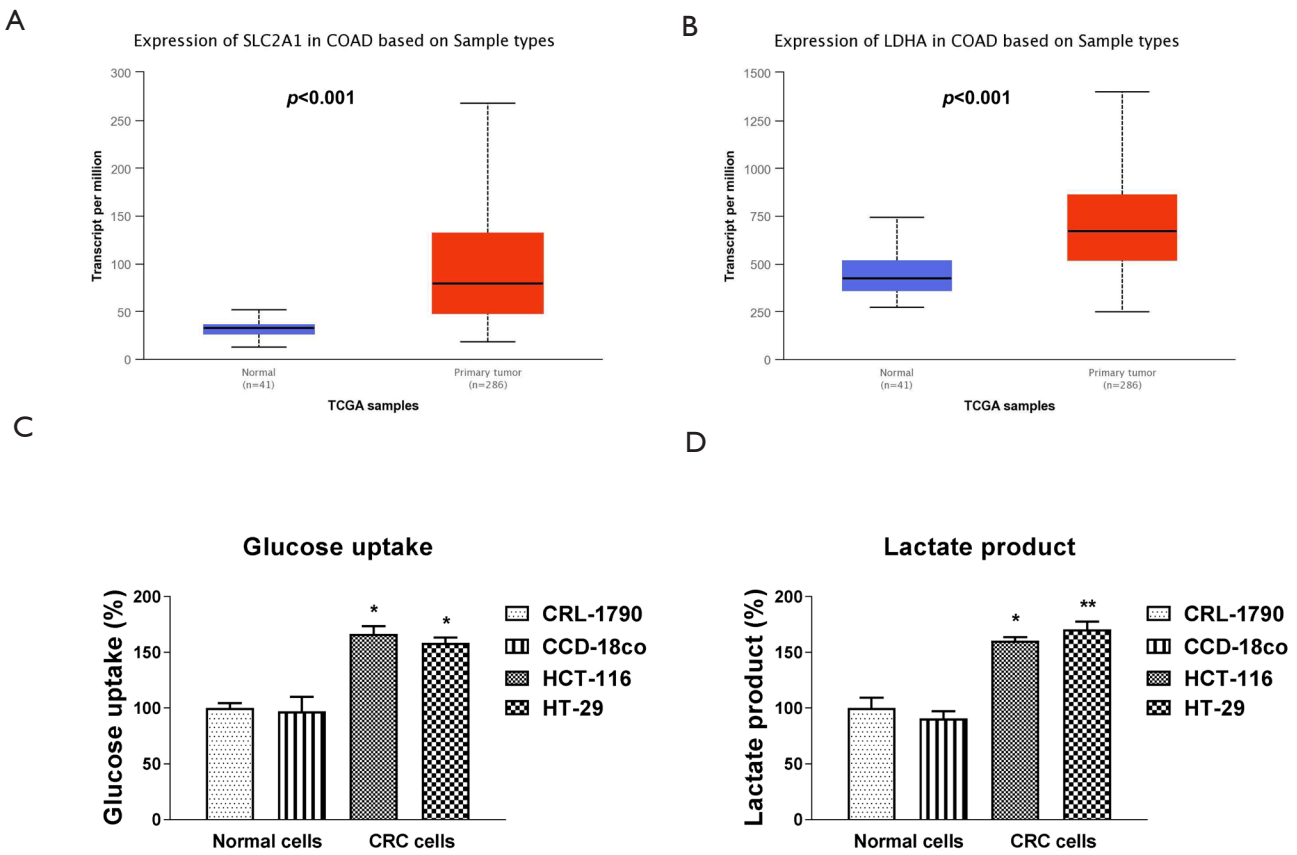

Figure S1 Comparison of glucose metabolism rates in normal colon tissues and CRC tissues. (A) The mRNA expression levels of *GLUT1* (*SLC2A1*) and (B) *LDHA* in normal colon tissues and CRC tissues. (C) Glucose uptake and (D) lactate production were assessed in normal colon cell lines and CRC cell lines. *, P<0.05; **, P<0.01. CRC, colorectal cancer.

http://dx.doi.org/10.21037/atm-21-2076

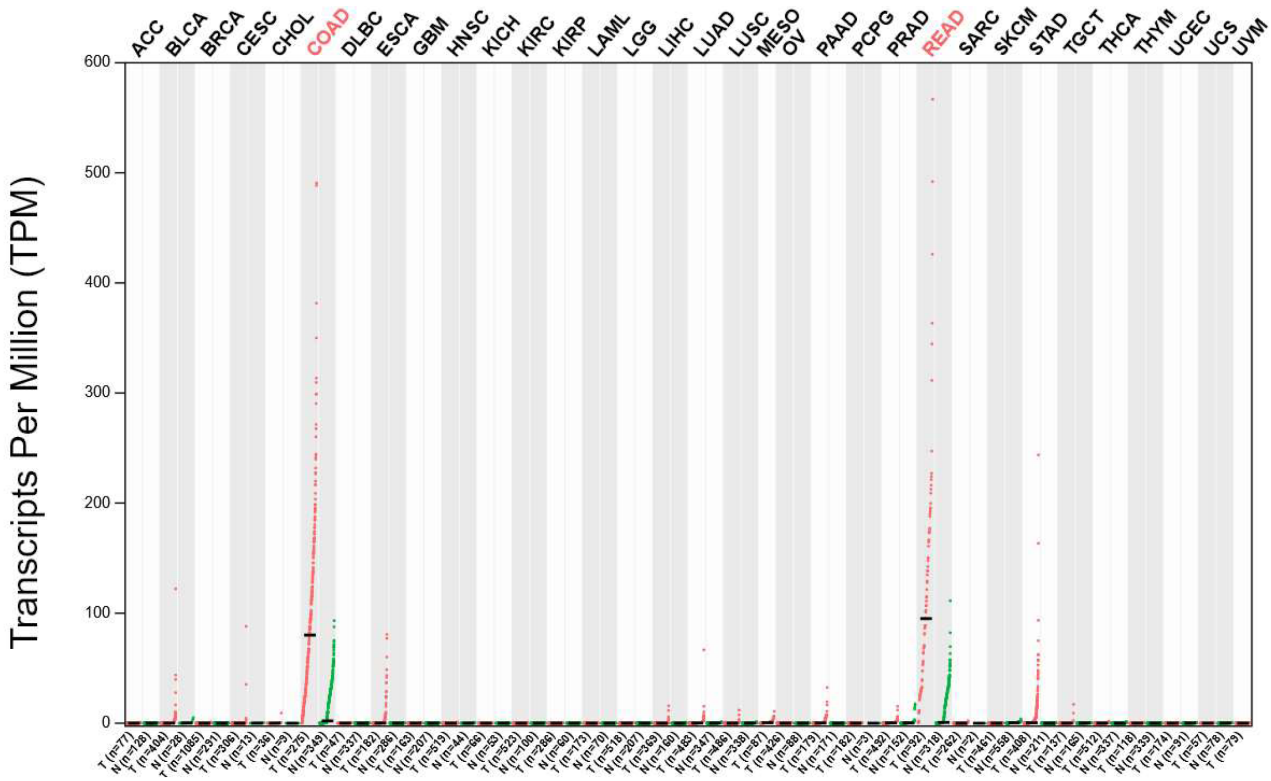

Figure S2 Expression of *Nox1* in cancer and normal tissues. The *Nox1* expression profiles were analyzed by gepia.cancer-pku.cn from TCGA database.

http://dx.doi.org/10.21037/atm-21-2076

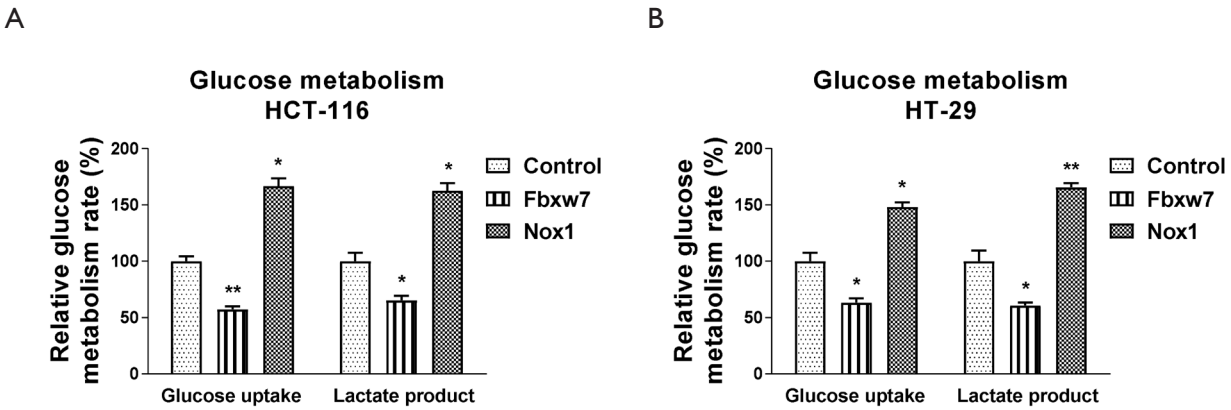

Figure S3 Modulation of glucose metabolism by *Fbxw7* and *Nox1* in colon cancer cells. (A) HCT-116 and (B) HT-29 cells were transfected with control, *Fbxw7*, or *Nox1* for 48 h, and glucose uptake and lactate production were then examined. *, P<0.05; **, P<0.01.

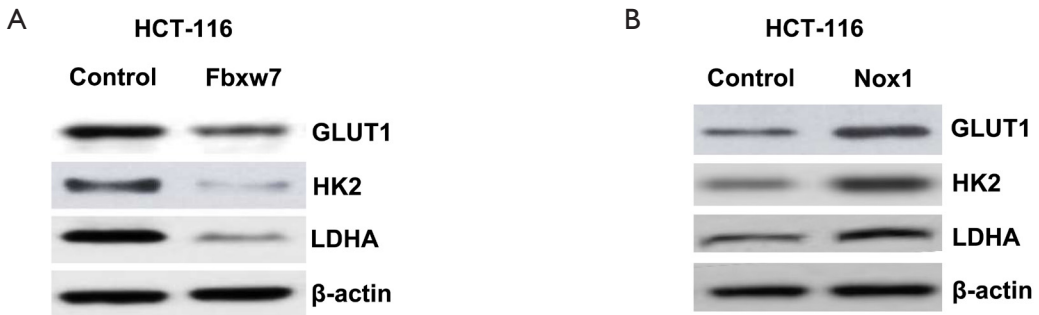

Figure S4 Modulation of glucose metabolism enzymes by *Fbxw7* and *Nox1* in colon cancer cells. (A,B) HCT-116 cells were transfected with control, *Fbxw7*, or *Nox1* for 48 h, and protein expression levels of *GLUT1*, *HK2*, and *LDHA* were examined by Western blot. β-actin was an internal control.

http://dx.doi.org/10.21037/atm-21-2076