LncRNA GALNT5 uaRNA promotes cisplatin resistance in colorectal cancer via NF-κB/MDR1/MRP1/ pathway

Zhiqiang Zhong (zhiqiang.zhong@163.com)
Capital Medical University
RiXing Bai
Capital Medical University
Jun Xu
Capital Medical University

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Abstract

To clarify the function of long non-coding RNA (lncRNA) GALNT5 uaRNA in cisplatin resistant colorectal cancer and its potential mechanism. The expression levels were also detected in cisplatin resistant colorectal cancer cell lines (HCT116/DDP and HT29/DDP cells) and sensitive colorectal cancer cell lines. The effects of GALNT5 uaRNA on apoptosis of HCT116/DDP and HT29/DDP cells were examined by flow cytometry assay respectively. The protein levels of drug resistance related genes influenced by GALNT5 uaRNA were detected by Western blot. The cells of siNC, sIGALNT5 uaRNA, Vector and GALNT5 uaRNA cells groups from HCT116/DDP and HT29/DDP cells were injected into dorsal flanks of the mice repectively. Higher GALNT5 uaRNA expression was observed in HCT116/DDP and HT29/DDP cells relative to that of their parental cells. The apoptotic rate increased in HCT116/DDP and HT29/DDP cells with GALNT5 uaRNA knockdown compared to the cells with control group. Furthermore, the expression levels of MDR1, MRP1 and p-NF-κB were decreased in HCT116/DDP and HT29/DDP cells with GALNT5 uaRNA knockdown compared to the cells with control group. The apoptotic rate decreased in HCT116/DDP and HT29/DDP cells with GALNT5 uaRNA overexpression. Furthermore, the expression levels of MDR1, MRP1 and p-NF-κB were increased in HCT116/DDP cells with GALNT5 uaRNA overexpression compared to the cells with control group. The tumors in the HCT116/DDP/siGANLNT5 uaRNAgroup were smaller and lighter than those in the HCT116/DDP/siNC. In a word GALNT5 uaRNA promotes cisplatin resistance by MDR1/MRP1/NF-κB pathway.

Introduction

Colorectal carcinoma (CRC) is the third most common cancer in the world[1]. CRC is caused by mutations that target oncogenes, tumour suppressor genes and genes related to DNA repair mechanisms[2]. Chemical drugs are used to CRC but can develop drug resistance to certain pathways. And some moleculars in the cells can influence the drug resistence. CD133 reverses drug resistance via the AKT/NF-κB/MDR1 pathway in CRC[3]. Although mammalian genomes are widely transcribed, only 1–2% of the genomic output encodes for proteins[4, 5]. Among the large of non-coding transcripts, the long noncoding RNAs with longer than 200 nts may present new challenge for cancer diagnosis and treatment. Extensive gene expression analyses have linked alteration of lncRNA expression to tumour development[6, 7]. Many studies reported that lncRNAs was also involved drug resistence in cancers. LncRNAs can be prospective candidates to develop new targeted therapies[8]. Exosome transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA[9]. LncRNA SNHG15 in promoting colon cancer and mediating drug resistance[10]. The precise anticancer mechanism of cisplatin (DDP) remains not completely understood. The efficacy is limited due to the development of drug resistance, leading to the treatment failure to cancer patients. If tumors become DDP resistance, they will easily develop cross resistance to other anti-cancer drugs[11]. Multiple drug resistance (MDR) is the most common reason for treatment failure in cancer patients. MDR-related proteins including MDR1 (also known as ABCB1, P-gp), multidrug resistance proteins (MRPs) are critically associated with MDR[12]. MDR1, a transmembrane transporter, is responsible for the efflux of anti-cancer drugs through the cell

membrane by ATP hydrolysis[13]. The MRPs, especially MRP1, also drive resistance to chemotherapeutics and the expression of MRPs is controlled by nuclear receptors, transcription factors and kinases[14]. The regulatory elements that are located in the 3'-UTR may participate in mRNA stability, transport, localization and translation[15, 16]. GALNT5 uaRNA were identified using a cDNA library[17]. GALNT5 uaRNA, an lncRNA that is derived from the 3'untranslated region (3'-UTR) of GALNT5 and is transcribed independently of the coding region of GALNT5, was greatly upregulated in gastric cancer and promotes gastric cancer progression through its interaction with HSP90[18]. If GALNT5 uaRNA influence the drug resistence need further be confirmed. In this study, we identify the role of GALNT5 uaRNA in upregulating MRP1 and MDR1 in HCT116/DDP cells and HT29/ DDP cells.

Methods

Cell culture and transfection

HCT116 cells and HT29 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. HCT116/DDP and HT29/DDP cells were induced in our laboratory and cultured in RPMI-1640 medium with 20 μg/mL cisplatin, respectively. Cells that were logarithmically grown and in good condition were inoculated into 6 well plates before transfection with 1×10^5 cells/well. Cell abundance during transfection was 60-70%. Transfection was performed for 48 h using Lipo fectamineTM 2000.

RNA extraction and real time polymerase chain reaction (RT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and quantified using UV spectrophotometer. D260 nm/D280 nm value was calculated for selecting qualified RNA samples, which were preserved at -20°C for use. Extracted RNA was reversely transcribed into cDNA and amplified by Real Time quantitative Polymerase Chain Reaction (RT-qPCR) using SYBR Premix Ex Taq II kit (TaKaRa, Otsu, Shiga, Japan). PCR reaction conditions were: pre-denaturation at 95°C for 30 s, 95°C for 5 s, and 60°C for 31 s, for a total of 40 cycles. The relative levels were quantitatively analyzed using the 2-ΔΔCt method. GAPDH was used as an internal reference. The experiment was repeated for three times.

Apoptosis determination

Cells collected were washed twice with phosphate-buffered saline (PBS) and stained using the Annexin V/fluorescein isothiocyanate (FITC) Apop-tosis Detection Kit (Beyotime, Shanghai, China). The single cell suspension was first incubated with 500 μL of 1×buffer, 5 μL of Annexin V and 5 μL of Propidium Iodide

(PI) at 37°C for 25 min in the dark. The above cells were examined by FACSAriaTM flow cytometry and analyzed by Win MDI. 2.9 software (TSRI Flow Cytometry Core Facility, La Jolla, CA). Every experiment was repeated three times.

Western blot

Total protein from cells was extracted with RIPA Lysis Buffer (RIPA; Beyotime, Shanghai, China). The protein were quantified by bicinchoninic acid (BCA) method (Pierce, Waltham, MA, USA) and loaded for electrophoresis. After transferring on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA), it was blocked in 5% skim milk for 2 hours, incubated with primary antibodies at 4°C overnight and secondary antibodies for 2 h. Bands were exposed by enhanced chemiluminescence (ECL) and analyzed by Image J Software (NIH, Bethesda, MD, USA).

Xenograft animal experiments

six weeks old nude mice were bought from Beijing HFK Bioscience (Beijing, China). The mices were randomized into four groups (n = 6 per group). The cells were injected subcutaneously into the right dorsal flanks of the mice at 6×10^6 cells per animal. The tumor sizes were measured every three days using a caliper. Tumor size was calculated according to the following formula: V (mm3) = length × width2/2. The mice were killed using CO2 inhalation followed by cervical dislocation. The animal experiments were performed according to the institutional guidelines for animal care and approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Medical Sciences.

Statistical analysis

Statistical analyses were performed using SPSS software (version 13.0). The t-test was used for analyzing the differences between the two groups. $p<0.05$ indicated the significant difference.

Results

High expression of GALNT5 uaRNA in cisplatin resistant colon cancer cells

The cellular expression of GALNT5 uaRNA was higher in cisplatin resistant HCT116 cells (Figure 1A) and HT29 cells (Figure 1B) compared with that of parental CRC cells by the qRT-PCR assay. GALNT5 uaRNA was localized mainly in the cytoplasmic region by the qRT-PCR assay (Figure 1C). The higher apoptotic rate was observed in HCT116/DDP cells (Figure 1D) and HT29/DDP cells (Figure 1E) with GALNT5 uaRNA knockdown.

GALNT5 uaRNA knockdown increased cisplatin sensitivity in colon cancer cells

To clarify the role of GALNT5 uaRNA in cisplatin resistant CRC, we examined cisplatin sensitivity in HCT116/DDP cells and HT29/DDP cells with GALNT5 uaRNA knockdown. First, the expression level of siGANLNT5 uaRNAwas determined. The expression level of GALNT5 uaRNA was decreased in siGANLNT5 uaRNAHCT116/DDP cells (Figure 2A) and HT29/DDP cells (Figure 2D) compared to the control group cells. But the expression level of GALNT5 was similar between siNC group and siGANLNT5 uaRNAgroup in HCT116/DDP cells (Figure 2B) and HT29/DDP cells (Figure 2E). The higher apoptotic rate was observed in HCT116/DDP cells (Figure 2C) and HT29/DDP cells (Figure 2F) with GALNT5 uaRNA knockdown. The above data showed that GALNT5 uaRNA silence enhanced cisplatin sensitivity in drug-resistant CRC.

High expression of GALNT5 uaRNA promoted cisplatin resistance in colon cancer cells

Furthermore, we detected the regulatory effect of GALNT5 uaRNA on parental CRC cells. GALNT5 uaRNA expression sufficiently upregulated in HCT116/DDP cells (Figure 3A) and HT29/DDP cells (Figure 3D) stably expressing GALNT5 uaRNA. But the expression level of GALNT5 was unchanged in GALNT5 uaRNA group compared to control group in HCT116/DDP cells (Figure 3B) and HT29/DDP cells (Figure 3E). Moreover, GALNT5 uaRNA overexpression greatly decreased cisplatin-induced CRC cell apoptosis in HCT116/DDP cells (Figure 3C) and HT29/DDP cells (Figure 3F). The results suggested that GALNT5 uaRNA overexpression attenuated cisplatin sensitivity of CRC cells.

GALNT5 uaRNA upregulates MRP1 and MDR1 via NF-κB activation

Western blot was conducted to observe changes in apoptosis specific and drug resistance specific genes in CRC cells. The protein levels of MDR1, MRP1 and p-NF-κB decreased by GALNT5 uaRNA knockdown and the protein levels of MDR1, MRP1 and p-NF-κB increased by GALNT5 uaRNA overexpression in HCT116/DDP cells (Figure 4A-D) and HT29/DDP cells (Figure 4E-H). These results demonstrated that GALNT5 uaRNA contributed to DDP resistance, while downregulation of GALNT5 uaRNA sensitized HCT116 and HT29 cells to DDP treatment, according to the NF-κB activation status.

GALNT5 uaRNA contributes to DDP resistence in vivo

To investigate the role of GALNT5 uaRNA in DDP resistance in vivo, DDP/ siNC, DDP/sIGALNT5 uaRNA, DDP/Vector and DDP/GALNT5 uaRNA cells were subcutaneously transplanted into nude mice

respectively. The results showed that the tumors in the HCT116/DDP/siGANLNT5 uaRNAgroup were smaller and lighter than those in the HCT116/DDP/ siNC group (Figure 5A, 5B, 5C). The tumors in the HCT116/DDP/Vector group were smaller and lighter than those in the HCT116/DDP/ GALNT5 uaRNA (Figure 5A, 5D, 5E). The tumors in the HT29/DDP/siGANLNT5 uaRNAgroup were smaller and lighter than those in the HT29/DDP/ siNC group (Figure 5F, 5G, 5H). The tumors in the HT29/DDP/Vector group were smaller and lighter than those in the HT29/DDP/ GALNT5 uaRNA (Figure 5F, 5I, 5J).

Discussion

Increasing evidence supports the role of lncRNAs in cancer development and progression. Additionally, their involvement in the development of drug resistance across various cancer entities are starting to be discovered. Consequently, lncRNAs have been suggested as factors in novel therapeutic strategies to overcome drug resistance in cancer[19]. lncRNA TUG1 promotes cisplatin resistance by regulating CCND2 via epigenetically silencing miR-194-5p in Bladder Cancer[20]. TGFbeta1 promotes gemcitabine resistance through regulating the LncRNA LET/NF90/miR-145 signaling axis in bladder cancer[21]. In our work, GALNT5 uaRNA was upregulated in cisplatin resistant CRC cells. The knockdown of GALNT5 uaRNA elevated cisplatin sensitivity in drug resistant CRC cells. Conversely, GALNT5 uaRNA overexpression could attenuate cisplatin sensitivity in ordinary CRC cells. Tumors in the siGANLNT5 uaRNAgroup were smaller and lighter than those in the control group. The tumors in the vector group were bigger and heavier than those in the control group.

The mechanism of MDR in tumors is very complex. ATP-binding cassette (ABC) proteins located on the cell membrane exert a crucial function, including MDR-associated proteins (MRP1/ABCC1) and MDR proteins (MDR1/Pgp/ABCB1)[22]. Juliano et al[23] first proposed that the drug pumping effect of transmembrane transporters markedly decreases the cellular concentration of antitumor drugs. They found MDR1in MDR cells. MDR1-transport drugs are usually lipophilic compounds with a large molecular weight[24]. MRP1 is an ABCC subfamily transporter discovered in MDR1-/- drug-resistant human-derived small cell lung cancer cell line H69AR. MRP1 mainly resistant to anthracyclines, vincristine, antifolate drugs, etc.[25]. In our work, GALNT5 uaRNA knocking down decreased the expression of MDR1 and MRP1 in HCT116/DDP cells and HT29/ DDP cells while GALNT5 uaRNA overexpression could upregulate the expression of MDR1 and MRP1.

NF-κB is a critical transcription factor. lncRNA-SRLR directly binds to NF-κB and promotes IL-6 transcription, leading to the activation of STAT3 and the development of sorafenib tolerance[26]. NF-κB plays an important role in transcriptional regulation of MDR1 and MRPs gene expressions. Inactivation of NF-κB may overcome drug resistance in some carcinomas. After NF-κB nuclear translocation and binding to its recognition site, the promoter of the MDR related genes are activated, and gene expression is induced[3, 27]. In our study the protein levels of p-NF-κB decreased by GALNT5 uaRNA knockdown and the protein levels of p-NF-κB increased by GALNT5 uaRNA overexpression in HCT116/DDP and HT29cells. These results demonstrated that GALNT5 uaRNA contributed to DDP resistance, while downregulation of GALNT5 uaRNA sensitized HCT116 and HT29 cells to DDP treatment, according to the

NF-κB activation status. Taken together, our results suggest that targeting GALNT5 uaRNA reverses cisplatin resistance via the NF-κB/MDR1/MRP1 pathway and that this pathway might serve as a potential therapeutic target to reverse DDP resistence in CRC.

Conclusions

We demonstrated that GALNT5 uaRNA was highly expressed in cisplatin resistant CRC. GALNT5 uaRNA promotes cisplatin resistance in CRC by regulating drug resistance related genes.

Declarations

Conflict of Interests

The authors declare that they have no conflict of interest.

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Figures

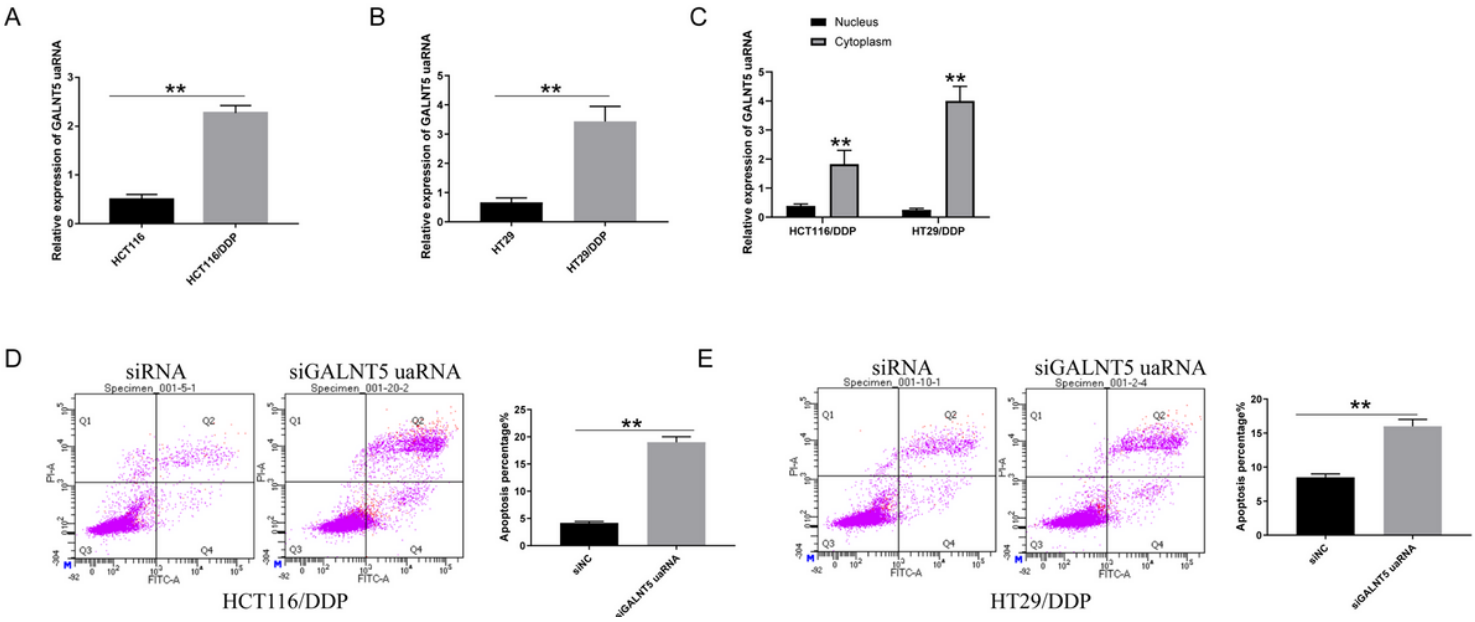

Figure 1

High expression of GALNT5 uaRNA in cisplatin resistant cells. Cellular expression of GALNT5 uaRNA was higher in cisplatin resistant HCT116 cells (A) and HT29 cells (B) compared with that of ordinary CRC cells. C, GALNT5 uaRNA was localized mainly in the cytoplasmic region in HCT116 and HT29 cells by qRT PCR assay. The higher apoptotic rate was observed in HCT116/DDP cells (D) and HT29/DDP cells (E) with GALNT5 uaRNA knockdown.

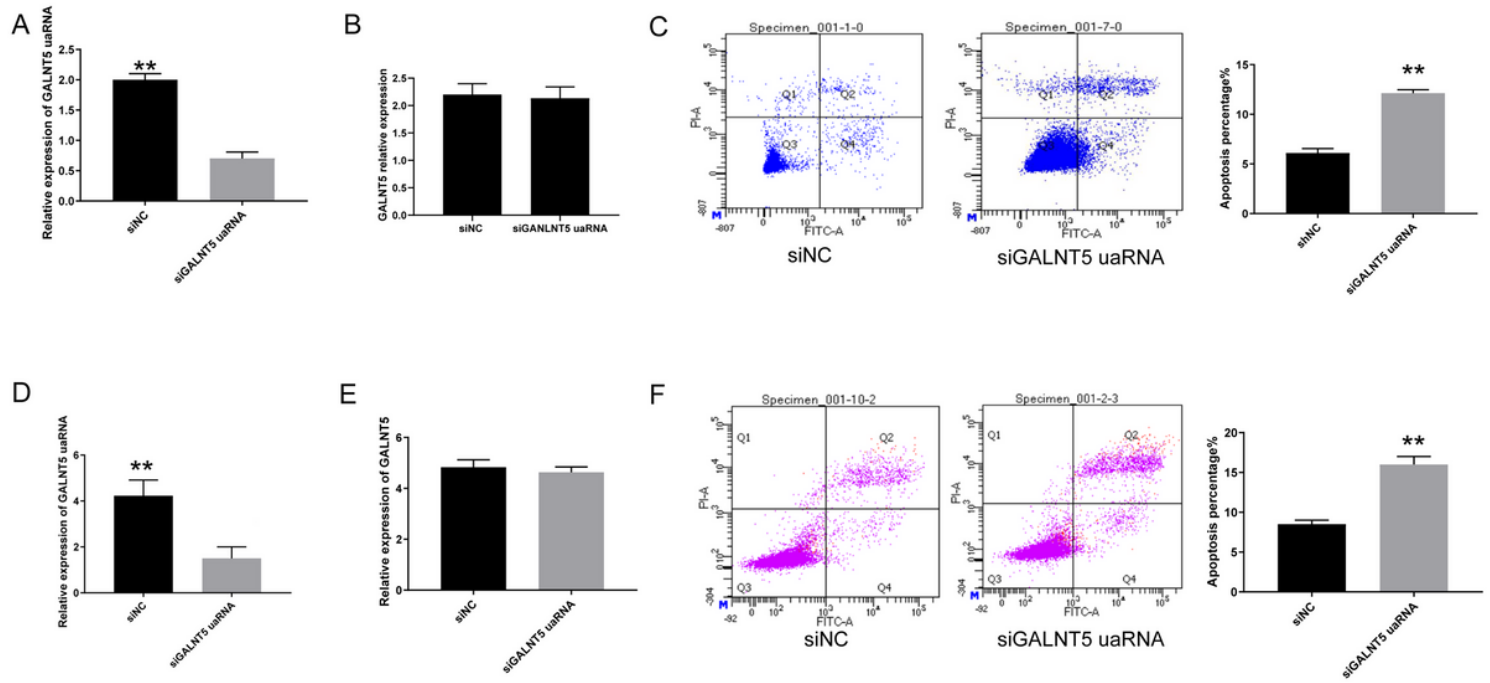

Figure 2

Low expression of GALNT5 uaRNA increased cisplatin sensitivity in HCT116 cells and HT29 cells. A, The expression of GALNT5 uaRNA in siGANLNT5 uaRNAHCT116/DDP cells. B, The expression of GALNT5 in siGANLNT5 uaRNAHCT116/DDP cells. C, Apoptotic rate in HCT116/DDP/ siGANLNT5 uaRNAcells and HCT116/DDP/siNC cells. D, The expression of GALNT5 uaRNA in siGANLNT5 uaRNAHT29/DDP cells. E, The expression of GALNT5 in siGANLNT5 uaRNAHT29/DDP cells. F, Apoptotic rate in HT29/DDP/ siGANLNT5 uaRNAcells and HT29/DDP/siNC cells.

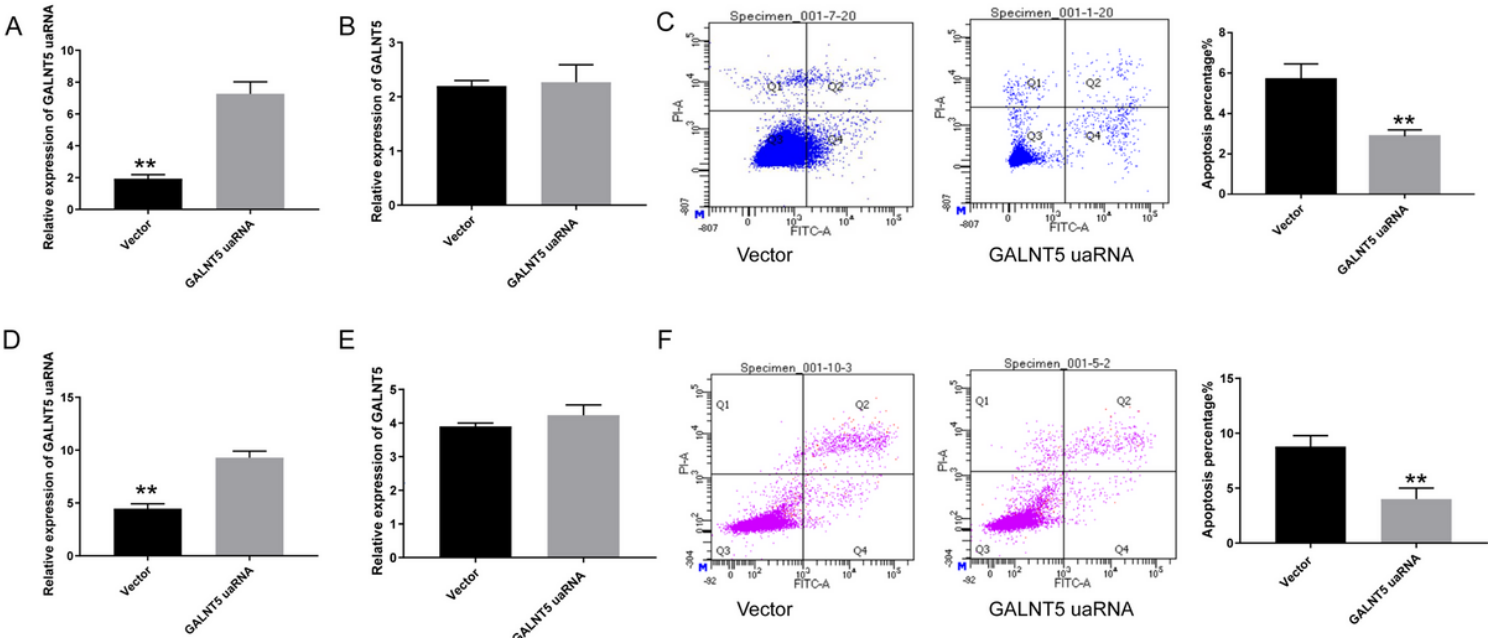

Figure 3

High expression of GALNT5 uaRNA promoted cisplatin resistance in HCT116/DDP cells and HT29/DDP cells. A, The expression of GALNT5 uaRNA in HCT116/DDP cells with over expression GALNT5 uaRNA. B, The expression of GALNT5 in HCT116/DDP cells with over expression GALNT5 uaRNA. C, Apoptotic rate in HCT116/DDP/GALNT5 uaRNA cells and HCT116/DDP/Vector cells. D, The expression of GALNT5 uaRNA in HT29/DDP cells with over expression GALNT5 uaRNA. E, The expression of GALNT5 in HT29/DDP cells with over expression GALNT5 uaRNA. F, Apoptotic rate in HT29/DDP/GALNT5 uaRNA cells and HT29/DDP/Vector cells.

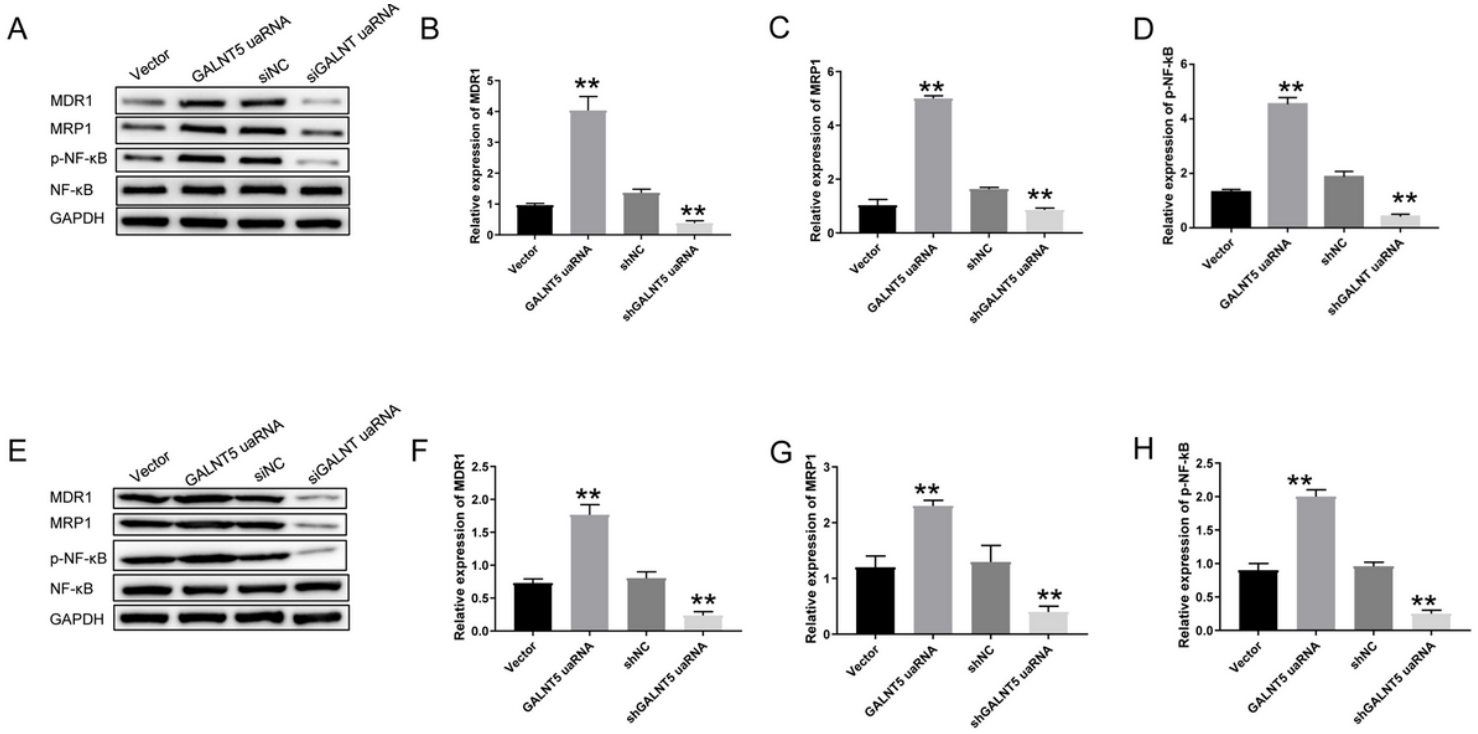

Figure 4

Western blot analysis showed that GALNT5 uaRNA upregulated MRP1 and MDR1 via NF-κB activation. The expression level of MDR1(A, B), MRP1(A, C)and p-NF-κB (A, D) in HCT116/DDP/Vector cells, HCT116/DDP/GALNT5 uaRNA cells, HCT116/DDP/siNC cells, HCT116/DDP/siGANLNT5 uaRNAcells respectively. The expression level of MDR1(E, F), MRP1(E, G)and p-NF-κB (E, H) in HT29/DDP/Vector cells, HT29/DDP/GALNT5 uaRNA cells, HT29/DDP/siNC cells, HT29/DDP/siGANLNT5 uaRNAcells respectively.**P < 0.01

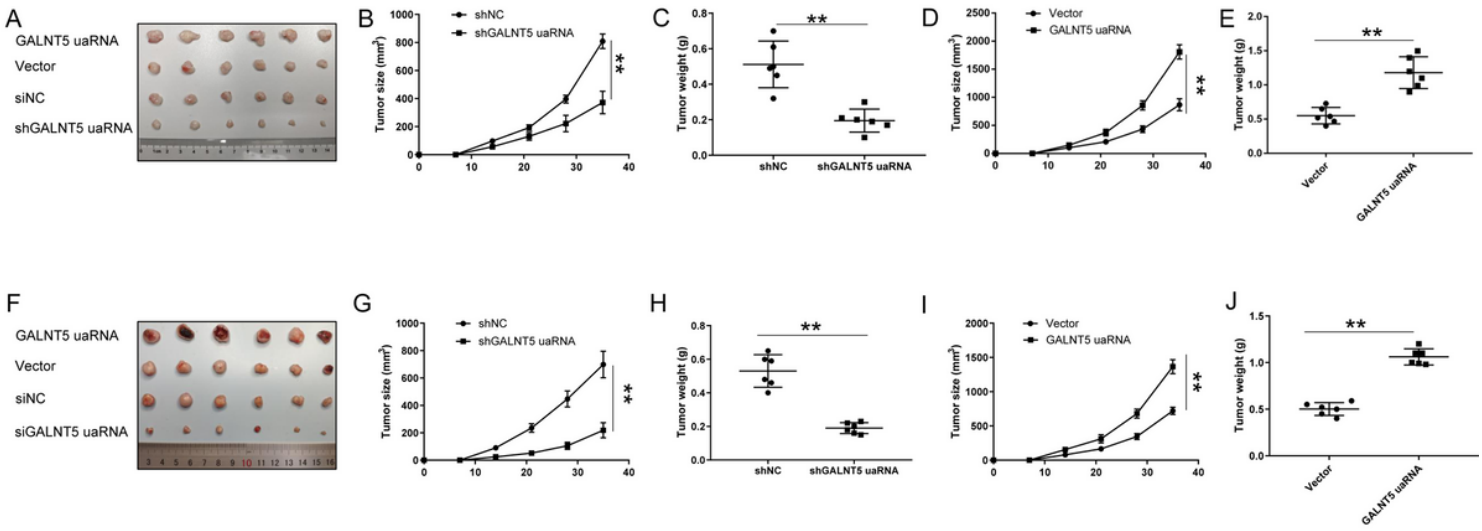

Figure 5

The influence of GALNT5 uaRNA on CRC cell xenograft in vivo. The exponential growing siGANLNT5 uaRNAcells, siNC cells, GALNT5 uaRNA cells and vector cells were injected subcutaneously into nude mice (n=6 every group) respectively. Tumor images of HCT116/DDP (A) and HT29/DDP (F) formed in the nude mice. Growth curves for the xenograft HCT116/DDP (B) and HT29/DDP (G) GALNT5 uaRNA knockdown tumors. Tumor weights of the xenograft HCT116/DDP (C) and HT29/DDP (H) GALNT5 uaRNA knockdown tumors were measured. Growth curves for xenograft HCT116/DDP (D) and HT29/DDP (I) GALNT5 uaRNA overexpression tumors. Tumor weights of xenograft HCT116/DDP (E) and HT29/DDP (J) GALNT5 uaRNA overexpression tumors were measured. **P < 0.01

Supplementary Files

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