

Original Article

CD47 blockade enhances cisplatin sensitivity by inhibiting DNA repair gene expression

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Abstract

CD47, a cell surface transmembrane glycoprotein, is an innate immune checkpoint that suppresses phagocytic clearance. Emerging evidence suggests that CD47 has noncanonical functions. However, its involvement in chemotherapy resistance is not well understood. Our study reveals that cisplatin treatment upregulates CD47 expression across multiple cancer cell lines. Cisplatin induces the expression of CD47 through the ATM/NF- κ B signaling pathway. Genetic ablation of CD47 dramatically sensitizes cancer cells to cisplatin. Mechanistically, CD47 depletion potentiates cisplatin-induced DNA damage, as demonstrated by elevated γ H2AX formation and ATM phosphorylation. Knockdown of *CD47* inhibits the expression of DNA repair genes *ERCC1*, *FANCA*, and *BRCA2* through the ATM/NF- κ B pathway. Remarkably, CD47 blockade with neutralizing antibodies recapitulates these effects, synergistically potentiating cisplatin's DNA-damaging capacity while suppressing DNA repair capacity. CD47 blockade also potentiates cisplatin's tumor inhibitory effect *in vivo*. These findings establish a novel mechanism whereby CD47 promotes cisplatin resistance through transcriptional regulation of DNA repair pathway, providing rationale for combining CD47-targeted therapies with conventional chemotherapy. This dual approach could simultaneously overcome immune evasion while enhancing treatment efficacy.

Key words CD47, cancer, chemotherapy resistance, DNA repair gene, cisplatin

Introduction

CD47 is a cell surface transmembrane glycoprotein. It binds to the signal regulatory protein (SIRP) on the surface of macrophages to prevent phagocytosis. It is widely expressed on many cells of epithelial and mesenchymal origin and is constitutively overexpressed in many tumors [1]. Expression of CD47 on cancer cells may allow cancer cells to escape macrophage phagocytosis and immune surveillance of the body [2–6]. High CD47 expression on tumor cells is associated with poor prognosis in human malignancies [7,8].

Blocking CD47 signaling or suppressing CD47 expression protects normal tissues from ionizing radiation (IR) injury [9–11]. CD47 deficiency protects irradiated T-cells and endothelial cells [12] and both hematopoietic and gastrointestinal tissue [11] from radiation by activation of autophagy, anabolic metabolites [10], transcription factors that support asymmetric stem cell self-renewal [13], and

SLFN11 expression [14]. It has been shown that non-malignant cells and Jurkat T cells lacking CD47 are protected from genotoxic stress induced by IR [10,12]. This protection is mediated in part by an enhanced protective autophagy response in cells lacking CD47 or with reduced CD47 expression [12]. Radioresistance in the CD47-deficient mutant is associated with global metabolic stabilization, including induction of nucleotide biosynthesis required to repair DNA damage induced by ionizing radiation [10]. Loss of CD47 or blocking its function with the CD47 function-blocking antibody B6H12 improved the ability of Jurkat T cells to restore genomic integrity after damage caused by IR [14]. However, the relationship between IR, DNA damage and CD47 expression, has not been studied yet. Ghantous *et al.* [15] demonstrated recently that IR and genotoxic agents-caused DNA damage induced CD47 expression in both non-malignant and malignant cells. The authors showed that genotoxic stress upregulated CD47 following DNA damage in a

MRE11-dependent manner.

Contrary to its protective effect in normal cells, CD47 loss has been shown to enhance radiosensitivity and chemosensitivity in cancer cells. Inhibition of CD47 sensitized the B16 mouse melanoma cells to IR injury *in vivo* [9]. CD47 expression was upregulated in chemoresistant hepatocellular carcinoma (HCC), and suppression of CD47 sensitized HCC to doxorubicin *in vivo* through blockade of CTSS/PAR2 signaling [16]. Knockdown of *CD47* in liver cancer Huh-7 and MHCC97L cells increased the sensitivity of the cells to both cisplatin and doxorubicin. CD47⁺ HCC cells derived from PLC/PRF/5 were more chemoresistant than CD47-null cells in response to cisplatin and doxorubicin treatments. Upregulated CD47 made HCC resistant to sorafenib, though the underlying mechanism is unknown [17]. Expression of CD47 in ovarian clear cell carcinoma correlated with chemotherapy resistance and prognosis [18]. Knockdown of *CD47* enhanced the sensitivity of breast cancer MDA-MB-231 cells to docetaxel [19]. These studies indicate that CD47 confers cancer cell resistance to chemotherapy. However, in B-cell chronic lymphocytic leukemia cells, CD47 activation induced cell apoptosis [20]. And CD47 activation mediated killing of breast cancer cells [21]. Analysis of B16 melanoma cells exposed to irradiation revealed that suppression of CD47 exerted a radioprotective effect for cell viability *in vitro*, which is different from that *in vivo* [9]. These results highlight the context-dependent functions of CD47.

Chronic DNA damage response in cancer cells might contribute to constitutive elevated expression of CD47 and promote immune evasion. However, the function of DNA damage reagent-induced CD47 in the survival of cancer cells remains largely unclear. Whether and how CD47 influences DNA damage repair and regulates survival of cancer cells need investigation. In this study we demonstrate that cisplatin induced the expression of CD47 through the ATM/NF- κ B pathway. Knockdown of *CD47* reduced the expression of DNA repair genes *ERCC1*, *FANCA*, and *BRCA2*, and enhanced DNA damage and cell death induced by cisplatin. Genetic ablation or blockade of CD47 significantly potentiated cisplatin's cytotoxic effects against malignant cells. Our results suggest that CD47 promotes chemoresistance through increasing DNA repair genes expression and CD47-targeting may potentiate the efficacy of chemotherapy.

Materials and Methods

Cell culture and treatment

Human liver cancer HepG2, Hep3B, and MHCC97L cells, colon cancer RKO and SW480 cells, breast cancer MCF-7 and MDA-MB-231 cells, and glioblastoma U87 and LN229 cells were from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and were maintained in Dulbecco's Modified Eagle's medium (Gibco, Grand Island, USA) with 10% fetal bovine serum. These cells were routinely tested for Mycoplasma infection. Cancer cells were treated with cisplatin in the presence or absence of NF- κ B inhibitor BAY 11-7082, TBK1 inhibitor GSK8612, or ATM inhibitor KU55933 for indicated hours. Cisplatin, BAY 11-7082, and GSK8612 were from MedChemExpress (Monmouth Junction, USA). KU55933 was a product of TargetMol (Nanjing, China).

Small interfering RNA (siRNA) experiment

Cancer cells were digested to form a single-cell suspension and plated in 6-well plates until the cells entered the logarithmic growth

phase. When the cell density reached ~80%, siRNA oligos (20 nM) were transfected into the cells using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, USA) as per the manufacturer's protocols. siRNA oligos were from GenePharma (Shanghai, China). These siRNA sequences are shown below: siATM-1: 5'-GAAA GAAAGAAGUUGAGAA-3', siATM-2: 5'-GAAAUUAUUCAGAAA GAA-3', sip65-1: 5'-AGCUGAUGUGCACCACGACAA-3', sip65-2: 5'-GGACAUUAUGAGACCUUCAA-3', and Control: 5'-UUCUCCGAAC GUGUCACGU-3'.

Overexpression experiment

Cells were seeded in 6-well culture plates at 50%–60% confluency. The following day, the culture medium was removed and replaced with a mixture of ERCC1- or FANCA-encoding vector (Youbio Biological Technology, Changsha, China) diluted in Opti-MEM serum-free medium complexed with lipofectamine 2000 (Thermo Fisher Scientific). After 6 h of transfection, the transfection mixture was replaced with fresh culture medium containing 10% serum.

Genomic editing

Single-guide RNAs were designed to target the CD47 using the CRISPR design tool (www.benchling.com). The annealed guide RNA oligonucleotides were inserted into a lentiCRISPR V2 vector (Addgene, Watertown, USA) digested with the *BsmBI* restriction enzyme. The lentiCRISPR V2-sgRNA together with psPAX2 and pMD2.G vectors were transfected into 293T cells, and the medium containing virus was collected at 48 h after transfection [22]. The cells grown in a 6-well plate were infected with the collected lentivirus in medium containing polybrene at a final concentration of 5 μ g/mL. After 48 h, the cells were selected in medium containing puromycin (1 μ g/mL). The sequences of sgRNAs targeting CD47 are as follows. sg1: 5'-TTGCACTACTAAAGTCAGTG-3'. sg2: 5'-ATGCTTTGTTACTAATATGG-3'.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, USA) and quantified using a NanoDrop 2000 (Thermo Fisher Scientific). First-strand cDNA was synthesized using the SuperScript II First-Strand Synthesis System (Invitrogen). SYBR® Green Real-time PCR Master Mix (ABclonal, Wuhan, China) was employed to measure the expression levels of target genes. The relative RNA expression levels were analyzed using the $2^{-\Delta\Delta C_t}$ method. *GAPDH* was used as the internal control. The primers used are listed in Table 1.

Western blot analysis

In brief, the proteins were extracted from cells using lysis buffer and quantitated using a BCA kit (GlpBio, Montclair, USA). Equal amounts of proteins were separated by 10% SDS-PAGE and transferred to a 0.45- μ m PVDF membrane. After being blocked with 5% skim milk at room temperature for 1 h, the membrane was incubated with primary antibody at 4°C overnight. The membrane was washed and then incubated with HRP-conjugated secondary antibody (Proteintech, Wuhan, China) at room temperature for 2 h. An enhanced chemiluminescence (ECL) kit (Meilunbio, Dalian, China) was used to visualize the protein bands. The β -actin antibody was obtained from Sigma (St Louis, USA). ATM and Cleaved Caspase 3 antibody were products of Zenbio (Chengdu,

Table 1. Sequences of primers used in this study.

Gene name	Primer sequence (5'→3')
CD47	Forward AGAAGGTGAAACGATCATCGAGC
	Reverse CTCATCCATACCACCGGATCT
FANCA	Forward GTTGCTCTAGCGTGGGAC
	Reverse GGAGAACATACTGTGTGCCAAT
BRCA2	Forward TGCCTGAAAACAGATGACTATC
	Reverse AGGCCAGCAAACCTCCGTTTA
ERCC1	Forward CTACGCCGAATATGCCATCTC
	Reverse GTACGGGATTGCCCTCTG
CSA	Forward AGCAAACACTGCTCATAATGGG
	Reverse GAACTATTCCAGAGCCTCATTCG
XRCC1	Forward CCTTTGGCTTGAGTTTGTACG
	Reverse CCTCCTTCACACGGAAGTGG
POLL	Forward GATGAAGGCATGGACTATGAGC
	Reverse TGAATCCAGCTACATCCACCAG
FAAP24	Forward TTAAGCTCATTTTCGAGGATGGC
	Reverse CCCGAACAAGCCTCTTTCTGT
REV3	Forward GTGGATGCTGTAGCTGCTGAT
	Reverse ATGGCCTGTAGACCAGGTTT
RAD50	Forward TTTGGTTGGACCCAATGGGG
	Reverse CAGGAGGGAAATCTCCAGTACAA
RAD51	Forward CAACCCATTTACGGTTAGAGC
	Reverse TTCTTTGGCGCATAGGCAACA
MRE11	Forward GGGGCAGATGCACTTTGTG
	Reverse GAAGCAAAACCGGACTAATGTCT
GAPDH	Forward CTGACTTCAACAGCGACACC
	Reverse TGCTGTAGCCAAATTCGTTGT

China). CD47 antibody was from Invitrogen. γ H2AX antibody was from Novus (Centennial, USA). p-ATM, p-p65, p65 and p-TBK1 antibodies were from Cell Signaling Technology (Beverly, USA). p-p53 and ERCC1 antibodies were purchased from Selleck (Shanghai, China). p53 antibody was purchased from Santa Cruz (Dallas, USA). FANCA and BRCA2 antibodies were from HUABIO (Hangzhou, China). TBK1 antibody was purchased from ABclonal.

Cell viability assay

A total of 2000 cells suspended in 100 μ L of medium were seeded into individual wells of a 96-well plate. Cell viability was measured using a CCK-8 kit (Beyotime Biotechnology, Shanghai, China) as previously described [22].

Flow cytometry assay

To detect the apoptosis rate of the cancer cells, we used an Annexin V-fluorescein isothiocyanate (FITC) assay kit (Beyotime Biotechnology). Briefly, the cells were washed twice with cold PBS and resuspended in 200 μ L of binding buffer. Then 10 μ L of Annexin V-FITC and 5 μ L of PI staining solution was added. The cells were vortexed, incubated for 15 min in the dark and detected with a flow cytometer (Beckman, Pasadena, USA). The mean fluorescent intensity of the Annexin V/PI double staining in the myocytes was

analyzed using FlowJo software version 10.8.1. The proportion of apoptotic cells (mortality) was defined as the percentage of Annexin V⁺-FITC⁺ cells.

Comet assay

The comet assay was performed using a Comet Assay Kit (Beyotime Biotechnology). In brief, agarose (0.1 g in 10 mL PBS) was melted in a microwave for 2–3 min. Next, the glass microscope slide was immersed in agarose and one side of the slide was wiped with a lint free cloth and dried on a flat surface. Cells (1×10^6 cell/mL in $1 \times$ PBS) were mixed gently with 0.7% molten low-melting-point agarose (pre-equilibrated to 37°C) at a 1:10 ratio, and 30 μ L of the cell mixture were immediately added onto a pre-warmed slide (37°C) to ensure the formation of a thin layer. The slide was immersed in lysis buffer for 1 h, followed by treatment in alkaline electrophoresis solution for 1 h at 4°C to allow DNA unwinding. The glass slide was electrophoresed in an alkaline electrophoresis solution, with a power supply voltage set to 1 V/cm (length between electrodes), and run at 4°C for 30 min. Then, the slide was immersed twice in a neutral buffer solution at 4°C for 5 min each time. About 20 μ L of propidium iodide solution was dropped onto the slide and stained in the dark for 10–20 min. Finally, the slide was washed three times with ultrapure water, covered with a cover glass, and observed under a fluorescence microscope. The proportion of cells with tails was calculated using CASP software (www.bio-launching.com).

Immunofluorescence staining

Immunofluorescence staining was performed using a DNA Damage Assay Kit (Beyotime Biotechnology). In brief, cells growing on coverslip in 6-well plate were washed with ice-cold PBS, followed by an incubation with fixative at room temperature for 15–20 min. The cells were then treated with blocking solution for 10–20 min, followed by an incubation with anti- γ H2AX antibody at room temperature for 1 h. After washing with washing buffer, the cells were incubated with anti-rabbit 488 secondary antibody for 1 h at room temperature. After washing, cell nuclei were stained with DAPI at room temperature for 10 min and the coverslip was mounted. Images were acquired using an immunofluorescence microscope and quantified using ImageJ software.

Animal experiments

An *in vivo* xenograft assay was performed as described previously [23]. Liver cancer MHCC97L cells (1×10^6) were subcutaneously implanted in the flank of male BALB/c nude mice (5-week-old, Beijing Vital River Laboratory Animal Technology, Beijing, China) which were maintained under specific pathogen-free conditions. The mice were randomly divided into 5 groups: control group, IgG group, anti-CD47 antibody group, cisplatin group, and cisplatin plus anti-CD47 antibody group. Five days post-injection, the tumor volumes were measured with a caliper, and nine days post-injection, the mice were treated as described. Cisplatin (2.5 mg/kg; MedChemExpress) and anti-CD47 antibody (200 mg/mice; Selleck) were injected i.p. to the mice every 3 days. The volume was calculated using the equation $V = ab^2/2$ (a, tumor length; b, tumor width). At the end of experiment, the mice were sacrificed and tumors were excised. All procedures were approved by the Ethics Committee of the Medical College of Qingdao University.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0; IBM Corp., Chicago, USA) and GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, USA). Differences between groups were analyzed using Student's *t*-test, one-way ANOVA, or the chi-square test. Correlations between groups were assessed using the Pearson correlation coefficient. Diagnostic value was evaluated using receiver operating characteristic curve analysis. The Kaplan-Meier method was used for survival analysis, and the log-rank test was used to determine statistical significance. Data are expressed as the mean $\pm$ SD. Statistical significance was set at $P < 0.05$.

Results

Cisplatin induces CD47 expression in cancer cells

We investigated the impact of cisplatin on CD47 expression in various cancer cell lines. Our findings revealed that cisplatin

treatment significantly upregulated both protein and mRNA levels of CD47 in liver cancer HepG2, Hep3B, and MHCC97L cells (Figure 1A,B). Furthermore, this induction of CD47 expression exhibited dose- and time-dependent patterns (Figure 1C,D). To extend our observations, we examined additional cancer cell types, including glioblastoma LN229 and U87, breast cancer MCF-7 and MDA-MB-231, and colon cancer RKO and SW480 cell lines. Consistent with our initial findings, cisplatin treatment similarly enhanced CD47 expression at both protein and mRNA levels across all tested cancer cell types (Figure 1E).

Cisplatin induces CD47 expression through the ATM/NF- κ B pathway

To elucidate the mechanistic basis of cisplatin-induced CD47 upregulation, we investigated potential regulatory pathways. Prior studies have established NF- κ B as a transcriptional regulator of

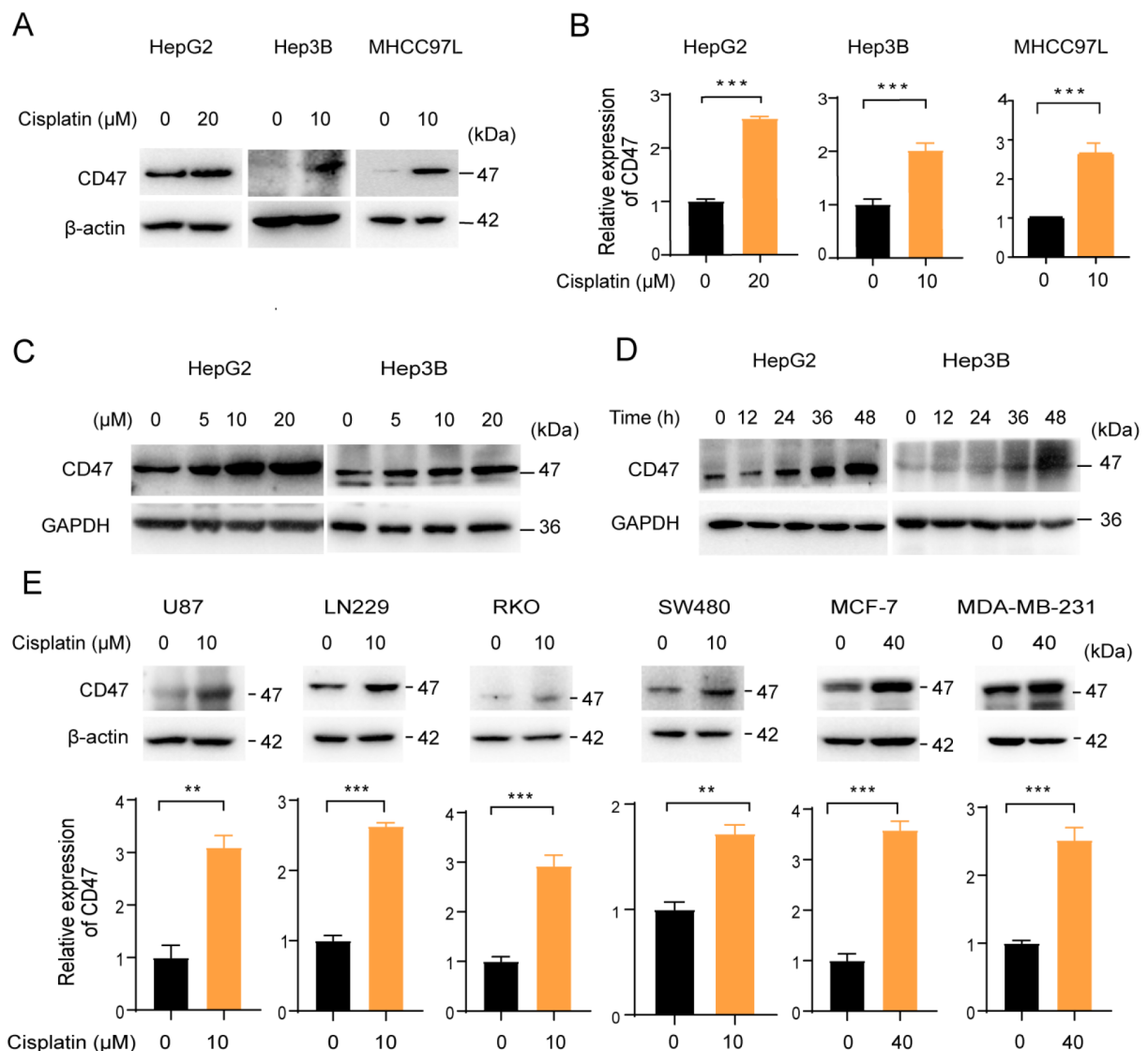


Figure 1. Cisplatin induces the expression of CD47 in cancer cells (A,B) HepG2, Hep3B, and MHCC97L cells were treated with cisplatin for 48 h, followed by determination of the protein (A) and mRNA (B) levels of CD47. (C) HepG2 and Hep3B cells were treated with different doses (0, 5, 10, 20 μM) of cisplatin for 48 h, followed by determination of CD47 protein levels. (D) HepG2 and Hep3B cells were treated with cisplatin (10 μM) for different time. The protein levels of CD47 were determined. (E) U87, LN229, RKO, SW480, MCF-7, and MDA-MB-231 cells were treated with cisplatin for 48 h, followed by determination of protein (upper panel) and mRNA (lower panel) levels of CD47. ** $P < 0.01$, *** $P < 0.001$.

CD47 expression [17]. Our data demonstrated that cisplatin treatment simultaneously upregulated expression of CD47 and induced phosphorylation of p65 in HepG2 cells (Figure 2A), indicative of possible activation and involvement of NF- κ B. As expected, pharmacological inhibition of NF- κ B using its specific inhibitor BAY 11-7082 abrogated cisplatin-mediated induction of CD47 expression (Figure 2A), confirming NF- κ B's essential role in this process.

Given that cisplatin-induced DNA damage activates both cGAS-STING-TBK1 axis [24] and ATM signaling [25], we systematically evaluated their contributions. While TBK1 inhibition with GSK8612

effectively blocked cisplatin-induced TBK1 phosphorylation, it only marginally affected p65 phosphorylation and CD47 expression in HepG2 cells (Figure 2B). In contrast, ATM inhibition with KU55933 potentially suppressed both ATM and p65 phosphorylation, accompanied by a dramatic reduction in CD47 expression induced by cisplatin (Figure 2C). To provide further evidence that cisplatin stimulates the expression of CD47 through ATM/NF- κ B pathway, we determined the effect of ATM knockdown or p65 knockdown on CD47 expression induced by cisplatin. Our results showed that knockdown of ATM significantly inhibited CD47 expression induced by cisplatin (Figure 2D). Similarly, depletion of p65 also

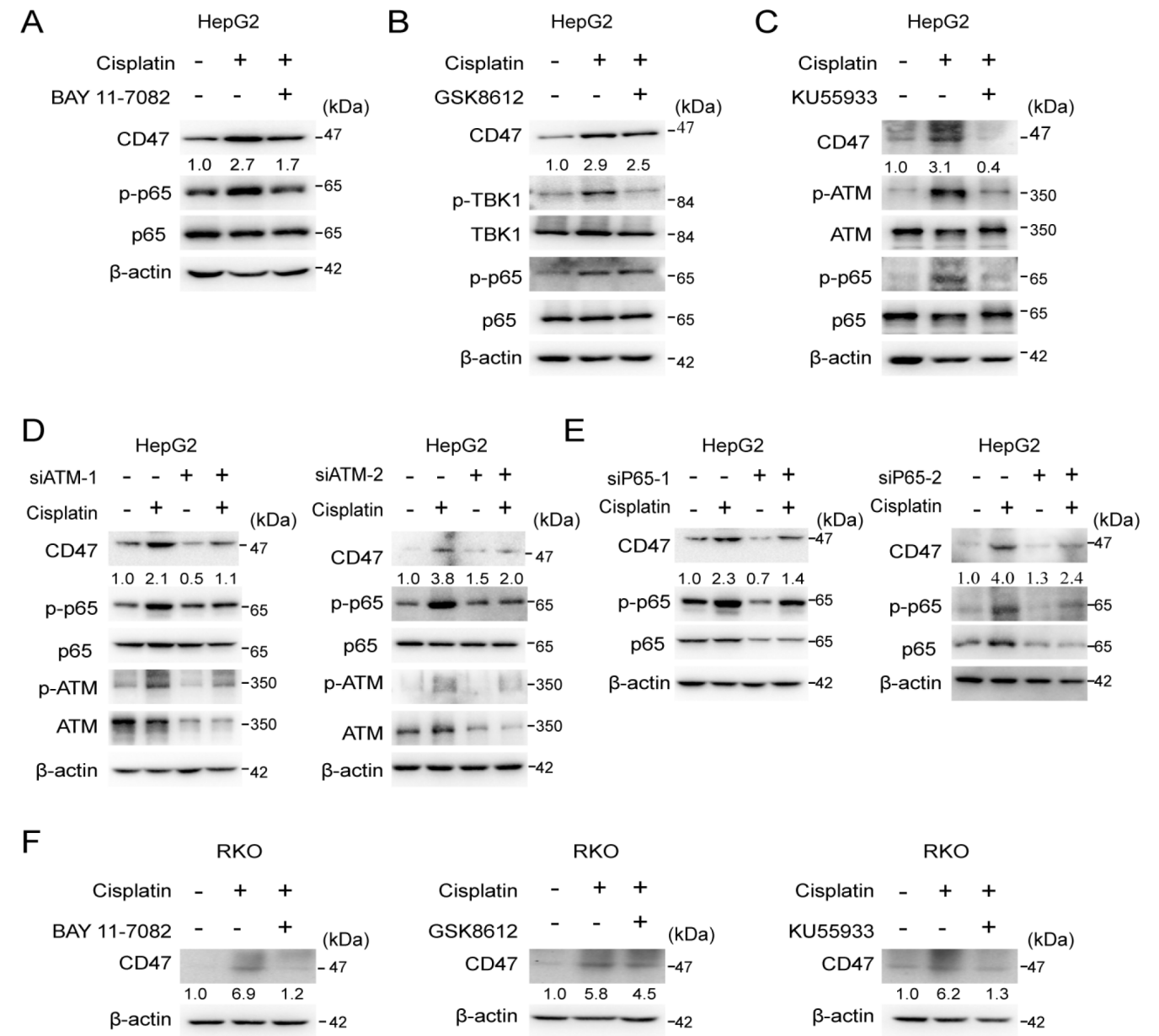


Figure 2. Cisplatin induces CD47 through the ATM/NF- κ B signaling (A) HepG2 cells were treated with 20 μ M cisplatin for 36 h, followed by a co-treatment with 5 μ M NF- κ B inhibitor BAY 11-7082 for 12 h. (B) HepG2 cells were exposed to 20 μ M cisplatin for 36 h, followed by a co-treatment with 10 μ M TBK1 inhibitor GSK8612 for 12 h. (C) HepG2 cells were incubated with 20 μ M cisplatin. After 36 h, 5 μ M ATM inhibitor KU55933 was added and the incubation continued for 12 h. (D,E) HepG2 cells transfected with siATM oligos (D) or si-P65 oligos (E) were treated with 20 μ M cisplatin for 36 h. (F) RKO cells were incubated with cisplatin (10 μ M) for 36 h, followed by treatment with BAY 11-7082 (5 μ M), GSK8612 (10 μ M), or KU55933 (5 μ M) for another 12 h.

blocked the cisplatin-stimulated expression of CD47 (Figure 2E). We also examined RKO cells and found that NF- κ B inhibitor BAY 11-7082 and ATM inhibitor KU55933, but not TBK1 inhibitor GSK8612, inhibited cisplatin-induced expression of CD47 significantly (Figure 2F). Together, these results suggest that cisplatin induced CD47 expression via the ATM/NF- κ B pathway.

Knockdown of CD47 sensitizes cancer cells to cisplatin

To investigate the role of CD47 in regulating cancer cell survival, we performed CD47 knockdown in HepG2 cells and treated them with or without cisplatin. Cisplatin treatment significantly induced cell death, while CD47 knockdown alone had minimal impact on cell survival (Figure 3A). Notably, CD47-deficient cells exhibited increased sensitivity to cisplatin compared to control cells. Similar results were observed in breast cancer MCF-7 cells and colon cancer RKO cells (Figure 3B). Flow cytometry analysis revealed that CD47 depletion alone did not induce apoptosis in HepG2, MCF-7, or RKO cells (Figure 4A,B). However, CD47 deficiency significantly enhanced cisplatin-induced apoptosis in these cell lines (Figure 4A,B). Western blot analysis further demonstrated that loss of CD47 markedly increased Cleaved Caspase 3 levels in the presence, but not in the absence, of cisplatin (Figure 4C). These findings collectively suggest that CD47 plays a crucial role in conferring cisplatin resistance to cancer cells.

Deletion of CD47 impairs DNA damage response

As a genotoxic compound, cisplatin induces DNA damage in cells [26]. To investigate whether CD47 influences cisplatin-induced DNA damage, we performed a comet assay in HepG2 cells. The results revealed that cisplatin treatment, but not CD47 knockdown,

caused DNA strand breaks (Figure 5A). However, CD47 knockdown further exacerbated the levels of DNA strand breaks induced by cisplatin. These findings suggest that loss of CD47 intensified DNA damage triggered by cisplatin.

Genotoxic compounds-caused DNA damage may induce phosphorylation of ATM at Ser1981 and the phosphorylated ATM then phosphorylates H2AX at Ser139 to form γ H2AX [27,28]. The formation of γ H2AX, a marker of DNA damage, is used to evaluate cell DNA damage [29]. We determined the effect of CD47 knockdown on the level of γ H2AX in HepG2 cells treated with or without cisplatin. Immunofluorescence staining of the cells with γ H2AX antibody indicated that cisplatin treatment, but not CD47 knockdown, induced the formation of γ H2AX, and loss of CD47 enhanced significantly the formation of γ H2AX induced by cisplatin (Figure 5B). Western blot analysis results also showed that loss of CD47 dramatically increased the formation of γ H2AX induced by cisplatin (Figure 5C).

Furthermore, we observed that cisplatin treatment, but not CD47 knockdown, significantly stimulated ATM phosphorylation at Ser1981 (Figure 5D). Loss of CD47 markedly enhanced the levels of p-ATM Ser1981 induced by cisplatin (Figure 5D). The tumor suppressor protein p53, a downstream substrate of ATM, is known to be activated and phosphorylated at Ser15 in response to DNA damage [30]. We found that cisplatin induced both the expression and phosphorylation of p53, whereas CD47 knockdown alone did not. Intriguingly, loss of CD47 dramatically enhanced p53 phosphorylation without affecting its expression levels in cisplatin-treated cells (Figure 5E). These data indicate that CD47 plays a critical role in facilitating DNA damage repair mechanisms activated by cisplatin.

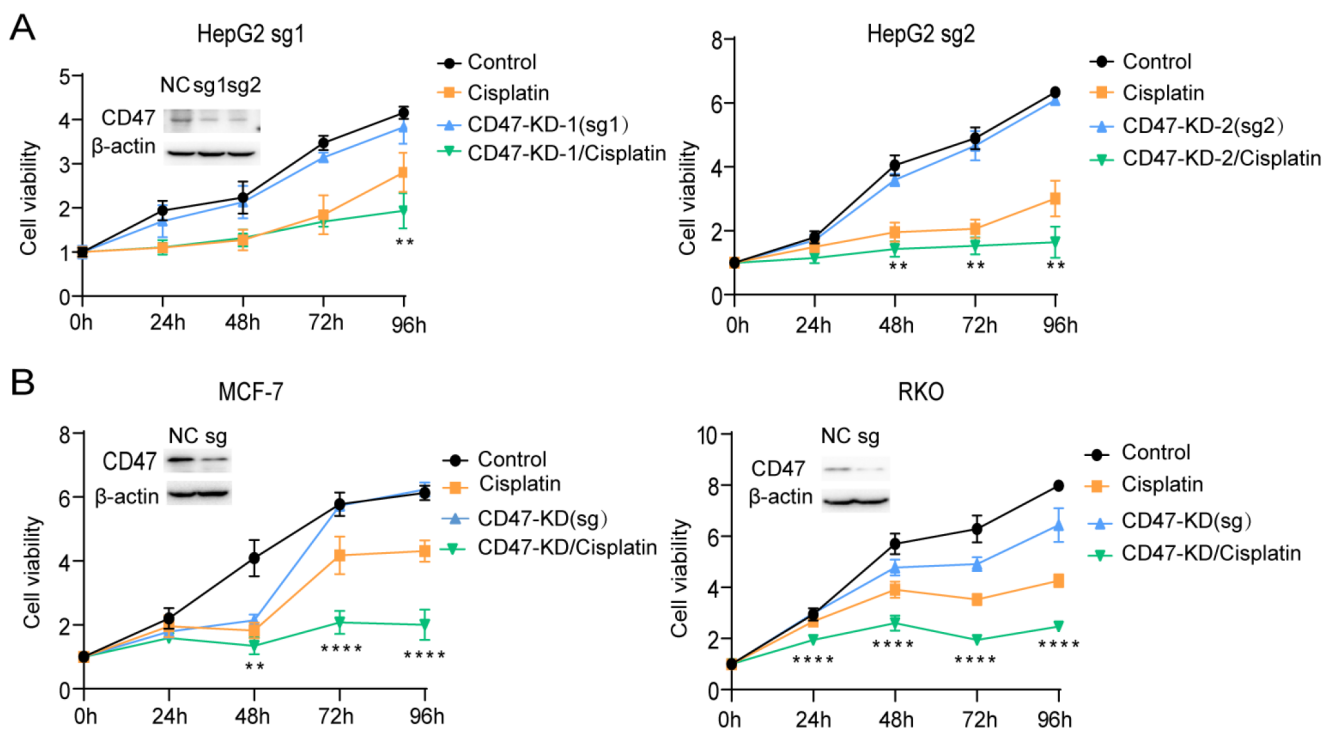


Figure 3. Depletion of CD47 potentiates cisplatin-induced loss of cell viability (A) The control and CD47-knockdown (CD47-KD) HepG2 cells (5×10^3) were seeded onto 96-well plates. After 24 h, cisplatin ($10 \mu\text{M}$) was added and incubation continued. Cell viability was determined at indicated time intervals. (B) MCF-7 and RKO cells were transfected as indicated. After 24 h, the cells were treated with cisplatin ($10 \mu\text{M}$) and cell viability was determined as described above. $**P < 0.01$, cisplatin vs CD47-KD/cisplatin, $****P < 0.0001$, cisplatin vs CD47-KD/cisplatin.

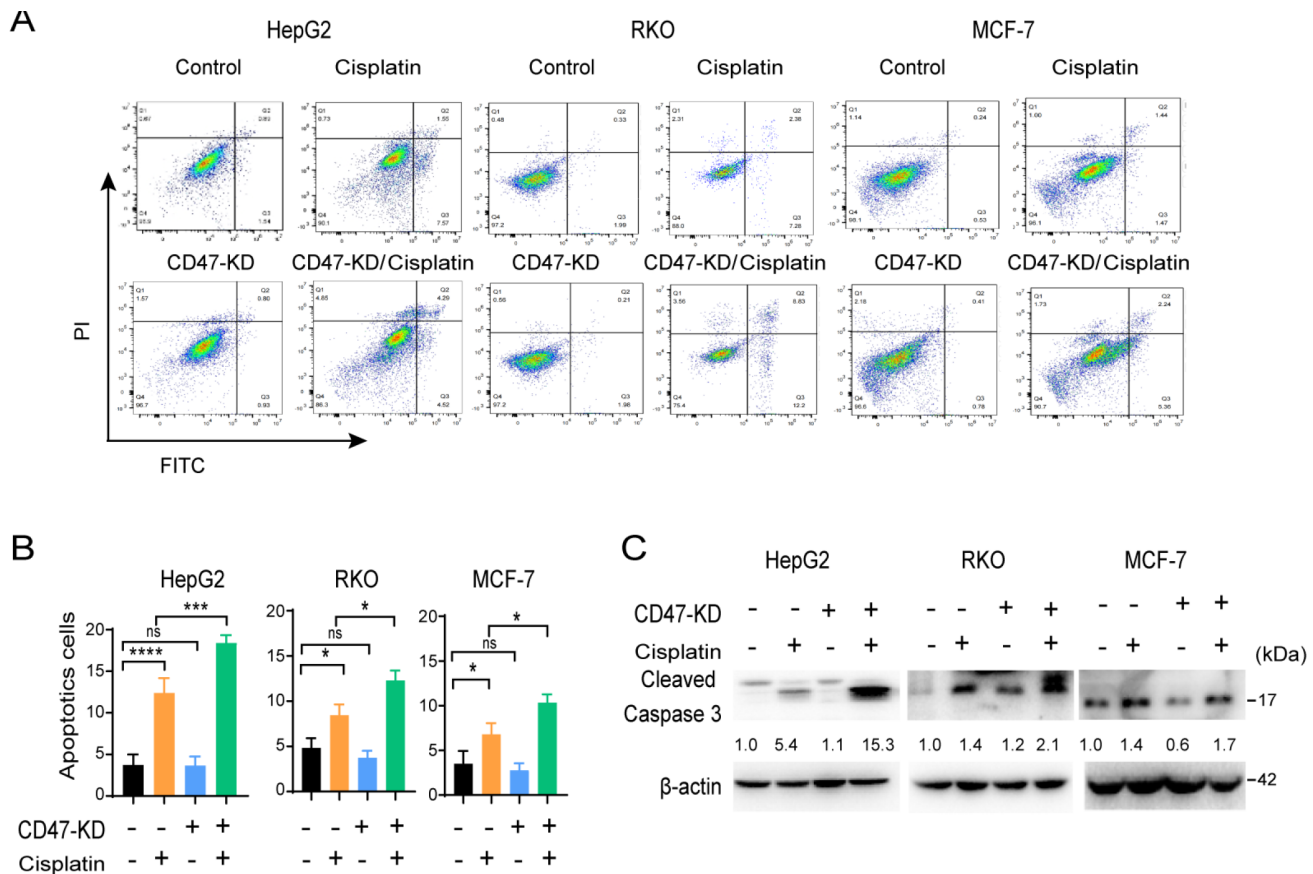


Figure 4. CD47 knockdown enhances cisplatin-induced apoptosis in cancer cells (A) The control and CD47-KD HepG2 cells, RKO cells, and MCF-7 cells (4.5×10^5) were respectively seeded onto 6-well plates. After 24 h, cisplatin (20 μ M) was added and incubation continued. After 48 h, apoptotic cells were detected by flow cytometry. (B) The calculated proportion of apoptotic cells. (C) CD47-KD increased the levels of Cleaved Caspase 3 induced by cisplatin in HepG2, RKO, and MCF-7 cells. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. ns, not significant.

CD47 regulates the expression of DNA damage repair genes

To elucidate the molecular mechanisms by which CD47 regulates the DNA damage response, we conducted qPCR to assess the expression of cisplatin-affected DNA damage repair genes, including *CSA*, *XRCC1*, *POLL*, *FAAP24*, *SLX4*, *FANCA*, *ERCC1*, *REV3*, *RAD50*, *RAD51*, *BRCA2*, and *MRE11* [31]. Our findings revealed that CD47 knockdown significantly suppressed the cisplatin-induced expression of *ERCC1*, *FANCA*, and *BRCA2* genes (Figure 6A,B). To investigate whether CD47 influences cell survival by modulating the expression of DNA repair genes, we overexpressed *ERCC1* and *FANCA* in HepG2 cells. The results demonstrated that overexpression of *ERCC1* and *FANCA* restored cell viability following cisplatin treatment (Figure 6C). Additionally, overexpression of these genes markedly decreased the cisplatin-induced formation of γ H2AX and p-ATM Ser1981 (Figure 6D). Collectively, these data indicate that CD47 promotes cancer cell survival by enhancing the expression of DNA repair genes.

CD47 blockade with neutralizing antibody sensitizes liver cancer cells to cisplatin

To investigate whether CD47 blockade sensitizes cancer cells to cisplatin, HepG2 cells were treated with cisplatin either in the presence or absence of a CD47 neutralizing antibody. Cisplatin treatment markedly induced cell death (Figure 7A). In contrast,

treatment with the anti-CD47 antibody alone had a minimal impact on cell survival. However, the combination of the anti-CD47 antibody with cisplatin significantly enhanced the cell death-inducing efficacy of cisplatin. Western blot analysis demonstrated that cells treated with anti-CD47 antibody and cisplatin exhibited elevated levels of γ H2AX, p-ATM Ser1981, and Cleaved Caspase 3 compared to those treated with cisplatin alone (Figure 7B). Additionally, anti-CD47 antibody treatment attenuated the expression levels of *ERCC1*, *BRCA2*, and *FANCA* upregulated by cisplatin (Figure 7C). These findings suggest that CD47 blockade potentiates cisplatin-induced cell death through reducing the expression of DNA repair genes.

We further performed *in vivo* xenograft growth assay. The mice bearing tumors were treated with cisplatin, anti-CD47 antibody, or cisplatin plus anti-CD47 antibody. The results showed that administration of anti-CD47 antibody to mice significantly enhanced the inhibitory effect of cisplatin on tumor growth (Figure 8A,B). Western blot analysis indicated that anti-CD47 antibody treatment enhanced the expression levels of Cleaved Caspase 3 and γ H2AX (Figure 8C) and decreased the expression levels of *FANCA*, *BRCA2*, and *ERCC1* (Figure 8D).

Discussion

In this study, we demonstrated that cisplatin induces the expression of CD47 through the ATM/NF- κ B pathway in cancer cells. Knock-

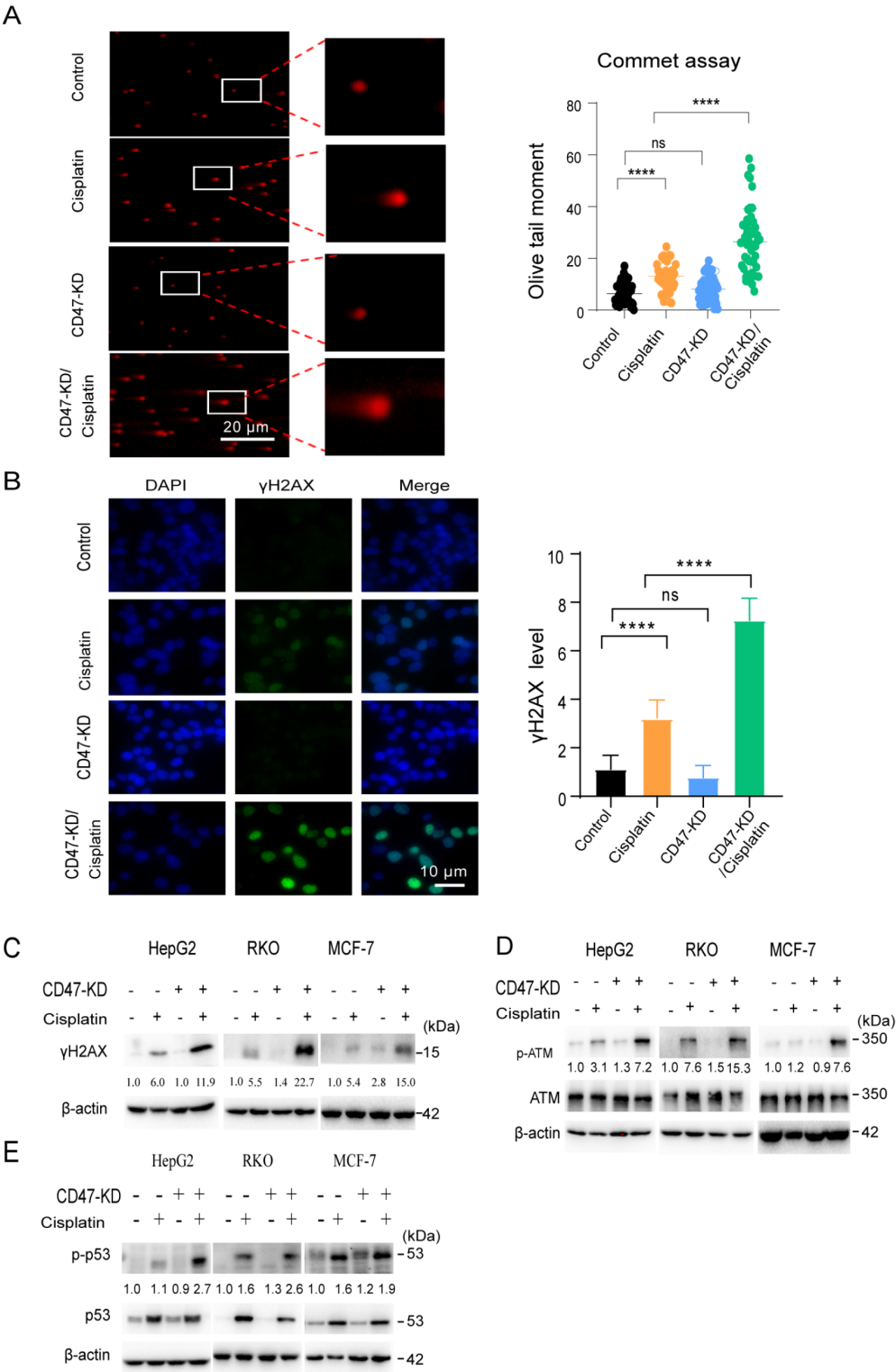


Figure 5. Deletion of CD47 impairs DNA damage response (A) Control and *CD47*-KD HepG2 cells (4.5×10^5) were seeded onto 6-well plates, respectively. After 24 h, cisplatin (20 μ M) was added and cell incubation continued. After 48 h, comet tails were photographed under a fluorescence microscope (left) and the proportion of cells with tails was calculated using CASP software (www.bio-launching.com) (right). (B) Control and *CD47*-KD HepG2 cells (4.5×10^5) were seeded onto 6-well plates covered with glass slides, respectively. After 24 h, cisplatin (20 μ M) was added and incubation continued. After 48 h, the cells were stained with anti- γ H2AX antibody and DAPI. The stained cells were observed under a fluorescence microscope (left) and the intensity of stained γ H2AX was measured using Image Lab (right). (C) *CD47*-KD increased the levels of γ H2AX induced by cisplatin in HepG2, RKO, and MCF-7 cells. (D) *CD47*-KD increased the levels of p-ATM induced by cisplatin. (E) *CD47*-KD increased the levels of p-p53 induced by cisplatin. **** $P < 0.0001$. ns, not significant.

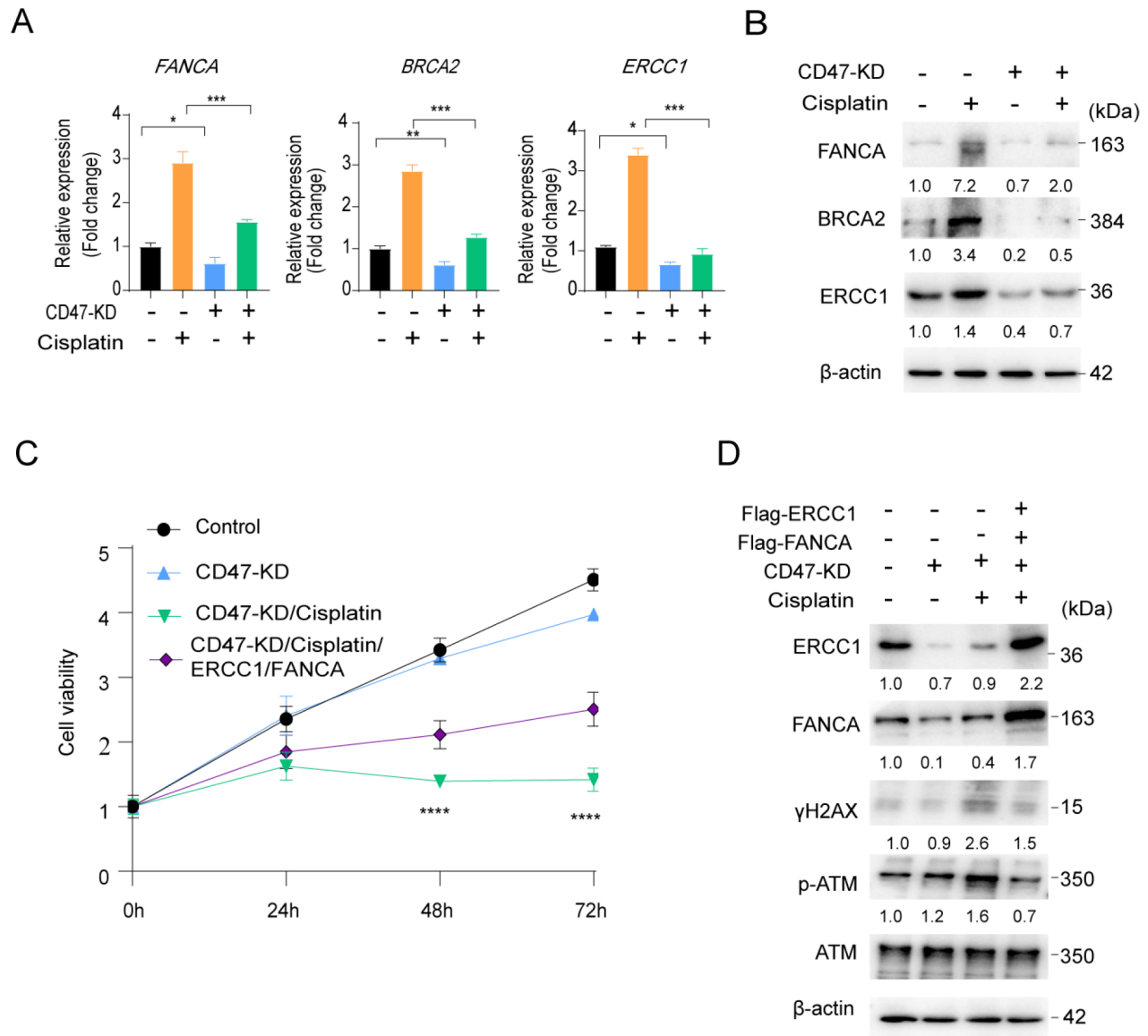


Figure 6. CD47 regulates the expression of DNA damage repair genes (A) HepG2 cells were treated with cisplatin (10 μ M) for 48 h. After treatment, the mRNA levels of indicated DNA repair genes were determined by qRT-PCR. (B) HepG2 cells were treated with cisplatin as described above. The protein levels of FANCA, BRCA2, and ERCC1 were determined. (C) Cells were seeded onto 96-well plates. After 24 h, cisplatin (10 μ M) was added and cell incubation continued. Cell viability was detected at indicated time points. (D) HepG2 control and CD47-KD cells expressing Flag-ERCC1 or Flag-FANCA were treated with cisplatin 10 μ M for 48 h, followed by western blot analysis. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, CD47-KD/cisplatin vs CD47-KD/cisplatin/ERCC1/FANCA.

down of *CD47* sensitizes cancer cells to cisplatin through inhibiting the cisplatin-induced expression of DNA repair genes *ERCC1*, *FANCA*, and *BRCA2*, impairing cellular DNA damage repair and increasing cell death. Notably, the anti-CD47 antibody treatment also reduced the expression of cisplatin-induced above mentioned genes and enhanced cell death. Our results suggest that CD47 functions to confer cancer cell resistance to cisplatin and CD47-targeting sensitizes cancer cells to chemotherapy (Figure 9)

We found that cisplatin induced the expression of CD47 across multiple cancer cell lines at the transcriptional level (Figure 1). Mechanistically, cisplatin induces phosphorylation of ATM and the activates ATM then phosphorylates and activates NF- κ B, leading to an increased expression of CD47 (Figure 2). ATM is a protein kinase

that activates checkpoint signaling upon DNA double strand breaks (DSBs) caused by genotoxic stresses. Our findings suggest that cisplatin upregulates CD47 expression through the activation of DNA damage response pathway. Ghantous *et al.* [15] reported that MRE11, a nuclear protein critical for DNA DBSs repair, mediated genotoxic stresses-induced CD47 expression in HEK293T cells, whereas NF- κ B was not involved. The difference between these findings indicate that the mechanisms by which genotoxic stresses activate CD47 expression may vary depending on cellular context.

Our study reveals that CD47 knockdown exacerbated cisplatin-induced DNA damage and enhanced the phosphorylation of ATM Ser1981 (Figure 5). Our findings indicate that CD47 is involved in the DNA damage repair process. By impairing the DNA damage

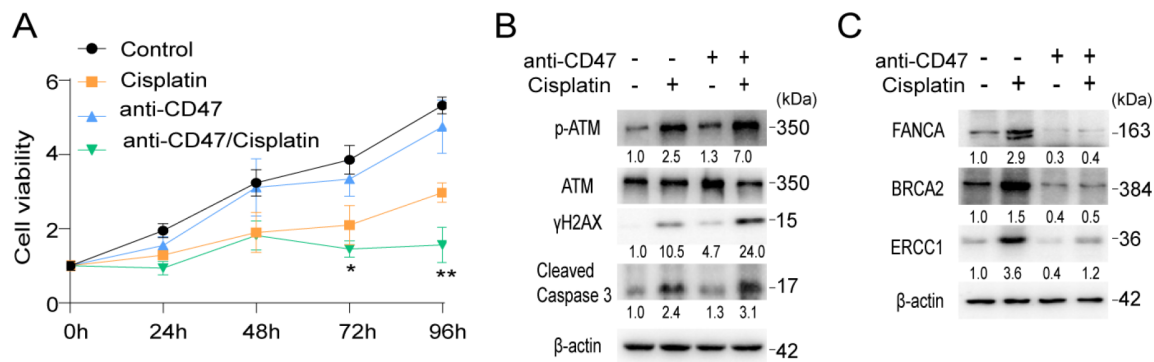


Figure 7. Blockade of CD47 sensitizes liver cancer cells to cisplatin (A) HepG2 cells (5×10^3) were seeded onto 96-well plates. After 24 h, cisplatin (10 μ M) and anti-CD47 antibody (2.5 μ g/mL) were added and incubation continued. Cell viability was detected at indicated time points. (B,C) HepG2 cells (4.5×10^5) were seeded onto 6-well plates and cultured for 24 h. Cisplatin (10 μ M) and anti-CD47 antibody (2.5 μ g/mL) were added and the cells were incubated for another 48 h. * $P < 0.05$, ** $P < 0.01$.

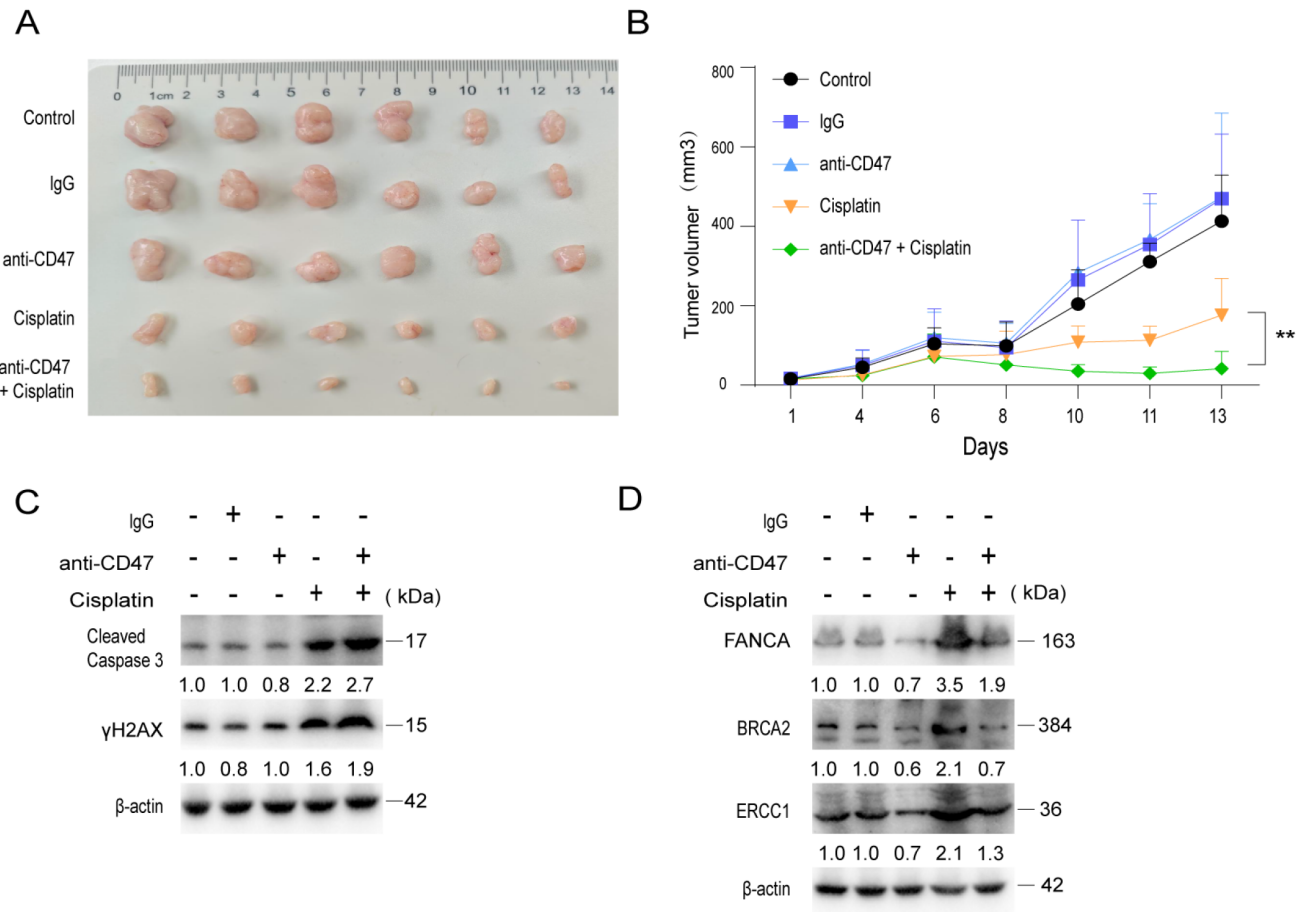


Figure 8. CD47 blockade enhances inhibitory efficacy of cisplatin on liver tumor growth (A,B) Liver cancer MHCC97 cells were implanted onto nude mice and the mice were treated as described in Methods. Tumors (A) and tumor growth kinetics (B) are shown. (C) Expression of Cleaved Caspase 3 and γ H2AX in tumors. (D) Expression of FANCA, BRCA2, and ERCC1 in tumors. ** $P < 0.01$, cisplatin vs anti-CD47/cisplatin.

response, *CD47* knockdown makes cancer cells more vulnerable to the genotoxic effects of cisplatin. This highlights a novel role for *CD47* in regulating DNA repair mechanisms, which could be exploited therapeutically to sensitize cancer cells to DNA-damaging agents like cisplatin.

This study identified that *CD47* knockdown suppressed the

expression of DNA repair genes including *ERCC1*, *FANCA*, and *BRCA2* induced by cisplatin (Figure 6). Among these three genes, *BRCA2* is a homologous recombination repair gene, *ERCC1* is a nucleotide excision repair gene, and *FANCA* belongs to Fanconi Anemia Pathway that plays a crucial role in the repair of DNA interstrand crosslinks [32]. Our results indicate that *CD47* mod-

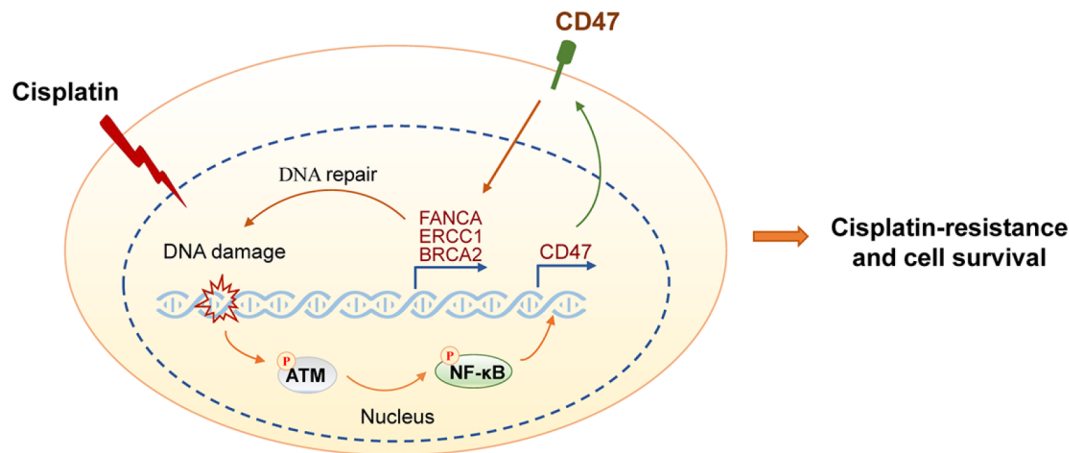


Figure 9. A working model for CD47-mediated chemoresistance in cancer cells

ulates DNA repair in a multiple ways. The mechanism by which CD47 regulates the expression of these DNA repair genes is still unknown. In addition to SIRP α , other molecules such as thrombospondin-1 (TSP-1) and integrins also interact with CD47 and affect CD47-expressing cells directly through closely related signaling pathways [33,34]. Thus, it is possible that CD47 may regulate the expression of DNA repair genes through these pathways, which warrant further investigation. The suppression of the expression of these genes upon CD47 knockdown further supports the idea that CD47 is a critical regulator of the DNA damage response. Targeting CD47 could thus disrupt the DNA repair machinery in cancer cells, making them more susceptible to cisplatin-induced cell death.

We demonstrated that blocking CD47 with an antibody sensitized HepG2 cells to cisplatin, leading to increased cell death, elevated levels of γ H2AX, p-ATM Ser1981, and Cleaved Caspase-3 (Figure 7). Additionally, CD47 blockade attenuated the expression of ERCC1, BRCA2, and FANCA that were upregulated by cisplatin. Similar results were obtained from *in vivo* xenograft assay (Figure 8). These results imply that CD47 blockade potentiates the cytotoxic effects of cisplatin. Given that CD47-inhibition enhances the phagocytic clearance of cancer cells, the dual effects make CD47 blockade a promising therapeutic strategy, particularly in combination with cisplatin-based chemotherapy. CD47 inhibitors are already being explored in clinical trials for their ability to enhance immune-mediated tumor clearance [35]. The results suggest that CD47 inhibitors could also be used to sensitize tumors to chemotherapy, particularly in cancers that are resistant to cisplatin.

Although our findings establish a crucial role for CD47 in mediating cisplatin resistance, several important questions warrant further investigation. First, translational validation in appropriate *in vivo* models and clinical cohorts will be essential to confirm the therapeutic potential of targeting this pathway. Second, while we have identified CD47's regulation of DNA repair genes, the precise molecular intermediaries connecting CD47 signaling to transcriptional control of DNA repair machinery remain to be fully characterized. Notably, our discovery raises the intriguing possibility that CD47 inhibition may represent a broad strategy for potentiating genotoxic therapies. Systematic evaluation of CD47 blockade in combination with other DNA-damaging agents (e.g., PARP inhibitors, platinum analogs) could reveal wider applications beyond cisplatin sensitization. Moreover, the intersection between

chemotherapy-induced immunogenic effects and CD47's immune checkpoint function presents an exciting avenue for further exploration. Future studies should examine potential synergies between cisplatin, CD47 inhibitors, and other immune modulators to develop comprehensive therapeutic strategies that simultaneously target, 1) tumor cell-intrinsic DNA repair pathways, 2) intrinsic/extrinsic immune evasion mechanisms, and 3) therapy-induced immunogenic cell death.

In summary, our study highlights the critical role of CD47 in mediating cisplatin resistance in cancer cells. By upregulating CD47, cancer cells not only evade immune surveillance but also enhance their ability to repair cisplatin-induced DNA damage. Targeting CD47 could therefore represent a promising strategy to overcome cisplatin resistance and improve therapeutic outcomes in patients with cancer.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Chan KS, Espinosa I, Chao M, Wong D, Ailles L, Diehn M, Gill H, *et al.* Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc Natl Acad Sci USA* 2009, 106: 14016–14021
2. Bian HT, Shen YW, Zhou YD, Nagle DG, Guan YY, Zhang WD, Luan X. CD47: beyond an immune checkpoint in cancer treatment. *Biochim Biophys Acta Rev Cancer* 2022, 1877: 188771
3. Jaiswal S, Jamieson CHM, Pang WW, Park CY, Chao MP, Majeti R, Traver D, *et al.* CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 2009, 138: 271–285

4. Barrera L, Montes-Servín E, Hernandez-Martinez JM, García-Vicente MÁ, Montes-Servín E, Herrera-Martínez M, Crispín JC, *et al.* CD47 overexpression is associated with decreased neutrophil apoptosis/phagocytosis and poor prognosis in non-small-cell lung cancer patients. *Br J Cancer* 2017, 117: 385–397
5. Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRPα signaling axis as an innate immune checkpoint in cancer. *Immunol Rev* 2017, 276: 145–164
6. Willingham SB, Volkmer JP, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, Wang J, *et al.* The CD47-signal regulatory protein alpha (SIRPα) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad Sci USA* 2012, 109: 6662–6667
7. Nagahara M, Mimori K, Kataoka A, Ishii H, Tanaka F, Nakagawa T, Sato T, *et al.* Correlated expression of CD47 and SIRPα in bone marrow and in peripheral blood predicts recurrence in breast cancer patients. *Clin Cancer Res* 2010, 16: 4625–4635
8. Yuan J, He H, Chen C, Wu J, Rao J, Yan H. Combined high expression of CD47 and CD68 is a novel prognostic factor for breast cancer patients. *Cancer Cell Int* 2019, 19: 238
9. Maxhimer JB, Soto-Pantoja DR, Ridnour LA, Shih HB, DeGraff WG, Tsokos M, Wink DA, *et al.* Radioprotection in normal tissue and delayed tumor growth by blockade of CD47 signaling. *Sci Transl Med* 2009, 1: 3ra7
10. Miller TW, Soto-Pantoja DR, Schwartz AL, Sipes JM, DeGraff WG, Ridnour LA, Wink DA, *et al.* CD47 receptor globally regulates metabolic pathways that control resistance to ionizing radiation. *J Biol Chem* 2015, 290: 24858–24874
11. Soto-Pantoja DR, Ridnour LA, Wink DA, Roberts DD. Blockade of CD47 increases survival of mice exposed to lethal total body irradiation. *Sci Rep* 2013, 3: 1038
12. Soto-Pantoja DR, Miller TW, Pendrak ML, DeGraff WG, Sullivan C, Ridnour LA, Abu-Asab M, *et al.* CD47 deficiency confers cell and tissue radioprotection by activation of autophagy. *Autophagy* 2012, 8: 1628–1642
13. Kaur S, Soto-Pantoja DR, Stein EV, Liu C, Elkhouloun AG, Pendrak ML, Nicolae A, *et al.* Thrombospondin-1 signaling through CD47 inhibits self-renewal by regulating c-myc and other stem cell transcription factors. *Sci Rep* 2013, 3: 1673
14. Kaur S, Schwartz AL, Jordan DG, Soto-Pantoja DR, Kuo B, Elkhouloun AG, Mathews Griner L, *et al.* Identification of schlafen-11 as a target of CD47 signaling that regulates sensitivity to ionizing radiation and topoisomerase inhibitors. *Front Oncol* 2019, 9: 994
15. Ghantous L, Volman Y, Hefez R, Wald O, Stern E, Friehmann T, Chajut A, *et al.* The DNA damage response pathway regulates the expression of the immune checkpoint CD47. *Commun Biol* 2023, 6: 245
16. Lee TKW, Cheung VCH, Lu P, Lau EYT, Ma S, Tang KH, Tong M, *et al.* Blockade of Cd47-mediated cathepsin s/protease-activated receptor 2 signaling provides a therapeutic target for hepatocellular carcinoma. *Hepatology* 2014, 60: 179–191
17. Lo J, Lau EYT, Ching RHH, Cheng BYL, Ma MKF, Ng IOL, Lee TKW. Nuclear factor kappa B-mediated CD47 up-regulation promotes sorafenib resistance and its blockade synergizes the effect of sorafenib in hepatocellular carcinoma in mice. *Hepatology* 2015, 62: 534–545
18. Wang H, Tan M, Zhang S, Li X, Gao J, Zhang D, Hao Y, *et al.* Expression and significance of CD44, CD47 and c-met in ovarian clear cell carcinoma. *Int J Mol Sci* 2015, 16: 3391–3404
19. Tan W, Tang H, Jiang X, Ye F, Huang L, Shi D, Li L, *et al.* Metformin mediates induction of miR-708 to inhibit self-renewal and chemoresistance of breast cancer stem cells through targeting CD47. *J Cell Mol Med* 2019, 23: 5994–6004
20. Mateo V, Lagneaux L, Bron D, Biron G, Armant M, Delespesse G, Sarfati M. CD47 ligation induces caspase-independent cell death in chronic lymphocytic leukemia. *Nat Med* 1999, 5: 1277–1284
21. Manna PP, Frazier WA. CD47 mediates killing of breast tumor cells via G1-dependent inhibition of protein kinase A. *Cancer Res* 2004, 64: 1026–1036
22. Lun J, Zhang Y, Yu M, Zhai W, Zhu L, Liu H, Guo J, *et al.* Circular RNA circHIPK2 inhibits colon cancer cells through miR-373-3p/RGMA axis. *Cancer Lett* 2024, 593: 216957
23. Wang Y, Lun J, Zhang Y, Yu M, Liu X, Guo J, Zhang H, *et al.* miR-373-3p promotes aerobic glycolysis in colon cancer cells by targeting MFN2. *Acta Biochim Biophys Sin* 2024, 56: 1498–1508
24. Miranda A, Shirley CA, Jenkins RW. Emerging roles of TBK1 in cancer immunobiology. *Trends Cancer* 2024, 10: 531–540
25. Wu ZH, Shi Y, Tibbetts RS, Miyamoto S. Molecular linkage between the kinase ATM and NF-κB signaling in response to genotoxic stimuli. *Science* 2006, 311: 1141–1146
26. Romani AMP. Cisplatin in cancer treatment. *Biochem Pharmacol* 2022, 206:115323
27. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: the trinity at the heart of the DNA damage response. *Mol Cell* 2017, 66: 801–817
28. Merighi A, Gionchiglia N, Granato A, Lossi L. The phosphorylated form of the histone H2AX (γH2AX) in the brain from embryonic life to old age. *Molecules* 2021, 26: 7198
29. Prabhu KS, Kuttikrishnan S, Ahmad N, Habeeba U, Mariyam Z, Suleman M, Bhat AA, *et al.* H2AX: a key player in DNA damage response and a promising target for cancer therapy. *Biomed Pharmacother* 2024, 175: 116663
30. Meek DW. Tumour suppression by p53: a role for the DNA damage response? *Nat Rev Cancer* 2009, 9: 714–723
31. Slysokova J, Sabatella M, Ribeiro-Silva C, Stok C, Theil AF, Vermeulen W, Lans H. Base and nucleotide excision repair facilitate resolution of platinum drugs-induced transcription blockage. *Nucleic Acids Res* 2018, 46: 9537–9549
32. Wang R, Sun Y, Li C, Xue Y, Ba X. Targeting the DNA damage response for cancer therapy. *Int J Mol Sci* 2023, 24: 15907
33. Ahvati H, Roudi R, Sobhani N, Safari F. CD47 as a potent target in cancer immunotherapy: a review. *Biochim Biophys Acta Rev Cancer* 2025, 1880: 189294
34. Polara R, Ganesan R, Pitson SM, Robinson N. Cell autonomous functions of CD47 in regulating cellular plasticity and metabolic plasticity. *Cell Death Differ* 2024, 31: 1255–1266
35. Lau APY, Khavkine Binstock SS, Thu KL. CD47: the next frontier in immune checkpoint blockade for non-small cell lung cancer. *Cancers* 2023, 15: 5229