Cisplatin Increased the Expression of PD-1 and PD-L1 by YAP1 in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) was one of the most malignant cancers in the world. Cisplatin (DDP) was one of the main chemotherapy drugs for HCC, but the mechanism of DDP treatment for HCC remains unclear. In this presentation, we found that DDP inhibited the growth of HCC cells and promoted the expression of PD-1 and its ligand PD-L1 in cancer cells. Meanwhile, flow cytometry analysis revealed that DDP enhanced PD-1⁺CD8⁺ T cells expression and decreased PD-1⁺CD8⁺ T cells expression. ELISA analysis suggested that DDP decreased TGF- β expression in vivo. Therefore, the study indicated that DDP enhanced PD-1 and PD-L1 expression and inhibited the growth of HCC.

Introduction

Hepatocellular carcinoma (HCC) was the third leading cause of cancer[1]. At present, surgery and chemotherapy were the main treatments for HCC. Chemotherapy drug cisplatin (DDP) was one of the most used drugs in the treatment of cancer. The tumor microenvironment inhibited the efficacy of DDP[2]. However, the effect of DDP on the tumor microenvironment and its mechanism remains unknown.

Tumor immune microenvironment includes tumor cells, lymphocytes (Th1, Th2, etc.), pro-inflammatory cytokines, free cytokines and membrane-expressed immunosuppressive molecule (PD-1 and PD-L1) [3, 4].

Programmed death protein 1 (PD-1), a type I transmembrane glycoprotein, was an essential negative regulator of immune responses. Under normal conditions, PD-1 inhibited the function of T lymphocytes by combining with its ligands PD-L1, leading to the tumor escape from the immune system[5].

Programmed death-ligand 1(PD-L1) was commonly upregulated in many cancer cells. Studies showed that the PD-1/PD-L1 pathway played a vital role in cancer immunity[6]. A previous study indicated that PD-1 and PD-L1 also expressed cancer cells. Over-expression of PD-1 and PD-L1 inhibited the progression of the cancer[7]. However, the effect and mechanism of DDP on PD-1 and PD-L1 remain unclear.

Yes-associated protein 1 (YAP1) was a critical downstream effector of the Hippo pathway[8]. A previous study revealed that YAP1 promoted the recruitment of immune cells. YAP1 also induced PD-L1 expression[9]. In this study, we also found that DDP promoted YAP1 expression promoted PD-L1 expression. It also increased CD8⁺ T cells[10]. We reported that DDP promoted the expression of PD-1 and PD-L1 by increasing the expression of YAP1 in HCC. Therefore, this study provided the mechanism for the liver cancer microenvironment with DDP.

Materials And Methods

Cell lines and drug treatment

HepG2215 and HepG2 cells were purchased from the American Type Culture Collection. HepG2215 and HepG2 cells were identified by Shanghai FuHeng BioLogy Co., Ltd. (www.fudancell.com) and Shanghai Biowing applied biotechnology Co., Ltd.(<http://www.biowing.com.cn/>), respectively, and cultured in

DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C and in an atmosphere with 5% CO₂ and 100% humidity. Cisplatin was purchased from Jinan Qilu Pharmaceutical Factory, Co., Ltd. (Jinan, China). HepG2 and HepG2215 cells were treated with cisplatin.

Lentivirus production and infection

The plasmids that encode the lentiviruses expressing shRNA molecules were obtained from the RNAi Consortium shRNA Library. The shRNA target 21-mer sequences were: shControl, CCTAAGGTTAAGTCGCCCTCG; human sh *YAP1*

#3CCGGGACCAATAGCTCAGATCCTTTCTCGAGAAAGGATCTGAGCTATTGGTCTTTTTG. HepG2215 cells were placed in a 24-well tissue culture dish (2×10^5 cells per well) and infected with 80 µL shRNA lentivirus supernatant and 4 µg/ mL polyene. The infected cells were placed in RPMI medium containing 10% fetal bovine serum and 3 µg/ mL purinomycin, and detected 72 h after infection. Western blot was used to detect shRNA knockout efficiency.

Human Protein Atlas database

Human Protein Atlas (HPA) (<https://www.proteinatlas.org/>) provided each protein in 64 cell lines, 48 kinds of normal human tissues, and 20 kinds of cancer tissues. We collected 365 patients from HPA database and plotted survival time curves.

Survival analysis of PD-1 and PD-L1

Kaplan-Meier Plotter (KM) (<https://kmplot.com/analysis/>) is a clinical database of survival analysis, Log rank p-value and hazard ratio (HR), and 95% confidence intervals to assess the relationship between PD-1 and PD-L1 and overall survival.

Animal experiment

All animals were fed in the SPF with constant temperature (22-24 °C) and a dark–light cycle of 12 h/12 h, and housed in plastic cages. The protocol was approved by the Ethics Committee for Animal Experiment of Hebei University of Chinese Medicine (Permit number: YXLL2018002).

Immunofluorescence assay

All fluorescence images were observed under a confocal instrument. The primary antibodies were rabbit anti-CD4 (GB11064, Seville Biological) and rabbit anti-CD8 (GB13429, Seville Biological). The secondary antibody was FITC goat anti-rabbit IgG-HRP (GB22303, Seville Biological). Nucleus was blue by labeling with DAPI. Positive cells were green or red according to the fluorescent labels used. The image was captured using a fluorescence microscope (Olympus BX51, Japan).

Flow cytometry analysis

Spleens of mice were removed and placed in 5 mL PBS+1%FBS+2 mM EDTA solution. The spleen was ground and filtered to obtain spleen cells; Lyse the erythrocytes, count the cells, adjust to 1×10^6 cells/ml; Adding the antibody according to the antibody instruction, stained and tested on the machine. Finally, the results were analyzed.

Hematoxylin and eosin (H&E) staining

All organs were removed quickly, and the liver tissues were fixed with 4% formaldehyde for 24-48 h and then embedded with paraffin. The section thickness was 5 μ m. The sliced sections were stained with haematoxylin and eosin (H&E), and change of histopathological was obtained using a microscope (Leica DM2500, Germany).

Immunohistochemistry (IHC)

Immunohistochemical staining was performed according to the instructions of Nakasi Jinqiao Kit. Finally, sections were determined by microscopic observation of the brown peroxidase in liver tissue at a 200 $\times$ and 400 $\times$ magnification. The results of immunohistochemistry were examined by 2 senior histopathologists. The cytomembrane/cytoplasm stained with light yellow or tan were regarded as positive cells. The optical density was measured by an Image-Pro Plus v6.0 software (Media Cybernetics, USA).

Measurement of inflammatory cytokines

IL-10 (ml002285, mlbio), IL-4 (ml002149, mlbio), IL-7 (ml002212, mlbio), IL-2

(ml002295, mlbio), IL-1 β (ml063132, mlbio), CCL-2 (ml037533, mlbio), TGF- β (ml057830, mlbio) and IFN- γ (ml063095, mlbio) were determined using ELISA kits according to the manufacturer's instructions.

Western blot analysis

HepG2215 and HepG2215 sh *YAP1* cells were seeded in 6-well plates (3×10^5 cells/well) and treated as described above. HepG2215 and HepG2215 shYAP1 cells were treated with DDP for 24 h. After a brief clean up at PBS, the cells were lysed directly in the SDS sample buffer (50 mM Tris-HCl pH 6.8, 1% SDS, 10% glycerol, 5% β -mercaptoethanol, 0.01% bromophenol blue). The primary antibodies were rabbit YAP1 (D8H1X) XP monoclonal antibody (CST, 14074s, diluted by 1:1000), rabbit PD-1 polyclonal antibody (Proteintech, 18106-1-AP, diluted by 1:2000), mice PD-L1 monoclonal antibody (Abcam, ab269674, diluted by 1:2000), rabbit GAPDH monoclonal antibody (Abways, AB0037, diluted by 1:5000). The secondary antibody was goat anti-rabbit IgG-HRP (Absin, abs20002, diluted by 1:10,000). The bands were detected by the ECL (enhanced chemiluminescence) detection system (Vilber, Fusion FX5 Spectra, France). The band intensity was measured by an Image-Pro Plus v6.0 software (Media Cybernetics, USA).

Ultrasonic testing

At the beginning and end of the experiment, ultrasound was used to measure the number of liver tumors in the mice using an imaging system (Vevo 2100, Visual Sonics Inc., Toronto, Canada) with an MS250 ultrasound transducer. Soon, the mice were anesthetized and their abdomens shaved. M-mode recording of short and long axis views of planed abdominal wall was performed.

DEN/TCPOBOP-induced HCC model in C57BL/6 mice

Yap1^{LKO} mice have been successfully constructed by Guangzhou Cyagen Biosciences (Guangzhou, China). The mice were identified by our group. The modeling method was introduced as previously described[11]. Yap1^{flx/flx} were used as the control. 3-week-old male C57BL/6 mice were injected intraperitoneally with 25 mg/kg bodyweight N-nitrosodiethylamine (DEN). Then at the age of 4 weeks, the mice were injected with a dose of 3 mg/kg body weight TCPOBOP. The mice were divided into four groups after the model was successfully built. For the DDP group, the DDP dissolved in saline. The control mice were injected with saline. The Ethics Committee approved all experimental animal protocols for the Animal Experiment of Hebei University of Chinese Medicine (Permit number: YXLL2018002).

Statistical analysis

All statistical tests were performed by SPSS23.0 statistics software (SPSS, Chicago, IL). All the *in vitro* experiments were repeated for at least three times. Data were presented as Mean±SD. When more than two groups were enrolled, the means were compared between each two groups with one-way ANOVA. Differences were considered statistically significant when $P<0.05$.

Results

DDP inhibited the liver cancer growth in DEN/TCPOBOP-induced HCC model in C57BL/6 mice.

DDP was one of the most commonly used chemotherapy drugs for the treatment of HCC. To further evaluate the effect of DDP *in vivo*, the mice model of liver carcinoma *in situ* was established successfully. We found that the mice's left and right liver lobes had tumors in the saline (NS) group. But there were no apparent tumors on the liver surface of the DDP group (Fig.1A). Further statistics found that DDP decreased the number of tumors ($P<0.05$). DDP also reduced the volume of tumor, but not significantly ($P>0.05$) (Fig.1A). Moreover, the development of tumors could be monitored by small animal ultrasound imaging. The results showed that on the 1st day, in the transverse section, tumor area in the NS group was 19.5 mm², the long diameter was 2.431 mm, and the short diameter was 2.38 mm; in the vertical section, tumor area in the NS group was 5.686 mm², the long diameter was 2.094 mm, and the short diameter was 1.525 mm. The transverse section area of the tumor in the DDP group was 22.093 mm², the long diameter was 3.97 mm, and the short diameter was 2.302 mm; the vertical section area of the tumor in the DDP group was 10.217 mm², the long diameter was 3.05 mm, and the short diameter was 2.28 mm. On the 25th day, in the transverse section, the area of the tumor in the NS group was 13.132 mm², the long diameter was 4.841 mm, and the short diameter was 3.837 mm; in the vertical section, the area

of the tumor in the NS group was 19.916 mm², the long diameter was 4.887 mm, and the short diameter was 3.805 mm. The transverse section area of the tumor in the DDP group was 8.502 mm², the long diameter was 1.093 mm, and the short diameter was 0.567 mm; the vertical section area of the tumor in the DDP group was 7.67 mm², the long diameter was 1.29 mm, and the short diameter was 0.459 mm (Fig. 1B). These results showed that DDP significantly inhibited the growth of liver tumor in C57BL/6 mice.

However, DDP also showed adverse reactions in tumor inhibition. Compared with NS group, DDP decreased the weight of the thymus ($P=0.04$) and kidney ($P=0.04$). It had little effect on the weight of liver and spleen. These results suggested that DDP decreased the weight of thymus and kidney, did not affect organ index (Fig. 1C).

DDP increased PD-1⁻CD8⁺ T cells and reduced PD-1⁺CD8⁺ T cell in cancer cells.

As the component of the immune system, CD8⁺ T cells played an important role in anti-cancer[12, 13]. To further study the expression of CD8⁺ T cells after the DDP treated hepatic carcinoma in situ C57BL/6 mice, immunofluorescence assay and flow cytometry were used for detection. The immunofluorescence assay results suggested that in the NS group, the number of CD8⁺ T cells decreased, while the DDP group had highly CD8⁺ T cells expression (Fig. 2A). Consistent with this, the previous study showed that compared with the control group, the DDP group increased CD8⁺ T-cell infiltration into tumor tissues[14]. Moreover, DDP or DDP combined with anti-PD-1 failed to inhibited the growth of tumor in the depletion of CD8⁺ T cells *in vivo*[14]. These findings indicated that CD8⁺ T cells played a vital role in the anti-tumor effect of DDP. Previous studies showed that high levels of PD-1⁺CD8⁺T cells were associated with a poor prognosis for a range of cancers, including liver, pancreatic, and head and neck cancers[15-18]. In our study, further study showed that DDP also significantly reduced exhausted CD8⁺T lymphocytes (PD-1⁺CD8⁺ T cell) in the blood (Fig. 2C) and spleen ($P<0.05$) (Fig. 2B). It also increased non - draining CD8⁺T lymphocytes (PD-1⁻CD8⁺T cells) in blood ($P<0.05$) (Fig. 2C) and spleen ($P<0.05$) (Fig. 2B). These results showed that DDP inhibited the growth of HCC by increasing the number of PD-1⁻CD8⁺ T cells and decreasing the number of PD-1⁺CD8⁺ T cells. In general, Th1 cells secrete Interleukin-2 (IL-2) and Interferon- γ (IFN- γ) to inhibit tumor growth, while Th2 cells release immunosuppressive factors Interleukin-4 (IL-4) and Interleukin-10 (IL-10) to produce immunosuppression. Chemokines recruited bone marrow-derived suppressor cells and promoted cancers. Interleukin-1 beta (IL-1 β), as proinflammatory cytokines, was elevated in cancers [19]. In our study, we found that DDP had almost no effect on IL-2, IFN- γ , IL-4 and IL-10 (Fig. 2D). Transforming growth factor- β (TGF- β) played crucial role in liver cancer [20]. A study showed that overactivation of TGF- β signaling led to progression of tumor [21]. In our study, ELISA results showed that DDP decreased the expression of TGF- β in liver tumor tissues. TGF- β was significantly lower in the DDP group than that in the NS group (Fig. 2D). The results suggested that DDP decreased the expression of TGF- β . In summary, these results showed that DDP reduced the expression of TGF- β , but did not affect the expression of Th1- type cytokines and Th2- type cytokines, IL-1 β , and chemokines in the liver cancer.

DDP significantly increased the expression of PD-1 in HepG2 and HepG2215 cells.

PD-1 receptor on the surface of immune cells was an immune checkpoint molecule that mediated immune escape from tumor cells. The study revealed that PD-1 expressed in a wide range of cancer cells[7]. To further verify the effect of DDP on PD-1 on hepatocellular carcinoma cells, immunofluorescence assay and Western blot were performed. Immunofluorescence assay results showed that in the NS group, PD-1 had less expression. DDP group significantly improved the expression of PD-1 compared with the NS group (Fig. 3A). The results of Western blot and statistical analysis also showed that DDP increased the PD-1 expression in HepG2 and HepG2215 cells ($P<0.05$) (Fig. 3B-C). PD-L1 was a major ligand [22]. PD-L1 and PD-1 had a similar effect in tumors. A previous study showed that PD-L1 was expressed on these cancer cell lines[7]. Indeed, in our previous study, we found that DDP promoted the PD-L1 expression on tumor cells by promoting YAP1 expression [23]. In summary, these results showed that DDP increased the PD-1 and PD-L1 expression in HepG2 and HepG2215 cells.

Low expression of PD-L1 and PD-1 had poor overall survival time in HCC.

To illustrate PD-1 and PD-L1 overall survival times in HCC, we analyzed clinical information on 365 patients from the HPA database and plotted survival time curves. The result showed that high expression of PD-1 had 255 patients and low expression of PD-1 had 110 patients. Moreover, low expression of PD-1 had poor overall survival time (Fig. 4A). The results showed that high expression of PD-L1 had 211 patients and low expression of PD-L1 had 154 patients. Moreover, low expression of PD-L1 had poor overall survival time compared with a high expression of PD-L1 (Fig. 4A). These results showed that low expression of PD-1 and PD-L1 had poor overall survival time in HCC. Moreover, we also used Kaplan-Meier Plotter database. These results were consistent with the HPA database. The results suggested that low expression of PD-1 (HR=0.68(0.46-0.99), log-rank $P=0.043$) had poor overall survival time in HCC. The expression of PD-L1(HR=0.71(0.47-1.06), log-rank $P=0.095$) had poor overall survival time in HCC (Fig. 4B). These results showed that low expression of PD-L1 and PD-1 had poor overall survival time in HCC

DDP increased the expression of PD-1 and PD-L1 by promoted YAP1 expression in HepG2215 cells.

YAP1 is a critical molecule in the Hippo pathway. The Hippo pathway was primarily known for its role in cell proliferation and differentiation, but some studies suggested its importance in tumor immunity. Studies showed that YAP1 was positively correlated with tumor-infiltrating cells, including CD8⁺ T cells and CD4⁺ T cells, in various cancers, including liver cancers[10]. This result indicated that the elevation of YAP1 was related to the infiltration of some immune cells and had an anti-tumor function. Our previous research showed that compared with the NS group, DDP improved the expression of YAP1 in HepG2215 cells in a dose-dependent manner and promoted the expression of YAP1 in BALB/c mice liver H22 cells[23]. We did immunohistochemical staining. We found that DDP promoted the high expression of YAP1. In this study, the result of immunohistochemical staining suggested that compared with the NS group, DDP improved the presentation of YAP1 in C57BL/6 mice (Fig. 5A).

However, it was unclear whether DDP induced PD-1 and PD-L1 through YAP1 in HepG2215 cells. Previous studies also showed that YAP1 directly bond to the PD-L1 primer to promote the transcription of PD-L1 in PC9 cells of lung cancer.[24]. The study also showed that PD-1 and PD-L1 had a similar effect on the cancer. We further explored the impact of YAP1 on PD-1 and PD-L1. The HepG2215 sh *YAP1* cells were constructed. Meanwhile, in our study, the results of Western blot indicated that compared with HepG2215 shCont cells, knockdown of YAP1 decreased the expression of PD-L1 and PD-1 in HepG2215 cells (Fig. 5B). In summary, DDP promoted the expression of PD-L1 and PD-1 through encouraged YAP1 expression.

DDP increased PD-L1 expression by promoted Yap1 expression in C57BL/6 mice.

To explore the DDP effect on PD-L1 and PD-1, we performed an experiment in C57BL/6 mice. Liver tissue-specific *Yap1* knockout (*Yap1*^{LKO}) mice successfully modeled for HCC. In *Yap1*^{flox/flox} group, DDP reduced the number of tumors (Fig. 6A). In *Yap1*^{LKO} group, DDP reduced the number of tumors. PCR was used to verify the expression of flox and cre (Fig. 6B). Western blot results showed that DDP increased the Yap1 expression compared with *Yap1*^{flox/flox} treated with NS group. Compared with *Yap1*^{flox/flox} treated with the NS group, DDP combined with *Yap1*^{LKO} reduced YAP1 expression. This suggested that DDP could not compensate for Yap1 expression in *Yap1*^{LKO} mice. Compared with *Yap1*^{flox/flox} treated with the NS group, DDP combined with *Yap1*^{LKO} reduced PD-L1 expression (Fig. 6C). These results showed that Yap1 affected the expression of PD-L1. Besides, we found that Yap1 knockout reduced PD-L1 and PD-1 expression. However, DDP had no significant effect on PD-1 in C57BL/6 mice (Fig. 6C). The reason could be that PD-1 was expressed in a variety of tissues, including cancer tissues.

Discussion

DDP was one of the most widely used drugs in the treatment of cancers. It was used in many cancers, such as liver cancers[25] and lung cancers[26]. However, the mechanism of DDP treatment for HCC in the microenvironment remains unclear. In this study, we showed that YAP1 expression promoted the expression of PD-1 and PD-L1 in liver cancer cells. Interestingly, DDP increased the expression level of PD-1 and PD-L1 by YAP1 expression. DDP also decreased the percentage of PD-1⁺CD8⁺ T cells in blood and spleen, and it decreased TGF- β expression level *in vivo*. This study provides a mechanism for the treatment of liver cancer with cisplatin.

PD-1 promoted cancer growth by activation of mTOR in the cancer[27]. Anti-PD-1 treatment reduced the growth of bladder and ovarian cancer cells [28]. These results revealed that PD-1, inherent in cancer cells, was a pro-tumor factor. A study showed that silencing PD-1 or PD-L1 promoted cell proliferation and colony formation *in vitro*, In contrast, overexpression of PD-1 and PD-L1 inhibited the proliferation of cancer and colony formation[7]. Consistently, our studies found that DDP increased the expression of PD-1 and PD-L1, and inhibited liver cancer growth in mice. Anti-PD-1 and anti-PD-L1 have been applied in many cancers by activating T cells. However, a considerable number of patients failed to block the response to the PD-1/PD-L1 axis or relapsed after response. Paradoxical progressive disease(PPD) and hyperprogressive disease (HPD) were gradually recognized in people [7, 29]. However, if the antibody-

activated T cell level is insufficient, cancer grows faster by activating the PD-1/PD-L1 function inherent to the tumor, and then degenerates after T cell overactivation (PPD). In contrast, antibody-mediated therapy enhanced cancer cell growth and suppressed anti-cancer immunity, leading to the development of HPD in the case of immunocompromised or antibody-activated T cells, and appropriately elevated PD-1/PD-L1 expression on cancer cells [30]. A previous study also indicated that the subpopulation of tumor cells expressing both PD-1 and PD-L1 reduced tumor growth [7]. A similar study found that transarterial chemoembolization (TACE) increased the expression of PD-1 and PD-L1 in HCC, optimizing tumor response in selected cases, which used TACE in combination with immunotherapy [31]. At the same time, we also found that DDP increased the expression of PD-1 and PD-L1 on HCC cells and high expression of PD-1 and PD-L1 had a longer overall survival time.

YAP1, as a transcriptional co-activator, was a key downstream effector of the Hippo pathway [32]. YAP1 promoted the recruitment of immune cells [33]. Pancancer analysis found that YAP1 activity was strongly positively correlated with many types of tumor-infiltrating cells in various cancers [10]. Interestingly, this our study also found that DDP promoted YAP1 expression and increased CD8⁺ T cells in C57BL/6 mice. Our previous study showed that DDP promoted YAP1 expression in HepG2215 cells, and YAP1 expression was restored with DDP in shYAP1 HepG2215 cells [23]. YAP1 also directly bond to the PD-L1 primer promote the transcription of PD-L1[24]. Our previous study suggested that overexpression YAP1 promoted PD-L1 expression. Consistently, a study showed that DDP induced the over-expression of PD-L1 in H22 hepatoma cells[34]. In this study, we also found that YAP1 knockdown and YAP1 knockout also reduced the expression of PD-1 and PD-L1 in liver cancer cells *in vivo* and *in vitro*.

CD8⁺ T cells played a vital role in the anti-cancer effect. Accordingly, other studies showed that CD8⁺ T cell infiltration into cancer tissues increased during treatment, including in the DDP alone group, compared to the control group, and the therapeutic effect diminished as the number of CD8⁺ T cells decreased[14]. Consistently, one study showed that DDP exhibited CD8⁺ T cell-mediated therapeutic effect on tumor-bearing mouse models [35]. Studies suggested that PD-1 expression was related to T cell exhaustion. In general, without immunotherapy, high expression of PD-1⁺CD8⁺ T cell was associated with poor prognosis in various tumors, including liver cancer [36]. In our study, we found that DDP significantly reduced exhausted CD8⁺ T lymphocytes in blood and in spleen. It also increased non-draining CD8⁺ T lymphocytes in the blood and in spleen. Previous studies showed that TGF- β and IL-2 and IFN- γ expressing CD8⁺ T cells contributed to an anti-cancer immune reaction[37]. TGF- β played a crucial role in liver cancer. The overactivation of TGF- β signaling may lead to the progression of the cancer. In general, Th1 cells secreted IL-2 and IFN- γ to inhibit cancer growth, while Th2 cells released immunosuppressive factors IL-4 and IL-10 to produce immunosuppression. Furthermore, CD8⁺ T cells expressed by IL-2 played an essential role in the response of anti-cancer immune. The study showed that the treatment of IL-2 reversed the exhaustion of CD8⁺ T cells and significantly increased the IFN- γ in malignant pleural effusion[38]. IL-2 promoted CD8⁺ T cells and natural killer cells responding to antigens[39]. Treatment of IL-2 reversed the exhaustion of CD8⁺T cells and significantly increased the IFN- γ in malignant pleural effusion. IFN- γ had direct anti-proliferation, pro-apoptotic and anti-angiogenesis effects on cancer

cells[40]. Studies showed that chemokines recruited bone marrow-derived suppressor cells and promoted cancers [41]. IL-1 β was a pro-inflammatory cytokine that promoted the growth of breast cancer cells through the P38/MAPK and PI3K/Akt pathways [19]. In our study, DDP decreased TGF- β expression, but it did not affect the expression of Th1- type cytokines and Th2- type cytokines, IL-1 β , and chemokines.

In conclusion, our study found that DDP inhibited the growth of HCC. It increased the expression of PD-1 and PD-L1 by YAP1 expression in the cancer. DDP increased the CD8⁺T cell number in tumor microenvironment. Meanwhile, DDP decreased the percentage of PD-1⁺CD8⁺ T cells in blood and spleen. DDP decreased TGF- β expression level in liver cancer. Therefore, this study provided the mechanism for the liver cancer microenvironment with DDP.

Declarations

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Author contribution

X.S. designed research; L.H., Y.G., Q.P., Z.Z., S.L., J.J., Y.X., Y.L., and C.L. performed the experiments; L.H. and X.S. wrote the manuscript with contributions from all authors. All the authors analyzed the data, and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

All authors declared that they have no completing interests.

Compliance with ethical standards

Conflict of interest. The authors declared no conflict of interest

All authors read and approved the initial manuscript.

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Figures

Figure 1

Cisplatin inhibited the growth of HCC. A. The organ of C57BL/6mice in the NS group and DDP group. Blue and red arrowheads indicate liver and spleen. The white circles are tumors. B. When the mice developed tumors, they were enrolled as individuals. Two successive ultrasonic measurements were carried out for detection. Ultrasonography was performed on the first day and 25 days after medication intervention. Ultrasonography was performed on the transverse and longitudinal sections of the lesion. The white

dotted line represents the tumor. C. Effect of cisplatin on weight and index of liver, spleen, kidney, and thymus. D. * indicates $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with the saline group.

Figure 2

Cisplatin increased PD-1- CD8+ T cells and reduced PD-1+ CD8+ T cell. A. Cells were treated with DDP and 0.1% DMSO (NC), respectively, for 24 h. CD8+cells were stained with green by immunofluorescence, and nuclei were counter-stained with DAPI (blue). The upper and bottom panels were respectively 100 ×.

B. Cisplatin increased PD-1- CD8+T cells and reduced PD-1+ CD8+T cells in the spleen. C. Cisplatin increased PD-1- CD8+ T cells and reduced PD-1+ CD8+T cells in the blood. D. Effect of cisplatin on immune factor. * indicates $P<0.05$, ** $P<0.01$, *** $P<0.001$.

expression of PD-1 in HepG2 cells. * indicates $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with the saline group. C. Western blot was detected the expression of PD-1 in HepG2215 cells.

Figure 4

Survival analysis of PD-1 and PD-L1. A. Low expression of PD-1 and PD-L1 had poor overall survival time in the HPA database. B. Low expression of PD-1 and PD-L1 had poor overall survival time in the KM database.

Figure 5

DDP increased PD-1 and PD-L1 expression in HCC by promoting the expression of YAP1. A. The analysis of YAP1 expression levels after cisplatin treatment was detected by Image J. In the cisplatin-treated group, the expression of YAP1 was significantly higher than that in the saline group. B. Western blot analysis of PD-1 and PD-L1 expression in YAP1 knockdown in HepG2215 cells. β -actin was used as a loading control. The expression of YAP1 in C57BL/6 mice was examined by immunohistochemical staining. And * indicates $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with the saline group.

Figure 6

DDP increased PD-L1 expression by promoting the expression of YAP1 in C57BL/6 mice. A. The liver figure showed that DDP reduced the number of tumors compared with the NS group. B. Mouse hepatocellular carcinoma modeling by DEN/TCPOBOP. PCR was used to verify the expression of flox and cre. C. Western blot analysis of YAP1, PD-1 and PD-L1 expression in vivo. And * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the saline group.

Figure 7

DDP inhibited the growth of HCC. Cisplatin promoted the expression of PD-1 and PD-L1 on HCC cells by promoting YAP1 expression, and inhibited the growth of HCC cells. DDP decreased the percentage of PD-1⁺CD8⁺ T cells in blood and spleen in tumor environment.