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Prognostic and Therapeutic Values of Tumor Necrosis Factor-Alpha in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) causes many deaths worldwide every year, especially in Asia. It is characterized by high malignancy, recurrence, and short survival time. Inflammation is closely related to the initiation and development of HCC. Tumor necrosis factor-α (TNF-α), an essential inflammatory mediator, has been studied as a potential therapy target in many cancers. However, its potential role in HCC diagnosis and therapy is still unclear.

Material/Methods: In our study, we detected the TNF-α expression in both human HCC tumor tissue and HCC cell lines HepG2 and HuH7. Then, we detected the effect of anti-TNF-α treatment and it synergistic function with 5-FU in an HCC xenograft mouse model and in HCC cell lines.

Results: Survival analysis and Cox regression analysis based on 97 HCC patients indicated that a high level of TNF-α is an independent predictor of poor survival in HCC patients. Anti-TNF-α treatment by infliximab synergizes with Fluorouracil (5-FU) by promoting apoptosis of HCC tumor cells through complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) effects.

Conclusions: Based on these data, we conclude that anti-TNF-α treatment could be a good way to increase the effect of classic chemotherapy of HCC patients, especially for the patients who have modest response to classic chemotherapy, such as 5-FU. TNF-α could also be used as a biomarker to help in early diagnosis of HCC.

MeSH Keywords: Antineoplastic Agents • Carcinoma, Hepatocellular • Inflammation • Survival Analysis • Tumor Necrosis Factor-alpha

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Background

Hepatocellular carcinoma (HCC), accounting for about 90% of primary liver cancers, is one of the leading killers among cancers worldwide, especially in Asia and Africa [1–3]. The incidence and mortality of HCC are still increasing in recent decades [2,4]. HCC usually occurs in the context of chronic liver diseases and cirrhosis. Major risk factors of HCC include virus infection (e.g., HBV and HCV), alcohol, fatty liver diseases, and toxic exposures (e.g., aflatoxin and vinyl chloride) [3]. Multiple therapies have been developed to treat HCC patients, such as surgical resection, radiofrequency ablation, chemotherapies, and orthotopic liver transplantation. Although the survival time has been prolonged for patients at early stages, overall survival is still low due to late diagnosis, lack of effective targets for therapies, and tumor metastasis and recurrence.

TNF-α was originally found to induce tumor cell lysis and is widely known as an important inflammatory mediator that induces immune responses. The downstream effects of TNF-α are mainly mediated by its 2 types of receptors: TNF-α receptor 1 (TNFR1) and TNFR2 [5,6]. The activation of these receptors initiates various intracellular signal transduction pathways [7], among which NF-κB and AP-1 transcription factor complexes have been recognized as 2 key intracellular links between TNF-α and inflammation [7]. Increased inflammatory cytokines, chemokines, and immune cells (e.g., macrophages and neutrophils) were observed in patients with high levels of TNF-α [7,8]. Although TNF-α was named for its ability to induce tumor cell lysis *ex vivo*, mounting evidence shows that TNF-α plays important roles in tumor initiation and tumor development [9–11]. Altered expression of TNF-α have been detected in many cancer tissues, such as ovarian and renal cancers [9–12], as well as in the serum of cancer patients, but not in serum of healthy individuals [7,13]. Pre-clinical studies in cancer models also showed that interruption of the TNF-α signaling pathway by antibodies or using TNFR1–/–mouse models was able to reduce tumor metastasis and prolong survival [10,14,15].

Inflammation has been established to be an important factor in promoting tumor development in various cancers, including HCC [16]. Tumors and infectious or inflammatory liver diseases are closely related as well [17]. In HCC, inflammation is a risk factor, suggesting that inflammation may play essential roles in HCC development. Increased inflammation mediators, such as TNF-α, C-reactive protein (CRP), and interleukin 6 (IL-6), have been detected in HCC patient serum [16]. Higher serum CRP and IL-6 levels in HCC patients are well correlated with shorter survival [16], indicating the value of targeting inflammatory mediators in HCC. TNF-α is an ideal candidate for inhibiting the inflammation in HCC patients due to its essential position in the network of inflammation and immune response. However, the exact role of TNF-α in HCC development is still unknown. Previous studies showed the improvement of overall survival was not apparent after anti-TNF-α treatment [11]. Infliximab is an FDA-approved anti-TNF-α chimeric monoclonal antibody that neutralizes the biological activity of TNF-α by binding with high-affinity TNF-α, thus preventing the effective binding of TNF-α with its receptors [18,19]. Based on these facts, we hypothesized that a combination of classic chemotherapy (which is used as a standard treatment in HCC patients) and infliximab treatment may produce better response. Therefore, our study evaluated the effects of combination treatment of 5-flurouricil (5-FU) and infliximab in HCC pre-clinical models.

Material and Methods

Cell culture

Human HCC cell lines HuH7 and HepG2 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, IL, USA) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin was used to culture these cells. All cells were cultured in a humidified incubator with 5% CO_2 at 37°C.

Patient sample analysis

This study was approved by the Medical Ethics and Human Clinical Trial Committee of the First Affiliated Hospital of Liaoning Medical University. We collected 97 formalin-fixed, paraffin-embedded (FFPE) HCC tumor samples diagnosed from November 2008 to June 2014 at the First Affiliated Hospital of Liaoning Medical University. Written informed content was signed by each patient or their legally authorized representatives. These tissues were collected from surgery resection before chemotherapy or radiotherapy. Immunohistochemistry staining was conducted to detect TNF-α expression. Clinicopathological features of these patients are summarized in Table 1. Grading of HCC samples followed the Edmondson and Steiner system, which grouped HCC into 4 grades from I to IV based on histological differentiation [20]. TNM classification was according to the AJCC Cancer Staging Manual, 7th edition.

Follow-up of these HCC patients started from the date of surgery to May 2015. The interval between the date of surgery and death was defined as overall survival (OS). Patients who died due to causes other than HCC were excluded from the cohort.

Immunohistochemistry

Standard immunohistochemistry (IHC) was conducted to detect TNF-α expression in HCC tumor tissues. Briefly, FFPE tissue

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Table 1. Relationship between TNF-α expression and clinicopathological features of HCC.

Parameters	TNF-α expression				P-Value
	Low (%)		High (%)		
Sex					
Male	37	(50.7%)	36	(49.3%)	0.314
Female	15	(62.5%)	9	(37.5%)	
Age					
<63	29	(55.8%)	23	(44.2%)	0.646
≥63	23	(53.6%)	22	(46.4%)	
T Stage					
T1+T2	20	(64.5%)	11	(35.5%)	0.140
T3+T4	32	(48.5%)	34	(51.5%)	
Lymph node					
N0–N2	38	(55.1%)	31	(44.9%)	0.650
N3–N4	14	(50%)	14	(50%)	
Metastasis					
Negative	51	(53.1%)	45	(46.9%)	0.350
Positive	1	(100.0%)	0	(0.0%)	
TNM Stage					
I+II	20	(51.3%)	19	(48.7%)	0.706
III+IV	32	(53.6%)	45	(46.4%)	
HBV or HCV					
Negative	20	(69.0%)	9	(31.0%)	**0.048**
Positive	32	(47.1%)	36	(52.9%)	
Edmondson Grade					
I+II	20	(46.5%)	23	(53.5%)	0.211
III+IV	32	(59.3%)	22	(40.7%)	
Survival status					
Alive	21	(75.0%)	7	(25.0%)	**0.007**
Dead	31	(44.9%)	38	(55.1%)	

sections were first deparaffinized by incubating with xylene using 3 changes for 5 min each. Then, tissue sections were rehydrated in gradient ethanol. Antigen retrieval was conducted by incubating in 1×Reveal Decloaker (Biocare Medical, CA, USA) at 120°C for 45 min. After washing with PBST once, slides were incubated for 15 min with 3% H_2O_2 in methanol in the dark to quench endogenous peroxidase. Then, slides were washed in PBST and blocked with 5% bovine serum albumin at room temperature for 15 min. Primary antibodies of TNF-α (Abcam, CA, USA) and cleaved caspase 3 (Abcam, CA, USA) were diluted by 1:100 and added for incubation overnight at 4°C. Then, horseradish peroxidase (HRP)-conjugated secondary antibody was added and incubated for 1 h at room temperature. After washing in PBST 3 times, DAB was added. Slides were immediately washed under tap water after color development and counterstained by hematoxylin. Finally, slides were mounted and observed under a light microscope. The scoring was based on positive area proportion identified in Image J software: 1 for 0–30% positive area, 2 for 30–60% positive area, and 3 for more than 60% positive area.

Cytokine assay

Bead-based assay was conducted to measure the expression level of inflammatory cytokines (TNF-α, IL-6, IL-17A, IL-17F, IFN-γ, IL-2, and IL4) in HCC xenograft tumor tissues. Cytokine beads were purchased from Biolegend (CA, USA). HCC xenograft nude mice were treated with saline, 5-FU, or infliximab, as detailed below. After harvesting from these mice, HCC tumor tissues were cut into small pieces, then minced and filtered

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for cell precipitation. RIPA buffer with protease inhibitor was added for protein extraction. Subsequently, BCA protein assay was performed to measure the protein concentration. After normalization of protein concentration, the bead-based assay was performed to measure the cytokine levels following the manufacturer's instructions. Each measurement was repeated 3 times, and the mean of the 3 values was used for final analysis.

Cell viability assay

A cell-counting kit (CCK-8) (Sigma Aldrich, MO, USA) was used for cell viability assay. All the steps were performed following the manufacturer's instructions. Equal numbers of cells were seeded in 96-well plates with 100 µl culture medium plus 5-FU and/or infliximab (Janssen Biotech Inc, USA) treatment for 48 h. Then, CCK-8 (10 µl/well) solution was added to incubate for 40 min, followed by absorbance (450 nm) measurement using an MRX II microplate reader (Dynex Technologies, VA, USA). The final cell viability was calculated according to the formula: [OD (treatment) – OD (blank)]/[OD (control) – OD (blank)].

Fluorescence-activated cell sorter analysis

TNF-α expression in HCC cell lines HepG2 and HuH7 was measured by florescence-activated cell sorter analysis (FACS). Cells were harvested and centrifuged to cell pellets. TNF-α antibody (Abcam, MA, USA) was added into cell pellets for incubation for 30 min at 4°C, followed by washing with PBS. Fluorescence-labeled secondary antibody was incubated for 15 min at room temperature. Then, cells were washed in PBS 3 times and analyzed using a FACSCanto II machine (Becton Dickinson, NJ, USA). Flow Jo software was used for data visualization.

Antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity assay

HCC cell lines HepG2 and HuH7 and macrophages isolated from peritoneal cavity flushing fluid of BALB/c mice were used to conduct antibody-dependent cellular cytotoxicity (ADCC) assay. Target cells (HepG2 and HuH7) were seeded into 96-well plates (2×10^3/well). Then, infliximab (16 µg/ml) was added for incubation for 1 h at 37°C. Effect cell macrophages (4×10^4/well) were then added into each well for 48 h at 37°C. For complement-dependent cytotoxicity (CDC) assay, HepG2 and HuH7 cells were seeded in 96-well plates at a density of 5×10^3/well. Fresh guinea pig serum containing active complements and infliximab was added for incubation for 6 h at 37°C. Then, cell viability of ADCC and CDC assay was measured by CCK-8 assay (Sigma Aldrich, MO, USA), as detailed above. Calculation of the cytotoxicity followed methods published previously [21].

Xenograft mouse model

All animal studies were approved by the Experimental Animal Committee of the First Affiliated Hospital of Liaoning Medical University. HCC xenograft mouse model was established using BALB/c nude mice (7 weeks old, 22–24 g weight) obtained from the Shanghai SLAC Laboratory Animal Center of the Chinese Academy of Sciences). HuH7 cells (10^6) were injected into the back of each nude mouse. A total number of 30 nude mice with tumors were obtained 1 week after injection and randomly assigned into 3 groups to accept different treatments: a) 5-FU (20 mg/kg/week); b) 5-FU (20 mg/kg/week) + infliximab (10 mg/kg/week); and c) saline. Tumor growth was checked and recorded every 2 weeks. Survival time of each mouse was recorded. Tumor volume was calculated based on length and width: $\text{width}^2 \times \text{length} \times \varpi/6$. After each mouse was sacrificed, xenograft tumor tissues were collected for further study. All nude mice were raised in a standard specific pathogen-free environment throughout the study.

Apoptosis assay

Apoptosis in HCC cell lines HepG2 and HuH7 was evaluated by measuring caspase 3 (cleaved form) level using the Pierce Colorimetric In-Cell ELISA Kit (Thermo Fisher Scientific, CA, USA). The experiment was conducted following the instructions of the manufacturer.

Statistical analysis

The software used for statistical analysis in this study included SPSS 17.0 (Chicago, IL, USA) and Graph Pad (CA, USA). One-way ANOVA, *t* test, chi-square analysis, and Bonferroni's pairwise comparisons were used to analyze the difference between individual groups. An ROC curve was used to determine the cutoff points that have best specificity and sensitivity. Kaplan-Meier survival analysis was used to plot the survival curves of HCC patients with different TNF-α levels, followed by log-rank test to evaluate the difference between these 2 groups. Multivariate analysis was conducted using the Cox proportional hazards regression model as reported before [22]. A 2-tailed $P<0.05$ was considered as statistically significant.

Results

Expression of TNF-α is an independent prognostic marker of HCC patients

As shown in Figure 1 A and 1B, we measured TNF-α expression in HCC patient tumor tissues via IHC. TNF-α was expressed in tumor cells and tumor stroma. TNF-α expression was divided into high expression (staining score ≥2) and low expression

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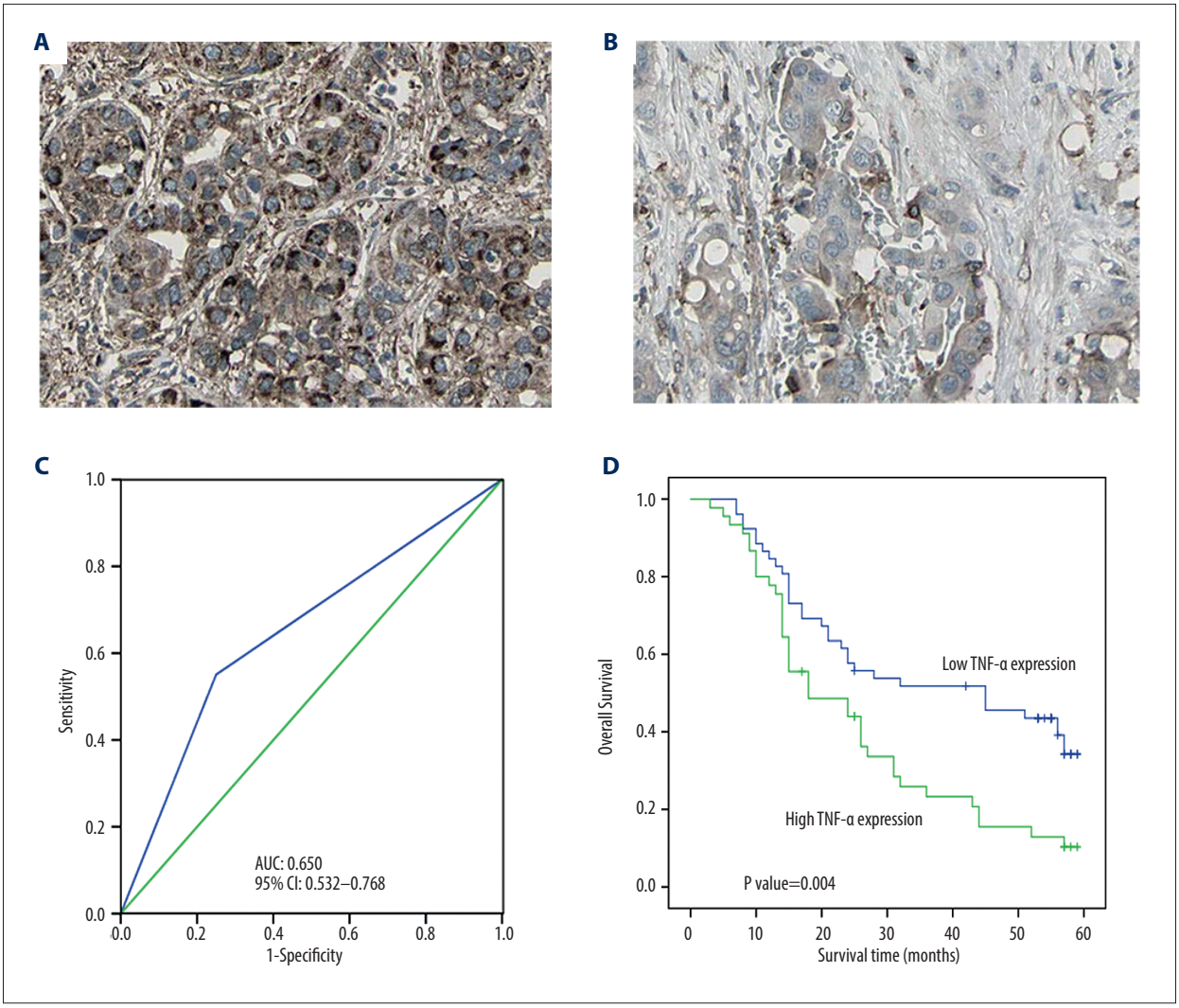

Figure 1. TNF-α is overexpressed in HCC tumor tissues and predicts poor survival of HCC patients. (**A, B**) representative pictures showing high TNF-α expression and low TNF-α expression in tumor cells and tumor stroma of human HCC tumor tissue; (**C**) ROC curve shows the sensitivity and specificity of the selected cutoff point (staining score ≥2 means high expression) of IHC scoring, with the area under the curve (AUC) is 0.650 (95% CI: 0.532–0.768); (**D**) Kaplan-Meier survival analysis indicated that HCC patients with high TNF-α expression have better survival than patients with low TNF-α expression.

(staining score <2) based on the cutoff point determined by the ROC curve (Figure 1C). Then, the relationship between TNF-α and clinicopathological features of HCC patients were tested by chi-square analysis (Table 1). Hepatitis B virus (HBV) or HCV infection and high survival rate are significantly related to TNF-α high expression (*P*-value=0.048 and 0.007, respectively). Patients with high T stage (T3+T3) tended to have high expression of TNF-α, but no statistical significance was observed. Survival analysis showed that HCC patients with high TNF-α have shorter survival time than those with low TNF-α expression (*P*=0.004), suggesting that TNF-α promotes HCC development. Consistently, as shown in Table 2, Cox regression modelling indicated that TNF-α is an independent predictor of poor survival of HCC patients (P<0.05, HR=1.689, 95%CI: 1.015–2.810). Other adverse independent predictors of survival included TNM stage (P<0.05, HR=2.132, 95%CI: 1.094–4.153), T stage (P<0.05, HR=7.561, 95%CI: 2.926–19.541), HBV or HCV infection (*P*<0.05, HR=2.393, 95%CI: 1.010–5.674), and Edmondson grade (*P*<0.05, HR=3.048, 95%CI: 1.702–5.456).

TNF-α is highly expressed in HCC cell lines and can be targeted by infliximab

As shown in Figure 2A–2D, high TNF-α expression was detected by FACS analysis in HCC cell lines HepG2 and HuH7. We treated these cell lines with gradient concentrations of infliximab, but no significant effect was detected (Figure 2E, 2F). Interestingly, infliximab treatment combined with co-culture

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Table 2. Multivariate Cox proportional hazard models of overall survival of CC patients.

Factors	P-value	HR (95%CI)
Sex (Male *vs.* Female)	0.582	1.171 (0.667–2.054)
Age (≥63 *vs.* <63)	0.133	1.542 (0.877–2.710)
TNM stage (III–IV *vs.* I–II)	**0.026**	**2.132 (1.094–4.153)**
T stage (T3–T4 *vs.* T1–T2)	**0.000**	**7.561 (2.926–19.541)**
Lymph node (N3–N4 *vs.* N0–N2)	0.841	1.071 (0.546–2.101)
Metastasis (Yes *vs.* No)	0.827	0.793 (0.099–6.351)
Edmondson Grade (III+IV *vs.* I+II)	**0.000**	**3.048 (1.702–5.456)**
HBV/HCV (Positive *vs.* Negative)	**0.047**	**2.393 (1.010–5.674)**
TNF-a Expression (High *vs.* Low)	**0.044**	**1.689 (1.015–2.810)**

macrophages resulted in significant inhibition of both HepG2 and HuH7 growth (Figure 2E, 2F), and infliximab treatment combined with active complement incubation also results in obvious inhibition of cell viability. These data suggest that anti-TNF-α treatment induces ADCC and CDC effects, thereby inhibiting HCC growth.

Infliximab synergizes with 5-FU *in vitro*

5-FU has been widely used in chemotherapy of various digestive system cancers, including liver cancers. We treated HCC cell lines HepG2 and HuH7 with infliximab combined with 5-FU to determine if it would have better effects. As shown in Figure 3, the viability of HCC cell lines treated with 5-FU and infliximab in the presence of complement was lower than that of the 5-FU single-treatment group, suggesting that infliximab has a synergic function with 5-FU in treating HCC.

Infliximab acts synergistically with 5-FU in an HCC animal model

To further confirm the synergistic effects of infliximab and 5-FU, we established an HCC xenograft nude mouse model using the HuH7 cell line treated with 5-FU and/or infliximab. As shown in Figure 4, tumor growth was inhibited in the 5-FU single-treated group and 5-FU+infliximab-treated group, but the tumor growth of the 5-FU+infliximab-treated group was much slower than that of the 5-FU single-treatment group. The survival analysis shows that mice receiving infliximab+5-FU treatment had longer survival than in the 5-FU single-treatment group. These results indicate that infliximab and 5-FU work synergistically to inhibit HCC growth.

Infliximab boosts apoptosis induction role of 5-FU

5-FU is known to induce apoptosis in tumor cells, but whether anti-TNF-α can promote this process is still unclear. We evaluated cleaved caspase-3 level in HCC cell lines and tumor tissues from an HCC xenograft mouse model. As shown in Figure 5, 5-FU treatment induced expression of cleaved caspase-3, but complement alone did not promote this effect. Interestingly, addition of infliximab increased 5-FU-induced cleaved caspase-3 level in the presence of active complement (Figure 5A, 5B). Consistently, cleaved caspase-3 level was also higher in the 5-FU + infliximab treatment group than in the5-FU single-treatment group *in vivo* (Figure 5C).

Infliximab synergizes 5-FU anti-tumor effects by down-regulating tumor-promoting cytokines

In addition to the synergistic effect of 5-FU and infliximab in enhancing apoptosis, the inflammation cytokine regulation role of infliximab was also investigated. We detected pro-inflammatory cytokines (TNF-α, IL-6, IL-17A, IFN-γ, IL-2, and IL-4) in HCC tumor tissue from the xenograft model. As shown in Figure 6, these cytokines were decreased in the 5-FU+infliximab-treated group, but not in the 5-FU-treated or vehicle control group. This evidence suggests that infliximab acts synergistically in promoting 5-FU anti-tumor effects by inhibiting inflammatory cytokine production and the subsequent tumor-promoting effects in HCC.

Discussion

Anti-TNF-α treatment has been widely studied in inflammatory diseases such as rheumatoid arthritis [19] as well as in many cancers such as skin cancer, breast cancer, ovarian cancer, and renal cell carcinoma [11,23–26]. Accumulating evidence

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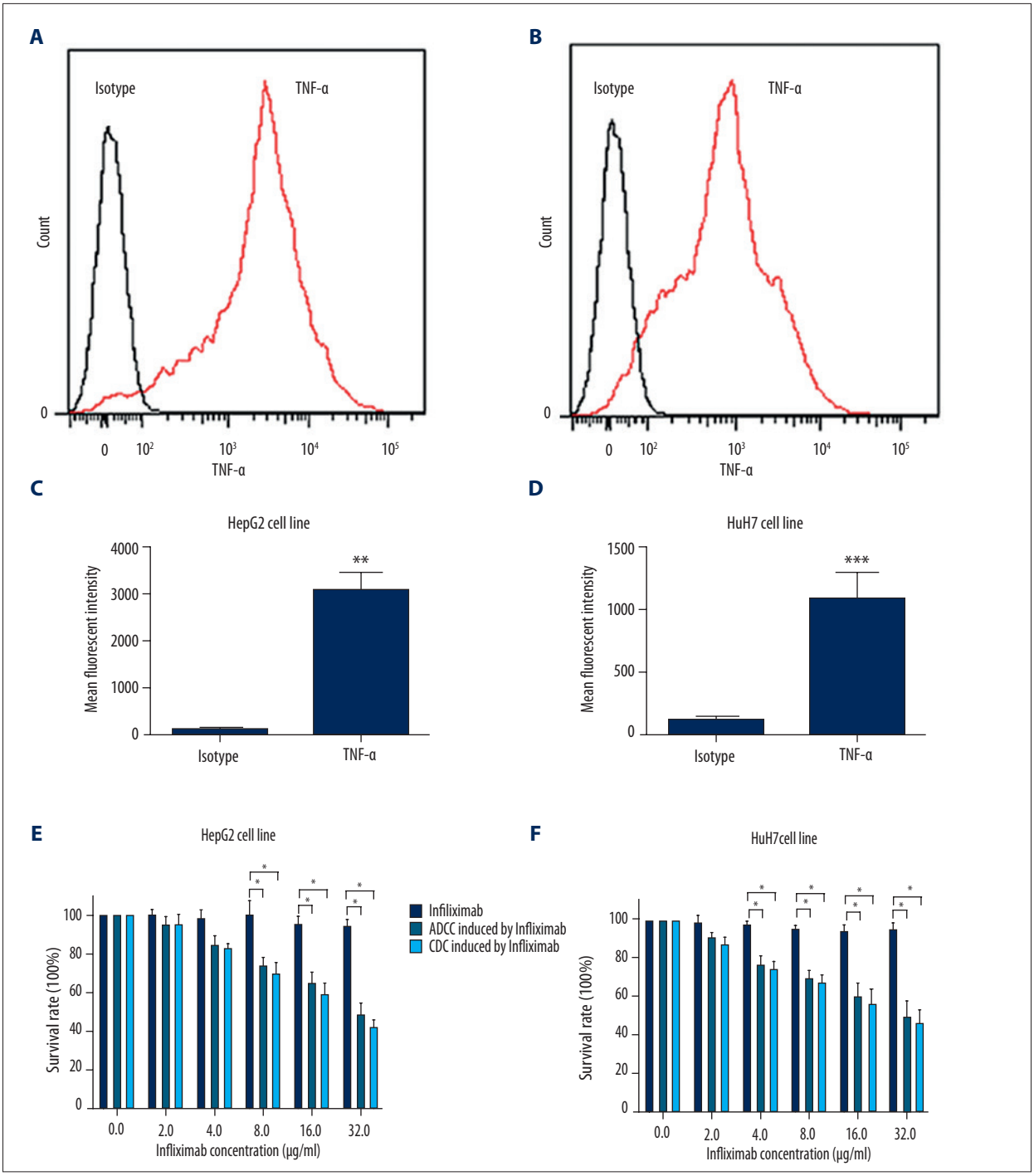

Figure 2. TNF-α was highly expressed in HCC cell lines HepG2 and HuH7, and anti-TNF-α treatment can inhibit cell survival through ADCC and CDC effects. (**A, D**) TNF-α expression in HCC cell lines HepG2 and HuH7, respectively; (**B, E**) the quantification of TNF-α expression in HCC cell line HepG2 and HuH7, respectively. TNF-α was highly expressed in these HCC cell lines; (**C, F**) Anti-TNF-α treatment by infliximab alone did not induce direct inhibition of cell survival, but under the presence of macrophages or active complement, infliximab treatment caused significant decrease of survival of HCC cell lines HepG2 and HuH7, respectively.

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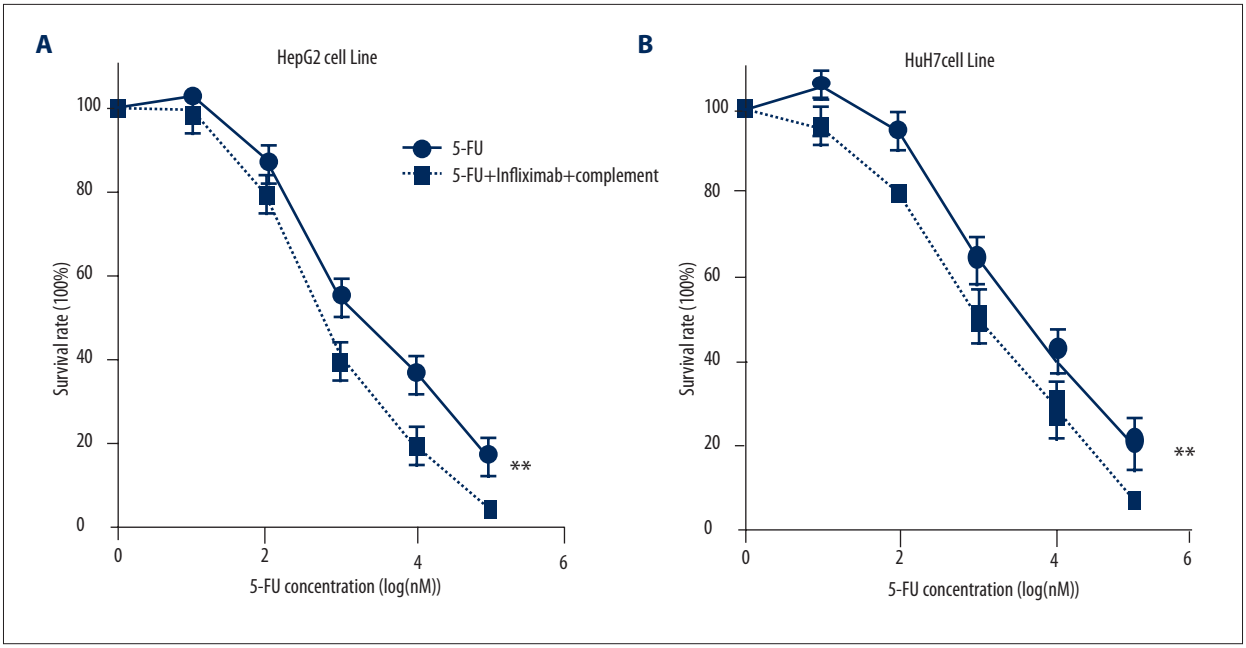

Figure 3. The synergetic function of infliximab and 5-FU in HCC cell lines. (**A**) In HepG2 cell line, infliximab + 5-FU treatment caused more inhibition of cell survival than 5-FU single treatment; (**B**) In HuH7 cell line, infliximab + 5-FU treatment also caused more sever inhibition of cell survival than in the 5-FU single-treatment and saline-treated group.

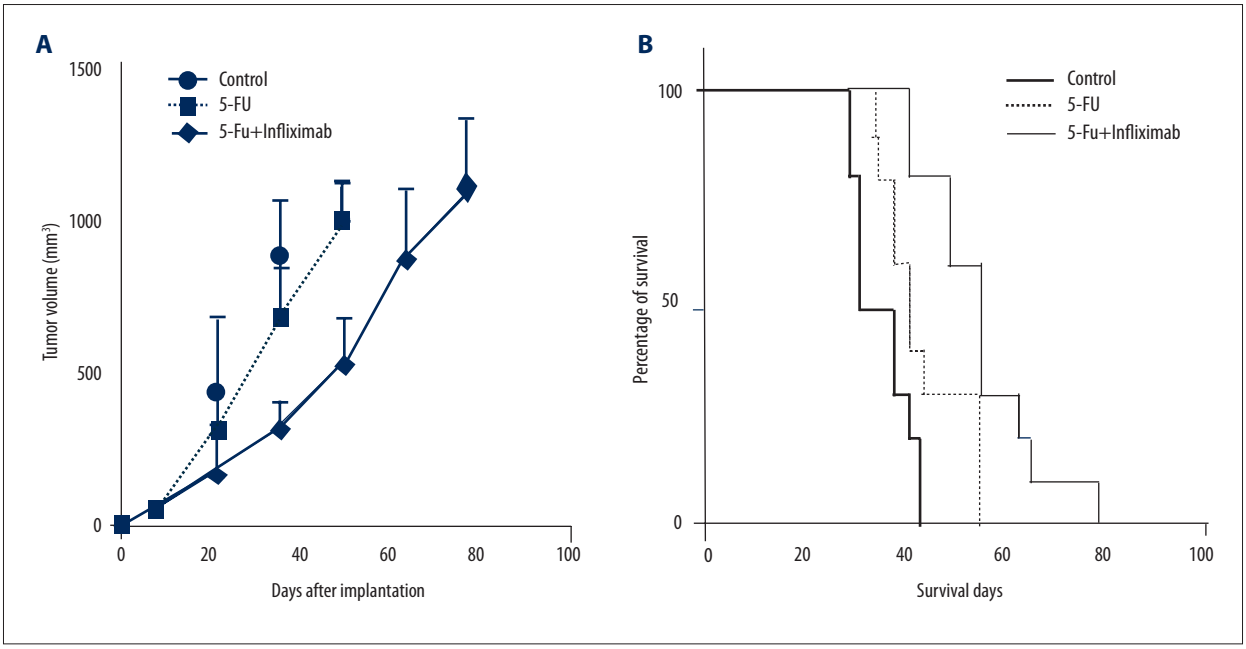

Figure 4. The synergetic function of infliximab and 5-FU in an HCC xenograft mouse model. (**A**) In the infliximab + 5-FU treatment group, the HCC xenograft tumor volume increased at a lower rate compared with the 5-FU single-treatment group and the control group. (**B**) Survival analysis of HCC xenograft mice. The survival in the infliximab+5-FU treatment group was longer than that of 5-FU treatment group and the control group.

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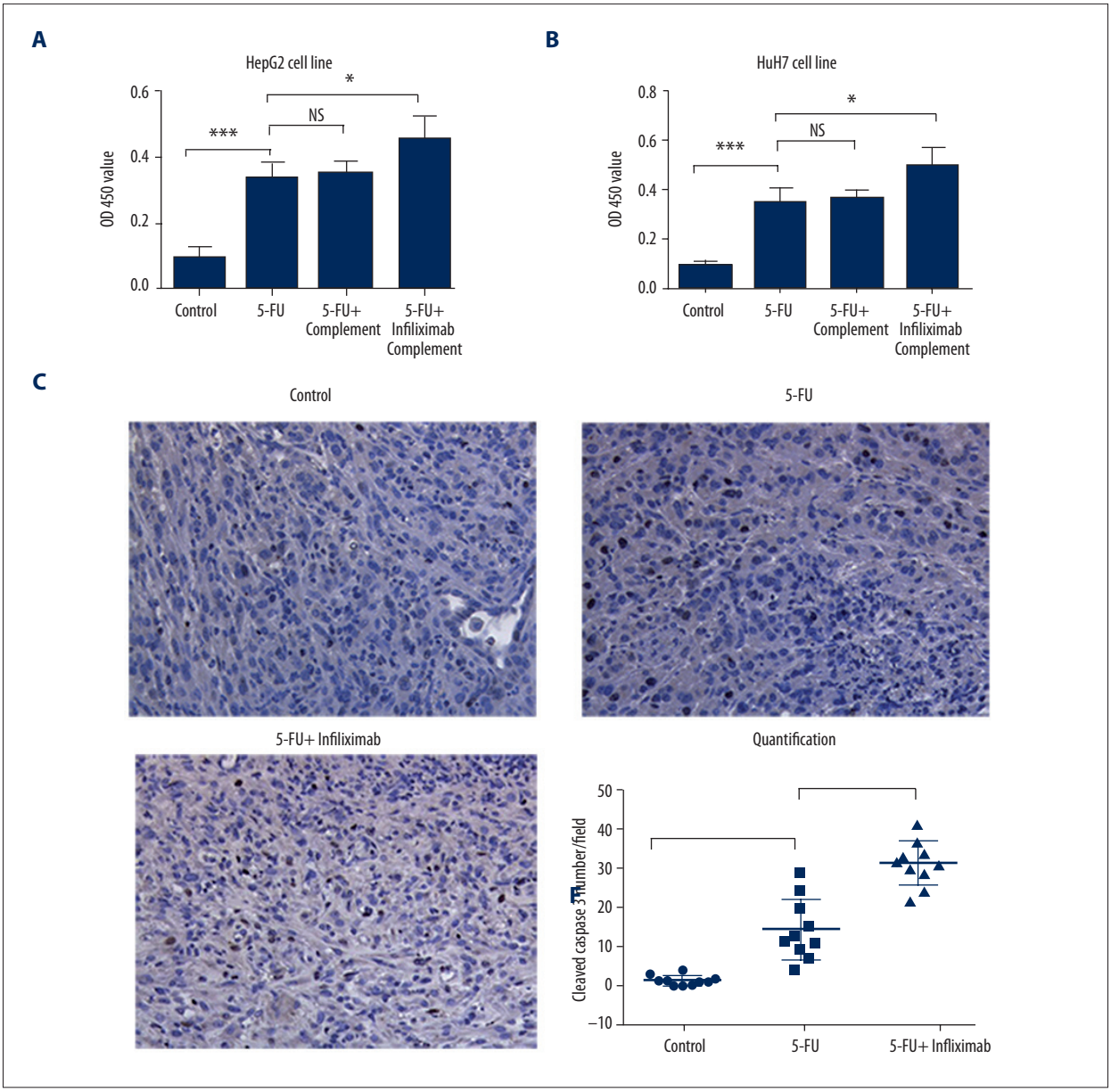

Figure 5. The synergetic function of infliximab and 5-FU in inducing apoptosis through CDC effect in HCC cell lines and xenograft tumor tissue. (**A, B**) infliximab treatment promoted apoptosis in HCC cell lines treated with 5-FU in the presence of active complement, while 5-FU alone did not induce apoptosis of HCC cell lines through CDC effect. (**C**) the representative pictures of cleaved caspase-3 expression in HCC xenograft tumor tissue from mice treated by 5-FU, 5-FU+ infliximab, and saline, and the quantitative results. Cleaved caspase-3 was increased more in the 5-FU+ infliximab treatment group than in the 5-FU-treated and control groups.

indicates that anti-TNF-α treatment using monoclonal antibodies such as infliximab has promising effects in pre-clinical/clinical studies [26,27]. For HCC, a leading killer among cancers in Asia and Africa, infliximab treatment is rarely studied and its effects are still unknown. Although there are some studies on the potential effects of anti-TNF-α treatment with or without combination with other drugs in HCC patients, its specific effect has not been deeply discussed [28,29]. A tumor is a super-complex "society" and the initiation of tumors also involves multiple factors. Most of the factors that influence the initiation and development of HCC are closely related to anti-apoptosis and inflammation, and TNF-α is an important factor involved in regulating these processes [7]. Thus, in the present study we investigated the effects of anti-TNF-α treatment using infliximab in combination with classic chemotherapy drug 5-FU of HCC tumor *in vitro* and *in vivo*.

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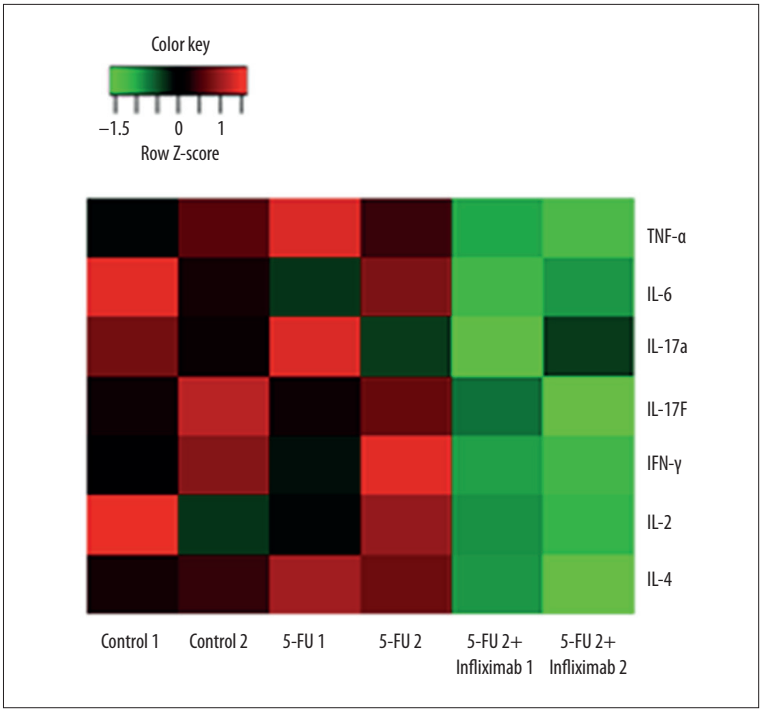

Figure 6. Infliximab synergizes with 5-FU by inhibiting pro-inflammatory cytokines expression. Red color indicates high expression, green color indicates low expression, and black color indicates average expression level. In the 5-FU + infliximab treatment group, all these pro-inflammatory cytokines (TNF-α, IL-6, IL-17A, IL-17F, IFN-γ, IL-2, and IL-4) were decreased compared with the 5-FU-treated group and the control group.

Under physiological conditions, TNF-α is ubiquitously expressed at low levels in many different tissues, but its expression is usually altered under pathophysiological conditions. Here, we observed that the TNF-α expression was increased in both human HCC tumor tissues and cell lines HepG2 and HuH7. Interestingly, high TNF-α expression was associated with HCC development. Patients with higher T stages tended to show high expression of TNF-α. Most importantly, survival analysis and Cox regression model analysis indicated that high TNF-α expression is an independent predictor of poor survival in HCC patients. This evidence indicates the prognostic value and targeting value of TNF-α for HCC patients. HCC is known for being difficult to diagnose early, as well as its high rates of metastasis and recurrence and poor patient survival. Thus, finding novel biomarkers for diagnosis and prognosis, as well as targets for therapy, is necessary. Our results indicate that TNF-α is a candidate for use in HCC prognosis, diagnosis, and targeting therapy.

TNF-α antagonists such as infliximab have been widely used in treatment of various inflammatory diseases, including rheumatoid arthritis and Crohn's disease [8]. Previous studies have shown that infliximab can inhibit cell survival through inducing ADCC and CDC effects [8,23]. In line with previous studies, we also found that infliximab treatment inhibits survival of HCC cell lines through ADCC and CDC effects. 5-FU is usually a first-line treatment for advanced HCC, but drug resistance is a major cause of treatment failure. Thus, using other drugs in combination with 5-FU could be a way to overcome this resistance. Our results indicate that infliximab acts synergistically with 5-FU *in vitro* and *in vivo*. This result is explained by the increased apoptosis in infliximab+5-FU-treated HCC cell lines and xenograft mouse models. The development of HCC is associated with accumulation of inflammatory cytokines [27,30,31]. TNF-α important in cytokine network regulation. In addition to the cell death effects of TNF-α, our data also revealed the cytokine-regulating roles of TNF-α. The survival analysis from the HCC xenograft mouse model also indicates that infliximab+5-FU treatment can prolong overall survival time. Taken together, our results show that anti-TNF-α plus 5-FU treatment is a potential therapeutic strategy for HCC.

Conclusions

Chemo-resistance remains the major challenge of HCC-treated patients with advanced disease. Results of the present study show the synergetic role of anti-TNF-α treatment with 5-FU chemotherapy in HCC. Clinical investigations combining chemotherapy and anti-TNF-α treatment in HCC are urgently needed.

Disclosure of conflict of interest

None.

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