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Synergistic effect of bromocriptine and tumor necrosis factor-α on reversing hepatocellular carcinoma multidrug resistance in nude mouse MDR1 model of liver neoplasm

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Abstract

AIM: To investigate the effect of bromocriptine (BCT) and tumor necrosis factor-α (TNF-α) on hepatocellular carcinoma (HCC) multidrug resistance (MDR) in nude mouse MDR model of liver neoplasm.

METHODS: Human hepatocarcinoma cell line $HepG_2$, drug resistant hepatocarcinoma cell line $HepG_2$/adriamycin (ADM) and hepatocarcinoma cell line transfected with TNF-α gene $HepG_2$/ADM/TNF were injected into the liver of nude mice via orthotopic implantation and MDR model of liver neoplasm *in vivo* was established ($HepG_2$, ADM, TNF, BCT groups). Among these groups, BCT group and TNF group were treated with BCT through gastric canal. Each group was divided into control group and chemotherapy group. Size and weight of the tumor were measured. Furthermore, tumor histological character and growth of the nude mice were observed and their chemosensitivity was tested. MDR-associated genes and proteins (MRP, LRP) of implanted tumors were detected by immunohistochemistry, reverse transcriptase polymerase chain reaction, and apoptosis rate of hepatocarcinoma cells was detected by TUNEL assay.

RESULTS: The nude mouse model of each cell line was inoculated successfully. The tumor growth rate and weight were significantly different among groups. After chemotherapy, abdominal cavity tumor growth inhibition rate was higher in BCT group (67%) compared to ADM and TNF groups, and similar to $HepG_2$ group (54%). MDR1 and LRPmRNA could be detected in all groups, but TNF-α was detected only in TNF and BCT groups. Furthermore, MDR1 and LRP protein expression of tumors in TNF and BCT groups was low similar to $HepG_2$ group. The apoptosis rate of hepatocarcinoma cells was much higher in BCT group than in other groups with TUNEL assay.

CONCLUSION: BCT and TNF-α can reverse HCC MDR in nude mouse MDR1 model of liver neoplasm.

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Key words: Bromocriptine; Tumor necrosis factor-α; Hepatocellular carcinoma

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INTRODUCTION

The development of multidrug resistance (MDR) in human tumors may be a major obstacle to successful cancer chemotherapy[1]. MDR is often associated with increased expression of the MDR1 (also known as P-glycoprotein) gene, encoding P-glycoprotein (Pgp)[2]. Since the MDR1 gene appears to represent a major cause of MDR in tumor cells, MDR1 messenger RNA (mRNA) and Pgp may be important targets in MDR reversal strategies. But almost all MDR reverse studies are only performed *in vitro*[3-5], and few are performed *in vivo*. A study[6] indicates that overexpression of Pgp, a product of MDR1, is intimately associated with liver cancer development and therefore inhibitors of Pgp should inhibit the development of liver cancer. Accordingly, we determined the effect of bromocriptine (BCT), a potent inhibitor of Pgp[7,8], and tumor necrosis factor-a (TNF-α)[9-11] which could downregulate expression of the MDR-associated genes LRP and MRP in multidrug resistant model of nude mice via orthotopic implantation of multidrug resistant human hepatocellular carcinoma (HCC) cells $HepG_2$/adriamycin (ADM).

TNF is a cytokine playing an important role in immunoregulation and antitumor mechanisms, and has been extensively tested in *in vitro* and *in vivo* investigations as well as in clinical trials for immunotherapy of malignant diseases[12,13]. More recently, it has been demonstrated that TNF can modulate

MDR1 expression and enhance cytotoxicity of certain MDR-related drugs to various cell lines[10,14]. The ability of cytokines to influence MDR phenotype is strongly supported by a previous study[15].

BCT, as a D_2 dopaminergic receptor agonist, has been reported to inhibit activity of ATPase and function of Pgp[7]. Moreover, because of its slight side-effects and the MDR-reversing effect[8], we chose BCT and TNF-α to detect their synergistic antitumor effects.

MATERIALS AND METHODS

Reagents

1640 medium, fetal bovine serum (FBS), lipofectamine 2000, and G418 were purchased from Invitrogen Inc., USA. Upstream and downstream primers were produced by Shanghai Boya Biosynthesis Co., Shanghai, China. dNTPs, RNasin, AMV reverse transcriptase, Taq and DNA enzymes were purchased from Sigma Company, USA. TRIzol reagent was purchased from Gibco Company, USA. BCT mesilate was purchased from Novartis, Italy; 5-fluorouracil (5-FU), ADM, mitomycin (MMC) were from Pharmacia & Upjohn, Italy; and S-P immune test kit was from Beijing Zhongshan Company, Beijing, China. LRP mouse antibody, P170 mouse antibody and Pan actin antibody-5 were purchased from Neomarker Inc., Germany.

Cell lines and cell culture

Human HCC cell line, $HepG_2$ (GDC024), was purchased from the China Center for Type Culture Collection (Wuhan University, Hubei). $HepG_2$ was induced to form a multidrug resistant cell line ($HepG_2$/ADM) by exposure to gradually increased concentration of ADM. Firstly, eukaryotic expression vector pBK-TNF-α was constructed by recombinant DNA technique, and transfected into multidrug resistant cell line ($HepG_2$/ADM) to obtain cell line $HepG_2$/ADM/TNF and cultured in the presence of 800 mg/mL G418. All cells were maintained in 1640 medium containing 10% heat-inactivated FBS at 37 ℃ in a humid atmosphere of 50 mL/L CO_2.

Multidrug resistant model establishment and grouping

BALB/c, nu/nu, nude mice, male and female from Centers for Disease Control and Prevention of Hubei province, age ranging 4-5 wk, weighing 12.1-15.0 g, bred in specific pathogen-free condition, were used in this study. Firstly, a left subcostal incision was made under anesthesia with 20% urethane, then left lobe of the liver was exposed, and each kind of cells was injected into mice liver and an orthotopic MDR1 hepatoma was obtained successfully ($HepG_2$, ADM, TNF, BCT groups respectively). Each group contained 16 nude mice inoculated with each kind of cell lines, and was divided into control group and experiment group, 8 in each group. Chemotherapy was performed when tumor diameter was 1.5 cm. Among these groups, BCT group and TNF group were treated with BCT (6.25 mg/kg·d) through gastric canal. Association of 5-FU (0.15 g/kg·d)+MMC (1.5 mg/kg·d) +ADM (10 mg/kg·d) were administered through intraperitoneal injection in experiment groups, but 0.9% NS 15 μL/g was given to control groups for 3 d. Nude mice were weighed and the size of tumors was measured every week by B-ultrasound after orthotopic implantation, when the mice were killed 14 d after chemotherapy. Furthermore, some specimens were fixed in 40 g/L formaldehyde and embedded with paraffin for immunohistochemistry (IHC), and other markers were detected by molecular biological techniques.

Evaluation of growth and sensitivity of chemotherapy

The living condition, diet and reaction with stimuli from environment were observed everyday from the first day of chemotherapy. The size of tumors was measured every week by B-ultrasound after chemotherapy using the formula $V = a \times b^2/2$ (V: volume; a: long diameter; b: short diameter). Meanwhile xenograft tumors were observed and weighed until the mice were killed. Lastly, the inhibition rate of volume and weight was calculated by the formula (inhibition rate of volume or weight = [1-volume or weight of chemotherapy group/volume or weight of control group]×100%).

HE staining and immunohistochemistry (IHC) for detection of MDR1 expression

Firstly, each kind of orthotopic tumor tissues was stained with HE, and fixed in formalin and embedded in paraffin. The expression of Pgp protein was studied using mouse anti-human MDR1 C219 and sections of 5 μm from the tumor tissues were implanted in each kind of cell lines and MDR1 expression was assayed by immunoperoxidase staining with the SP method (SP kit, Zhongshan Biocompany). IHC process was strictly done as the indication of SP kit. In summary, endogenous peroxidase was blocked with H_2O_2 and non-specific antigens were blocked with normal serum. The primary antibodies were diluted to 1∶100 for Pgp, then incubated at 4 ℃ overnight, washed thrice with PBS. The secondary antibody (1∶500) was incubated for 15 min at 37 ℃, sections were stained with diaminobenzidine tetrahydrochloride (DAB) and restained with hematoxylin for visualization of nuclei. For negative controls, primary antibodies were substituted by PBS instead of Pgp antibodies. The Pgp protein expression intensity was observed through ocular lens and the positive cells per square millimeter were calculated and expressed as mean±SD.

Expressions of TNFmRNA, MDR1 mRNA and LRP mRNA

Total RNA was respectively extracted from tumors in nude mice with TRIzol according to the manufacturer's instructions. Reverse transcription (RT) was performed with random primers by a complementary DNA (cDNA) synthesis kit (Promega). RT-reaction reagents (25 μL) were added as follows: 0.1 μg OligdT (100 g/L), 25 U rRNasin, 1.25 μL dNTP (10 mmol/L), 5 μL M-MLV 5×buffer, 200 U M-MLVRT. After all the components were mixed, the mixture was incubated at 42 ℃ for 1 h in a DNA thermal cycler (PTC-100, MJ Research Inc., Watertown, MA, USA), and the reverse transcriptase was inactivated by heating the reaction mixture to 95 ℃ for 5 min. Then each tube was kept at -20 ℃ until PCR was performed. The primers of the target genes MDR1, LRP, and the endogenous reference β-actin were designed using the Primer Express Software (Applied Biosystems) (Table 1). The final volume of the reaction (25 μL) of PCR included: 25 μL transcription solution containing 15 pmol upstream and downstream respectively, 2.5 μL 10×buffer, 2 μL cDNA, 0.5 μL dNTP (10 mmol/L),

Table 1 MDR1, LRP primers

Gene	Quantification method	Sequence (5′-3′)	Size (bp)
MDR1	Forward primer	CATTGGTGTGGTGAGTCAGG	176
	Reverse primer	CTCTCTCTCCAACCAGGGTG	
LRP	Forward primer	TAAGGGCTTCCAGCACCAAC	237
	Reverse primer	GGAGTTCTCGCTTCTCGTCC	
TNF-α	Forward primer	GC*GGATT*CATGAGCACTGAAAGCATGATCC	725
	Reverse primer	CCC*AAGCTT*TCACAGGGCAATGATCCCAAAG	
β-actin	Forward primer	GTGCGTGACATTAAGGAG	530
	Reverse primer	CTAAGTCATAGTCCGCCT	

2 μL $MgCl_2$ (25 mmol/L), 1 μL Taq polymerase (10×10^6 U/L), ddH_2O to a final volume of 25 μL. Thirty-five PCR cycles were performed, for each cycle, the sample was denatured at 94 ℃ for 1 min, annealed at 55 ℃ for 1 min, extended at 72 ℃ for 1 min, and finally extended at 72 ℃ for 10 min.

TUNEL assay for apoptosis detection

The paraffin-embedded specimens were cut into 5-μm-thick sections, rehydrated and then incubated with protease K solution for 30 min at room temperature. After being washed twice with PBS, the sections were incubated with TUNEL reaction solution at 37 ℃ for 60 min. The transforming solution (POD) was added and incubated at 37 ℃ for 30 min. The sections were stained with DAB for 10 min. Then counterstained with hematoxylin for 10 min, dehydrated in graded alcohol and covered with resin. The criterion of positive staining was that the nuclei were stained pale brown.

Statistical analysis

Data were presented as mean±SD, significance of statistical difference was assessed by Student's *t*-test and analyzed with SPSS11.0. $P<0.05$ was considered statistically significant.

RESULTS

TNF-α mRNA expression in orthotopic tumor tissues

TNF-α mRNA could be amplificated only from the tumors of TNF and BCT groups in the orthotopic tumor tissues injected into three kinds of cell lines, and no positive band could be seen from the other two groups. Electrophoresis showed that there was a positive band at 725 bp in the tissues injected into multidrug resistant cells transfected with TNF-α gene (Figure 1) and no band was found, indicating TNF-α mRNA was integrated into the nude mice implanted with multidrug resistant cells transfected with TNF-α gene. Thus, the model of implanted orthotopic tumor was successfully established.

Evaluation of growth and sensitivity of chemotherapy

No death occurred. The successful rate of tumor implantation was 100%, and tumor could be seen via B ultrasound after 10 d. The rate of tumor growth in the group transfected with TNF-α was lower compared to other groups ($P<0.05$). Low appetite and emotion occurred in the mice receiving chemotherapy, especially in BCT group. The tumor volume and weight of BCT group decreased compared to the other group ($P<0.01$), and tumor growth inhibition rate in $HepG_2$ group was similar to TNF group ($P>0.05$), but lower than that in BCT group ($P<0.05$, Table 2, Figures 2 and 3).

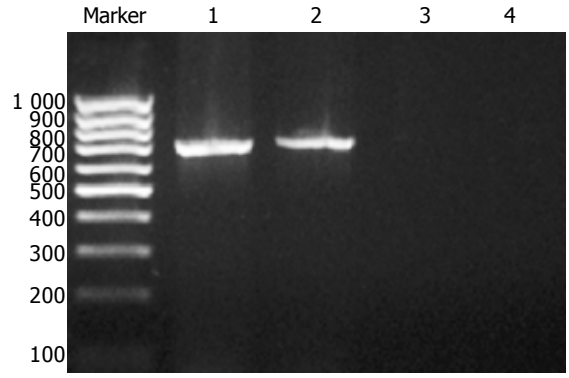

Figure 1 RT-PCR of TNFmRNA. M: Marker; lanes 1 and 2: TNF group, BCT group; lanes 3 and 4: $HepG_2$ group, ADM group.

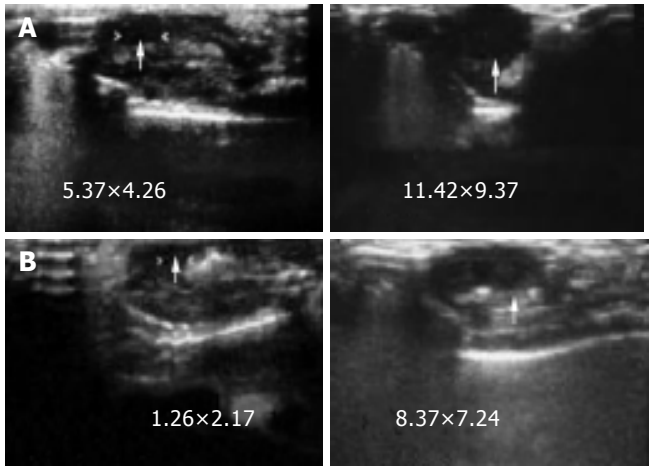

Figure 2 Size of tumor after chemotherapy in groups of $HepG_2$ (**A**) and BCT (**B**).

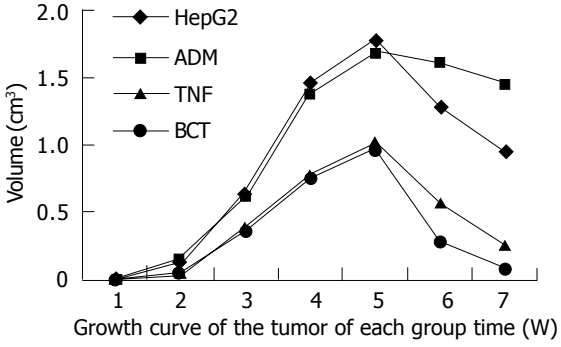

Figure 3 Growth curve of tumor in each group after chemotherapy.

Table 2 Volume and size of orthotopic tumor tissues (mean±SD)

Groups	Mice and incidence of tumor, *n* (%)	Volume (cm^3)	Rate of volume inhibition (%)	Weight (g)	Rate of weight inhibition (%)
$HepG_2$			57		54
Experiment	8 (100)	1.25±0.13		0.72±0.12	
Control	8 (100)	2.92±0.21		1.55±0.17	
ADM			14		18
Experiment	8 (100)	2.03±0.11		1.22±0.11	
Control	8 (100)	2.37±0.18		1.48±0.21	
TNF			58[a]		57
Experiment	8 (100)	0.67±0.22		0.45±0.16	
Control	8 (100)	1.37±0.18		0.93±0.18	
BCT			66[c]		67
Experiment	8 (100)	0.49±0.17[b]		0.29±0.21	
Control	8 (100)	1.45±0.26		0.88±0.17	

[a]$P>0.05$ *vs* $HepG_2$ group, $\chi^2=4.32$; [b]$P<0.01$ *vs* ADM group, $t=2.38$; [c]$P<0.05$ *vs* $HepG_2$ group, $\chi^2=2.49$.

Histologic characteristics and Pgp protein expression in orthotopic tumor tissues

Histological characteristics of the tumor model in different groups were similar (Figure 4). Pgp protein expression was low. Pgp protein expression was lower in $HepG_2$ group, but high in ADM group, and lower in groups TNF and BCT than in ADM group. There was a significant difference between the groups ($P<0.01$), but no difference among $HepG_2$, BCT and TNF groups ($P>0.05$, Figure 5)

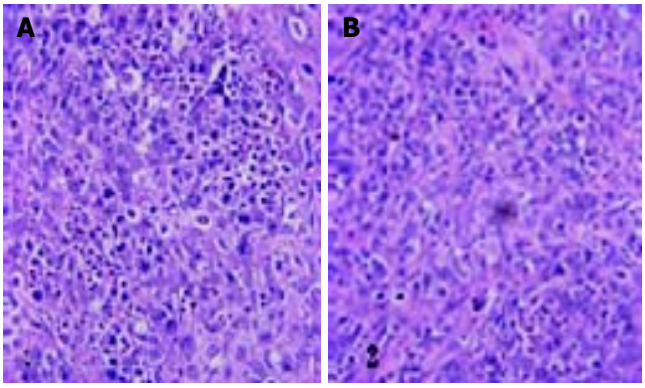

Figure 4 Histological characteristics of tumor in $HepG_2$ group (**A**) and BCT group (**B**).

Downregulation of MDR1mRNA expression in orthotopic tumor tissues transfected with TNF-α

Electrophoresis in each group showed 176, 237, and 530 bp bands, which were coincident with the amplification fragments of designed MDR1mRNA, LRPmRNA and β-actin genes. The relative quantity of MDR1mRNA in groups of BCT, TNF, $HepG_2$ was 0.21±0.02, 0.15±0.03, 0.13±0.02 respectively. The relative quantity of LRPmRNA in groups BCT, TNF, $HepG_2$ was 0.17±0.04, 0.15±0.01, 0.11±0.01. There was no difference among these groups ($P>0.05$), but the relative quantity of MDR1mRNA, LRPmRNA was significantly lower in groups of BCT, TNF, and $HepG_2$ than in ADM group ($P<0.05$, Figure 6, Table 3).

Apoptosis of tumor cells

After intra-abdominal chemotherapy with ADM, 5-FU, and mitomycin, the apoptotic rate of tumor cells expressing TNF-α was significantly higher compared to those not expressing TNF-α. The average of apoptotic cells in TNF-α and BCT group was (21.9±2.1) and (30.5±4.2) per field of high-power microscope respectively. There was a difference between the groups ($P<0.05$). TNF-α could reduce the tolerance of hepatoma cells to chemotherapy and increase apoptosis of tumor cells. BCT and TNF-α could induce apoptosis of HCC (Figure 7, Table 4).

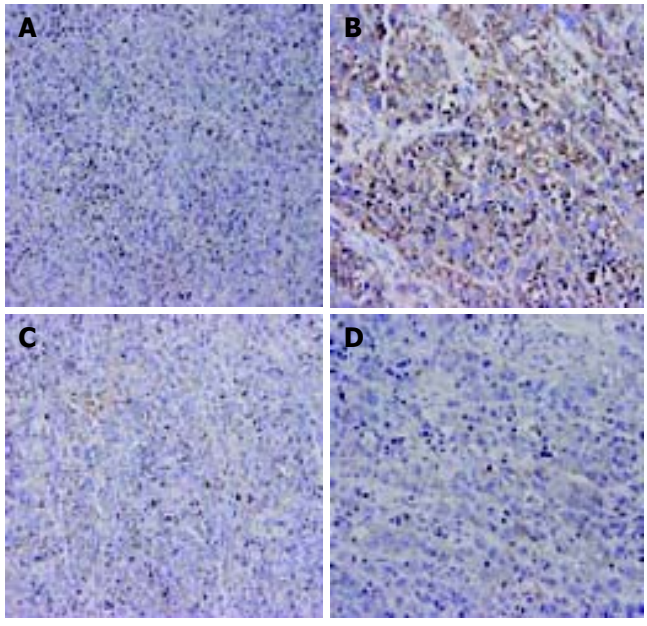

Figure 5 Pgp protein expression in groups of $HepG_2$ (**A**), ADM (**B**), BCT (**C**), and TNF (**D**).

DISCUSSION

Since MDR is the leading cause of failure of comprehensive therapy for HCC, investigations aimed at circumvention of intrinsic tumor-mediated drug resistance are warranted. But the efficacy of some drugs used to reverse MDR is limited due to their side-effects[16-18]. BCT has been used to treat hyperprolactinemia for more than two decades because of its slight side-effects[19,20]. It was reported that BCT can

Table 3 mRNA level of MDR-associated genes in response to TNF-α gene induction (mean±SD)

Groups	MDR1mRNA	LRPmRNA
BCT	0.21±0.02[a,c]	0.17±0.04
TNF	0.15±0.03[a,c]	0.15±0.01
ADM	0.89±0.15	0.83±0.12
$HepG_2$	0.13±0.02[c]	0.11±0.01

[a]P>0.05 *vs* $HepG_2$ group; [c]P<0.05 *vs* ADM group.

Table 4 Apoptosis index of each kind of tumors (mean±SD)

Group	Mice (no)	Experimental (index of apoptosis)
BCT	8	30.5±4.2[a,b,c]
TNF	8	21.9±2.1[b,c]
ADM	8	2.3±1.8
$HepG_2$	8	23.7±2.9

[a]P<0.05 *vs* TNF group; [b]P<0.01 *vs* ADM group; [c]P>0.05 *vs* $HepG_2$ group.

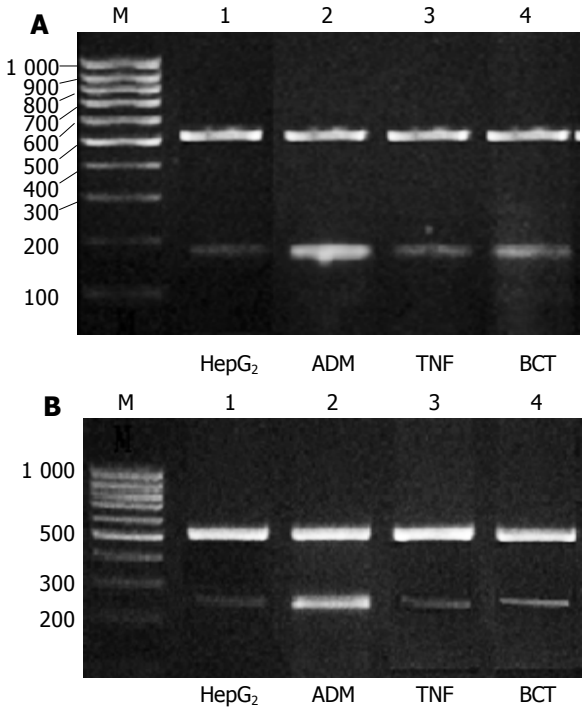

Figure 6 Expression of MDR1mRNA (**A,** 176 bp) and LRPmRNA (**B,** 237 bp).

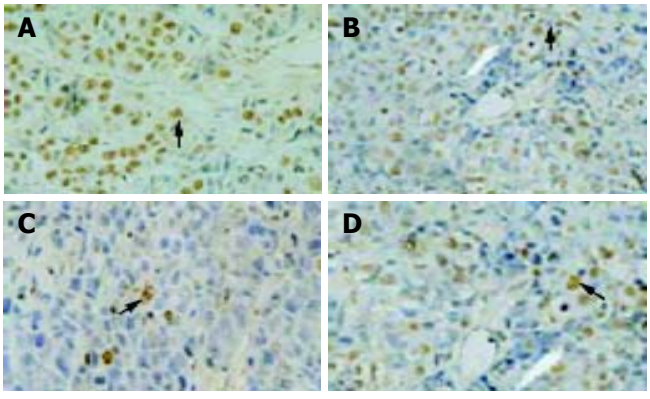

Figure 7 HHC cell apoptosis in groups of BCT (**A**), TNF (**B**), ADM (**C**), and $HepG_2$ (**D**).

be used as a chemosensitizer against MDR tumor cells[7,8] and a current study showed that TNF-α may play a role in MDR[21].

In this study, we first demonstrated that TNF-α was expressed in the orthotopic tumor tissues injected into $HepG_2$/ADM cells transfected with TNF-α. Moreover, tumor inhibition rate after treatment with 5-FU, ADM, MMC through intraperitoneal injection was higher in BCT group (67%, P<0.05) than in TNF group (57%) and ADM group (18%), and similar to $HepG_2$ group (54%). As the data shows, the drug-resistant hepatocarcinoma cell line $HepG_2$/ADM transfected with TNF-α could excrete cytokine TNF-α which could induce cancer cell apoptosis and inhibit the growth of tumor[22,23]. The result is in coincidence with Stein *et al*.[24]. Furthermore, TNF-α gene in combination with BCT could reverse HCC MDR. Moreover, IHC and reverse transcriptase polymerase chain reaction (RT-PCR) showed that transduction and expression of human TNF-α in drug-resistant HCC cell line $HepG_2$/ADM could reverse expression of MDR1mRNA, LRPmRNA, and Pgp, but had no synergistic effect compared to BCT, suggesting that TNF-α can downregulate the expression of MDR-associated genes and proteins[25], and BCT may act on for its potential interaction and transport by Pgp[26-31], but has no effect on MDR-associated genes. Additionally, an interesting discovery indicate that the rate of apoptosis after chemotherapy in BCT group is the highest in all experiment groups, and BCT and TNF-α can reverse HCC MDR in nude mice MDR1 model of liver neoplasm.

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REFERENCES

1 **Germann UA,** Pastan I, Gottesman MM. P-glycoproteins: mediators of multidrug resistance. *Semin Cell Biol* 1993; **4**: 63-76
2 **Marzolini C,** Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; **75**: 13-33
3 **Chan JY**, Chu AC, Fung KP. Inhibition of P-glycoprotein expression and reversal of drug resistance of human hepatoma HepG2 cells by multidrug resistance gene (mdr1) antisense RNA. *Life Sci* 2000; **67**: 2117-2124
4 **Huesker M**, Folmer Y, Schneider M, Fulda C, Blum HE, Hafkemeyer P. Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-MDR1 ribozymes. *Hepatology* 2002; **36** (4 Pt1): 874-884
5 **Wang H**, Chen XP, Qiu FZ. Overcoming multi-drug resistance by anti-MDR1 ribozyme. *World J Gastroenterol* 2003; **9**: 1444-1449
6 **Grude P**, Conti F, Mennecier D, Louvel A, Houssin D, Weill B, Calmus Y. MDR1 gene expression in hepatocellular carcinoma and the peritumoral liver of patients with and without cirrhosis. *Cancer Lett* 2002; **186**: 107-113
7 **Orlowski S,** Valente D, Garrigos M, Ezan E. Bromocriptine modulates P-glycoprotein function. *Biochem Biophys Res Commun* 1998; **244**: 481-488
8 **Shiraki N,** Okamura K, Tokunaga J, Ohmura T, Yasuda K, Kawaguchi K, Hamada A, Nakano M. Bromocriptine reverses

P-glycoprotein-mediated multidrug resistance in tumor cells. *Jpn J Cancer Res* 2002; **93**: 209-215

9 **Stein U,** Walther W, Laurencot CM, Scheffer GL, Scheper RJ, Shoemaker RH. Tumor necrosis factor-alpha and expression of the multidrug resistance-associated genes LRP and MRP. *J Natl Cancer Inst* 1997; **89**: 807-813

10 **Walther W,** Stein U, Pfeil D. Gene transfer of human TNF alpha into glioblastoma cells permits modulation of mdr1 expression and potentiation of chemosensitivity. *Int J Cancer* 1995; **61**: 832-839

11 **Hirsch-Ernst KI**, Ziemann C, Foth H, Kozian D, Schmitz-Salue C, Kahl GF. Induction of mdr1b mRNA and P-glycoprotein expression by tumor necrosis factor alpha in primary rat hepatocyte cultures. *J Cell Physiol* 1998; **176**: 506-515

12 **Gilboa E,** Lyerly HK, Vieweg J, Saito S. Immunotherapy of cancer using cytokine gene-modified tumor vaccines. *Semin Cancer Biol* 1994; **5**: 409-417

13 **Uckert W,** Walther W. Retrovirus-mediated gene transfer in cancer therapy. *Pharmacol Ther* 1994; **63**: 323-347

14 **Walther W,** Stein U. Influence of cytokines on mdr1 expression in human colon carcinoma cell lines: increased cytotoxicity of MDR relevant drugs. *J Cancer Res Clin Oncol* 1994; **120**: 471-478

15 **Mitchell MS.** Combining chemotherapy with biological response modifiers in treatment of cancer. *J Natl Cancer Inst* 1988; **80**: 1445-1450

16 **Madureira AM**, Spengler G, Molnar A, Varga A, Molnar J, Abreu PM, Ferreira MJ. Effect of cycloartanes on reversal of multidrug resistance and apoptosis induction on mouse lymphoma cells. *Anticancer Res* 2004; **24**: 859-864

17 **Woehlecke H,** Osada H, Herrmann A, Lage H. Reversal of breast cancer resistance protein-mediated drug resistance by tryprostatin A. *Int J Cancer* 2003; **107**: 721-728

18 **Schuetzer-Muehlbauer M,** Willinger B, Egner R, Ecker G, Kuchler K. Reversal of antifungal resistance mediated by ABC efflux pumps from Candida albicans functionally expressed in yeast. *Int J Antimicrob Agents* 2003; **22**: 291-300

19 **Schlesinger I**, Ravin PD. Dopamine agonists induce episodes of irresistible daytime sleepiness. *Eur Neurol* 2003; **49**: 30-33

20 **Deleu D**, Northway MG, Hanssens Y. An evidence-based review of Dopamine receptor agonists in the treatment of Parkinson's disease. *Saudi Med J* 2002; **23**: 1165-1175

21 **Lee JS**, Song CH, Lim JH, Kim HJ, Park JK, Paik TH, Kim CH, Kong SJ, Shon MH, Jung SS, Jo EK. The production of tumour necrosis factor-alpha is decreased in peripheral blood mononuclear cells from multidrug-resistant tuberculosis patients following stimulation with the 30-kDa antigen of Mycobacterium tuberculosis. *Clin Exp Immunol* 2003; **132**: 443-449

22 **Corti A**. Strategies for improving the anti-neoplastic activity of TNF by tumor targeting. *Methods Mol Med* 2004; **98**: 247-264

23 **Kim HR**, Park HJ, Park JH, Kim SJ, Kim K, Kim J. Characteristics of the killing mechanism of human natural killer cells against hepatocellular carcinoma cell lines HepG2 and Hep3B. *Cancer Immunol Immunother* 2004; **53**: 461-470

24 **Stein U**, Walther W, Shoemaker RH. Reversal of multidrug resistance by transduction of cytokine genes into human colon carcinoma cells. *J Natl Cancer Inst* 1996; **88**: 1383-1392

25 **Walther W,** Stein U, Fichtner I, Alexander M, Shoemaker RH, Schlag PM. Mdr1 promoter-driven tumor necrosis factor-alpha expression for a chemotherapy-controllable combined *in vivo* gene therapy and chemotherapy of tumors. *Cancer Gene Ther* 2000; **7**: 893-900

26 **Samini M,** Fakhrian R, Mohagheghi M, Dehpour AR. Comparison of the effect of levodopa and bromocriptine on naloxone-precipitated morphine withdrawal symptoms in mice. *Hum Psychopharmacol* 2000; **15**: 95-101

27 **Teodori E,** Dei S, Scapecchi S, Gualtieri F. The medicinal chemistry of multidrug resistance (MDR) reversing drugs. *Farmaco* 2002; **57**: 385-415

28 **Wu JY,** Fong WF, Zhang JX, Leung CH, Kwong HL, Yang MS, Li D, Cheung HY. Reversal of multidrug resistance in cancer cells by pyranocoumarins isolated from Radix Peucedani. *Eur J Pharmacol* 2003; **473**: 9-17

29 **Marian T,** Szabo G, Goda K, Nagy H, Szincsak N, Juhasz I, Galuska L, Balkay L, Mikecz P, Tron L, Krasznai Z. *In vivo* and *in vitro* multitracer analyses of P-glycoprotein expression-related multidrug resistance. *Eur J Nucl Med Mol Imaging* 2003; **30**: 1147-1154

30 **Psarros T,** Zouros A, Coimbra C. Bromocriptine-responsive akinetic mutism following endoscopy for ventricular neurocysticercosis. Case report and review of the literature. *J Neurosurg* 2003; **99**: 397-401

31 **Yavuz D,** Deyneli O, Akpinar I, Yildiz E, Gozu H, Sezgin O, Haklar G, Akalin S. Endothelial function, insulin sensitivity and inflammatory markers in hyperprolactinemic pre-menopausal women. *Eur J Endocrinol* 2003; **149**: 187-193

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