Effects of p53 on apoptosis and proliferation of hepatocellular carcinoma cells treated with transcatheter arterial chemoembolization

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INTRODUCTION
Hepatocellular carcinoma (HCC) is one of the most common malignancies. Surgical resection has been recognized as the most effective method for the treatment of HCC[10,11], but it is only indicated for small number of HCC patients. Transcatheter arterial chemoembolization (TACE) has become one of the most popular and effective palliative methods for HCC. Various mixtures of anticancer drugs, Lipiodol and gelatin sponge have been used as TACE agents. There have been a few reports on comparison of the efficacy of different TACE regimens on HCC patients[12].

Cellular homeostasis in tissue depends on the balance between apoptosis and cell proliferation. Wild-type p53 protein inhibits the growth of tumor by arrest of cell proliferation and induction of apoptosis[13]. Bcl-2 and Bax protein are important regulators of apoptosis[5]. Bcl-2 proteins can prolong cell survival by suppressing apoptosis, and Bax proteins can enhance apoptosis[4]. PCNA and Ki-67 protein are useful markers for proliferative activity[6]. PCNA functions as a cofactor of DNA-polymerase and an important mark for evaluating the proliferation of colon cancer[14], gastric adenocarcinoma[7], H pylori associated gastric epithelial lesions[8], lung cancer[9], ovarian cancer[12], large intestine polyps[10] and HCC[11].

As far as we know, the effect of p53 on apoptosis and proliferation of HCC cells treated with different TACE regimens has not been investigated yet. In particular, it is unclear whether p53 can affect apoptosis and proliferation of HCC cells treated with TACE by modulating the expressions of Bcl-2, Bax, PCNA and Ki-67 proteins. In the present study, we examined the effects of p53 on apoptosis and proliferation of HCC cells treated with TACE alone or in combination with others.

MATERIALS AND METHODS
Patients
From February 1992 to February 2001, 136 patients with HCC were referred to our hospital for surgery, including 122 men and 14 women with a mean age of 45 years (range 20 to 70 years). Preoperative ultrasound (US), computed tomography (CT), magnetic resonance (MRI), digital subtraction angiography (DSA) and plasma AFP levels were used to diagnose the conditions and the diagnosis was finally confirmed with pathological biopsy.

Surgical procedure
The patients were divided into TACE or non-TACE group. In TACE group, 79 patients underwent 1-5 courses chemoembolization prior to liver resection. Of them, 11 patients had 1-4 courses of chemotherapy only (group A), 33 patients had 1-5 courses of chemotherapy combined with iodized oil (group B), 23 patients had 1-3 courses of chemotherapy, iodized oil and gelatin sponge (group C), 12 patients had 1-3 courses of chemotherapy combined with iodized oil, ethanol and gelatin sponge (group D). Considering the course of TACE, 50 patients underwent three or more courses. The interval from the last TACE to the surgery was 52.8±12.2 days (±s), 25 patients had...
P53 expression in different pathological grades

Pathological grades were divided into four groups. Expression of p53 protein in HCC cells increased as the increase of pathological grade in non-TACE or TACE group (P<0.05). P53 protein expression in grade II specimens in TACE group was significantly lower than that in non-TACE group (P<0.05, Table 2).

Table 2 Expressions of proteins in different grades of HCC

| Treat groups | I      | II     | III    | IV     |
|--------------|--------|--------|--------|--------|
| Non-TACE     | 60.9±4.305 | 67.2±15.53 | 72.9±20.60 | 93.4±0.00 |
| TACE         | 32.5±7.68 | 60.0±14.67 | 74.6±8.65  | 82.6±1.11 |
| P            | >0.05   | <0.05  | >0.05  | >0.05  |

P53 expression in HCC cells

Expression of p53 protein in HCC cells was 69.37±18.81% in the non-TACE group, 65.09±15.71% in TACE group, 75.34±5.36% in group A, 69.34±12.59% in group B, 60.94±17.24% in group C, and 53.41±18.13% in group D. Expression of p53 protein was significantly higher in group A than in groups B, C, D and non-TACE group, and was higher in group B than in groups C and D, and lower in group D than in non-TACE group (P<0.05, Figures 1-4).

Correlation between courses of TACE and p53 protein expression

Expression of p53 protein in HCC cells was 69.37±18.81% in non-TACE group, 65.76±13.96% in one-course of TACE group, 64.09±19.81% in two-courses of TACE group, and 65.19±17.80% in three or more courses of TACE group. No statistical difference was found among groups (P>0.05).

Correlation between interval of TACE and p53 protein expression

Considering the interval from the last TACE to the operation, expression of p53 protein was 69.37±18.81% in non-TACE group, 63.54±13.72% in TACE group with an interval ≤1 month, 61.17±18.32% in TACE group with a 1-2 months interval, 70.87±15.06% in TACE group with a 2-3 months interval, and 73.67±5.87% in TACE group with an interval >3 months. P53 protein expression was significantly lower in patients with an interval ≤2 months than that in patients with an interval >3 months (P<0.05).

Correlation of p53 expression with Ki-67, PCNA, Bax, Bcl-2 protein expressions and AI

In non-TCAE group, p53 expression (69.37±18.81%) had a positive correlation with expressions of Ki-67 protein.
(44.43±20.70%, $P<0.05$), PCNA (62.92±17.21%, $P<0.05$), and Bax (44.29±23.73%, $P>0.05$), and a negative correlation with the ratio of Bcl-2 to Bax (0.48±0.64, $P<0.05$), AI (5.71±1.38, $P>0.05$) and Bcl-2 (12.72±4.92%, $P>0.05$) (Table 3).

In TACE group, expression of p53 protein (65.09±15.71%) had a positive correlation with expression of Ki-67 (40.24±16.59%, $P<0.05$), PCNA (59.95±17.75%, $P<0.05$), and Bcl-2 (7.47±6.41%, $P>0.05$), Bcl-2 to Bax ratio (0.21±0.29, $P>0.05$), and a negative correlation with AI (14.69±6.29%, $P<0.05$) and Bax (54.59±23.63%, $P>0.05$) (Table 3).

DISCUSSION

HCC is one of the most common malignant neoplasms. Most HCC patients are treated palliatively to improve the resectable rate and prolong survival. The best therapeutic method for HCC with tumor thrombi in portal vein (PVTT) has been regional hepatic TACE treatment after hepatic resection with removal of tumor thrombi\cite{16}. TACE has become one of the most common and effective palliative approaches. The prognosis of patients treated with TACE was dependent on both the effect of TACE and tumor factors\cite{17}.

To our knowledge, few data regarding the molecular mechanism of TACE treatment for HCC are available, the current study is the first report to describe the correlations between p53 expression and different TACE regimens.

Our study showed that the frequency of p53 expression was higher in group A than in group B, non-TACE group and TACE group, the lowest in group C and D ($P<0.05$). Our previous study showed that multidrug resistant gene product Pgp protein was significantly increased in chemotherapy group alone\cite{18}, suggesting that p53 expression increased after chemotherapy with the development of chemoresistance. p53 status might be an important determinant of tumor response to chemotherapy. Tumors with P53 (-) expression might respond to chemotherapy. Inversely, chemotherapy was not effective in patients with P53 (+) expression\cite{19}. This study demonstrated that p53 protein expression had no significant difference between the TACE and non-TACE groups\cite{20}.

The reverse correlation between AI and p53 protein expression of HCC cells was found in both TACE and non-TACE groups in this study. Most p53 protein measured was the mutant-type. AI was significantly positively correlated with wild-type p53 which enhanced apoptosis by activating proapoptotic Bax and by down-regulating antiapoptotic Bcl-2\cite{21}.

The current study demonstrated that p53 expression was positively related to Ki-67 and PCNA protein expression in the non-TACE and TACE groups, suggesting that the mutated p53 protein could enhance the growth of HCC. Our previous study showed both PCNA and p53 expressions had a significantly parallel correlation, the overexpression of mutated p53 resulted in cancer immortalization, high tumor recurrence risk, more aggressive growth and poor survival\cite{22} and Kieser et al found that a mutated p53 gene could provide an advantage for tumor proliferation\cite{23}. Igarashi et al reported that expression of p53 protein like Ki-67 labeling index was a useful indicator for high proliferative activity\cite{24}. A mutated p53 gene provided an advantage for tumor proliferation not only by allowing escape from apoptosis, but also by leading to formation of a vascular-rich microenvironment\cite{25}. However, it has been known that wild-type p53 could inhibit the expression of PCNA\cite{26}.

Wild-type p53 is a positive transcriptional activator for human Bax gene and a negative transcriptional activator for human Bcl-2 gene. The activation of p53 pathway could lead to the down-regulation of Bcl-2 and up-regulation of Bax\cite{27}. The current study demonstrated that p53 protein expression was positively related to Bax expression, and negatively to Bcl-2 expression in the non-TACE group. Bcl-2 expression had an inverse correlation with mutant p53, and wild-type p53
We need further study to clarify the correlation between p53 expression and suppression of HCC cells after TACE. P53 protein expression enhances proliferation of HCC cells, but did not increase in tumors with mutant p53 gene.

This study demonstrated that discrepancy of histological types and pathological grades in p53 protein expression is existed in both non-TACE and TACE groups. Zhao et al reported that p53 gene mutation varied in histological types and the mutated rate was 10.5% in trabecular cells, 37.5% in pseudoglandular cells, 60.0% in solid cells and 33.3% in sclerosis (P<0.05) [30]. Our previous study showed that trabecular and clear cells were more sensitive to TACE than solid, poorly or undifferentiated, and small cells of HCC [14]. Wang et al also found that the clear cells of HCC were more sensitive to TAE than small cells of HCC and poorly differentiated or undifferentiated HCCs [31]. Yamashita et al found that most HCCs responded to TACE well [32]. These suggest that p53 protein expression would influence the effects of TACE on HCC. Further study is needed to clarify the correlation between p53 protein expression and tumor necrosis and patient survival.

This study also demonstrated that p53 protein expression increased as grades increased in both non-TACE and TACE groups (P<0.05), which was inconsistent with the study of Zhao et al [30]. P53 protein expression of grade II HCC was significantly lower in TACE group than in non-TACE group, suggesting that influences of the molecular markers were very complicated and discrepancy in therapeutic effect of TACE on different histopathological types of HCC was existed. It would be the best choice to use TACE in an individualized mode.

The best interval of treatment for repeated TACE or second stage resection is controversial. In this study, we found that p53 protein expression of HCC cells was significantly lower in the group with a 2 months or less than in the group with an over 3 month treatment interval (P<0.05). We found that the best interval between treatment with TACE or second stage resection was 2-3 months.

In conclusion, discrepancy of histological types and pathological grades in p53 protein expression is existed in HCC. P53 protein expression can enhance proliferation of HCC cells and suppress apoptosis of HCC cells after TACE. P53 protein expression would influence effects of TACE on HCC. We need further study to clarify the correlation between p53 protein expression, tumor necrosis and patient survival.

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