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ORIGINAL PAPER

Clinical study of recombinant adenovirus-p53 combined with fractionated stereotactic radiotherapy for hepatocellular carcinoma

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Abstract
Objective The purpose of our study was to evaluate the feasibility and treatment outcomes of recombinant adenovi- rus-p53 (rAd-p53, trademarked as Gendicine) combined with fractionated stereotactic radiotherapy (fSRT) in treat- ment of primary hepatocellular carcinoma (HCC).
Methods We randomly enrolled 40 patients with HCC treated by fSRT alone (fSRT group) or rAd-p53 combined with fSRT (combined group). Tumor size was 2–5.2 cm (average 3.2 cm). We prescribed 50 Gy in 10 fractions at the 50%–80% isodose line of the planning target volume for 2 weeks in two groups. The combined group was treated with two intratumoral injections of rAd-p53 on day 1 and 8 while fSRT started on day 3. Tumor response was assessed after treatment using modiWed WHO criteria. The follow- up period was 11–44 months (median 35 months).

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Results The overall response rate of fSRT group was 70%, with 4 patients showing complete response (20%), 10 partial response (50%) and 6 stable disease (30%). Corre- spondingly the overall response rate of combined group was 85%, with 7 patients showing complete response (35%), 10 partial response (50%) and 3 stable disease (15%). The 1-year survival rates of fSRT group and combined group were 70.0% and 90.0%, respectively. The 1-year disease-free survival rates of fSRT group and com- bined group were 65% and 85%, respectively. These treat- ments were well tolerated, because grade 3 or 4 toxicity was not observed.
Conclusion These results suggest that rAd-p53 combined with fSRT is a relatively safe and eVective method for treat- ing primary hepatocellular carcinoma compared with only fSRT. Thus, rAd-p53 combined with fractionated SRT may be preferred as a choice of local treatment for primary HCC when the patients are inoperable or when the patients refuse operation.

Keywords Hepatocellular carcinoma ·Fractionated stereotactic radiotherapy ·Recombinant adenovirus-p53

Introduction

Hepatocellular carcinoma (HCC) is the Wfth most common malignancy worldwide, with approximately 500,000 new cases per year, of which approximately 75% cases were from Asian countries mainly due to chronic hepatitis B virus (HBV) infection and may progress to death within a few months if treated improperly (Cook and Moosa 1985). Surgical resection and liver transplantation are considered the cures for HCC. However, in most patients primary HCC is often accompanied by chronic viral hepatitis or liver

cirrhosis, and curative surgery may not be indicated in such circumstances. Classical treatment methods except surgical resection include transarterial chemoembolization, percuta- neous ethanol injection, radiofrequency ablation and radia- tion therapy (Yu et al. 1993). Although there are many treatment options, the standard treatment modality for pri- mary HCC is not yet established. Recently, with develop- ment of RT techniques including three-dimensional conformal radiotherapy (3D-CRT), stereotactic radiother- apy (SRT), proton therapy and cyber-knife, radiotherapy is becoming one of the most important tools for treatment of HCC (Zhou et al. 2007). But it is also used primarily for palliative purposes. Now, it is well known that gene therapy by recombinant adenovirus-mediated wild-type p53 gene (rAd-p53) transfer has been successfully inhibiting growth of many tumors including head–neck cancer, lung cancer and prostate cancer by intratumoral injection (Neyns and Noppen 2003; Frank 2002), but there is no report on role of rAd-p53 in combination with SRT in treating HCC. There- fore, the aim of this study is to investigate the eYcacy and safety of rAd-p53 (Gendicine) combined with SRT in treat- ment of HCCs and evaluate its clinical application.

Patients and methods

Eligibility

The study retrospectively and consecutively analyzed 40 patients who were histologically diagnosed having primary HCC from August 2004 to May 2007 in Daping Hospital, Third Military Medical University, Chongqing, China. All 40 patients were randomly divided into two groups, frac- tionated SRT alone and rAd-p53 (trademarked as Gendi- cine) combined with fSRT. The criteria for selecting patients for the study were as follows: (1) showing no extrahepatic metastasis, (2) belonging to a group lower than Grade B on Child-Pugh classiWcation, (3) ECOG score < 2,
(4) no experience of radiation treatment and (5) having a single lesion. The median age was 56 years (range from 43 to 72 years), and there were 32 male and 8 female patients. Using the orthogonal diameter estimated by computed tomography (CT), the tumor size was measured, which ranged from 2 to 5.2 cm (average 3.2 cm). All patients in combined group had signed the agreement to use the rAd- p53 (Table 1).

P53 gene therapy and fractionated stereotactic radiotherapy

The patients treated with rAd-p53 combined with fSRT (combined group) were given two intratumoral injections of rAd-p53 on day 1 and 8 while fSRT started on day 3. Guided by B-ultrasound, we carried the recombinant ade-

Table 1 Patient characteristics

Characteristics	fSRT group	Combined group
Gender (number)		
Male	15	17
Female	5	3
Age (years)		
Range	43–68	45–72
Median	53	57
Child-Pugh class (number)		
A	12	15
B	8	5
Tumor size (cm)		
Range	2.1–4.8	2–5.2
Median	3.2	3
ECOG performance status (number)		
0–1	16	15

2	4	5
Liver cirrhosis (number)		
Yes	10	12
No	10	8

ECOG Eastern Cooperative Oncology Group

novirus vector containing the wild-type p53 gene into liver cancer target using the method of percutaneous multiplicity of injection. Each injection of virus titer was (1–3) 10^{12} of viral particles (VP).

We used the body gamma knife treatment system (OUR company, Shenzhen, China), where a stereotactic three-dimensional coordinate was used to maximize the precision of the treatment target and also minimize the injury to nor- mal tissues in the vicinity. In brief, the procedures of the radiation therapy were as follows: Wrst, each patient was immobilized in the supine position or face lying position with shallow respiration using a vacuum cushion during the course of the treatment planning and fSRT. Then, the abdominal CT was also performed at 3–5 mm intervals under these conditions. The CT images were transferred to the three-dimensional treatment planning system (SGI, Southeast University, China) and reconstructed as a three- dimensional image for dose planning. For fractionated SRT, irradiation of 5 Gy was used per dose based on the planning target volume (PTV). Dose prescription was nor- malized at 50–80% isodose line. To assess the accuracy of patient position and target volume localization, veriWcation Wlms were taken before CRT. Radiation therapy was per- formed after the error range was <5 mm by repeating these localization and veriWcation procedures. We used rotation focus beams generated by gamma knife treatment system and used target fusion technique to Wt the target shape. At the time of treatment planning, the dose volume histogram of target volume and the adjacent organs, including remaining

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1 3

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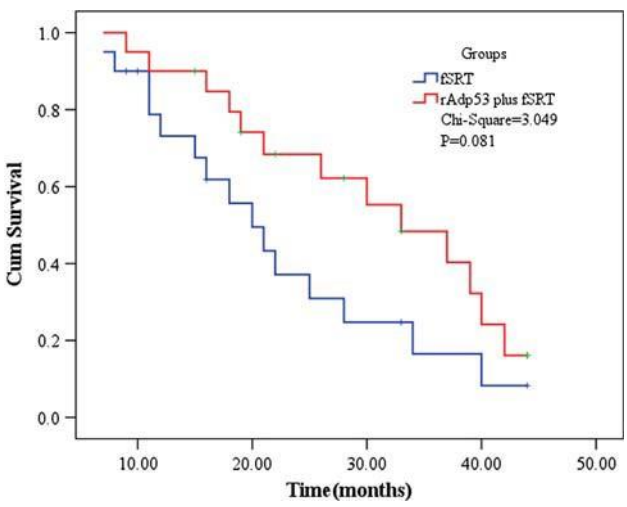
normal liver, was conWrmed to minimize the side eVects. By the body gamma knife treatment system and radiation dose of 5 Gy per fraction, fractionated SRT was performed 5 times per week for 2 weeks, with the total irradiation dose of 50 Gy.

Table 2 Treatment outcomes of fractionated SRT alone and rAd-p53 combined fSRT therapy (n = 20)

Groups	Treatment outcomes				
	CR	PR	SD	PD	RR (%)
fSRT	4/20	10/20	6/20	0/20	14/20 (70%)
Combined	7/20	10/20	3/20	0/20	17/20 (85%)



Response and toxicity evaluation

All patients were evaluated for response and toxicity. Treatment response was determined by measuring the largest size on the abdominal CT scans checked at 1 month after completion of fSRT or rAd-p53 intratumoral injection and subsequently at 2–3 month intervals. The WHO clinical response criteria were applied. A complete response (CR) was deWned as complete disappearance of the lesion, a partial response (PR) was deWned as a decrease in the tumor size or necrosis by >50% of the initial lesion size, a stable disease (SD) was deWned as a decrease in the tumor size or necrosis by <50% of the initial lesion size, and a progressive disease (PD) was deWned as a progression of the initial lesion. Toxicity was evaluated according to the NCI common toxicity criteria.

Follow-up and statistical analysis

Analysis was performed after 40 patients had been enrolled. In this study, the survival time was measured as the period from

the date of fSRT to the date of death or the last follow- up, and disease-free survival time as the period from the date of fSRT to the date of disease progression or death. The survival rate and disease-free survival rate were deter- mined using the Kaplan–Meier method by using SPSS 12.0.

Results

Treatment outcomes

All patients were followed up for a period of 11–44 months (median 35 months). Among 40 patients, the overall response rate of fSRT group was 70%, with 4 patients showing complete response (20%), 10 partial response (50%) and 6 stable disease (30%), and no progressive dis- ease was detected. Correspondingly the overall response rate of rAd-p53 combined with fSRT group was 85%, with 7 patients showing complete response (35%), 10 partial response (50%) and 3 stable disease (15%), and no progres- sive disease was detected (Table 2).

During 44 months of follow-up, the 1-year survival rates of alone and combined group were 70.0% and 90.0%, respectively. The 1-year disease-free survival rates were

Fig. 1 Overall survival of patients treated with SRT and rAd-p53 plus SRT

65% and 85%, respectively. Figure 1 shows the overall sur- vival of patients treated with fSRT alone and rAd-p53 com- bined groups. The median survival was 20 months and 33 months, respectively. Two typical cases are shown in Figs. 2 and 3.

Toxicity

All patients tolerated these treatments. Treatment-related toxicities were negligible in all patients. None of the patients was observed to have more than grade-3 toxicity. Fever and gastrointestinal toxicity was observed in 10 patients of alone group and 12 patients of combined group, respectively. Seven patients of fSRT group and eight patients of combined group showed abnormal liver func- tions without evidence of tumor progression. Liver func- tions showed abnormality developed 2–4 weeks after completion of fractionated SRT, but recovered or stabilized into at least the upper limit of normal function treatment with drugs of protecting liver within 2–3 months. In addi- tion, hematological toxicity was thrombocytopenia in 6 patients of alone group and 8 patients of combined one and leukopenia in 3 patients of alone group and 3 patients of combined one. Nevertheless, all patients recovered follow- ing supportive therapy, with no fatal toxicities (Table 3).

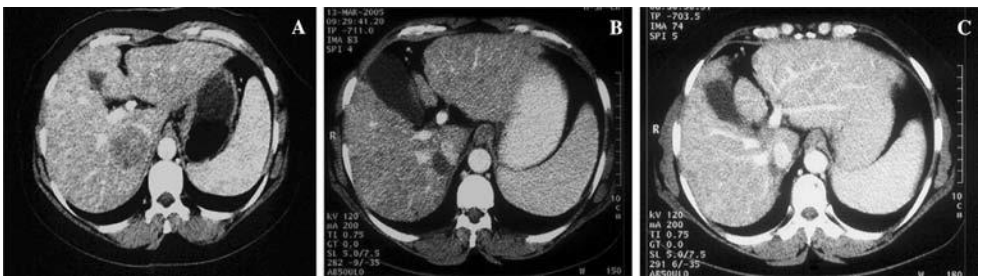


Fig. 2 Abdominal CT scans of typical case 1. **a** Before rAd-p53 plus fSRT; **b** one month after treatment; **c** 3 months after treatment. Tumor disappears and the treatment response recovers to be as CR

Fig. 3 Abdominal CT scans of a typical case 2. **a** Before rAd-p53 plus fSRT; **b** 3 months after treatment. Tumor miniWcates and the treatment response is conWrmed as PR

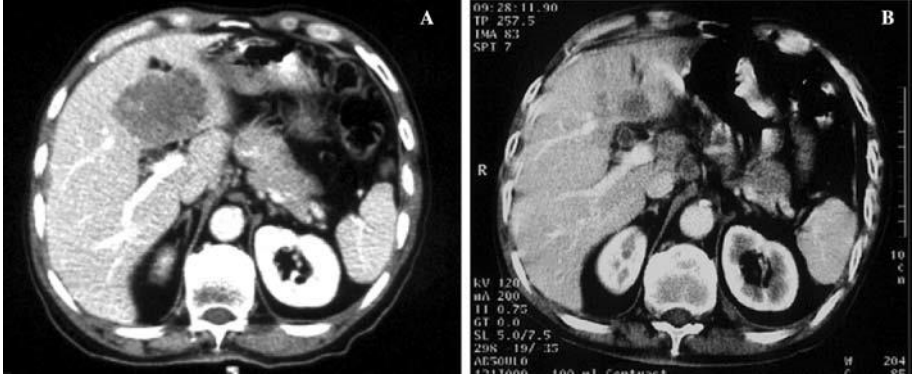


Table 3 Toxicity of fractionated SRT alone and SRT combined p53 gene therapy (n = 20)

Group	Toxicity grade			
	1	2	3	4
SRT alone				
Gastrointestinal	6/6	4/6	0/0	0/0
Liver	4/5	3/3	0/0	0/0
Thrombocytopenia	3/4	3/4	0/0	0/0
Gastrointestinal	6/6	4/6	0/0	0/0
Combined				
Liver	4/5	3/3	0/0	0/0
Thrombocytopenia	3/4	3/4	0/0	0/0
Leukopenia	2/1	1/2	0/0	0/0
Gastrointestinal	6/6	4/6	0/0	0/0

Discussion

This study was performed on patients with a comparatively small tumor containing a single lesion. Surgical resection was the most common method for the early stage liver can- cer. But when the patients refused surgery or when the patients were medically inoperable due to chronic viral hepatitis or liver cirrhosis, we had to select the invasive techniques. It is well reported that there are transarterial chemoembolization, percutaneous ethanol injection, radio-

frequency ablation for HCC. Each of these local treatments has advantages as well as disadvantages. For example, tran- sarterial chemoembolization is ineVective if the tumor has a collateral blood supply (Miyayama et al. 2006). It is uneasy to apply ethanol evenly to the entire tumor when perform- ing percutaneous ethanol injection due to unsatisfactory distribution, and the use of radiofrequency ablation is lim- ited to tumors that are not near the blood vessels or the sur- face of the liver.

Now using stereotactic radiotherapy (SRT) some of these problems were easily avoidable. Radiotherapy for the treatment of HCC has been attempted for decades. Early trials applied whole liver irradiation but used an inadequate radiation dose. Because of hepatic toxicity and ineVective- ness of such low-dose whole-liver irradiation, radiotherapy has not been considered for the treatment of HCC for some time (Seong et al. 2001). However, recently, partial hepatic irradiation has been performed with 3D-CRT by several investigators who have found that high doses of radiation can be safely delivered to a portion of the liver (Krishnan et al. 2008; Tse et al. 2008). Although the low whole-organ tolerance of the liver had previously limited radiation to a palliative role, 3DCRT treatment planning allows signiW- cant portions of normal liver to be excluded from the treatment volume when hepatic involvement is not diVuse. Because normal liver is spared, a potentially tumoricidal dose of radiation (much higher than whole-liver tolerance) can be administered with acceptable complications.

Recently, the application of SRT has also expanded to can- cers including lung, liver and pancreas, and results of the clinical use of fractionated SRT for lung cancer have been frequently reported (Senan et al. 2004; Trodella et al. 2003). And reports showed that a high local elimination rate might be anticipated if a high single fractionation dose, such as 5 Gy or

larger fraction dose, is used for the treat- ment. Méndez Romero et al. (2006) observed the feasibil- ity, toxicity and tumor response of stereotactic radiation therapy for treatment of primary and metastatic liver tumors. Patients with HCC 4 cm and cirrhosis received
5 5 Gy or 3 10 Gy. Local control rates at 1 and 2 years for the whole group were 94% and 82%. In the report of Wulf et al. (2006), a high-dose fraction group also showed stereotactic irradiation of primary liver cancer and hepatic metastases oVer a locally eVective treatment without sig- niWcant complications in patients. Takeda et al. (2008) found that stereotactic radiotherapy (a total dose of 35– 50 Gy was delivered in 5–7 fractions over 5–9 days) was feasible for HCC and could provide good local control with a short treatment period. Tse et al. (2008) reported that individualized six-fraction SRT is a safe treatment for unre- sectable HCC. These reports strongly support the impor- tance of dose fraction in inducing tumor regression and ultimate success in terms of increased survival. In our study, treated with fractionated SRT alone, the overall response rate was 70%, with 4 patients showing complete response (20%), 10 patients showing partial response (50%) and 6 patients showing stable disease (30%), and progressive disease was not detected. The results were sim- ilar to corresponding clinical trial. In the treatment of HCC by fSRT, there is also a need to establish a thorough treat- ment plan that includes objective criteria, such as dose per irradiation, number of fractionations and total radiation dose. At the same time, no patient developed grade 3–4 gastrointestinal complications including gastroduodenal ulcer and bleeding in the present study. Our study had no treatment-related deaths.

However, HCC was a highly resistant solid tumor, and
HCC cells could overexpress the multidrug resistance gene. It was reported that p53 inactivation in some cancers likely led to selective resistance to chemotherapeutic agents because of up-regulation of MDR1 expression (Thottassery et al. 1997). The p53 gene like the Rb gene was a tumor suppressor gene. Its activity stops the formation of tumors. However, mutations in p53 gene were found in most tumor types including HCC (Bourdon 2007; Spandidos 2007), and so contributed to the complex network of molecular events leading to tumor formation. With the development of novel gene targeted therapies, there is an opportunity to evaluate the most common gene agents—the recombinant adenovirus vector containing the wild-type p53 in HCC. The recombinant adenoviral-p53(rAd-p53, Gendicine) had

multiple anti-tumor eVects. It was reported that the combi- nation of adenovirus p53 gene therapy with irradiation has remarkable synergistic eVects in many solid tumors, includ- ing non-small lung cancer, prostate cancer, esophagus car- cinoma and nasopharyngeal carcinoma (Neyns and Noppen 2003; Frank 2002). The results indicated that the molecular mechanisms of enhanced anti-tumor eVect of rAd-p53 com- bined with radiotherapy may include cell cycle arrest, apop- tosis/necrosis and upregulation of innate immune defenses. As the control factor of DNA damage-induced apoptosis, loss or malfunction of this p53-mediated apoptotic pathway has been proposed as one mechanism by which tumors become resistant to chemotherapy or radiation (Timiryas- ova et al. 2003; Shinoura et al. 2000). The systemically delivered adenoviral wide-type p53 gene therapeutics resulted in eYcient expression of functional wild-type p53, sensitizing the tumors to chemotherapy and radiotherapy. From our results, the overall response rate of fSRT com- bined with p53 gene group was 85%, with 7 patients show- ing complete response (35%), 10 partial response (50%) and 3 stable disease (15%), and progressive disease was also not detected. During the 44 months of follow-up, the 1-year survival rate of combined group was 90.0% com- pared with 70.0% of fSRT group. And the 1-year disease- free survival rate was 85% to 65%. The median survivals of fSRT group and combined group were 20 months and 33 months, respectively. The survival rates were signiW- cantly increased compared with treatment by fractionated SRT alone although ignoring other therapies during the fol- low-up period. And all patients tolerated these treatments. Although treatment-related toxicities were negligible, none of the patients was observed to have more than grade-3 tox- icity or fatal situation.

In conclusion, it is conWrmed that fractionated SRT com-
bined with p53 gene therapy is a relatively safe and eVec- tive local treatment for primary HCC and is also useful for patients who are medically inoperable or who refuse sur- gery. Although we do not know whether there is a survival beneWt through the use of this treatment, SRT combined with p53 gene therapy seems to be a practical method of salvage for this subset of patients. Further study is needed to evaluate the survival of such patients with or without this treatment. Analysis with a larger number of patients using the combined therapy method is required to validate whether results identiWed in this study is prospective to pre- dict response to p53 gene in HCC. Of course, the prognos- tic factors of HCC reported in the literature include tumor size, tumor type, tumor stage, portal vein thrombosis, serum AFP status and several serum parameters related to hepatic function. Some authors have also advocated that a combination of several factors can deWne prognostic groups. Evaluation of p53 gene in combination with anti- tumor agents in HCC is worthy of further study.

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