P53 Gene Therapy for Pulmonary Metastasis Tumor from Hepatocellular Carcinoma

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Objectives: To explore safety, efficacy and administration method of recombinant adenovirus p53 gene (rAd-p53, or Gendicine®) in treatment of pulmonary metastasis tumor from advanced hepatocellular carcinoma (HCC).
Methods: Pulmonary metastasis tumor from HCC in 20 patients were treated using trans-catheter bronchial arterial Gendicine infusion combined with transcatheter arterial embolization (TAE) and intratumor injection of Gendicine if the maximal diameter of a metastatic tumor is ≥3cm. Three patients received 3 times, 7 received 2 times and 10 received 1 time of combined therapy. Eighteen patients were followed up for 2-12 months after treatment and 2 patients lost follow-up. Spiral CT was performed during follow-up visits to monitor tumor progress.
Results: Lung metastasis tumor disappeared in 4 patients and the tumor size decreased in 6 patients, un-changed in 5 and increased in 3 patients. Overall clinical symptoms alleviated in 16 patients (88.9%) and exacerbated in 2 patients. New metastatic lesions were found in 8 patients. There were no serious adverse events except for self-limited fever (38 ºC-39.5 ºC) was found in 16 patients.
Conclusion: Trans-catheter bronchial arterial Gendicine infusion combined with transcatheter arterial embolization （TAE）, with or without intratumor injection of Gendicine is a safe, effective therapy for treatment of pulmonary metastasis tumor from HCC.

Key Words: P53; Hepatocellular carcinoma; Pulmonary metastasis; Gene therapy;

Hepatocellular carcinoma is one of most common malignant tumors and the lungs are most common site of metastasis when HCC progesses to an advanced stage. After comprehensive treatment of advanced hepatocellular carcinoma, patients' survival time is prolonged but companied with a late higher rate of pulmonary metastasis developed. Thus, treatment to pulmonary metastasis tumor may further prolong survival time of the HCC patients. A few papers reported it was effective and safe using p53 gene therapy to treat primary lung cancer. But there is no report using p53 gene therapy to treat pulmonary metastatic tumor from HCC. In this report we demonstrated p53 gene therapy is a safe and effective method for treatment of pulmonary metastatic tumor from HCC.

Methods

The rAd-p53, or Gendicine® was from SiBiono GeneTech Co. Ltd (19 First Science & technology Middle Road, Shenzhen Guangdong 518057, P. R. China).From January 2008 to September 2009, we treated 20 HCC patients with pulmonary metastatic lesions. All of 20 cases were diagnosed as advanced hepatocellular carcinoma by fine needle aspiration biopsy and by MRI or MSCT, 8 of them with pulmonary metastasis at primary diagnosis and 12 patients developed pulmonary metastasis after hepatic TACE (10 patients) and surgical resection (2 patients). Patients were from 48 to 80 years old with a median age of 52 years. Fifteen patients were male and 5 patients were female. Using Liver Function Child-pμgh Grade, 7 patients are grade A, 5 patients are grade B, and 8 patients are grade C. Patients' characteristics are summarized in table 1.

Table 1 Patients' Characteristics

Characteristics	Statistics
Age (years old)	53±24.4 (48-80)
Gender	
Male	15 (75%)
Female	5 (25%)
Number of metastatic noduls in lungs at treatment	
1	15(75%)
2	4(20%)
≥3	1(5%)
Metastatic tumors in right or/and left lungs	
Right	12
Left	8
Both	3
Metastatic tumor size (cm)	3.1±3.8 (0.8-6.4)
≤3	15
≥3	13
Primary HCC therapy	
Partial hepatic resection	5(25%)
TACE	15(75%)

Pulmonary metastatic tumors were treated using trans-catheter bronchial arterial Gendicine infusion combined with transcatheter arterial embolization（TAE）, and Intratumor injection of Gendicine if the maximal diameter of metastasis tumor is ≥3cm. The volume of 2 ml-20 ml lipiodol emulsion was injected after Gendicine infusion.

Before p53 gene therapy, all patients took routine blood test, liver and kidney function tests, as well as the chest and upper abdomen CT or MRI horizontal scan and

enhanced scanning. If the diameter of a single lung metastatic tumor nodule was ≥3cm, CT guided multi-points and multi-directions intra-tumor Gendicine injection was performed. The 10E12 virus particle (vp) diluted in 2 ml physical saline was injected if the tumor diameter is between 3-6 cm and 2 x 10E12 vp was injected if tumor diameter greater than 6 cm. After 3-5 days of intratumor injection, 2 x 10E12 vp were injected throμgh a tumor branch of the bronchial artery and TAE was applied after the injection. The catheter was inserted to a bronchial artery throμgh one femoral artery under CT guidance. The contrast agents were injected to find the tumor arterial branch.

We monitored progress of the pulmonary metastatic tumors using spiral CT or MRI examination. If the metastatic tumor disappeared, or tumor size decreased, or tumor size kept stable without new metastatic tumor for 4 weeks, these patients would be considered effective. Liver and kidney functions, blood routine (bilirubin, aminotransferase, blood urea nitrogen, creatinine, blood cells, and platelets) were tested at each visit. The clinical symptoms such as coμgh, chest pain, loss of appetite, fatigue, weight loss, etc. were evaluated.

Results

Three patients received 3 times of combined therapy, 7 received 2 times and 10 received only 1 time of therapy. Eighteen patients were followed up for 2-12 months after therapy and 2 patients lost follow-up. Gendicine intratumoral injection was applied in 13 patients, and Gendicine bronchial intra-arterial injection combined with TAE was conducted 20 patients. Pneumothorax occurred in 2 patients after the intratumoral injection and was completely absorbed by closed drainage. The other patients did not have any complications. Sixteen patients had a fever of (38 ºC-39.5ºC) for 1 to3 days after the treatment. Two patients with body temperature of 39.5ºC or higher were treated using i.v. 10 mg of Dexamethasone diluted in 500 ml of 5%Glucose and Sodium Chloride. Their body temperature decreased to normal in one day. Eighteen patients were followed up for 2-12 months after the treatment and 2 patients lost follow-up. Lung metastasis tumor disappeared in 4 patients and the tumor size decreased in 6 patients, un-changed in 5 and increased in 3 patients. Figure 1 shows a typical patient after 3 times of intratumor injection and one time of TAE plus bronchial arterial Gendicine infusion, left lung metastasis tumor completely disappeared at the one year follow-up of after treatment.

A B

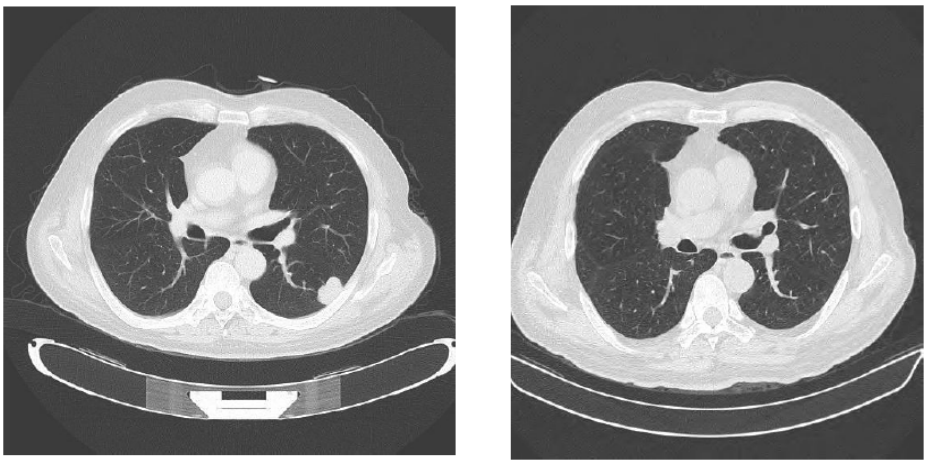

Fig. 1 A: Left lung 3 x 4.5 cm nodule diagnosed by needle aspiration biopsy as pulmonary metastatic tumor from HCC; B: Pulmonary metastatic tumor completely disappeared.

Overall clinical symptom alleviated in 16 patients (88.9%) and exacerbated in 2 patients. New metastatic lesions were found in 8 patients. There were no serious adverse events except for fever.

Discussions

The human tumor suppressing p53 gene is located at the short arm of the 17 chromosome (17p13.1).The gene is 20kb long consisted of 11 exons and 10 introns. The transcript of p53 gene is the 2.5kb mRNA and the translation product is a protein with a molecular weight of 53 kD. P53 protein involve in cell cycle control, DNA repair, apoptosis, and cell differentiation [1]. Its antitumor functions include cell cycle arrest and DNA repair [2], apoptosis [3], inhibition of angiogenesis, by-stander effect and inhibition of cell adhesion, invasion and metastasis [4]. Studies reported that about 50% of human tumors have various types of p53 gene mutation [5]. The loss or mutation of p53 gene results in a dysfunctional protein and loss of the function in inhibition of cancer development. Gendicine, the first antitumor gene product registered for marketing in the world, is a recombinant adenoviral p53 gene, in which, a wild type p53 gene was inserted in a defect adenovirus type 5. Several studies reported p53 gene therapy is effective for primary lung cancer [6, 7]. Tian and other studies sμggested that p53 gene has a strong inhibition to the hepatic tumor cells. The Inhibitory effect was associated with the percentage of transfected cells [8]. The intra-arterial p53 gene injection combined with TAE was used to treat patients with hepatocellular carcinoma and showed a great efficacy result [9]. We treated 20 patients using trans-catheter bronchial arterial Gendicine infusion combined with transcatheter arterial chemoembolization （TAE）, and intratumor injection of Gendicine if the maximal diameter of metastasis tumor is ≥3cm. Eighteen patients were followed up for 2-12 months after the gene therapy. Lung metastasis tumor

disappeared in 4 patients and the tumor size decreased in 6 patients, un-changed in 5 and increased in 3 patients. Overall clinical symptom alleviated in 16 patients (88.9%) and aggravated in 2 patients. New metastatic lesions were found in 8 patients. There were no serious adverse events except for fever, which may relate to immuno-response to adenovirus.

Compared with chemo- or radio-therapy, p53 gene therapy has much less adverse events and improves the quality of patients' life, which is more important for cancer patients with an advanced stage. In this report, except for self-limited fever no other significant adverse events were observed, which is consistent with other reports about using Gendicine to treat malignant tumors [8-10]. We performed 13 CT-guided intratumor injections throμgh the pleural space and only two patients developed pneumothorax. This complication was not commonly observed as concerned before. Accurate direction and location of the injection are key to avoid pneumothorax and injury to other important tissue structures.

Most tumors in advanced stages become resistant to the standard therapies such as chemo- or radio-therapy, or some patients are not able to tolerate side effects from the standard therapies. Gene therapy will be an alternative method for these patients. Gene therapy has been proved as an effective method for many chemo- or radio-resistant tumors [10, 11]. One of advantages of p53 gene therapy is its synergic effect with both chemo- and radio- therapy. Combination of p53 gene therapy with other tumor treatments might be more effective.

The longest follow-up time for this group of patients is 12 months. Long term results need to be confirmed by longer follow-up and a well-designed random clinical trial.

Conclusion: Trans-catheter bronchial arterial Gendicine infusion combined with transcatheter arterial chemoembolization （TACE）, or/and Intratumor injection of Gendicine is a safe and effective therapy for treatment of pulmonary metastasis tumor from HCC.

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