Journal of Hainan Medical University

http://www.hnykdxxb.com

Effect of surgical resection combined with transcatheter arterial chemoembolization on postoperative serum tumor marker levels and stem cell characteristics during tumor recurrence

Sen Yang✉, Zhao Lai, Xue−Wen Wang, Jie Chen, Teng−Gang Deng

Department of Hepatobiliary Surgery, Zigong Fourth People's Hospital in Sichuan Province, Zigong 643000, China

ARTICLE INFO

Article history:
Received 14 Apr 2017
Received in revised form 18 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Liver cancer
Transcatheter arterial chemoembolization
Tumor stem cell
Proliferation

ABSTRACT

Objective: To study the effect of surgical resection combined with transcatheter arterial chemoembolization (TACE) on postoperative serum tumor marker levels and stem cell characteristics during tumor recurrence. **Methods:** A total of 98 patients with liver cancer who received radical resection in our hospital between May 2013 and July 2015 were reviewed and divided into TACE group and control group according to whether they received TACE within two months after surgical resection. Serum levels of tumor markers were detected 4 weeks after operation; the tumor recurrence was followed up within 3 years after operation, and the expression of stem cell marker molecules and cell proliferation molecules in recurrent lesions were detected. **Results:** 4 weeks after radical hepatectomy, serum AFP, AFP-L3, GP73 and GPC3 levels in TACE group were significantly lower than those in control group; Nanog, CD133, EpCAM, PICK1, CyclinD1, C-myc and Survivin expression in surgically removed lesions of TACE group were not different from those of control group while Nanog, CD133, EpCAM, PICK1, CyclinD1, C-myc and Survivin expression in recurrent lesions were significantly lower than those of control group. **Conclusion:** Surgical resection combined with TACE can more effectively remove liver cancer lesions, reduce the tumor marker levels and inhibit the tumor stem cell characteristics and cell proliferation activity in recurrent lesions.

1. Introduction

Liver cancer is one of the most common malignant tumors of digestive system, the liver tissue accepts two sets of blood supply from the portal vein and hepatic artery, so the liver cancer is with rich blood supply during the development, the illness progresses quickly, and both metastasis and recurrence rates are higher[1,2]. Surgical excision is still the main method for treating hepatocellular carcinoma, and the postoperative recurrence and metastasis are the main factors affecting the prognosis of patients. Transcatheter arterial chemoembolization (TACE) is important means of non-surgical treatment, which is mainly used for liver cancer that cannot be surgically removed, can form a high concentration of chemotherapy drugs within the lesions and kill cancer cells, and can also block the blood supply to the cancer cells and then control the disease development[3,4]. In recent years, some scholars have reported that surgical resection combined with TACE can reduce the long-term recurrence rate of liver cancer to a certain extent[5], but there is no report about the changes of combination therapy for the levels of molecules. In the following study, the effect of surgical resection combined with transcatheter arterial chemoembolization on postoperative serum tumor marker levels and stem cell characteristics during tumor recurrence was analyzed

2. Subjects and methods

2.1 Research subjects

A total of 98 patients with liver cancer who received radical resection in our hospital between May 2013 and July 2015 were selected as the research subjects, and all the patients were with preoperative liver function Child-Pugh A-B grade, diagnosed with hepatocellular carcinoma by postoperative pathology and with

✉Corresponding author: Sen Yang, Department of Hepatobiliary Surgery, Zigong Fourth People's Hospital in Sichuan Province, Zigong 643000, China.
Tel: 13778502576
Fund Project: Zigong Key Science and Technology Planning Self-financed Projects No: 2015ZC14.

complete disease and follow-up data; patients associated with distant metastases, lymph node metastases as well as portal vein tumor thrombus and postoperative tumor residues were eliminated. The patients' medical history data were reviewed, and then they were divided into TACE group and control group according to the use of TACE after radical excision. TACE group (n=42) received TACE within two months after surgical resection, including 29 male cases and 13 female cases that were 42-59 year old; control group (n=56) only received radical operation for liver cancer, including 38 male cases and 18 female cases that were 43-58 years old. There was no significant difference in general information between the two groups of patients (P>0.05).

2.2 Therapy

Both groups of patients received radical hepatectomy under general anesthesia, hepatic inflow was selectively blocked, the liver tissue ischemia line was observed, the corresponding parts of the liver tissue were removed, the incision was properly sutured, and the abdomen was closed after the abdominal cavity was flushed. TACE group received TACE within 2 months after surgery, and the method was as follows: Seldinger method was used for right femoral arterial puncture cathetering, angiography was conducted, the catheter was super-selectively inserted to proper hepatic artery, chemotherapy drugs pirarubicin 40 mg, cisplatin 30 mg and fluorouracil 0.75 g were mixed with ultra-fluid lipiodol 5 mL and injected into proper hepatic artery through catheter, and the puncture point was under pressure dressing for 24 h after the catheter was removed.

2.3 Postoperative serum tumor marker detection

4 weeks after radical hepatectomy, 5 mL peripheral venous blood was collected from the two groups and centrifuged to get serum, and enzyme-linked immunosorbent assay kits were used to determine the levels of alpha fetoprotein (AFP), alpha fetoprotein variant (AFP-L3), Glypican-3 (GPC3) and golgin protein-73 (GP73).

2.4 Molecule expression detection in postoperative recurrent lesions

Tumor recurrence was followed up for 3 years after operation, the basis of recurrence was biopsy and pathological examination, biopsy recurrent lesions were collected, added in the protein lysis buffer and fully homogenized, the homogenate was centrifuged in centrifuge for 20 min at a speed of 12 000 r/min to separate supernatant, and then enzyme-linked immunosorbent assay kits were used to detect Nanog, CD133, EpCAM, PICK1, CyclinD1, C-myc and Survivin levels.

2.5 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis between two groups was by t test and P<0.05 meant statistical significance in differences.

3. Results

3.1 Serum tumor marker levels

4 weeks after radical hepatectomy, analysis of serum tumor markers AFP, AFP-L3, GP73 and GPC3 levels between two groups of patients was as follows: serum AFP, AFP-L3, GP73 and GPC3 levels in TACE group were significantly lower than those in control group. Differences in serum AFP, AFP-L3, GP73 and GPC3 levels were statistically significant between two groups of patients 4 weeks after radical hepatectomy.

Table 1.

Serum tumor marker levels in two groups of patients after operation (ng/mL).

Groups	n	AFP	AFP-L3	GPC	GP73
TACE group	42	52.68±7.81	71.32±9.24	7.96±0.93	76.67±9.31
Control group	56	93.25±10.36	127.62±15.62	12.32±1.54	115.36±14.26
T		9.125	7.864	8.236	7.316
P		<0.05	<0.05	<0.05	<0.05

3.2 Stem cell marker molecule expression in surgically removed lesions and recurrent lesions

Analysis of stem cell marker molecules Nanog, CD133 and EpCAM expression in surgically removed lesions and recurrent lesions between two groups of patients was as follows: Nanog, CD133 and EpCAM expression in surgically removed lesions of TACE group were not different from those of control group while Nanog, CD133 and EpCAM expression in recurrent lesions were significantly lower than those of control group. Differences in Nanog, CD133 and EpCAM expression in surgically removed lesions were not statistically significant between two groups of patients (P>0.05) while differences in Nanog, CD133 and EpCAM expression in recurrent lesions were statistically significant between two groups of patients (P<0.05).

Table 2.

Stem cell marker molecule expression in surgically removed lesions of two groups of patients (ng/mL).

Groups	n	Nanog	CD133	EpCAM
TACE group	42	3.56±0.42	11.32±1.75	7.84±0.89
Control group	56	3.71±0.39	11.09±1.35	7.91±0.93
T		0.328	0.187	0.103
P		>0.05	>0.05	>0.05

Table 3.

Stem cell marker molecule expression in recurrent lesions of two groups of patients (ng/mL).

Groups	n	Nanog	CD133	EpCAM
TACE group	12	1.93±0.22	8.56±0.98	6.24±0.82
Control group	26	4.55±0.69	13.77±1.86	10.33±1.36
T		0.328	0.187	0.103
P		<0.05	<0.05	<0.05

3.3 Cell proliferation molecule expression in surgically removed lesions and recurrent lesions

Analysis of cell proliferation molecules PICK1, CyclinD1, C-myc and Survivin expression in surgically removed lesions and recurrent lesions between two groups of patients was as follows: PICK1, CyclinD1, C-myc and Survivin expression in surgically removed lesions of TACE group were not different from those of control group while PICK1, CyclinD1, C-myc and Survivin expression in recurrent lesions were significantly lower than those of control group. Differences in PICK1, CyclinD1, C-myc and Survivin expression in surgically removed lesions were not statistically significant between two groups of patients (P>0.05) while differences in PICK1, CyclinD1, C-myc and Survivin expression in recurrent lesions were statistically significant between two groups of patients (P<0.05).

Table 4.

Cell proliferation molecule expression in surgically removed lesions of two groups of patients (ng/mL).

Groups	*n*	PICK1	CyclinD1	C-myc	Survivin
TACE group	42	7.51±0.89	15.23±1.99	9.33±1.03	4.78±0.55
Control group	56	7.74±0.93	15.62±1.78	9.71±0.98	4.82±0.57
T		0.228	0.194	0.352	0.103
P		>0.05	>0.05	>0.05	>0.05

Table 5.

Cell proliferation molecule expression in recurrent lesions of two groups of patients (ng/mL).

Groups	*n*	PICK1	CyclinD1	C-myc	Survivin
TACE group	12	4.03±0.55	2.77±0.34	7.38±0.93	3.16±0.47
Control group	26	11.32±1.46	8.93±1.03	16.47±1.93	5.52±0.78
T		13.485	20.385	9.395	8.033
P		<0.05	<0.05	<0.05	<0.05

4. Discussion

Surgical resection is a major means for clinical treatment of liver cancer, but liver cancer is with rich blood supply and rapid growth, so there are some small lesions in liver tissue that are hard to be removed by surgery. In the development of the postoperative disease, the residual of the small lesions will become the pathological basis of tumor recurrence[6,7]. TACE is an important interventional therapy for hepatocellular carcinoma that cannot be surgically removed, and it has a direct killing effect on cancer cells[8]. In recent years, studies have shown that radical resection combined with postoperative TACE therapy has exact curative effect for liver cancer, and the results showed that combination of TACE can reduce the long-term recurrence of hepatocellular carcinoma[5]. The prominent value of surgical resection combined with TACE is to form a relatively high concentration of chemotherapeutic drugs in the liver tissue, also cause liver tissue ischemia hypoxia, thus implement killing effect on small hepatocellular carcinoma lesions that cannot be removed by operation, and reduce the long-term tumor recurrence caused by the residual of tiny lesions[9,10]. However, the change in gene expression levels is not clear during the postoperative recurrence of surgical resection combined with TACE.

In the development and change of liver cancer, tumor cells can synthesize and secrete a variety of proteins, peptides polysaccharides, which are secreted into the blood circulation and become the markers to reflect tumor load degree. After hepatectomy, the residual tiny lesions will continue to synthesize and release tumor markers, so postoperative serum levels of tumor markers can reflect whether there is residual tumor. AFP, AFP-L3, GP73 and GPC3 are the common liver cancer markers. AFP is the first tumor marker to be used in the screening of liver cancer, and the isomer AFP-L3 has a strong specificity for the diagnosis of liver cancer[11]; GPC3 is connected to the cell membrane structure through C-terminal glycophosphatidyl inositol, GP73 locates in the cis-face of golgi apparatus, and the GPC3 and GP73 will be secreted into the blood circulation in the process of tumor development[12,13]. In order to define the residual of tiny tumor lesions after radical hepatectomy, the postoperative serum levels of tumor markers were analyzed in the study, and the results showed that serum AFP, AFP-L3, GP73 and GPC3 levels in TACE group were significantly lower than those in control group. This suggests that surgical resection combined with TACE can be more effective in removing liver cancer lesions, reducing the residual of small lesions, and reducing the levels of tumor markers.

Postoperative residual of tiny liver cancer lesions is the pathological basis to cause postoperative recurrence of the tumor, studies have confirmed that surgical resection combined with TACE treatment can more effectively prevent tumor recurrence, but the molecule expression change in the process of recurrence is not clear. The tumor stem cells in the remaining small lesions are a type of cells with self-renewal and pluripotency, which play a critical role in the recurrence of the tumor. Nanog, CD133, and EpCAM are the markers of tumor stem cells in liver cancer tissue, and their expression can reflect stem cell characteristics. Nanog is a transcription factor that maintains stem cell pluripotency and self-renewal[14]; CD133 and EpCAM are the stem cell markers that are expressed in the surface of the cell membrane, which allow the tumor cells to acquire stem cell characteristics and enhance their ability to proliferate[15]. In the study, analysis of above stem cell molecule expression in surgically removed lesions and recurrent lesions showed that Nanog, CD133 and EpCAM expression in surgically removed lesions of TACE group were not different from those of control group while Nanog, CD133 and EpCAM expression in recurrent lesions were significantly lower than those of control group. This suggests that at the time of surgery, the tumor stem cell characteristics of the two groups of hepatocellular carcinoma are comparable; during the postoperative recurrence of liver cancer,

combined TACE therapy can inhibit tumor stem cell characteristics in recurrent lesions.

Tumor stem cells are characterized by self-renewal and pluripotency, and they can be differentiated into malignant tumor cells and continue to proliferate, leading to tumor recurrence. The proliferation of liver cancer cells is regulated by various proliferation molecules, and PICK1, CyclinD1, C-myc, Survivin, etc., have all been linked to the proliferation of liver cancer cells. PICK1 is a kind of protein kinase that regulates cell proliferation, which can adjust the CyclinD1 expression by PDZ domain, promote the cell cycle transition from G1 phase to S phase, and accelerate the process of cell cycle; PICK1 can also increases the expression of c-myc and increases cell proliferation activity[16]. Survivin is the most powerful anti-apoptotic molecule in cells, which can antagonize the pro-apoptotic effect of multiple caspase molecules and promote cell proliferation[17]. In the study, analysis of above cell proliferation molecule expression in surgically removed lesions and recurrent lesions showed that PICK1, CyclinD1, C-myc and Survivin expression in surgically removed lesions of TACE group were not different from those of control group while PICK1, CyclinD1, C-myc and Survivin expression in recurrent lesions were significantly lower than those of control group. This suggests that at the time of surgery, the cell proliferation characteristics of the two groups of hepatocellular carcinoma are comparable; during the postoperative recurrence of liver cancer, combined TACE therapy can inhibit cell proliferation in recurrent lesions.

To sum up, it is believed that surgical resection combined with TACE can more effectively remove liver cancer lesions, reduce tumor marker levels, and inhibit tumor stem cell characteristics and cell proliferation activity in recurrent lesions.

References

[1] Testino G, Leone S, Patussi V, Scafato E, Borro P. Hepatocellular carcinoma: diagnosis and proposal of treatment. *Minerva Med* 2016; **107**(6): 413-426.

[2] Gallicchio R, Nardelli A, Mainenti P, Nappi A, Capacchione D, Simeon V, et al. Therapeutic strategies in hcc: radiation modalities. *Biomed Res Int* 2016; **2016**: 1295329.

[3] Kim JY, Sinn DH, Gwak GY, Choi GS, Saleh AM, Joh JW, et al. Transarterial chemoembolization versus resection for intermediate-stage (BCLC B) hepatocellular carcinoma. *Clin Mol Hepatol* 2016; **22**(2): 250-258.

[4] Facciorusso A, Serviddio G, Muscatiello N. Transarterial radioembolization vs chemoembolization for hepatocarcinoma patients: A systematic review and meta-analysis. *World J Hepatol* 2016; **8**(18): 770-778.

[5] Liu Guang-hua, Xia Fang, Ouyang Qiang. The effect of transcatheter arterial chemoembolization on the prevention of recurrence in hepatocellular carcinoma following hepatectomy. *J Interv Radiol* 2015; **24**(2): 125-129.

[6] Ali MA, Li WF, Wang JH, Lin CC, Chen YJ, Lin TL, et al. Impact of pathological features of primary hepatocellular carcinoma on the outcomes of intrahepatic recurrence management: single center experience from Southern Taiwan. *HPB (Oxford)* 2016; **18**(10): 851-860.

[7] Panwar R, Pal S, Dash NR, Shalimar, Sahni P, Acharya SK, et al. Hepatic resection for predominantly large size hepatocellular carcinoma: Early and long-term results from a tertiary care center in India. *Indian J Gastroenterol* 2016; **35**(4): 280-286.

[8] Wang FY, Meng W, Li Y, Li T, Qin CY. Comparison of overall survival in patients with unresectable hepatic metastases with or without transarterial chemoembolization: a propensity score matching study. *Sci Rep* 2016; **13**(6): 35336.

[9] Zhang YF, Wei W, Wang JH, Xu L, Jian PE, Xiao CZ, et al. Transarterial chemoembolization combined with sorafenib for the treatment of hepatocellular carcinoma with hepatic vein tumor thrombus. *Onco Targets Ther* 2016; **12**(9): 4239-4246.

[10]Hatooka M, Kawaoka T, Aikata H, Morio K, Kobayashi T, Hiramatsu A, et al. Comparison of outcome of hepatic arterial infusion chemotherapy and sorafenib in patients with hepatocellular carcinoma refractory to transcatheter arterial chemoembolization. *Anticancer Res* 2016; **36**(7): 3523-3529.

[11]Ding Cai-zhi, Ren Li, Li Jian-feng, Zhang Chuan-yuan. Study on contents of serum tumor markers in hepatocellular carcinoma patients and its correlation with apoptosis molecular and immune molecules in epatocellular carcinoma tissue. *J Hainan Med Univ* 2015; **21**(5): 607-610.

[12]Pang Min-hong, Lu Zhi-hong. Diagnostic value of the combined detection of multiple tumor markers in the primary hepatic cancer. *J Hainan Med Univ* 2015; **21**(11): 1581-1583.

[13]Cao Fenghua, Wang Shuncai, Ma Jie, Zhu Mengqi, Ma Jiuming, Zhou Hua, et al. The diagnostic value of serum phosphatidylinositol proteinglycan 3 and Golgi protein 73 in hepatocellular carcinoma. *Acta Universitatis Medicinalis Nanjing (Natural Science)* 2015; **35**(3): 393-397.

[14]Chen CL, Uthaya Kumar DB, Punj V, Xu J, Sher L, Tahara SM, et al. NANOG metabolically reprograms tumor-initiating stem-like cells through tumorigenic changes in oxidative phosphorylation and fatty acid metabolism. *Cell Metab* 2016; **23**(1): 206-219.

[15]Cheung PF, Cheung TT, Yip CW, Ng LW, Fung SW, Lo CM, et al. Hepatic cancer stem cell marker granulin-epithelin precursor and β-catenin expression associate with recurrence in hepatocellular carcinoma. *Oncotarget* 2016; **7**(16): 21644-21657.

[16]Textor S, Bossler F, Henrich KO, Gartlgruber M, Pollmann J, Fiegler N, Arnold A, et al. The proto-oncogene Myc drives expression of the NK cell-activating NKp30 ligand B7-H6 in tumor cells. *Oncoimmunology* 2016; **5**(7): e1116674.

[17]Tang B, Liang X, Tang F, Zhang J, Zeng S, Jin S, et al. Expression of USP22 and Survivin is an indicator of malignant behavior in hepatocellular carcinoma. *Int J Oncol* 2015; **47**(6): 2208-2216.