RESEARCH ARTICLE

Association Between Insulin-like Growth Factor-2 Expression and Prognosis after Transcatheter Arterial Chemoembolization and Octreotide in Patients with Hepatocellar Carcinoma

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Abstract

Objective: To investigate the association between the change of IGF-2 level in serum after transcatheter arterial chemoembolization (TACE) and hepatocellular carcinoma (HCC) progression, especially in relation to metastasis. Methods: IGF-2 in serum was measured by quantitative sandwich enzyme-linked immunosorbent assaybefore, 3 days and 4 weeks after TACE in 60 patients with HCC. The occurrence of HCC metastasis was also evaluated, 3 months after TACE. Results: (1) The average serum level of IGF-2 in the 60 patients with HCC was 136.5 ± 87.3 pg/ml; (2) A tendency for increase was observed with heterogenous uptake of octreotide and portal vein thrombosis. Metastatic foci were found in 37/38 patients in the group with IGF-2 increasing (97.0%), in contrast to 3/22 (13.6%) patients with IGF-2 decrease. Conclusion: The increase of IGF-2 level in serum appears to be associated with the occurrence of metastatic HCC after TACE and chemotherapy.

Keywords: Octreotide - HCC - IGF-2 - TACE - metastasis - prognosis

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Introduction

In recent years, the incidence of primary liver cancer shows a rising trend, and about one million people die from liver cancer in an average year in the world. The liver cancer cases in China accounted for about 43.7% of the total number of the world with the male to female ratio about 3:1. Mortality rate in man is only after gastric cancer and in the second place of the malignant tumor. Also the morbidity has a regionalism which the incidence of Asian men (35.5/10,000) was significantly higher than the Nordic (2.6/10 million) and North America (4.1/10 million). Transcatheter arterial chemoembolization (TACE) is the preferred treatment method for unresectable hepatocellular carcinoma (HCC).

As a rich blood supply of solid tumors, HCC grows fast and is easy to form new blood vessels, establish collateral circulation, recurrence and distant metastasis. As a result, it is the largest difficulty in the treatment. Somatostatin can combine with somatostatin receptor (SSTR) expressed by tumor cells and then inhibit promoting tumor growth hormone or cytokine's production and adjust tumor blood supply (Hasskarl et al., 2011; Jiang et al., 2011; Zhang et al., 2011; Laznicek et al., 2012; Niu et al., 2012). Insulin-like growth factor-2 (IGF-2) is an important angiogenic factor during the process which HCC induced neovascularization (El Tayebi et al., 2011; Rehem et al., 2011; Wachter et al., 2012). It can strongly induce angiogenesis and stimulate recurrence and metastasis of HCC. At present we have not yet found the study of IGF-2 expression in serum and prognostic impact which brought by the treatment of octreotide peptide combined with TACE to liver cancer.

By the treatment of Primary Liver Cancer using somatostatin octapeptide analogue octreotide (El Tayebi et al., 2011; Ji et al., 2011) (OCT) combined with TACE, this study was designed to study the changes of IGF-2 expression in serum and the relationship between the level of IGF-2 in serum and recurrence and metastasis of HCC.

Materials and Methods

Conditions of selected patients

60 cases of liver cancer patients which were sequence diagnosed as IV III level from January 2010 to March 2011 and the classification standard is in accordance with the TNM method of international UICC 1997. Patients in the study signed the informed contents and were agreed by the Hospital Ethics Committee.

Diagnostic basis was referenced to the "Chinese criteria for diagnosis and treatment of common malignant tumors". The clinical statistics of patients see Table 1.

Methods

TACE: For the 60 liver cancer cases, we intubated the PiGu artery catheter to the hepatic artery to do angiography and then clearly further ultra-selectively inserted into Tumor-burdened artery using the Seldinger technique

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Table 1. 60 cases of HCC Patients Clinical Baseline

Clinical variable index	result
Age (mean ± standard deviation)	47.64±21.46
Sex (male/female)	Aug-52
epidemiology (HBV/HCV)	48/0
Child-Pugh classification (A/B)	34/26
Serum IGF-2 (pg/ml)	136.53±87.25
Tumor Type (sn/mn)a	14/46
Tumor size (cm)	10.71(2.8-20.3)
portal vein tumor thrombus	22
Hepatic artery fistula portal vein	18

a, sn, single nodules mn, multi-nodules and diffuse multiple

Table 2. The Preoperative Serum IGF-2 Baseline Expression and Clinical Relationship

Clinical index	Serum IGF-2 level (pg/ml) Mean ± standard deviation	T value	P value
Serum AFP(ng/ml)		0.4	P1=0.7
< 400 (cases 24)	180.2±70.6		
≥ 400 (36)	168.1±95.3		
Child-pugh classification		0.4	P2=0.7
A (34)	167.9±103.0		
B (26)	179.4±57.4		
Tumor size (cm)		0.9	P3=0.3
≤ 3 (4)	117.1±47.9		
> 3 (56)	176.8±86.4		
Tumor type		0.2	P4=0.8
Single nodules (14)	166.4±60.5		
multi-nodules (46)	174.8±92.4		
portal vein tumor thrombus		2. 5	P5=0.01
negative (38)	145.8±49.8		
positive (22)	221.1±112.1		

under DSA. Poured antitumor drugs via the catheter with the specific circumstances were as follows: 5-FU:1.00 g; NDP:60~100 mg; and lipiodol added EADM: 40 mg emulsion after embolization. The Lipiodol dosage depended on tumor size as well as general condition and the common was 10-30 ml.

Octreotide administration: Combined treatment TACE group were subcutaneous injected octreotide from preoperative 3 days (Sinopharm as one, 0.2 mg/support) with a dose of 0.2 mg 3 times a day, 1-5 days, interval of two days and four weeks as 1course of treatment.

Serum sample collection and IGF-2 measurement

Take 4ml morning fasting cubital vein blood sample of all patients and collect in a sterile tube. The blood samples were 3000 rpm centrifuged 15 minutes at 4 °C.Then separated the serum and store it at -20 °C. Serum IGF-2 expression was measured by enzyme-linked immunosorbent sandwich assay (ELISA). All tests were repeated once and averaged using imported kits with manufacturers guidance. Among measurement board, coefficient variation was less than 9.5%.Sensitivity:15 pg/ml.

Analysis of serum IGF-2 expression changes

We divided serumIGF-2 changes in amplitude expression in the preoperative and postoperative TACE three days and four weeks into two groups. Group1.

Table 3. Serum IGF-2 Expression Changes and the Clinical Relationship

Clinical index	IGF-2l decrease group1	IGF-2 level increase group 2(38)	P value
Serum AFP(ng/ml)			0.6
< 400 (24 cases)	10(42%)	14(58%)	
≥ 400 (36)	12(33%)	24(67%)	
Child-pugh classification			0.9
A (34)	12(35)	22(65)	
B (26)	10(39)	16(61)	
Tumor type			0.7
Single nodules (14)	6(43)	8(57)	
multi-nodules (46)	16(35)	30(65)	
portal vein tumor thrombus PVTT			0.1
negative (38)	10(26)	28(74)	
positive (22)	12(55)	10(45)	
Iodine oil distribution			0
equality 20	16(80)	4(20)	
asymmetry 40	6(5)	34(95)	
TACE reaction			0.5
local (38)	16(42)	22(58)	
No change (10)	4(40)	6(60)	
advanced (12)	2(17)	10(83)	

Postoperative Serum IGF-2 expression decreased. Group2. Postoperative IGF-2 expression increased. Clinical statistical index and IGF-2 changes were analyzed.

Statistical analysis

Get statistical package using SPSS 10.0 for windows, use T-test, chi-square test and analysis of variance for different data, and considered P value < 0.05 as statistically significant.

Results

The HCC patients serum IGF-2 expression baseline. 30 cases of HCC patients were randomly selected in the preoperative treatment of TACE combined with octreotide and 30 cases of non-tumor healthy volunteers were considered as control. The IGF-2 expression was detected. The former was 136.53 ± 87.25 pg/ml while the latter 22.82 ± 10.95 pg/ml (P= 0.001). SIGF-2 expression of liver cancer patients before TACE was significantly higher than that of healthy adults.

SIGF-2 expression and AFP, child stage, tumor size and type,

Portal vein tumor thrombus analysis of HCC patients preoperative TACE, group design the T-test results were shown in Table 2.

The serum IGF-2 baseline expression of positive preoperative portal vein embolization patients were significantly higher than that of negative patients, and the difference was statistically significant (p<0.05).

The IGF-2 expression changes in serum and clinical relationship analysis three days before and after the TACE. The IGF-2 expressions in serum of 22 cases of patients were decreased and 38 cases were increased among 60 cases of HCC patients. Only 20 percent of cases (4/20) expression increased in the iodine oil uniform distribution group after surgery while 85% in the uneven distribution of

Table 4. The Related Analysis of Intro-Extrahepatic Relapse and Metastasis Risk Factors After HCC 3 Months

Clinical index	Relapse and metastasis number	P value
Child-pugh classification		P2=0.178
A (34)	24	
B (26)	16	
Tumor size(cm)		P3=0.038
≤ 3 (4)	0	
> 3 (56)	40	
Tumor type		P4=0.001
Single nodules (14)	2	
multi-nodules (46)	38	
Serum level change		P8=0.010
IGF-2 decrease group (22)	3	
IGF-2 increase group (38)	37	

group (34/40). There was a significant difference between the iodine oil uniform distribution group and uneven distribution group after TACE ($p < 0.001$).

Serum IGF-2 change and the prognosis of patients with details after TACE

The table suggested that IGF-2 expression in serum, tumor type and tumor size are postoperative risk factors for metastasis and recurrence.

Discussion

Clinically, HCC has a high incidence and death rate. So it is more beneficial to explore more effective means of treatment for middle and advanced HCC, carry comprehensive treatment and achieve complementary advantages. Octreotide is widely used in the treatment of acute pancreatitis. In recent years, it was found that octreotide can also significantly inhibit the liver, stomach cancer and other digestive solid tumor growth in vitro and in vivo (Eli et al., 2011; Ji et al., 2011). Domestic and international studies (Barbare et al., 2009; Guo et al., 2009; Salvatore et al., 2010)showed that somatostatin and analogues affect intracellular signal transduction pathways or induce apoptosis of tumor cells and other ways to inhibits tumor cell growth mainly through somatostatin receptor. The combined application of octreotide and chemotherapy drugs has a synergistic inhibition of cell proliferation. It is applied to cancer comprehensive treatment drug because of its small side effects. Also researches (Prete et al., 2009) showed octretide combined with sorafenib has a significantly synergistic anticancer effect of liver cancer. Therefore in this research we chose octretide peptide joint TACE as a treatment for HCC.

The prognosis related to cancer recurrence and metastasis and liver function condition is a vital part of the clinical management of patients with liver cancer. Neovascularization is the critical step for the cancer cells forming local mass. Blood vessel formation is regulated by the expression balance adjustment of positive and negative regulator. Under the conditions of hypoxic microenvironment, cancer cells can produce HIF-1factor through the signaling to stimulate the angiogenesis. The positive factors include IGF-2, bFGF, pIGF, HIF, VEGF and so on (Wang et al., 2009; Xianget al., 2011). Negative regulatory factors include a variety of angiostatin.

Studies have (El Tayebi et al., 2011) discussed the importance of IGF-2 in HCC recurrence and prognosis, which has a high expression in liver cancer tissue. IGF-II was influenced and regulated by IGF binding protein-3, matrix metalloproteinases (MMP) and tissue inhibitor of metalloprotienase-1 (TIMP-1). The determination of IGF-2 is a new molecular marker of HCC diagnosis. IGF-2 is one of the most important promoting angiogenesis factors. It can be secreted by tumor cell and effect on the FLK-1/fl-1 receptors on vascular endothelial cells, leading to vascular endothelial cell proliferation, migration and germination and may also promote vascular permeability. Some research showed that IGF-I, IGF-II and IGFBP-3 expression in cirrhosis patients is lower than in normal man and this is may be due to hepatic insufficiency (Rehem et al., 2011) Expression of IGF-I and IGFBP-3 is higher in HCC patients than in normal and cirrhosis patients. But the expression of IGF-II and AFP is reverse, which illustrate that serum IGF-I, IGF-II and AFP Joint C-reactive protein is more effective for predicting liver dysfunction and severity comparing individual C-reactive protein. Serum IGF-II expression can be used as serological marker for identifying HCC from cirrhosis.

There is some research proceed the clinical and pathological analysis of insulin-like growth factor II mRNA-binding protein 3 (IMP3) expression. HCC nuclear needle biopsy experiment showed that IMP3 expressing application is limited, while joint with other markers can be helpful (Wachter et al., 2012). Qian J and other research suggests liver IGF-II gene which expressed in HCC 100%; cancerous peripheral tissue 54.3% and without expression in normal tissue is located in cancerous tissue (Qian et al., 2010). Also there is differential expression in the peripheral blood, 61.6 percent of HCC patients are high-expressed and 100% in extrahepatic metastasis.

Blood line planting is the most common way of intrahepatic and extrahepatic recurrence and metastasis of HCC patient tumor cell. In this study, recurrence and metastasis were more common in the large mass, multiple nodules, high serum IGF-2 expression and postoperative lipiodol uneven distributed patients. Since tumor cells was not completely deactivation after TACE, pathology conform that tumor cells don't fully necrosis and still a part of the cancer cells were still alive. As a result, TACE combined with other local ablation therapy can reduce the risk of recurrence (Finn et al., 2012). Diffused and multiple nodular liver cancer got poor effect of TACE and increase the trend of recurrence and metastasis after TACE. In this study, the increased expression of IGF-2 was related with the intrahepatic and extrahepatic recurrence and metastasis after TACE. Considering cancer cells' local hypoxia after embolization which strongly stimulate and induct IGF-2 and make serum IGF-2 expression increased, many recent studies (Bao et al., 2012; Facciorusso et al., 2012) focus on the anti-angiogenic therapy and multi-kinase molecular targeted therapies. IGF-2 can increase the infiltration capacity of the blood vessels. Matrix of tumor blood vessels is not complete, endothelial cell gap wide and tumor cell is easy escape into the blood circulation,

which all result in the transfer. Therefore, non-thorough palliative embolization for hepaticarteriovenous fistula, massive type, tumor diffused middle and advanced HCC seems to be of limited importance and it may enhance the occurrence of liver cancer recurrence and metastasis. So TACE should be accurate complete embolization as possible. Data of this study showed that serum IGF-2 expression is often increased in the large mass and iodized oil uneven distributed tumor after TACE. We concluded that serum IGF-2 was from the remnants of live cancer cells because of local anoxia and ischemia inducing after TACE. Local anoxia and ischemia induced IGF-2 increase may affect the recurrence and metastasis of liver cancer by autocrine, paracrine or endocrine manner. Current research showed when perform TACE in the mass and high serum AFP expression, IGF-2 as well as serum IGF-2expression would increase and the remaining cancer cells are often potential highly and metastatic relapse and metastasis which related with HCC recurrence and metastasis.

References

Bao Y, Feng WM, Tang CW, et al (2012). Endostatin Inhibits angiogenesis in hepatocellular carcinoma after transarterial chemoembolization. *Hepatogastroenterology*, **28**, 59.

Barbare JC, Bouché O, Bonnetain F, et al (2009). Treatment of advanced hepatocellular carcinoma with long-acting octreotide: a phase III multicentre, randomised, double blind placebo-controlled study. *Eur J Cancer*, **45**, 1788-97.

El Tayebi HM, Salah W, El Sayed IH, et al (2011). Expression of insulin-like growth factor-II, matrix metalloproteinases, and their tissue inhibitors as predictive markers in the peripheral blood of HCC patients. *Biomarkers*, **16**, 346-54.

Facciorusso A, Nacchiero MC, Rosania R, et al (2012). Pathways and gene expression profiles in hepatocellular carcinoma. *Minerva Gastroenterol Dietol*, **58**, 35- 48.

Finn RS (2012). Advanced HCC: emerging molecular therapies. *Minerva Gastroenterol Dietol*, **58**, 25-34.

Guo TK, Hao XY, Ma B, et al (2009). Octreotide for advanced hepatocellular carcinoma: a meta-analysis of randomized controlled trials. *J Cancer Res Clin Oncol*, **135**, 1685-92.

Hasskarl J, Kaufmann M, Schmid HA (2011). Somatostatin receptors in non-neuroendocrine malignancies: the potential role of somatostatin analogs in solid tumors. *Future Oncol*, **7**, 895-913.

Jiang J, Deng L, He L, et al (2011). Expression, purification, refolding, and characterization of octreotide-interleukin-2: a chimeric tumor-targeting protein. *Int J Mol Med*, **28**, 549-56.

Ji XQ, Ruan XJ, Chen H, et al (2011). Somatostatin analogues in advanced hepatocellular carcinoma: an updated systematic review and meta-analysis of randomized controlled trials. *Med Sci Monit*, **17**, 169-76.

Laznicek M, Laznickova A, Maecke HR (2012). Receptor affinity and preclinical biodistribution of radiolabeled somatostatin analogs. *Anticancer Res*, **32**, 761-6.

Niu J, Su Z, Xiao Y, et al (2012).Octreotide-modified and pH-triggering polymeric micelles loaded with doxorubicin for tumor targeting delivery. *Eur J Pharm Sci*, **45**, 216-26.

Prete SD, Montella L, Caraglia M, et al (2010). Sorafenib plus octreotide is an effective and safe treatment in advanced hepatocellular carcinoma: multicenter phase II study. *Cancer Chemother Pharmacol*, **66**, 837-44.

Qian J, Yao D, Dong Z, et al (2010). Characteristics of hepatic igf-ii expression and monitored levels of circulating igf-ii mRNA in metastasis of hepatocellular carcinoma. *Am J Clin Pathol*, **134**, 799-806.

Rehem RN, El-Shikh WM (2011). Serum IGF-1, IGF-2 and IGFBP-3 as parameters in the assessment of liver dysfunction in patients with hepatic cirrhosis and in the diagnosis of hepatocellular carcinoma. *Hepatogastroenterology*, **58**, 949-54.

Salvatore DP, Liliana M, Michele C, et al (2010). Sorafenib plus octreotide is an effective and safe treatment in advanced hepatocellular carcinoma: multicenter phase II So. LAR. study. *Cancer Chemother Pharmacol*, **66**, 837- 44.

Wachter DL, Kristiansen G, Soll C, et al (2012). Insulin-like growth factor II mRNA-binding protein 3 (IMP3) expression in hepatocellular carcinoma. A clinicopathological analysis with emphasis on diagnostic value. *Histopathology*, **60**, 278-86.

Wang Z, Zhou J, Fan J, et al (2009). Sirolimus inhibits the growth and metastatic progression of hepatocellular carcinoma Journal of Cancer Research and Clinical. *Oncology*, **135**, 715-22.

Xiang ZL, Zeng ZC, Fan J, et al (2011). Gene expression profiling of fixed tissues identified hypoxia-inducible factor-1α, VEGF, and matrix metalloproteinase-2 as biomarkers of lymph node metastasis in hepatocellular carcinoma. *Clin Cancer Res,* **17**, 5463-72.

Zhang Y, Zhang H, Wang X, et al (2012). The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. *Biomaterials*, **33**, 679-91.