RESEARCH ARTICLE

Asparagus Polysaccharide and Gum with Hepatic Artery Embolization Induces Tumor Growth and Inhibits Angiogenesis in an Orthotopic Hepatocellular Carcinoma Model

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Abstract

Liver cancer is one of leading digestive malignancies with high morbidity and mortality. There is an urgent need for the development of novel therapies for this deadly disease. It has been proven that asparagus polysaccharide, one of the most active derivates from the traditional medicine asparagus, possesses notable antitumor properties. However, little is known about the efficacy of asparagus polysaccharide as an adjuvant for liver cancer chemotherapy. Herein, we reported that asparagus polysaccharide and its embolic agent form, asparagus gum, significantly inhibited liver tumor growth with transcatheter arterial chemoembolization (TACE) therapy in an orthotopic hepatocellular carcinoma (HCC) tumor model, while significantly inhibiting angiogenesis and promoting tumor cell apoptosis. Moreover, asparagine gelatinous possessed immunomodulatory functions and showed little toxicity to the host. These results highlight the chemotherapeutic potential of asparagus polysaccharide and warrant a future focus on development as novel chemotherapeutic agent for liver cancer TACE therapy.

Keywords: Hepatocellular carcinoma (HCC) - asparagus gum - asparagus polysaccharide - TACE therapy

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Introduction

Liver cancer, or hepatocellular carcinoma (HCC), is one of the leading causes of cancer-related death worldwide. It has a long latency, and most patients are often diagnosed at late stages when tumors are of high grade and progress rapidly (Siegel et al., 2014). Based on the clinical performance, it was characterized as "Ganji, liver obstruct, fertilizer gas, ruffian, product gas, bloating, hypochondriac pain, jaundice" and other features in traditional Chinese medicine (TCM). To date, surgical approaches including liver resection and liver transplantation are currently regarded as potentially curative treatments for HCC (Graf et al., 2014; Ravaioli et al., 2014). However, nonsurgical approaches are necessary, since only a small minority of patients is suitable for surgical therapy due to numerous lesions, extrahepatic metastases as well as early vascular liver cancer. Transcatheter arterial chemoembolization (TACE) is a minimally invasive technique to treat liver tumors and is performed in interventional radiology to restrict a tumor's blood supply. Recently, TACE has been used extensively in the palliative treatment of unresectable HCC and been proved to result in regression of HCC and reduction of systemic toxicity (Buijs et al., 2008), thereby to improve the therapeutic effects. However, we should pay much attention to both the elimination of the tumor itself and the protection of the patient's liver function, reducing liver damage during the procedure.

An ideal embolic agent may need to own the following characteristics: excellent anti-cancer effect, low toxicity, improving liver function, and sound immunomodulatory function. These features are particularly important for patients carrying hepatitis, cirrhosis and liver cancer at the same times. Recently there is an increasing interest in developing novel anticancer agents from traditional Chinese medicine as which have better efficacy and safety profiles than conventional synthetic compounds. Interventional treatments of Chinese medicine for liver cancer also has draw people's great attention. Currently, the most focused-on anti-tumor embolization agents of researches are javanicaoi, Curcuma oil, Baiji particles, Baiji glue and so on, while all of which are with marginal therapeutic effect. Novel anti-tumor embolization agents are hence urgently needed for clinical cancer therapy.

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Asparagus is a traditional Chinese medicine used for the treatment of multiple types of tumors and chronic inflammation treatment and antioxidant(Chun et al., 2011; Thakur et al., 2012; Samad et al., 2014). Asparagus polysaccharide, an extract from Asparagus, is a polysaccharide protein which has been proved to be the main active ingredient of Asparagus in the respect of anti-cancer effects and immunity-enhancing activities. Many studies reported that the Asparagus polysaccharide can inhibit tumor cell proliferation and prolong survival of tumor-bearing animals (Yu Bin et al., 2010; Zhao et al., 2012; Xiang et al., 2014). Clinically, it has been used to treat many malignancies, such as breast cancer, malignant lymphoma, leukemia and lung cancer. Preliminary studies showed that the Asparagus gum, derivated from asparagine polysaccharide, is a very good embolic agent (Min-guang, 2003; xing, 2005). Asparagus gum has many advantages as follows: of certain viscosity, water-soluble and slow-released effect, no significant hemolytic reaction, and can be absorbed or biodegradable in a certain period of time (about two weeks) and can markedly induce tumor cell apoptosis in HCC treatment. However, few experimental or clinic reports have been reported on the combination of TACE with Asparagus polysaccharide.

In the present study, we systemically evaluated the toxicity and antitumor effect of Asparagus polysaccharide and Asparagus gel-like substance via intervention treatment on liver cancer using an orthotopic HCC tumor-bearing rat model, to elucidate its chemotherapeutic potential of being development as novel chemotherapeutic agents for future liver cancer TACE therapy.

Materials and Methods

Cell lines and reagents

Walker-256 cells were purchased from Institute of Oncology, Chinese Academy of Sciences and were cultured in RPMI 1640 medium, supplemented with 10% FBS and 1% penicillin/streptomycin. Mitomycin and iodized oil were obtained from Shanghai Xudong Haipu Pharmaceutical Co., Ltd (Shanghai, China). Asparagus polysaccharide was purchased from Xi'an Si Raadt company (Xi'an, China), Asparagus gum was our patent protected agent with a patent number (CN200410025494.5). Antibodies against Bcl-2 and Cleaved-caspase-3 were from Santa Cruz (Santa Cruz Biotechnology CA, USA), Antibodies against VEGF, CD34, and α-Tubulin were from Cell Signaling (Cell Signaling Technology, MA, USA).

Orthotopic HCC tumor model

Male wistar rats (50-100g for young; 180±30g for adult) were purchased from Hercynian Pool-Rubicam experimental animals Ltd (Shanghai, China). The animals were kept under conventional conditions at a temperature of 22±2°C with a relative humidity of 55±10% and a dark/light rhythm of 12h. All surgical procedures and care administered to the animal were approved by the institutional ethics committee of Shanghai University of Traditional Chinese Medicine.

For subcutaneous HCC tumor model establishment, Walker-256 carcinoma cells were firstly injected into the backs of rats to obtain subcutaneous xenograft tumors (tumor size of each rat was measured by a caliper, and calculated by the formula: $L \times W^2/2$, where 'W' was the shortest dimension and 'L' was the longest dimension in centimeters). For orthotopic HCC tumor model, the Walker-256 xenograft tumors tissue, obtained from the subcutaneous tumor-bearing rats 2 weeks after implantation, was cut into small cubes of about 1-2 mm3. A small subscapular incision on the left lateral lobe of the liver was made in the recipient Wistar rats under anesthesia. The implanted site was gently compressed for 15-20 s with a small cotton swab on the liver surface, and the abdominal wall was then closed.

For intervention therapies, by using abinocular operative microscope, the catheter was inserted retrogradely into the gastroduodenal artery and pushed forward to the common hepatic artery under laparotomy. Non-superselective embolization was possible due to small hepatic artery branches. To ensure that the main agent flow was directed into the left hepatic artery, the right hepatic artery was always compressed manually. The placement of the line under the hepatic artery during the injection of the agents could be regarded as central embolization and prevented a reflux of the agents to the common hepatic artery and the coeliac trunk. In total, 160 rats were randomly assigned to eight groups (see Table 1), each comprising 20 animas. All animals underwent transarterial chemoembolization (TACE) with different applying agents (Table 1).

Drug preparation

Asparagus polysaccharide was made as reported (Xiang et al., 2014). Asparagus polysaccharide was dissolved in iodine fluoride alcohol as a solvent to prepare the medicine asparagus gum. In the group treated with low dose of asparagus gum, the drug contained 2000 mg asparagus polysaccharide, per 100 ml of the iodine fluoride alcohol. In the group with high-dose asparagus gum, the drug contained 4000 mg of asparagus polysaccharide. They were mixed, wrapped with aluminum foil and, swelled temperature for 24-48 hours. Finally, the agents were packed in glass bottles with high pressure steam sterilization, and then stored under the condition of 4 degrees.

Life span and survival rate

The life span and survival rate of experiment animal was defined as follows: Life span was calculated from the next day after the interventional treatments were administrated to the date when death appeared (ten animals in each group).

The survival rate was calculated basing on the following formula: Survival rate = (the treatment group average survival time - the control group average survival time) / the control group average survival time × 100%.

Hematoxylin-eosin (HE) staining and immunohistochemical (IHC) assay

For HE staining, all HCC specimens were fixed in 10% formalin for 24 hours and then embedded in paraffin and

sectioned into 4-mm slices. The histological sections were stained with HE and observed under a light microscope.

For immunohistochemical assay, unstained 4.0 μm sections of clinical specimens were deparaffinized with xylene and rehydrated with ethanol, followed by staining with primary antibodies against CD34 (diluted at 1:100) and VEGF (diluted at 1:100). Then secondary antibody was incubated for 15 min. The immunohistochemical staining was analyzed using an Olympus microscope.

Positive VEGF expression in the cytoplasm appeared pale yellow, brown or brownish yellow granules. Subsequently, five staining fields from each section were analyzed by using an image analyzer to read the positive area and the OD value under high magnification (200×). Microvessel density (MVD) was counted as described by Weidner et al (1991). Briefly, all dyed brown individual endothelial cells or endothelial cell clusters separated from adjacent blood vessels, tumor cells and stoma phase were counted. The capillaries were firstly observed under low magnification (100×) to watch the entire film, and then five non-repetition horizons vision were selected to count microvessels under high magnification (200×), seeking its average value of MVD.

Western blotting

Western Blotting experiments were carried out as reported(Chen et al., 2011). In Brief, HCC tumor tissues were homogenized in protein lysate buffer, and the debris was removed by centrifugation. Aliquots containing identical amounts of protein were resolved on 10% polyacrylamide-SDS gels, and electrophoretically transferred to the methanol pre-activated polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% BSA, incubated with primary Antibodies, and subsequently with an alkaline phosphatase-conjugated secondary Antibody.

Table 1. The Different Groups of Treatment in the Intervention Therapies in the HCC Orthotopic Rat Model (n=20)

Groups	Drugs and Dosage	Volume (mL)
NS	0.9% normal saline	0.1
L-Asp	20mg/ml Asparagus polysaccharide	0.1
H-Asp	40mg/ml Asparagus polysaccharide	0.1
L-Asg	20mg/ml aspartame gum	0.1
H-Asg	40mg/ml aspartame gum	0.1
L-Mito	0.2mg/kg Mitomycin	0.1
H-Mito	0.4mg/kg Mitomycin	0.1
Iodized oil	Iodine 0.296~0.328g/ml	0.05

Statistical analysis

All the data were present as mean values ± standard deviation (SD). Comparisons among multiple groups were made with a one-way analysis of variance (ANOVA), and the differences between two groups were analyzed by a Least-Significant Difference (LSD) test. All statistical analyses were performed by using SPSS 18.0 with "$p<0.05$" as statistical significance.

Results

Asparagus polysaccharide or Asparagus gum intervention therapy significantly inhibites HCC tumor growth and prolonged the survival time

After orthotopic liver tumor modeling, the rats became apathetic with a tendency of laziness, dislike of moving and anorexia, and the fur also turned boring, and lack luster two to three days later. At the fourth day, the mental condition gradually improved, but the weight of rat continued to decline and the subcutaneous fat of the rats turned thinner. The rats were respectively given the intervention therapies with normal saline (NS), Iodized oil, lower or higher dose of Asparagus polysaccharide (L-Asp or H-Asp), lower or higher dose of Asparagus gum (L-Asg or H-Asg), lower or higher dose of mitomycin (L-Mito or H-Mito) on the eighth day.

The body weights of rats in each group were compared during therapy. As shown in Figure 1A, although the body weights of rats in all groups were significantly decreased, as observed in different four time ($p<0.01$), they did not exhibit significant difference between the control group and the treated groups($p>0.05$). These results indicated that loss of body weight is due to the impact of liver cancer progression but not the toxicity of drugs.

The tumor volume of rats was determined 7 days after hepatic artery intervention treatment. As shown in Figure 1B, by comparison with control group, the tumor growth was more significantly inhibited in a dose dependent manner in rats treated with Asparagus polysaccharide or Asparagus gum. ($p<0.001$). Similar inhibitory effects were also found in rats that treated with Mitomycin or Iodized oil ($p<0.001$). We also evaluated the effect of different

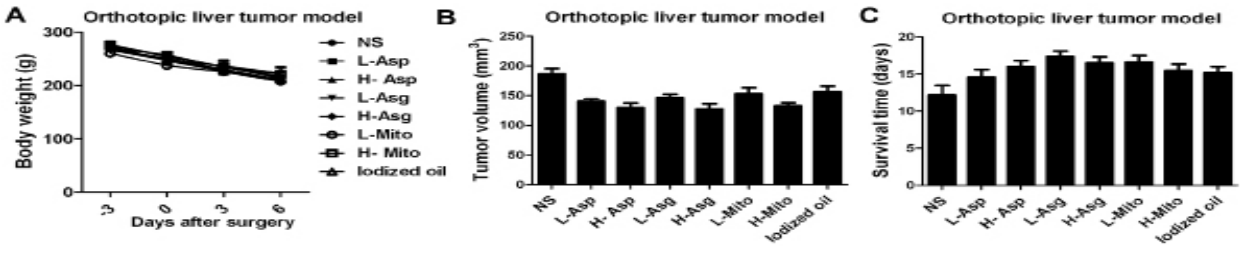

Figure 1. The Body Weight, Tumor Volume and Survival Time of Orthotopic Hepatocarcinoma-bearing Rats After Different Intervention Therapies. A) Changes of the body weight of rats during the different treatments. The body weight of rats was determined respectively at four different time-points before or after the orthotopic liver tumor modeling. **B)** The tumor volume of the tumor-bearing rats (mm3) was determined by vernier caliper 7 days after hepatic artery intervention treatments (n=10). **C)** The survival time of rats in different groups was recorded

TACE therapies on rat overall survival. Compared with the control group, the survival time of rats was significantly prolonged for at least one week in groups with H-Asp, L-Asg, or H-Asg intervention treatment (Figure 1C, P<0.0001 for all three). These data indicate that Asparagus polysaccharide and Asparagus gum significantly inhibited orthotopic liver tumor growth and prolonged the survival time of tumor-bearing rats.

Table 2. The Changes of General Blood Cell Contents in Rats 8 Days After TACE Therapies

Groups	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	HB (g/L)	PLT ($\times10^9$/L)
NS	17.5±0.6*	8.9±1.1	153.6±6.3	576.2±62#
H- Asp	7.3±3.4▲*	8.7±0.7	170.4±14.7	462.2±197.8#
L-Asg	7.0±3.2▲*	7.0±0.7	136.0±13.3	1106.8±138.1
L-Mito	2.2±0.5▲	7.7±0.9	151.2±10.8	552.4±111.5#
H- Mito	2.6±0.7▲	7.7±1.0	150.0±19.5	536.6±138.8#
Iodized oil	12.6±2.2▲*	9.9±1.0	178.8±12.2	448.6±53.5#

WBC, white blood cells; RBC, red blood cells; hb, hemoglobin; PLT, platelet. ▲compared to NS, $p<0.05$; *compared to mitomycin, $p<0.05$; #compared toL-ASG, $p<0.05$.

Asparagus polysaccharide or Asparagus gum induces Embolization and tumor tissue necrosis

After the intervention therapy, the rats were sacrificed and the localized pathologic changes of the liver tissue were observed by HE staining. As shown in Figure 2, hepatocytes with marked pleomorphism were observed in all groups, with cloudy swelling, cytoplasm osteoporosis or water degeneration in some regions of the liver cells, indicating the successful establishment of the orthotopic tumor model (Figure 2A-H). It also showed that conspicuous focal necrosis was appeared in tumors after the intervention therapies with all drugs (B-H) compared to the control (A).

Asparagus polysaccharide treated tumors (B-C) has the least necrosis which may be induced by apoptosis(Xiang et al., 2014). While Asparagus gum induced the most necrosis which may be due to the embolization and apoptosis (D-E). Hypoxia of embolization in Iodized oil (H) treated group induced more necrosis than the mitomycin treated groups (F-G). Moreover, lung metastasis more seriously observed in the control group than the treated groups under anatomy (data not shown), whereas other organs had no observable pathologic changes.

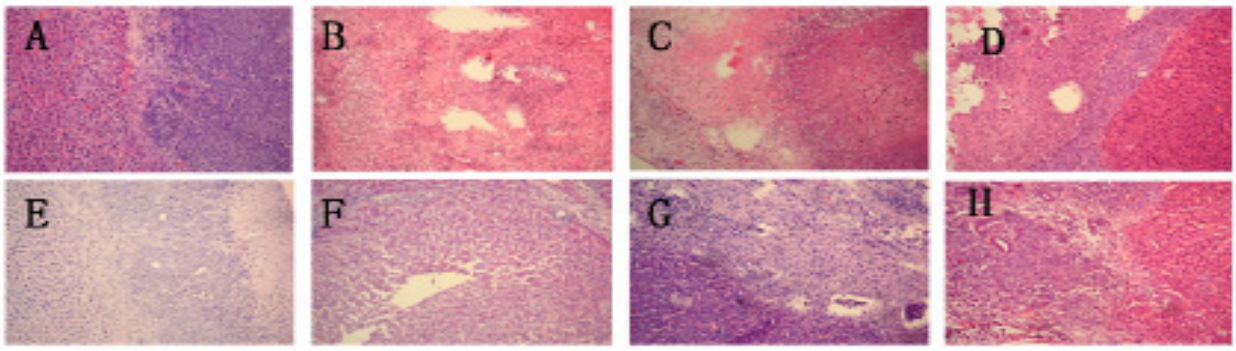

Figure 2. Localized Pathologic Changes After Hepatocarcinoma Intervention Therapies. The HE staining and histopathologic feature of liver tissue in rats respectively treated with saline **(A)**, L-Asp **(B)**, H- Asp **(C)**, L-Asg **(D)**, H-Asg **(E)**, L-Mito **(F)**, H- Mito **(G)**, Iodized oil **(H)**. All images are 40× magnified

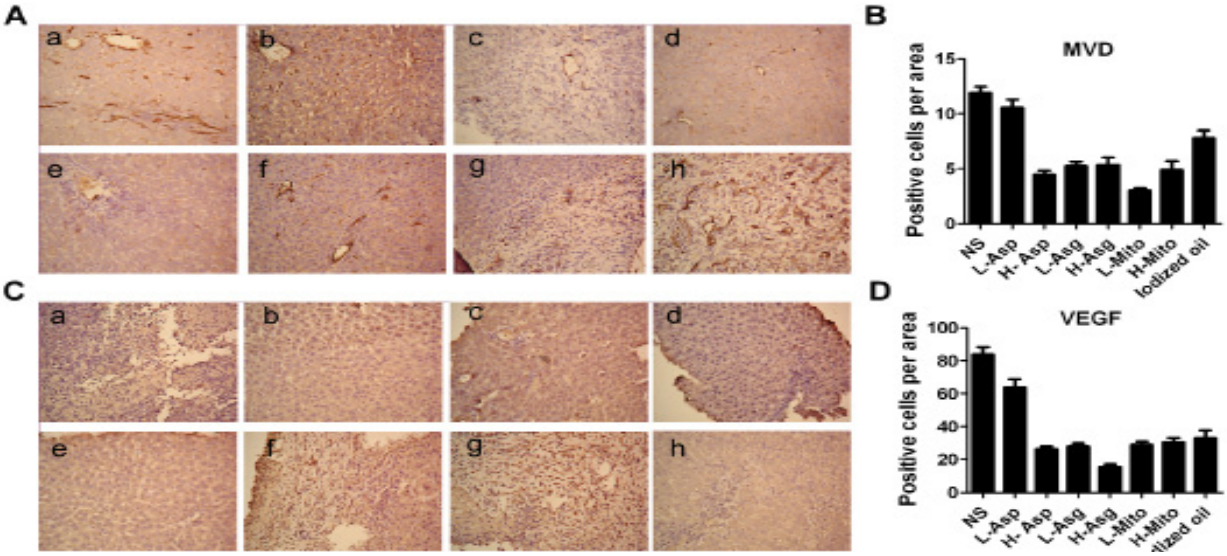

Figure 3. The Immunostaining of MVD (CD34) and VEGF in Tumors with Different Treatments. A) The immunostaining of MVD in liver tumors respectively treated with saline (a), L-Asp (b), H- Asp (c), L-Asg (d), H-Asg (e), L-Mito (f), H-Mito (g), Iodized oil (h). **B)**The quantification of positive MVD staining cell in each group. C The immunostaining of VEGF in liver tumors respectively treated with saline (a), L-Asp (b), H- Asp (c), L-Asg (d), H-Asg (e), L-Mito (f), H- Mito (g), Iodized oil (h). D. The quantification of positive VEGF staining cell in each group

Table 3. The Changes of General Blood Biochemical Index in Rats 8 Days After TACE Therapies

Groups	TP(g/L)	sALB(g/L)	A/G	ALT(U/L)	BUN(U/L)
NS	47.4±4.0	17.3±2.1	0.57±0.06	25.4±18.8*	13.0±8.4
Iodized oil	46.0±6.4	15.0±2.5	0.48±0.03	450.8±244.1*	11.1±3.6▲
L- Asp	49.2±10.4	16.2±3.0	0.49±0.02	108.2±64.2	7.8±2.7
H- Asp	44.20±7.0	14.2±2.2	0.47±0.01	126.0±23.8*	7.8±1.5
L-Asg	40.8±16.9	14.6±6.5	0.55±0.08	110.4±43.4*	5.2±1.7
H-Asg	48.4±5.4	16.4±3.5	0.51±0.09	112.2±60.5*	8.0±0.9
L- Mito	47.0±6.9	17.9±3.7	0.62±0.13	120.1±41.7*	12.1±5.6▲
H- Mito	44.4±8.6	15.7±3.5	0.55±0.06	77.6±26.2*	8.7±0.9

*Compared to Iodized oil group, $p<0.05$; ▲Compare to L-Asg, $p<0.05$

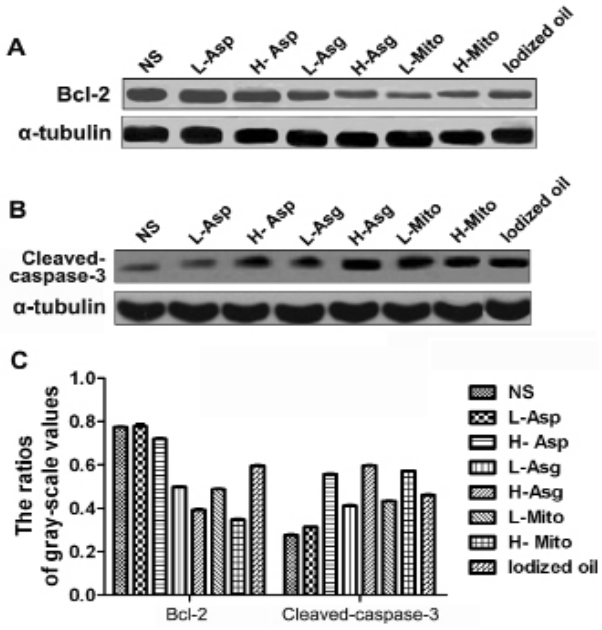

Figure 4. Expression of Bcl-2 and Caspase-3 Activation in Tumor Tissues After Different Treatments.
Western blotting showed the expression of Bcl-2 (A) and Caspase-3 cleavage (B) in tumor with different TACE therapies (α-tubulin was chosen as control): saline (NS), L-Asp, H- Asp, L-Asg, H-Asg, L-Mito, H- Mito, Iodized oil. C. Quantification of Bcl-2 and Cleaved-caspase-3 was determined by gray scale of each band

Asparagus polysaccharide or Asparagus gum is of less toxicity and has immuno-protecting effects.

Leukocytopenia, anemia, thrombocytopenia, and prolonged activated partial thromboplastin time are the most common hematologic toxic effects in clinical responses. One of the major problems in administration of anticancer drugs is a decrease of peripheral blood leucocytes, which leads to the reduction of the ability to defense against various infections in patients. To evaluate the potential hematologic toxic effects after the TACE with asparagus polysaccharide and asparagus gum, the hematologic values including white blood cells (WBC), red blood cells (RBC), hemoglobin (HB), and platelet (PLT) were determined after TACE therapies. Mitomycin, the clinical used anticancer drug, was used as a positive control. As presented in Table 2, the number of leucocytes (WBC) in rats with high (H-Mito) or low (L-Mito) dose of mitomycin treatment was significantly decreased, compared with control group or other groups ($p<0.05$). These results were resulted from the known clinical side effect of mitomycin on hematopoietic cells in terms of leukocytopenia and thrombocytopenia. As comparison, a significant recovery of leucocytes was observed in rat receiving asparagus polysaccharide ($p<0.05$), indicating its immuno-protecting effects. We observed a marked increase of platelet in rats with L-Asg treatment ($p<0.001$), but not in other groups. We did not find obvious changes of red blood cells and hemoglobin among the groups.

Moreover, the blood biochemical test was also evaluated as shown in Table 3, including total protein (TP), soluble albumin (sALB), the ratio of albumin and globulin (A/G), aspartate aminotransferase (ALT) and urea nitrogen (BUN). Administration dugs on rats had no marked influenced on the TP, sALB and A/G in each group. Compared with the saline group, ALT was greatly increased in other groups among which rats treated with Iodized oil had the highest level of ALT ($p<0.05$). The values of BUN in iodized oil and H-Mito groups were significantly higher than other groups. Generally, we observed a lower level of ALT and BUN in rats treated with asparagus polysaccharide or asparagus gum than that treated with Iodized oil and mitomycin. These observations indicated that deproteinized asparagus polysaccharide may decrease toxicity in the hepatocellular carcinoma.

Asparagus polysaccharide or Asparagus gum significantly reduces CD34 and VEGF expressions.

Angiogenesis plays an important role of in liver cancer progression, we continued to analyze some angiogenesis markers such as CD34 and VEGF on the tumor tissues after therapies by IHC staining. As presents in Figure 3A and B, the expression of MVD markers was apparently reduced in asparagus polysaccharide or mitomycin treated liver tumors, but not in that with Iodized oil treated. Among them, tumors with H-Asg treatment showed the lowest level of CD34 expression (Figure 3, A and B). Similarly, we also found a lower expression of VEGF in asparagus polysaccharide or mitomycin treated liver tumors (Figure 3, C and D). These results suggested that TACE with asparagus polysaccharide or asparagus gum might inhibit the tumor angiogenesis in vivo.

Asparagus polysaccharide or Asparagus gum induces cell apoptosis in HCC tumor tissue

We also investigated the expression of cell apoptosis related proteins Bcl-2 and Caspase-3 activation in HCC tumor tissues by Western blot analysis (Figure 4). As

shown, Bcl-2 was significantly decreased in tumors treated with asparagus polysaccharide, asparagus gum or mitomycin in a dose dependent manner in comparison to that NS group and Iodized oil group (Figure 4, A and C), whereas Caspase-3 was activated with accordingly significantly increased Cleaved-caspase-3 (Figure 4 B and C). These results suggested that asparagus polysaccharide or asparagus gum could result in tumor cell apoptosis in vivo, which may partly explain the mechanism of their anti-liver tumor mechanism.

Discussion

The most important factors in choosing optical stratery for TACE are to obtain favorable therapeutic effects and to reduce adverse side-effects (Xu et al., 2014). With regard to the embolic agent used in TACE for HCC, several studies have analyzed the efficacy of lipiodol (Idee and Guiu, 2013; Peng et al., 2014). Howerve, the adverse of TACE using lipiodol for HCC can not be ignored, due to the potential impairment of hepatic function among patients with poor liver function or a huge HCC that would require a large volume of lipiodol. Asparagus polysaccharide is deproteinized extract from Asparagus which has been reported to have immunomodulatory function and anti-tumor effect. In this study, the antitumor effect and potent toxic effect of Asparagus polysaccharide were evaluated after hepatic arterial chemoembolization on rat. Our experiments showed that Asparagus polysaccharides and its embolizing form Asparagus gum could significantly inhibit liver tumor growth and prolonged survival time of tumor-bearing rats in vivo, with less toxic effect on rats in terms of multiple blood routine and blood biochemical markers.

Asparagus polysaccharide has been used in the clinical treatment of breast cancer, malignant lymphoma, lung cancer, leukemia, etc. Many evidence suggest that the anti-tumor mechanism of Asparagus polysaccharides may be associated with promoting apoptosis (Xiang et al., 2014). Apoptosis refers to the activation of cell suicide mechanism is triggered as a kind of inherent pyknotic cells as the main morphological changes of programmed cell death. Apoptosis can be broadly divided into the following stages: receiving apoptotic signal, intermolecular interaction of apoptosis, caspase enzyme activation, triggeri cascading waterfall effect and causing the DNA fragmentation, macromolecular synthesis, eventually leading to cell death(Boatright and Salvesen, 2003; Deng et al., 2011). caspase-3 is one of key initiator caspases to cleave and activate effector caspases, resulting in the cleavage of a variety of cellular protein substrates and ultimately leading to apoptosis (Kuida et al., 1996; Kumar, 2007). Bcl-2, a famous apoptosis inhibitory genes, inhibits various factors on the induction of apoptosis, and thus prolongs the cell survival and inhibit cell apoptosis (Czabotar et al., 2013; Volkmann et al., 2014). Our study showed that the cleavage (or activation) of Caspase-3 was apparently increased and Bcl-2 were significantly decreased in group Asp and Asg as compared with those in groups NS and group Iodized oil, suggesting that the Asparagus polysaccharides can induce tumor cell apoptosis by reducing the Bcl-2 expressions and enhancing the Caspase-3 expression.

Transcatheter arterial chemoembolization (TACE) is an accepted treatment option for unresectable hepatocellular carcinoma (HCC) (Peng et al., 2014; Xu et al., 2014), of which equential TACE could an effective method to improve resection opportunity, expand the scope of surgical resection, as well as to greatly reduce postoperative intra- and extrahepatic metastasis (Xu et al., 2014). However, the long-term effectiveness of TACE remains limited. There are many obstacles that may deter TACE from eradicating the tumor (Rou et al., 2014). It has been reported that the tumor produces a series of changs (such as angiogenesis, apoptosis, etc.) in order to adapt to a hypoxic environment after embolization. Ischemic necrosis has been proved to be associated with angiogenic activities after TACE (Sergio et al., 2008; Wang et al., 2008). Hypoxia tension is a key factor in the gene expression of angiogenic factors, such as vascular endothelial growth factor (VEGF) [21]. CD34 and VEGF are the most important quantitative indicators of tumor blood vessels for microvascular density (MVD) (Weidner et al., 1991; Horak et al., 1992). Abnormal increased expression of CD34 and VEGF has been correlated with tumor growth and metastasis and the patient's prognosis(Poon et al., 2004; Shim et al., 2008). In our research, VEGF and CD34 expressions were significantly decreased when treated with Asparagus polysaccharides and its embolizing form Asparagus gum, suggesting that Asparagus polysaccharide or Asparagus gum can treat HCC by blocking the VEGF expression and tumor angiogenesis.

In summury, we showed that aspartame polysaccharide and aspartame polysaccharide gel-like material had a certain anti-cancer effect in a TACE therapy through blocking tumor angiogenesis and promoting cell apoptosis. More importantly, it had lower toxic effects and reduced liver and kidney functional damage. These results highlighted the potent of aspartame polysaccharides and aspartame gum as the novel emboliaztion agents for clinical liver cancer TACE therapies. As combined chemotherapy drug with TACE (lobaplatin) can obtain significant clinical efficacy, and TACE combined with 125I seed implantation can better control the lesions and protect liver function for advanced HCC (Peng et al., 2014), it is worthy to expect the effective combinational usage of aspartame polysaccharides or aspartame gum for HCC TACE therapy.

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