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Prognostic value of serum soluble Fas in patients with locally advanced unresectable rectal cancer receiving concurrent chemoradiotherapy

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Abstract: Objective: This study was designed to detect the changes of serum soluble Fas (sFas) levels in patients with locally advanced unresectable rectal cancer (LAURC), and to explore its prognostic value of response. Methods: Soluble samples were obtained from LAURC subjects, treated by concurrent chemoradiotherapy, before treatment and one month after treatment. Healthy donor serum samples were used as controls. sFas concentration was measured by enzyme-linked immunosorbent assay (ELISA). Results: The sFas levels before treatment and one month after treatment were both significantly higher in LAURC subjects than in healthy controls [(8.79±1.39) and (7.74±1.32) vs. (5.53±1.13) ng/L, P<0.01]. The sFas levels before treatment and one month after treatment were significantly lower in the response group (complete and partial responses) than in the non-response group (stable and progressive diseases) [(8.50±1.25) vs. (10.17±1.26) ng/L, P<0.01 and (7.50±1.24) vs. (8.90±1.13) ng/L, P<0.01, respectively]. The one-year survival rate was 54.2% and 82.6% in those with sFas levels >8.79 ng/L and <8.79 ng/L before treatment (P<0.02), respectively, 50.0% and 87.0% in those with sFas levels >7.74 ng/L and <7.74 ng/L one month after treatment (P<0.01), respectively. Conclusions: The sFas level is higher in LAURC subjects than in healthy controls. Concurrent chemoradiotherapy can reduce sFas levels in LAURC patients. The monitoring of sFas may provide prognostic information for LAURC patients.

Key words: Soluble Fas (sFas), Rectal cancer, Concurrent chemoradiotherapy, Prognosis
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1 Introduction

The morbidity and mortality of rectal cancer have been increasing worldwide in recent years (Liang *et al*., 2009). Surgical resection was the first choice for patients with early stage cancer; however, five-year overall survival rate was only about 60% after radical resection (Bosset *et al*., 2004), and the overall recurrence rate was 25%–40% (Havenga *et al*., 1999; Nesbakken *et al*., 2002). The result in locally advanced unresectable rectal cancer (LAURC) patients with chemotherapy or radiotherapy alone has been disappointing, but chemotherapy combined with radiotherapy made a remarkable improvement (Kachnic, 2007). The advantages of concurrent chemoradiotherapy might be: (1) synergistic effect between radiotherapy and chemotherapy; (2) no delayed treatment time; (3) reduction of the chance of drug-cross resistance. Research has shown that concurrent chemoradiotherapy can improve local control rates in patients with rectal cancer, thus improving the survival rate (Klautke *et al*., 2005); however, there is still a lack of accurate biological indicators in diagnosis, treatment, and prognosis. Fas is a key receptor triggering apoptosis in cells. Studies have reported that

soluble Fas (sFas), to a certain extent, reflects the biological behavior of tumors (Liang *et al*., 2002), and it can be a predictive factor during radiotherapy (Zhu *et al*., 2006) and chemotherapy (Shimizu *et al*., 2005; Chaudhry *et al*., 2008), but there is still little evidence confirming whether concurrent chemoradiotherapy declines sFas levels and whether sFas levels have a prognostic value in LAURC. Thus, the aim of our study was to detect the changes of serum sFas level in patients with LAURC, and to explore its prognostic value of response.

2 Materials and methods

2.1 Patients' characteristics

From Jan. 2006 to Aug. 2008, the serum samples of 47 patients with LAURC and 31 healthy donors were collected from the Department of Oncology in Affiliated Hospital of Guangdong Medical College, Zhanjiang, China. Inclusion criteria: age <75 years, histologically confirmed, presented LAURC with site, clinical stages III and IV (including recurrence) who were unwilling to have an operation or for whom radical resection was too difficult (seriously infiltrating pelvic wall, main vessels, or involving S3–S5), no evidence of distant metastases, Karnofsky performance status (KPS) score >60, expected survival time ≥3 months, and routine blood examination, renal and liver functions, and electrocardiogram all normal. Clinicopathologic data are shown in Table 1.

Table 1 Patients' characteristics (*n*=47)

Characteristic	Value[a]
Gender	
Male	27 (57.4%)
Female	20 (42.6%)
Age (year)	58.6 (23–66)[b]
Pathologic type	
Poorly differentiated adenocarcinoma	10 (21.3%)
Moderately differentiated adenocarcinoma	15 (31.9%)
Well differentiated adenocarcinoma	16 (34.0%)
Mucinous adenocarcinoma	6 (12.8%)
Clinical stage	
III	29 (61.7%)
IV	18 (38.3%)
Sphincter-preserving	
Yes	16 (34.0%)
No (artificial anus)	31 (66.0%)
Tumor recurrence type	
Anastomotic recurrence	12 (25.5%)
Pelvic recurrence (including the pelvic lymph nodes, uterus and accessories, vagina, presacral space)	24 (51.1%)
Perineal recurrence	11 (23.4%)

[a] Value are expressed as number (percentage) of patients; [b] Value is expressed as median (range)

2.2 Treatments

Radiotherapy was delivered with photons from a linear accelerator with energy >6 MV. Computed tomography-assisted three-dimensional (3D) planning of radiation therapy was employed. The planning target volume was comprised of: upper border at the L5/S1 junction, inferior border 3 cm below the primary tumor or at the inferior aspect of the obturator foramina, anterior border 1 cm anterior to the vagina wall or bladder, posterior border 1 cm behind the anterior bony sacral margin, and lateral border 1.5 cm lateral to the widest bony margin of the true pelvic side wall. Radiotherapy was delivered with three or four fields using an isocentric technique with individually collimated field portals. The total radiation dose was 66–68 Gy (Spindler *et al*., 2006) in 2 Gy per fraction per day, 5 d per week. The first 46 Gy was given to the planned field, and 20–22 Gy was given to the reduced field (after reviewing magnetic resonance imaging (MRI) or computed tomography (CT), if the tumor size decreased more than 50%, consideration was given to reducing radiation field).

Radiotherapy was administered with concurrent chemotherapy with FOLFOX regimen: including 5-fluorouracil (5-Fu) 400 mg/m^2, iv, Day 1; 5-Fu, 3.0 g/m^2, ivgtt for 46 h, Days 1–2; leucovorin (LV), 400 mg/m^2, iv, Day 1; and oxalipatin, iv, 100 mg/m^2, Day 1, every 3 weeks. Chemotherapy was administered on radiotherapy Days 1–2 and 22–23, for a total of two cycles and after 3–4 weeks of concurrent radiotherapy, two or three more cycles consolidated chemotherapy.

2.3 Serum sFas level detection

Serum sFas level was detected one week before starting chemoradiotherapy and one month after finishing all the treatments. The concentration of sFas was measured by enzyme-linked immunosorbent assay (ELISA; Genzyme Corporation, USA) according to the manufacturer's instruction.

2.4 Follow-up

The patients were followed up every month for 16 months by mail, telephone, and outpatient interviews, and all patients were hospitalized and evaluated every two or three months after treatment. The total survival time was defined as the period from beginning treatment to death or the 16th month.

2.5 Evaluation criteria

The evaluation criteria were based on response evaluation criteria in solid tumor (RECIST) criteria: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR plus PR cases were defined as the overall response rate. SD and PD cases were summed to calculate the overall non-response rate. Toxicity assessment was according to the World Health Organization (WHO) standards.

2.6 Statistical analysis

All analyses were performed using the SPSS 18.0 software package for Macintosh. The statistical significance of differences for the mean values of sFas concentrations was determined by Student's *t*-test. The one-year survival rate was analyzed with the Kaplan-Meier method. The accumulative survival rates were compared with the log-rank test. Differences with $P<0.05$ were considered significant.

3 Results

3.1 Therapeutic efficacy

The overall response rate was 66.0% (31/47), CR 10 cases, PR 21 cases, SD 9 cases, PD 7 cases, and the one-year survival rate was 68.1% (32/47).

3.2 Adverse events

Treatment-related adverse reactions were neutropenia, thrombocytopenia, nausea and vomiting, radiation proctitis, nerve cacesthesia, liver function lesion, and oral mucositis, most of I–III degrees, with no treatment-related deaths (Table 2).

Table 2 Adverse effects of treatment

Adverse event	Number of patients				
	0*	I	II	III	IV
Neutropenia	0	16	26	3	2
Thrombocytopenia	29	12	6	0	0
Nausea and vomiting	9	15	17	6	0
Radiation proctitis	22	10	8	5	2
Nerve cacesthesia	16	13	10	6	2
Liver function lesion	25	11	10	1	0
Oral mucositis	32	8	6	1	0

* 0–IV degrees according to WHO standards

3.3 sFas levels

The mean sFas levels before and one month after treatments were: (8.79±1.39) and (7.74±1.32) ng/L in the subjects with LAURC, respectively, which were higher than that in the healthy control (5.53±1.13) ng/L ($P<0.01$). Comparing the sFas level before treatment with that one month after treatment, the difference was also significant ($P<0.01$). There were 39 cases in the response group (CR+PR) and 8 cases in the non-response group (SD+PD). The comparisons of the sFas levels between two groups before treatment and one month after treatment both had significant differences ($P<0.01$) (Table 3).

Table 3 Comparisons of the changes in sFas levels in subjects with LAURC

Group	*n*	sFas level* (ng/L)	*P*
Pre-treatment			
Control	31	5.53±1.13	<0.01
LAURC	47	8.79±1.39	
Response (CR+PR)	39	8.50±1.25	<0.01
Non-response (SD+PD)	8	10.17±1.26	
Post-treatment			
Control	31	5.53±1.13	<0.01
LAURC	47	7.74±1.32	
Response (CR+PR)	39	7.50±1.24	<0.01
Non-response (SD+PD)	8	8.90±1.13	

* Values are expressed as mean±SD

According to the changes of sFas levels before and after treatments, the subjects were further divided into four groups: <8.79 ng/L (23 cases), >8.79 ng/L (24 cases), <7.74 ng/L (23 cases), and >7.74 ng/L (24 cases), respectively, and their one-year survival rates were: 82.6% (19/23), 54.2% (13/24), 87.0% (20/23), and 50.0% (12/24) (Fig. 1).

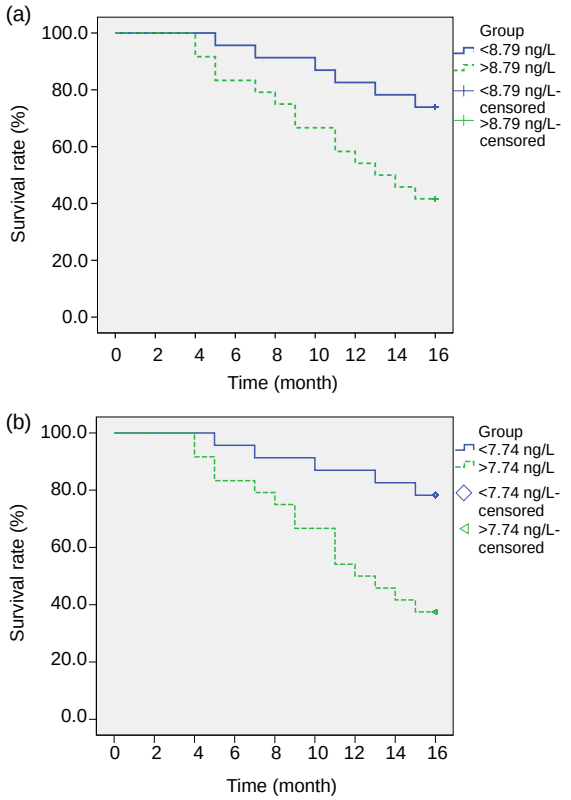

Fig. 1 Overall survival curves of the LAURC subjects
(a) sFas levels <8.79 ng/L and >8.79 ng/L before treatment (log-rank test: $P<0.02$); (b) sFas levels <7.74 ng/L and >7.74 ng/L one month after treatment (log-rank test: $P<0.01$)

4 Discussion

The Fas/Fas ligand (Fas/FasL) system plays an important role in regulating apoptosis. FasL (Apo-1/CD95 ligand) is a type II membrane protein having homology with tumor necrosis factor receptor (TNFR) family (Suda *et al*., 1993; Boroumand-Noughabi *et al*., 2010), which triggers target cells into apoptosis in several hours by combining with Fas or Fas monoclonal antibody inducing Fas trimerization or oligomerization (Griffith *et al*., 1995). Fas (Apo-1/CD95) is a type I cellular membrane protein, which belongs to the tumor necrosis factor receptor superfamily/the nerve growth factor receptor (Pan *et al*., 2007). Fas has two forms, a transmembrane form and a soluble form. Serum sFas is generated from cell surface that lacks 21 amino acid residues containing the transmembrane domain by alternative splicing (Petak *et al*., 2000). sFas inhibits the cell surface signal transduction by competitively binding and neutralizing FasL (Suda *et al*., 1993; Cascino *et al*., 1995; Natoli *et al*., 1995), which may correlate with cancer escaping immune surveillance and progression. Increased serum sFas level has been observed in patients with hepatocellular (Jodo *et al*., 1998), breast (Bewick *et al*., 2001), small cell lung cancer (SCLC) (Shimizu *et al*., 2005), esophageal (Gratas *et al*., 1998) and epithelial ovarian cancers (Chaudhry *et al*., 2008).

Radiotherapy plays an important role in the process of tumor apoptosis. Fas is the key element of radiation inducing apoptosis. Kimura and Gelmann (2000) have reported that proper γ-irradiation up-regulated cell's surface Fas antibody and enhanced Fas receptor's sensitivity, promoting tumor cell into apoptosis. Zhu *et al*. (2006) used immunohistochemistry and TdT-mediated dUTP nick end labeling (TUNEL) techniques to detect the changes of Fas, p53, Bcl-2, and apoptosis in 40 patients with uterine cervix carcinoma during radiotherapy, indicating a positive apoptosis rate, increased p53 and Fas expression, and significantly decreased Bcl-2 after radiotherapy. Several studies have demonstrated that apoptosis induced by chemotherapy involved the Fas/FasL system (Bewick *et al*., 2001; Shimizu *et al*., 2005; Naumnik *et al*., 2007; Chaudhry *et al*., 2008). Cytotoxic drugs lead to increasing FasL expression, which triggers tumor cells into apoptosis by binding Fas (Eichhorst *et al*., 2001). Debatin and Krammer (2004) expressed a different view that the Fas/FasL system may amplify the mitochondrial pathway to induce apoptosis. Many studies have confirmed that chemotherapy decreases serum sFas (Shimizu *et al*., 2005; Naumnik *et al*., 2007; Chaudhry *et al*., 2008), which relieves the inhibition of Fas/FasL system, and amplifies the apoptotic signal leading to tumor shrinkage.

The concentration of sFas in colorectal cancer correlated with the clinicopathological stage and treatment response (Kushlinskii *et al*., 2001). Abbasova *et al*. (2009) found that the sFas median concentration increased with tumor progression, and sFas levels showed significant differences between cases of regional metastases and no metastases in colorectal cancer. In the present study, the sFas levels before and after treatment in those with LAURC were higher than those in healthy controls. Furthermore, the sFas level after treatment was significantly lower

than that before treatment. This significant change may be a result of concurrent chemoradiotherapy. In order to further investigate the prognostic value of sFas, according to mean sFas levels before and after treatments, subjects were divided into four groups, and followed up for 16 months. As a result, the low sFas level groups before and after treatment both had significant differences compared with the high sFas level groups in their one-year survival rates. In other words, low sFas levels were associated with a better prognosis.

In conclusion, concurrent chemoradiotherapy can decrease serum sFas level in LAURC patients. The monitoring of sFas may provide prognostic information for these patients.

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