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BRIEF ARTICLE

Expression and significance of cyclooxygenase-2 mRNA in benign and malignant ascites

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Abstract

AIM: To investigate the mRNA expression of cyclooxygensae-2 (COX-2) in benign and malignant ascites, and to explore the difference in COX-2 mRNA expression among different diseases.

METHODS: A total of 36 samples were collected from the Fifth Affiliated Hospital of Sun Yat-Sen University and divided into two experimental groups: benign ascites (n = 21) and malignant ascites (n = 15). Benign ascites included cirrhotic ascites (n = 10) and tuberculous ascites (n = 5). Malignant ascites included oophoroma (n = 7), cancer of colon (n = 5), cancer of the liver (n = 6), gastric cancer (n = 2), and bladder carcinoma (n = 1). The mRNA expression of COX-2 in ascites was examined with reverse transcriptase polymerase chain reaction (RT-PCR) technology, and the positive rate of COX-2 mRNA was compared between different diseases.

RESULTS: The positive rate of COX-2 mRNA in malignant ascites was 42.9% (9/21), which was significantly higher than in benign ascites, 6.7% (1/15), difference being significant between these two groups (χ^2 = 4.051, P = 0.044). The proportion of the positive rate in the malignant ascites was as follows: ovarian cancers 57.1% (4/7), colon cancer 40.0% (2/5), liver cancer 33.3% (2/6), gastric cancer 50.0% (1/2), and bladder cancer 0.00% (0/1). However, there was no significant difference in COX-2 mRNA expression among various tumors with malignant ascites (χ^2 = 1.614, P = 0.806). Among the benign ascites, COX-2 mRNA levels were different between the tuberculous ascites (0/5) and cirrhotic ascites (1/10), but there was no significant difference (P = 1.000).

CONCLUSION: COX-2 mRNA, detected by RT-PCR, is useful in the differential diagnosis of benign and malignant ascites, which also has potential value in the clinical diagnosis of tumors.

Key words: Ascites; Cyclooxygensae-2 mRNA; Reverse transcriptase polymerase chain reaction; Malignant tumor

Core tip: Ascites is a common symptom caused by a variety of diseases, the differential diagnosis between benign ascites and malignant ascites is one of the most important issues in clinical practice. Cytologic examinations and ascites tumor markers can provide important evidence, but their sensitivity and specificity are far from satisfactory. Our study aimed to explore the difference in cyclooxygensae-2 (COX-2) mRNA expression among different diseases. Our research suggests that COX-2mRNA can be detected by reverse transcriptase polymerase chain reaction, but there are no significant differences in the expression of COX-2 mRNA among

various disease types with benign or malignant ascites.

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INTRODUCTION

Ascites is a gastroenterological term for an accumulation of fluid in the peritoneal cavity, which might result from cirrhosis, tuberculous peritonitis or a malignant tumor.

The detection of exfoliated ascites cells is often used to identify these diseases in the clinic, but there is no economic, practical, or effective index with a high specificity. Therefore, it is important to discriminate benign from malignant ascites for clinical diagnosis and treatment[1,2].

In recent years, cyclooxygensae-2 (COX-2) has been extensively studied as an inducible expression protein, and has been detected in various tumor tissues in epidemiologic and cytologic researches[3,4], such as pancreatic cancer, colorectal carcinoma, non-small lung cancer and so on. Research has found that COX-2 expression is unregulated in precancerous lesions and preinvasive carcinoma and positively correlates with tumor invasion and lymphatic metastasis[5,6]. Therefore, increasing expression of COX-2 might occur in the early stages of the tumor and the detection of COX-2 level is helpful for early diagnosis.

However, there are only few studies regarding COX-2 expression in benign and malignant ascites. We employed reverse transcriptase polymerase chain reaction (RT-PCR) technology to detect the expression level of COX-2 in benign and malignant ascites, and analyzed the difference in mRNA expression of COX-2 among different diseases.

MATERIALS AND METHODS

Subjects

A total of 36 patients with ascites who underwent abdominocentesis at the Fifth Affiliated Hospital of Sun Yat-Sen University between August 2011 and March 2012 were selected. The subjects were divided into benign and malignant groups according to medical history, physical examination, B ultrasound, computed tomography (CT), pathology and the presence of exfoliated tumor cells. There were 15 patients with benign ascites, including nine males and six females, aged 43-75 years with an average age of 62.5 ± 1.8 years; the patients consisted of 10 cases of cirrhosis and five cases of tuberculous peritonitis according to disease type. There were 21 patients with malignant ascites, including 11 males and 10 females, aged 41-79 years with an average age of 58 ± 2.3 years; the patients consisted of seven cases of ovarian cancer, five cases of colon cancer, six cases of liver cancer, two cases of gastric cancer and one case of bladder cancer according to disease type.

Table 1 Primer sequences of cyclooxygensae-2 and glyceraldehyde-3-phosphate dehydrogenase genes

Primer		Primer sequence	Product size
COX-2	Forward primer	5′-CTTGGGTGTCAAAGGTAA-3′	581 bp
	Reverse primer	5′-AGGGACTTGAGGAGGGTA-3′	
GAPDH	Forward primer	5′-GTGGGGCGCCAGGCACCA-3′	146 bp
	Reverse primer	5′-CTCCTATGTCACGCACATTC-3′	

COX-2: Cyclooxygensae-2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

The samples were centrifuged at 3000 r/min for 15 min immediately after collection from the patients, and the supernatant was removed. The pellet was stored in -80 ℃ refrigerator for total RNA extraction.

The sample collection was approved by the Ethics Committee of the hospital and all patients provided written informed consent.

RT-PCR

Primer design: The gene sequences of the primers were designed with Primer Premier 5.0 software according to the literature[5,6] and mRNA sequences for human COX-2 reported by NCBI; GAPDH was selected as the positive control primer for RT-PCR and synthesized by the Shanghai ShengGong Biological Engineering Co., Ltd., China (Table 1).

Total RNA extraction: Total RNA was extracted from benign and malignant ascites with RNA extraction reagent (Trizol, Invitrogen, United States), and its content and purity were measured by ultraviolet spectrophotometry [1.9 < D(260)/D(280) < 2.1].

Synthesis of cDNA by reverse transcription: Reverse transcription was performed to synthesize cDNA with the Avian Myeloblastosis Virus (AMV) Reverse Transcriptase Kit, which contained AMV reverse transcriptase and olig(dt)$_{18}$ primer. The RT-PCR reaction mixture of 10 μL contained 1 μL extracted total RNA, 2 μL $MgCl_2$, 1 μL 10 × RNA PCR buffer, 3.75 μL RNase free dH_2O, 1 μL dNTP mixture, 0.25 μL RNase inhibitor, 0.5 μL AMV reverse transcriptase and 0.5 μL Oligo dT-adaptor primer. The reaction conditions were set at 30 ℃ for 10 min, 50 ℃ for 30 min, 99 ℃ for 5 min and 5 ℃ for 5 min.

PCR amplification: After reverse transcription, cDNA was used as the template in PCR amplification with primers for COX-2 and glyceraldehyde-3-phosphate dehydro-

Table 2 mRNA expression of cyclooxygensae-2 *n* (%)

Group	COX-2 mRNA expression		χ^2	*P* value
	Positive	Negative		
In benign and malignant ascites[1]			4.051	0.044
Benign ascites	1 (6.7)	14 (93.3)		
Malignant ascites	9 (42.9)	12 (57.1)		
Among different disease types in benign group[2]				1.000
Cirrhosis with ascites	1 (6.7)	9 (60)		
Tuberculous ascites	0 (0.0)	5 (33.3)		
Among different disease types in malignant group[3]			1.614	0.806
Ovarian cancer	4 (19.0)	3 (14.3)		
Colon cancer	2 (9.5)	3 (14.3)		
Liver cancer	2 (9.5)	4 (19.0)		
Gastric cancer	1 (4.8)	1 (4.8)		
Bladder cancer	0 (0.0)	1 (4.8)		

[1]Comparison was made between groups using Yates' continuity correction; [2]Comparison was made between groups using Fisher's exact probability test; [3]Comparison between groups utilized χ^2 test; $P > 0.05$ was considered not statistically significant. $P < 0.05$ was considered statistically significant. COX-2: Cyclooxygensae-2.

genase (GAPDH); a negative control was established. The PCR reaction mixture of 50 μL contained 3 μL $MgCl_2$, 4 μL 10 × LA PCR buffer II (Mg^2 + free), 31.75 μL sterilized distilled water, 0.25 μL TaKaRa LA Taq and 1 μL the COX-2 or GAPDH primers. The PCR cycle consisted of the following steps: denaturing at 94 ℃ for 30 s, annealing at 60 ℃ for 30 s and elongation at 72 ℃ for 1.5 min, which was repeated for 30 cycles. Amplification products were utilized for electrophoresis in a 1.5% agarose gel, and were observed and photographed under ultraviolet light.

Statistical analysis

Statistical analysis was performed with SPSS 13.0 software, and qualitative data was described by frequency and rate; the comparison between groups of qualitative data was made using the χ^2 test with Yates' continuity correction and Fisher's exact probability test; $P < 0.05$ was considered significant.

RESULTS

mRNA expression of COX-2 in benign and malignant ascites

The positive rate of COX-2 mRNA in malignant ascites was 42.9% (9/21), which was significantly higher than in benign ascites, 6.7% (1/15), the difference being significant between the two groups ($\chi^2 = 4.051$, $P = 0.044$), (Table 2).

mRNA expression of COX-2 among different disease types in benign group

Among the benign ascites, COX-2 mRNA levels were different between the tuberculous ascites (0/5) and cirrhotic ascites (1/10), but the difference being not significant ($P = 1.000$), (Table 2).

mRNA expression of COX-2 among different disease types in malignant group

The proportion of the positive rate in the malignant ascites was as follows: ovarian cancers 57.1% (4/7), colon cancer 40.0% (2/5), liver cancer 33.3% (2/6), gastric cancer 50.0% (1/2), and bladder cancer 0.00% (0/1). However, there was no significant difference in COX-2 mRNA expression among various tumors with malignant ascites ($\chi^2 = 1.614$, $P = 0.806$; $P > 0.05$), (Table 2).

DISCUSSION

COX, or prostaglandin-endoperoxide synthase (PGH), is a major rate-limiting enzyme in the synthesis of prostaglandin, which is able to metabolize arachidonic acid into prostaglandin products[7-9]. COX-2, an inducible protein expression, is absent in normal cells and tissues, but is rapidly synthesized and expressed under pathological conditions or after stimulation (such as inflammation, hypoxia, laser radiation, ultraviolet radiation, *etc.*). COX-2 is involved in a variety of pathophysiological processes, such as the occurrence and development of inflammation and cancer[10]. At present, mRNA expression of COX-2 in various tumor tissues has been extensively investigated; increasing numbers of research have demonstrated that COX-2 expression is unregulated in precancerous lesions and preinvasive carcinoma and positively correlates with tumor invasion and lymphatic metastasis[11-14]. Therefore, increased expression of COX-2 might occur in the early stage of the tumor and the detection of COX-2 level is helpful for early diagnosis[15,16].

Ascites is a common symptom of many diseases and the differential diagnosis of benign and malignant ascites is important in clinical practice. So far, smear tests of exfoliated cells from ascites and the detection of tumor markers, such as CA-125, CA19-9 and AFP, have been employed to identify ascites induced by malignant tumors, but these indices are far from satisfactory in terms of sensitivity and specificity, so it is important to search for a new indicator of benign and malignant ascites[17-21] Since COX-2 has a close relationship with tumors, its expression in malignant ascites has become an issue that is worth exploring.

We used RT-PCR to assess the mRNA expression of COX-2 in 21 cases of malignant ascites. The positive rate of COX-2 mRNA was 42.9% (9/21), which was significantly higher than in benign ascites, 6.7% (1/15) ($P < 0.05$). This result indicated that the measurement of COX-2 mRNA facilitates the identification of benign and malignant ascites and has a potential value for clinical diagnosis and screening of tumors. In previous studies on COX-2, its expression was usually detected in malignant tumor tissues[22-24], but our experiment used ascites as the samples. They were convenient to collect from patients, with less pain and being easy for clinical application. In addition, COX-2 is absent in normal cells and tissues as an inducible expression protein with specificity, so is a

potential indicator for the identification of benign and malignant ascites, and an effective supplement to common indices, such as CA125, CA19-9 and AFP.

There were no significant differences in the expression of COX-2 mRNA among various disease types with benign or malignant ascites ($P > 0.05$), which was probably associated with the small number of samples and requires further confirmation. We employed one step RT-PCR, which was easy to perform, required little contact with experimental samples and avoided unnecessary contamination, and also facilitated further research and the development of clinical detection kits.

In conclusion, differential diagnosis between benign and malignant ascites is of importance and is helpful for designing a treatment plan. We hope our study can provide a new insight to explore this field in the future.

COMMENTS

Background

In recent years, cyclooxygensae-2 (COX-2) has been extensively studied as an inducible expression protein, and has been detected in various tumor tissues in epidemiological and cytological research. Therefore, increased expression of COX-2 might occur in the early stage of the tumor and the detection of COX-2 level is helpful for early diagnosis.

Research frontiers

At present, mRNA expression of COX-2 in various tumor tissues has been extensively investigated; more and more research has demonstrated that COX-2 expression is unregulated in precancerous lesions and preinvasive carcinoma and positively correlates with tumor invasion and lymphatic metastasis. Therefore, increased expression of COX-2 might occur in the early stages of the tumor and the detection of COX-2 level is helpful for early diagnosis.

Innovations and breakthroughs

This study employed RT-PCR to assess the mRNA expression of COX-2 in 21 cases of malignant ascites. The positive rate of COX-2 mRNA was 42.9% (9/21), which was significantly higher than in benign ascites, 6.7% (1/15), ($P < 0.05$). This result indicated that the measurement of COX-2 mRNA facilitates the differential diagnosis between benign and malignant ascites and has a potential value for clinical diagnosis and screening of tumors.

Applications

Differential diagnosis between benign and malignant ascites is of importance and is helpful for designing a treatment plan. The study can provide a new insight to this field in the future.

Peer review

This is an interesting manuscript about mRNA expression of COX-2 in benign and malignant ascites. The authors made a good research on this topic. Differences in COX-2 mRNA expression among different diseases were explored. The data is well present and discussed.

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