

# Expression of PCNA and CD44mRNA in colorectal cancer with venous invasion and its relationship to liver metastasis

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## Abstract

**AIM:** To investigate the expression of proliferating cell nuclear antigen (PCNA) and CD44mRNA in colorectal cancer with venous invasion and its relationship with liver metastasis.

**METHODS:** Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect the expression of PCNA and CD44mRNA in 31 cases of colorectal cancer with venous invasion.

**RESULTS:** Positive expression rates of PCNA and CD44mRNA in colorectal cancer were higher than those without liver metastasis ( $P < 0.05$  and  $P < 0.01$ ). In case of colorectal cancer with liver metastasis, strongly positive rates of PCNA and CD44mRNA were 94.1 % and 70.6 %, respectively, significantly higher than those without liver metastasis. There was a positive relationship between the expressions of PCNA and CD44mRNA ( $r = 0.67$ ,  $P < 0.05$ ).

**CONCLUSION:** Detection of PCNA and CD44mRNA expression in colorectal cancer may be useful for evaluating liver metastasis of cancer cells.

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## INTRODUCTION

Many mechanisms are involved in liver metastasis of colorectal cancer, of which venous invasion is considered to be the chief process<sup>[1-6]</sup>. Previous studies showed that the degree of venous invasion was positively related to the rate of liver metastasis. But it is unknown which factors participate in liver metastasis of colorectal cancer. PCNA is a chief marker reflecting the activity of cell proliferation, which is closely related to invasion and metastasis of malignant neoplasms and their prognosis<sup>[7-10]</sup>. Cell adhesion molecules (CAMs) correlate to the invasion and metastasis of tumor cells, and play an important role in occurrence, development and metastasis of neoplasms<sup>[11,12]</sup>. The goal of this study was to test the expression of PCNA and adhesion molecule CD44mRNA in colorectal cancer with venous invasion by RT-PCR and its relationships with liver metastasis.

## MATERIALS AND METHODS

### Materials

According to the pathological diagnosis standards, the severity of venous invasion was classified as V0- V3<sup>[13]</sup>. Thirty-one patients with severe venous invasion of colorectal cancer in V3 stage were chosen as study subjects (male 20, female 11), aged 44-82 years (average 66 years), of them 17 cases had liver metastasis. After operations, neoplasm samples were kept in liquid nitrogen. RNA extract reagent was purchased from Gibco Co., Taq enzyme from Takara Co., PCNA, CD44mRNA and  $\beta$ -actin primer were synthesized by BoYa Shanghai Co.. PCNA's sequence of up-stream primer was 5' -GCCGAGATC-TCAGCCATATT-3', that of down-stream primer was 5' -ATGTACTTAGAGGTACAAAT-3'. CD44's sequence of up-stream primer was 5' -CTTCATCCCAGTGACC-3', that of down-stream primer was 5' -TGCCACTGTTGATCAC-3'.  $\beta$ -actin's sequence of up-stream primer was 5' -CACCATGTACCCTGGCATTG-3', that of down-stream primer was 5' -TAACGCAACTAAGTCATAGT-3'. The size of anticipatively amplified products was 452 bp, 446 bp and 243 bp, respectively.

### Methods

Using TRIzol reagent kit, total RNA was extracted according to the method previously described<sup>[14-20]</sup>. The purity and content of RNA were measured by a spectrophotometer, and kept at -80 °C. Total RNA 5  $\mu$ g, 5 $\times$ reaction buffer 10  $\mu$ l, 10 mmol $\cdot$ L<sup>-1</sup> dNTPs 5  $\mu$ l, RNasin 20 U, oligo(dT)<sub>12-18</sub> 0.25  $\mu$ g, reverse transcriptase (M-MLV, Gibco) 200 U and 0.1 mol $\cdot$ L<sup>-1</sup> DTT 0.5  $\mu$ l were added to reaction volume of 50  $\mu$ l, incubated at 37 °C for 1 h, then heated at 65 °C for 5 min to stop reaction. cDNA 0.1  $\mu$ g, 10 $\times$ PCR buffer 2.5  $\mu$ l, 2 mmol $\cdot$ L<sup>-1</sup> dNTPs 2.5  $\mu$ l, 25 mmol $\cdot$ L<sup>-1</sup> MgCl<sub>2</sub> 2.5  $\mu$ l, PCR primer 20 pmol and Taq DNA polymerase (Takara) 5 U were added to reaction volume of 25  $\mu$ l. Using PTC-100 equipment (MJ Research), PCR conditions were as follows: pre-denaturing at 93 °C for 1 min, then 35 cycles at 93 °C for 30 s, at 52 °C for 30 s and 72 °C for 1 min, followed by extension at 72 °C for 8 min. Each amplified product of 10  $\mu$ l was detected via 3 g $\cdot$ L<sup>-1</sup> sepharose electrophoresis, bromide staining, and analyzed by using a UVP gel imaging system and Labworks software. The ratio of density of positive PCNA and CD44 to that of  $\beta$ -actin was considered to be PCNA and CD44 relative expression quantity<sup>[21-28]</sup>. Expression intensity was classified into 3 grades: +: 1-30 %  $\beta$ -actin density, ++: 31-65 %, +++: 66-100 %.

### Statistics

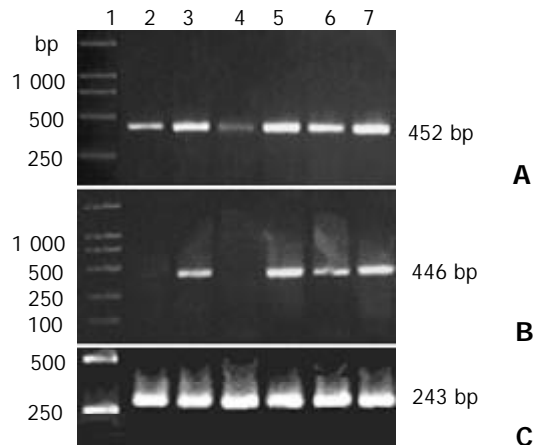
All the data were analyzed by  $\chi^2$ -test, and a value of  $P < 0.05$  was considered significant and  $P < 0.01$  very significant.

## RESULTS

### Expression of PCNA and CD44mRNA in colorectal cancer tissue

PCNA, CD44 and  $\beta$ -actin gene expressions were detected in colorectal cancer tissues by RT-PCR, the size of amplified fragment was coincident with that of anticipation (Figure 1). All cases had expression of PCNA, the positive rate was 100 %,

but the expression levels were different among different cases (Figure 1A). Twenty cases had positive expression of CD44 mRNA, the positive rate was 64.5%. The expression levels were different among different cases. As an inter-control, the expression level of  $\beta$ -actin was basically coincident among different cases (Figure 1C).



**Figure 1** Expression of PCNA (A), CD44 (B) and  $\beta$ -actin (C) in colorectal cancer by PCR amplification. 1: DL2000 DNA marker (Takara), 2-7: Colorectal cancer tissues at various venous invasion stages.

#### Relationship between expression of PCNA and CD44mRNA and liver metastasis

In 17 cases of liver metastasis, 16 cases had strongly positive expression of PCNA mRNA (++, +++) (94.1%), significantly higher than those without liver metastasis (28.6%,  $P < 0.01$ ). There was a positive relationship between the expression of PCNA and colorectal cancer with liver metastasis ( $r = 0.82$ ,  $P < 0.01$ ). In 17 cases of liver metastasis, 12 cases had strongly positive expression of CD44mRNA (70.6%), significantly higher than those without liver metastasis (21.4%,  $P < 0.01$ ). There was a positive relationship between the expression of CD44mRNA and colorectal cancer with liver metastasis ( $r = 0.82$ ,  $P < 0.01$ ).

#### Relationship between expressions of PCNA and CD44mRNA in colorectal cancer

Of the 16 cases with strongly positive expression of CD44mRNA, 12 cases had strongly positive expression of PCNA (75.0%). Of the 15 cases with weak or negative expression of CD44mRNA, 7 cases had strongly positive expression of PCNA (46.7%), and there was a significant difference ( $P < 0.05$ ). There was a positive relationship between the expressions of PCNA and CD44mRNA ( $r = 0.67$ ,  $P < 0.05$ ).

#### DISCUSSION

Venous invasion of cancer cells was the first step of neoplasm's liver metastasis<sup>[29]</sup>. However, in clinical cases, venous invasion by histological detection was not definitely related to liver metastasis. It implies that, besides venous invasion, other factors might also participate in the process of liver metastasis<sup>[30-32]</sup>.

PCNA is a kind of non-histone nuclear polypeptide with 36 000 molecular weight, as an assistant protein to DNA polymerase, its content could reflect the degree of cell proliferation<sup>[33-37]</sup>. Previous studies showed that there was a significant relationship between PCNA labelling and degree of malignancy, vessel invasion, distant metastasis and prognosis<sup>[38-42]</sup>. In this study, we found that under the condition of venous invasion, the strong expression rate of PCNA mRNA

in venous invasion of colorectal cancer with liver metastasis, was significantly higher than that without liver metastasis. This showed that there was a positive relationship between the strong expression of PCNA and colorectal cancer with liver metastasis. All these indicate that colorectal cancer cells with higher proliferating activity are much more easier to proceed liver metastasis.

CD44 is a kind of cell adhesion molecules. Under normal conditions, CD44 acts as the receptor of hyaluronic acid, and chiefly participates in intra-cells and cell-stroma specific adhesion<sup>[43-46]</sup>. Recent studies showed that CD44 could be expressed in different neoplasm tissues. Bhatavdekar *et al* found that over-expression of CD44 was associated with clinical staging of colorectal cancer. Furthermore, positive expression of CD44 is an important prognostic factor associated with colorectal cancer patients' relapse and total survival time. Further studies found that CD44 could make cancer cells be able to metastasize, adhere to vessel endothelium, accelerate cancer metastasis. We found that the strong expression rate of CD44 mRNA in colorectal cancer with liver metastasis was significantly higher than that without liver metastasis, further suggesting that CD44 might play an important role in colorectal cancer with liver metastasis. But it is still in argument whether CD44 can be taken as an independent marker for progress and metastasis of colorectal cancer.

We also found that under the condition of venous invasion in colorectal cancer patients with liver metastasis, the expression of PCNA and CD44mRNA was strong at the same time. All these indicate that there is a positive relationship between colorectal cancer with liver metastasis and expression of PCNA and CD44mRNA ( $r = 0.82$ ,  $P < 0.01$ ). Moreover, in colorectal cancer tissue with strong expression of CD44mRNA, PCNA mRNA was significantly higher than that with weak or negative expression, showing that there was a positive relationship between the expressions of CD44 and PCNA ( $r = 0.67$ ,  $P < 0.05$ ). Taken together, we conclude that detection of PCNA and CD44 expression in colorectal cancer may be useful for evaluating liver metastasis of cancer cells.

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