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ORIGINAL ARTICLE

# Expression and clinical significance of CD74 and MMP-9 in colon adenocarcinomas

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## *Summary*

***Purpose:*** *To detect the expressions of CD74 and matrix metalloproteinase-9 (MMP-9) in colon adenocarcinomas, and to explore the relationship between the expressions and clinicopathological characteristics and prognosis.*

***Methods:*** *98 cases of colon adenocarcinoma tissues from patients who underwent colon cancer resection in the Sixth Affiliated Hospital, Sun Yat-sen University from January 2013 to March 2015 comprised the experimental group, while 71 cases of colon mucosa tissues from patients who underwent colon polypectomy during the same period comprised the control group. qRT-PCR was used to detect the expressions of CD44 and MMP-9 mRNAs in the two groups, in order to analyze their correlation in colon adenocarcinomas, and to also analyze their relationship with clinicopathological characteristics and prognosis.*

***Results:*** *The expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues were significantly higher than those in normal colon mucosa tissues ($p<0.05$). The expressions of CD74 and MMP-9 mRNAs had no significant relationship with the patient's gender, age, differentiation grade and tumor type in colon adenocarcinoma tissues ($p>0.05$), but had significant correlation with lymph node metastasis and pathological stage ($p<0.05$). According to the average expressions of CD74 and MMP-9 mRNAs, the patients were divided into low and high expression groups. The 3-year survival rate of patients in the low expression group was significantly higher than that in the high expression group ($p<0.05$). Moreover, the expressions of CD74 and MMP-9 were positively correlated ($r = 0.853$, $p<0.001$).*

***Conclusion:*** *CD74 and MMP-9 are highly expressed in colon adenocarcinomas, and their expressions are closely related to the pathological stage, lymph node metastasis and prognosis of colon adenocarcinoma patients. Therefore, they can be used as important biological markers for diagnosis and prognosis prediction of colon adenocarcinoma.*

***Key words:*** *colon adenocarcinoma, CD74, MMP-9, clinicopathological characteristics, prognosis*

## Introduction

Colon cancer is a clinically common malignant tumor of the digestive tract, with a high and rising incidence rate [1]. Many colon cancer patients are prone to abdominal and pulmonary metastasis in advanced stage, with poor curative effect and prognosis [2]. Colon cancer is easy to be ignored because

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of its dormant onset and unobvious symptoms at the early stage. Therefore, many patients are in an advanced stage when they are first diagnosed, and miss the optimal treatment period, which is also the reason for high mortality rate of colon cancer patients [3]. Colon adenocarcinoma is the main pathological type of colon cancer, so the exploration on its pathogenesis and metastasis has an very important clinical significance for the early diagnosis and treatment of this disease [4].

CD74 is a type II transmembrane protein that binds to major histocompatibility complex (MHC) type II protein in an immune response as a molecular chaperone, and is mainly expressed in antigen presenting cells (APC) [5]. In recent years, studies have found that CD74 is highly expressed in some malignant tumors, such as cervical squamous cell carcinoma [6], bladder urothelial carcinoma [7] and others. Matrix metalloproteinases (MMPs) are zinc and calcium-dependent proteolytic enzymes that can degrade extracellular matrices and decline the defense function of tissue barriers such as basement membrane by destroying the degradation balance of matrices [8]. As an important member of MMPs family, MMP-9 is mainly derived from macrophages, and has an effect on degrading type IV, V collagens and gelatin [9]. Moreover, it can promote tumor invasion and metastasis by degrading extracellular matrix and basement membrane, and is considered as an important molecular marker [10]. In addition, some authors [11] found that interference with CD74 could inhibit the proliferation and migration of lung cancer cells, and the expression of MMP-9 was also inhibited when CD74 expression was down-regulated. So we guess that there might be a link between CD74 and MMP-9.

However, there are few studies [12,13] on the expressions of CD74 and MMP-9 in colon adenocarcinomas, and there is no relevant report on the correlation of their expressions. Therefore, the expression and clinical significance of CD74 and MMP-9 in colon adenocarcinomas were explored in this study to provide more biological reference indicators for the diagnosis and treatment of this malignancy.

## Methods

*General data*

98 cases of colon adenocarcinoma tissues from patients who underwent colon cancer resection in the sixth affiliated Hospital, Sun Yat-sen University from January

**Table 1.** General data

| *Factor* | *Experimental group (n=98) n (%)* | *Control group (n=71) n (%)* | $t/x^2$ | *p* |
|---|---|---|---|---|
| Gender | | | 0.036 | 0.849 |
| Male | 51 (52.04) | 38 (53.52) | | |
| Female | 47 (47.96) | 33 (46.48) | | |
| Age (years) | | | 0.009 | 0.922 |
| ≤52 | 38 (38.78) | 27 (38.03) | | |
| >52 | 60 (61.22) | 44 (61.97) | | |
| BMI ($kg/m^2$) | | | 0.058 | 0.810 |
| ≤ 21 | 46 (46.94) | 32 (45.07) | | |
| >21 | 52 (53.06) | 39 (54.93) | | |
| Lymph node metastasis | | | - | - |
| Yes | 62 (63.27) | - | | |
| No | 36 (36.73) | - | | |
| Type | | | - | - |
| Lump | 24 (24.49) | - | | |
| Infiltrative | 46 (46.94) | - | | |
| Ulcerative | 28 (28.57) | - | | |
| Pathological stage | | | - | - |
| I-II | 54 (55.10) | - | | |
| III-IV | 44 (44.90) | - | | |
| Differentiation grade | | | - | - |
| Well differentiated adenocarcinoma | 41 (41.84) | - | | |
| Moderately differentiated adenocarcinoma | 32 (32.65) | - | | |
| Poorly differentiated adenocarcinoma | 25 (25.51) | - | | |

2013 to March 2015 comprised the experimental group, including 51 males and 47 females with a mean age of 52.15±10.27 years. Meanwhile, 71 cases of colon mucosa tissues from patients with an average age of 52.33±10.29 years who underwent colon polypectomy during the same period of time comprised the control group with the consent of the patients. There was no significant difference in gender and age between the two groups (p>0.05) (Table 1). All tissues were immediately put into liquid nitrogen tank for preservation after resection.

*Inclusion criteria:* patients pathologically diagnosed with colon adenocarcinoma.

*Exclusion criteria:* patients with severe liver and kidney dysfunction; patients with other malignant tumors; patients who had received radiotherapy and chemotherapy before surgery; patients with communication and cognitive dysfunction; patients not cooperating with the experiment.

All subjects and their families agreed to participate in the experiment and signed an informed consent form. This experiment was approved by the Ethics Committee of the sixth affiliated Hospital, Sun Yat-sen University.

*Experimental reagents*

Real-time quantitative PCR instrument was purchased from BioRad Company, USA; qPCR kit was from Takara, Japan, RQ106, cDNA reverse transcription kit was from Thermo, EHS1031 and Trizol reagent were purchased from Applide Invitrogen Company, USA. Primer sequences of CD74 mRNA, MMP-9mRNA and β-actin were synthesized and designed by Shanghai Omicsspace Bioteh Co., Ltd.

*Experimental methods*

Part of the tissues identified by pathological examination were taken out from the liquid nitrogen tank, grinded, and then added with Trizol reagent to extract total RNAs. The purity and concentration of the RNAs were detected by ultraviolet spectrophotometer. Then, 2 μg of total RNA were reversely transcribed to cDNA according to the instructions of the kit. Reaction parameters: 37°C for 45 min, 95°C for 5 min. The transcribed cDNA was used for PCR amplification with β-actin being the internal reference. Reaction system: 1 μL of cDNA, 0.4 μL of each upstream and downstream primers, 10 μL of 2X TransScript® Tip Green qPCR SuperMix, Passive Reference Dye (50X), and Nuclease-free water was added to supplement up to 20 μL. The primer sequence is shown in Table 2. PCR reaction conditions: 94°C for 2 min, 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, for a total of 40 cycles. Real-time fluorescence quantitative PCR detection was performed by a PCR instrument, and the experiment was repeated 3 times.

*Outcome measures*

(1): The expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues and normal colon mucosa tissues were observed. (2): According to the average expressions of CD74 and MMP-9 mRNAs in cancer tissues, colon adenocarcinoma patients were divided into high and low expression groups, and then the correlation between expressions and clinicopathological characteristics was analyzed. (3): The relationship between the expressions of CD74, MMP-9 mRNAS and the 3-year survival rate in colorectal cancer tissues was analyzed.

*Statistics*

In this study, SPSS15.0 software (Bizin Sight (Beijing) Information Technology Co., Ltd.) was used for statistical analyses. Chi-square test was used for counting data analysis, and mean±standard deviation was used for measurement data. The comparison between the two groups was conducted with *t*-test, the comparison among multiple groups was conducted by one-way analysis of variance (ANOVA), represented by F, and protein correlation analysis was carried out with Pearson test. GraphPad Prism 6 software was used for drawing the experimental figures, and Kaplan-Meier method was used for survival analysis. A value of p<0.05 was considered as statistically significant.

## Results

*Expressions of CD74 and MMP-9 mRNAs*

The mean expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues were

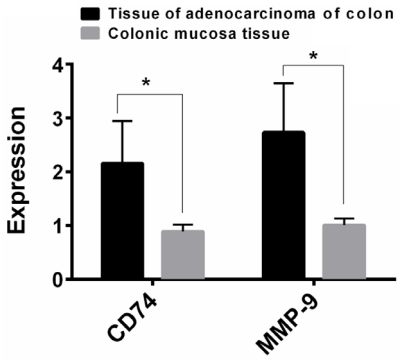


**Figure 1.** Expressions of CD74 and MMP-9 mRNAs. qRT-PCR showed that the expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues were significantly higher than those in normal colon mucosa tissue, with statistically significant difference (*p<0.05).

**Table 2.** Primer sequences

| *Gene* | *Upstream primer* | *Downstream primer* |
|---|---|---|
| CD74 | 5-'GAATGCTGACCCCCTGAAGGTGTA-3' | 5'-GGGGGCTGAAGGGAGCAAGAAAGC-3' |
| MMP-9 | 5'-TCCAGTACCAAGACAAAGC-3' | 5'-GAGCCCTAGTTCAAGGGCAC-3' |
| GAPDH | 5'-GCTGGGGCTCACCTGAAGGG-3' | 5'-GGATGACCTTGCCCACAGCC-3' |

2.15±0.79 and 2.73±0.91, respectively, while those in colon mucosa tissues were 0.89±0.13 and 1.01±0.12, respectively. Therefore, the expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues were significantly higher than those in normal colon mucosa tissues, with statistically significant difference (p<0.05), as shown in Figure 1.

*Correlation between expressions of CD74, MMP-9 mRNAs and clinicopathological characteristics*

There was no significant correlation between the expressions of CD74, MMP-9 mRNAs and gender, age, differentiation grade and tumor type in colon adenocarcinoma tissues (p>0.05), but there was significant correlation with lymph node metastasis and pathological stage, and the difference was statistically significant (p<0.05) (see Table 3 for details).

*Survival analysis in high and low expression groups*

In the low expression group, the CD74 mRNA expression was ≤2.15 (n=41) and MMP-9 mRNA expression ≤2.73 (n=39), whereas in the high expression group, the CD74 mRNA expression was

**Table 3.** Correlation between expressions of CD74 and MMP-9 mRNAs and clinicopathological characteristics in cancer tissues (mean±SD)

| *Factor* | *CD74 (n=98)* | *t/F* | *p* | *MMP-9 (n=98)* | *t/F* | *p* |
|---|---|---|---|---|---|---|
| Gender | | 0.140 | 0.889 | | 0.162 | 0.872 |
| Male | 2.14±0.68 | | | 2.71±0.93 | | |
| Female | 2.16±0.73 | | | 2.74±0.90 | | |
| Age (years) | | 0.138 | 0.891 | | 0.265 | 0.791 |
| >52 | 2.13±0.73 | | | 2.69±0.89 | | |
| ≤52 | 2.15±0.68 | | | 2.74±0.92 | | |
| Differentiation grade | | 0.655 | 0.425 | | 0.016 | 0.985 |
| Well | 2.04±0.65 | | | 2.70±0.88 | | |
| Moderately | 2.16±0.72 | | | 2.72±0.90 | | |
| Poorly | 2.19±0.81 | | | 2.74±0.93 | | |
| Lymph node metastasis | | 9.957 | <0.001 | | 4.006 | <0.001 |
| Yes | 2.93±0.56 | | | 3.14±0.82 | | |
| No | 1.63±0.72 | | | 2.41±0.95 | | |
| Tumor type | | 0.100 | 0.905 | | 0.034 | 0.966 |
| Lump | 2.05±0.85 | | | 2.66±0.89 | | |
| Infiltrative | 2.11±0.79 | | | 2.72±0.92 | | |
| Ulcerative | 2.15±0.79 | | | 2.69±0.95 | | |
| Pathological stage | | 8.451 | <0.001 | | 6.072 | <0.001 |
| I-II | 1.49±0.66 | | | 2.29±0.96 | | |
| III-IV | 2.85±0.93 | | | 3.41±0.84 | | |

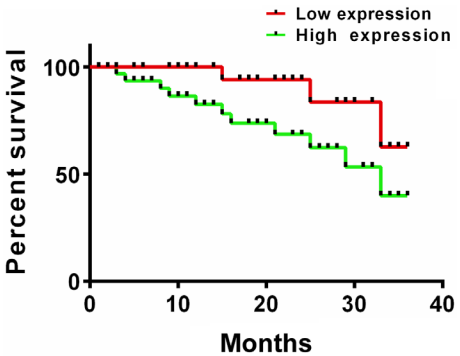


**Figure 3.** Survival analysis in MMP-9 mRNA low and high expression groups. The 3-year survival rate in the MMP-9 low expression group was significantly higher than in the high expression group, with statistically significant difference (p<0.05).

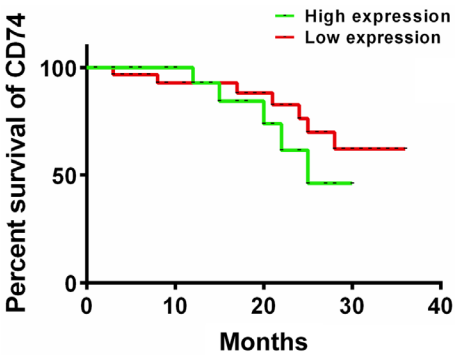


**Figure 2.** Survival analysis in CD74 low and high expression groups. The 3-year survival rate in the CD74 low expression group was significantly higher than that in the high expression group, with statistically significant difference (p<0.05).

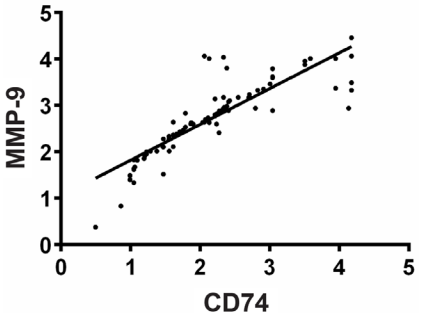


**Figure 4.** Correlation analysis between CD74 and MMP-9 expressions in colon adenocarcinoma. Pearson's correlation analysis showed that the expressions of CD74 and MMP-9 were positively correlated (r = 0.853, p<0.001).

>2.15 (n=57) and MMP-9 mRNA expression >2.73 (n=59). The 3-year survival rates in CD74 low and high expression groups were 65.85% (27 / 41) and 36.84% (21/57) respectively. So the 3-year survival rate in the low expression group was significantly higher than that in the high expression group, with statistically significant difference (p<0.05). The 3-year survival rate in MMP-9 mRNA low and high expression groups were 66.67% (26/39) and 37.29% (22/59) respectively. Therefore, the 3-year survival rate in the low expression group was significantly higher than in the high expression group, with statistically significant difference (p<0.05) (See Figures 2 and 3 for details).

*Correlation analysis between CD74 and MMP-9 expressions in colon adenocarcinoma*

The expressions of CD74 and MMP-9 were positively correlated (r = 0.853, p<0.001), as shown in Figure 4.

## Discussion

Colon cancer is one of the most common malignant tumors of the digestive tract and is also the one of the main causes of cancer-related death in the world [14]. As the most common pathological type of colon cancer, colon adenocarcinoma accounts for 98% of newly diagnosed colon cancer cases [15]. CD74, a type II transmembrane protein, has been shown to be abnormally expressed in various malignant tumors [16]. Besides, some studies show that the high expression of CD74 in malignant tumors is considered as a marker of tumor progression or poor prognosis [17]. As an important member of MMPs family, MMP-9 can be activated to form type IV collagenase, thus damaging the structure of extracellular matrix and basement membrane by degrading IV, V collagens, gelatin and other components in extracellular matrix, thus leading to invasion and metastasis of tumor cells [18]. However, there is little research on MMP-9 in colon adenocarcinoma.

In our study, the expressions of CD74 and MMP-9 in colon adenocarcinoma tissues and their correlation with clinicopathological characteristics of colon adenocarcinoma were discussed. The results showed that CD74 and MMP-9 mRNAs were highly expressed in colon adenocarcinoma tissues, and the expressions of CD74 and MMP-9 mRNAs in colon adenocarcinoma tissues were significantly related to lymph node metastasis and pathological stage (p<0.05). According to the average expressions of CD74 and MMP-9, the patients were divided into high and low expression groups, and it was found that the 3-year survival rate in the low expression group was significantly higher than in the high expression group. Moreover, there was a positive correlation between CD74 and MMP-9. The above results suggest that patients with high expressions of CD74 and MMP-9 usually have poor prognosis. A previous study [19] found that the increased expression of CD74 promoted the development of tumor cells when exploring the expression of CD74 in bladder urothelial carcinoma. In addition, an experimental study in mice [20] found that CD74 was expressed in CT26 colon cancer cell line, and its stimulation on migration inhibitory factors could lead to increased survival of colon cancer cells, as well as up-regulation of Akt phosphorylation and Bcl-2 expression. Another study [12] showed that the stronger the positive expression of CD74, the worse the prognosis of patients. The above results all confirmed our conclusion. At present, some authors have proposed that CD74 can be used as a new target for the treatment of malignant tumors, and the treatment of multiple myeloma with CD74 antibody also has achieved good therapeutic effects [21,22]. There are relatively few researches on the correlation between MMP-9 and colon adenocarcinoma, but some authors have found that the expression of MMP-9 in breast cancer patients is higher than that in healthy people, and the expression is closely related to lymph node metastasis and tumor stage, which also confirms our conclusion. A previous study [12] has found that the selective inhibition of COX-2 in tumor cells can reduce the expression of MMP-9 and inhibits tumor development. The relevant mechanisms of CD74 and MMP-9 in colon adenocarcinoma have not been explored, which will be a direction for our future research. Finally, through correlation analysis, a positive correlation between CD74 and MMP-9 expressions was found. There is no relevant literature to explain the correlation between CD74 and MMP-9 in the regulation of colon adenocarcinoma cells at present. However, we speculate

that CD74 may regulate the expression of MMP-9 through some signaling pathway or gene.

To sum up, CD74 and MMP-9 are highly expressed in colon adenocarcinoma tissues, and their expressions are closely related to the pathological stage, lymph node metastasis and prognosis of colon adenocarcinoma patients, which can be used as important biological markers for diagnosis and prognosis prediction of colon adenocarcinoma.

## Conflict of interests

The authors declare no conflict of interests.

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