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Expression changes in epithelial cell adhesion molecule during colorectal cancer tumorigenesis

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ABSTRACT. We investigated the relationship between the expression of epithelial cell adhesion molecule (EpCAM) and the occurrence and development of colon cancer. Fifty colon cancer tissues and adjacent normal tissues were collected, while 40 normal intestinal mucosa tissues were collected as the blank group. EpCAM expression was detected by immunohistochemistry and the patients were followed-up to evaluate the prognosis. The positive expression rate of EpCAM reached 93.7% in patients with colorectal carcinoma, which was significantly higher than that in the negative control group and blank group (14.8 and 12.7%, $P < 0.05$, respectively). There was no significant difference between the control group and blank group regarding EpCAM expression. No direct relationship was observed between EpCAM expression and patient age, gender, and other characteristics. EpCAM was overexpressed in colorectal cancer and had a high detection rate. EpCAM can be used as a diagnostic biomarker for clinical detection of colorectal cancer.

Key words: Colorectal cancer; Epithelial cell adhesion molecule; Immunohistochemistry

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INTRODUCTION

Colorectal cancer is a digestive system carcinoma that impairs human health and is highly lethal. Its pathogenesis is primarily caused by the abnormal expression of a variety of oncogenes and tumor suppressor genes induced by external stimulation (Carmon et al., 2011; Spizzo et al., 2011; Clevers and Nusse, 2012). The importance of early detection and timely treatment are well-known key factors in the effective control of tumors (Bowman and Nusse, 2011).

Epithelial cellular adhesion molecule (EpCAM) is a single transmembrane protein encoded by the tumor-associated calcium signal transducer 1 gene, and belongs to the adhesion molecule family. It can regulate cell adhesion functions and participate in signal transduction between cells. EpCAM has a direct relationship with cell proliferation, migration, and differentiation, but is only overexpressed in malignant tumor cells and in a few types of normal cells. Furthermore, EpCAM is a target for cancer diagnosis and treatment and has been considered as the main antigen in colon cancer since it was first discovered in the colon (Spizzo et al., 2011; van Amerongen et al., 2012). Under normal conditions, EpCAM is expressed in a variety of epithelial structures (excluding the squamous epithelium); however, the protein is abnormally expressed in many types of tumors under the pathological state, such as breast cancer and lung cancer. Although the exact regulatory mechanism remains unclear, EpCAM is mainly expressed in the cytoplasm, cell membrane, and tight junction (Maghzal et al., 2013). EpCAM can activate protocarcinogenic gene expression through the Wnt signaling pathway, resulting in tumorigenesis. In many tumorigenesis processes, overexpression of EpCAM often indicates poor prognosis (Yoon et al., 2011). EpCAM overexpression can activate c-Myc to accelerate the cell cycle, promote cell proliferation, and increase cell invasion ability.

EpCAM has a close relationship with the occurrence of numerous tumor types, cancer development, and patient prognosis. In addition, it is often used as a tumor biomarker. EpCAM is one of the most widely expressed surface antigens in tumors, and thus it is currently being investigated as a target for tumor therapy. However, few studies have examined the relationship between EpCAM and colorectal cancer development. We evaluated EpCAM expression using immunohistochemistry and determined the relationship between EpCAM and the occurrence and development of colorectal cancer, providing further support for clinical treatment.

MATERIAL AND METHODS

Patients

Colorectal cancer tissues and adjacent normal controls were collected from 50 patients between January 2013 and January 2014 in our institution. Of these patients, 22 were in stage I, 16 in stage II, and 12 in stage III, based on TNM staging. An additional 40 normal intestinal mucosa samples were selected as the blank control group. None of the patients had received chemoradiation therapy or other immune therapy before operation. Patient survival time was calculated as the surgery date and ended in death or the last follow-up. The protocol of this study was approved by the First Affiliated Hospital, Zhengzhou University (Zhengzhou, China). Informed consent was obtained from all subjects.

Reagents

The StreptAvidin-Biotin Complex immunohistochemical kit and DAB chromogenic

reagent kit were from Takara (Shiga, Japan). Primary antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Immunohistochemical detection

Sections (4-μm-thick) were cut from paraffin blocks and mounted onto adhesive-coated glass slides. Slides were placed in a xylene bath and incubated for 5 min. The xylene in the baths was changed, and the procedure was repeated twice. Slides were then placed in absolute ethanol for 3 min, which was repeated once. Slides were then placed in 95% ethanol for 3 min, and this was repeated once. Slides were finally placed in distilled water for a minimum of 30 s.

The specific program for EpCAM immunostaining consisted of the following steps: 1) endogenous peroxidase blocking by treating slides with 3% hydrogen peroxide and incubation for 5 min; 2) incubation with EpCAM primary antibody (concentration 1:100; R&D Systems, Minneapolis, MN, USA) overnight at 4°C; 3) incubation with peroxidase-labeled secondary antibody for 15 min; 4) incubation with the substrate-chromogen 3,3'-diaminobenzidine tetrahydrochloride (Liquid DAB+, code no. K3467, Dako, Glostrup, Denmark) for 8 min; 5) upon completion of the run, slides were removed from the staining machine and rinsed in Tris-buffered saline; 6) slides were placed in a bath of aqueous hematoxylin (code No. S3309) for 45 s; 7) slides were then gently rinsed in a distilled water bath; 8) slides were dehydrated using the following solutions: 95% ethanol over 3 min with 1 bath change, 100% ethanol over 3 min with 2 bath changes, and xylene over 5 min with 2 bath changes; 9) coverslips were then applied to specimens using routine pathological procedures. The positive EpCAM cells appeared to have yellow granular cytoplasm. EpCAM expression was evaluated as follows: strong positive, positive cells >50%; weak positive, positive cells 15-50%; negative, no positive cells (Spizzo et al., 2011).

Statistical analysis

Enumeration data were analyzed using the chi-square test, while measurement data were analyzed using the Mann-Whitney *t*-test. Data correlation was analyzed using the Spearman correlation test. All statistical analyses were performed using the SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA). P values < 0.05 were considered to be statistically significant.

RESULTS

EpCAM immunohistochemical staining

Pathological sections in each group were tested for EpCAM immunohistochemical staining following the manufacturer instruction. The results are shown in Figure 1.

EpCAM expression analysis

According to the immunohistochemical staining results, EpCAM expression was analyzed. EpCAM expression in cancer tissues was significantly higher than that in carcinoma adjacent tissues and normal tissues, while the positive rates were 92, 5, and 0%, respectively (Table 1).

After analyzing EpCAM expression levels in different cancer stages, we found that

as the tumor malignancy degree increased, the EpCAM expression level gradually increased (Table 2). This indicated that the expression level of EpCAM was positively correlated with tumor malignancy degree.

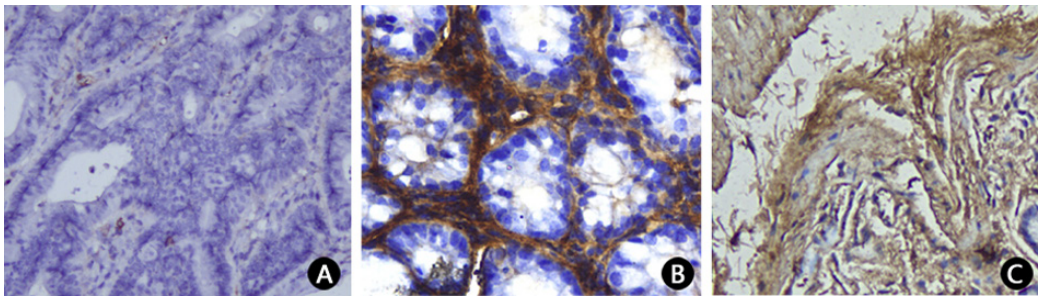

Figure 1. EpCAM immunohistochemical staining. **A.** Negative staining, significant positive particles were not observed on the membrane or in the cytoplasm. **B.** Weak positive staining, light brown particles were observed on the membrane or in the cytoplasm. **C.** Strong positive staining, deep maroon particles were observed in the tissue.

Table 1. Clinical pathological immunohistochemical analysis [number, (%)].

Group	Positive group		Negative group		Blank control	
	Negative	Positive	Negative	Positive	Negative	Positive
EpCAM	4 (8)	46* (92)	45 (90)	5 (10)	40 (100)	0 (0)

*P < 0.05 compared with the negative group and blank control.

Table 2. Immunohistochemical analysis based on different clinical staging [number, (%)].

Group	Stage I		Stage II		Stage III	
	Weak positive	Strong positive	Weak positive	Strong positive	Weak positive	Strong positive
EpCAM	18* (81.8)	4 (18.2)*	5* (31.3)	11 (68.7)*	0 (0)*	12 (100)*

*P < 0.05.

Follow-up statistics

Patient follow-up begun after operation. The survival rate was calculated each year for a total of 5 years (Table 3). Patients with a higher level of EpCAM showed poor prognosis, although no clear differences were observed during the first year between the 2 groups. Over time, the patient survival rate decreased sharply in the high EpCAM group, indicating a negative correlation between EpCAM expression level and patient prognosis.

Table 3. Comparison of 5-year survival rate [number, (%)].

Class	First year	Second year	Third year	Fourth year	Fifth year
EpCAM strong positive	25 (92.6)	18 (66.7)	16 (59.3)	12 (44.4)	10 (37.0)
EpCAM weak positive	21 (91.3)	20 (86.9*)	19 (82.6*)	18 (78.3*)	18 (78.3*)

*P < 0.05.

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DISCUSSION

EpCAM was the first tumor-associated antigen observed in the colorectal cancer tissue; this protein is also known as cluster of differentiation 326, membrane glycosylated protein, and tumor-associated calcium signal transducer 1. Its protein has 3 domains, including extracellular, single transmembrane, and intracellular structures. The extracellular region facilitates EpCAM adherence to other homologous cells, while the intracellular region can combine with actin and interact with the cytoskeleton (Terris et al., 2010). Under physiological conditions, EpCAM is primarily expressed between normal epithelial cells, except the squamous epithelium, but is not expressed in other regions such as the connective tissue. EpCAM is widely expressed in adenocarcinoma (Petsch et al., 2011), and this high expression is negatively correlated with patient prognosis, particularly when postoperative recurrence and lymph node metastasis occurs at a high rate (Lugli et al., 2010; Konigsberg et al., 2011; Imrich et al., 2012; Ni et al., 2013; Schnell et al., 2013). In our study, EpCAM was highly expressed in colorectal cancer tissue, whereas lower or even no expression was observed in adjacent tissue or normal controls, which correlates with EpCAM expression in other tumor types. Using current cancer treatment methods, monoclonal antibodies, double specificity antibodies, and tumor immunotherapy show good clinical curative effects and application prospects. These treatments were developed based on tumor-associated antigens. Thus, determining the correlation between an antigen and various tumors is very important (Benko et al., 2013; Lin et al., 2013; Torino et al., 2013). EpCAM was highly expressed in a variety of malignant tumors, indicating its potential in treatment as the target antigen. Antibody immune therapy has been used in the treatment of some malignant tumors, but the use of EpCAM in colorectal cancer treatment remains largely unexplored (Varga et al., 2004). Because treatment and clinical evidence remains unclear, we investigated EpCAM expression changes during colorectal cancer development and EpCAM expression in other tumors. Our results showed that EpCAM expression had no direct relationship with the colorectal cancer patient age and gender, which is consistent with the results of previous studies (Shiah et al., 2009; Yamashita et al., 2009); however, EpCAM expression is closely and positively related to tumor type. Patient follow-up showed that increased EpCAM expression greatly impacted prognosis, while the prognosis of patients with strong positive expression was significantly worse than those with weak positive expression. Thus, whether EpCAM is a key regulator of colorectal cancer requires further study. However, we speculate that EpCAM plays an important role in the process of colorectal cancer occurrence and development. Our results are significant for studies of EpCAM target therapy or for developing specific monoclonal antibodies for colorectal cancer treatment.

Conflicts of interest

The authors declare no conflict of interest.

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