Basic Research Paper

Expression and significance of adenomatous polyposis coli, β-catenin, E-cadherin and cyclin D1 in esophageal squamous cell carcinoma assessed by tissue microarray

Hui Peng,1 Xue-Yun Zhong,1,* Kun-Ping Liu2 and Su-Mei Li1

1Department of Pathology; Medical School; Jinan University; Guangzhou, Guangdong P.R. China; 2Department of Pathology; The Fifth Affiliated Hospital; Medical School; Jinan University; Qingyuan, Guangdong P.R. China

Key words: esophageal neoplasms, pathology, tissue microarrays, Wnt signaling transduction pathway, adenomatous polyposis coli (APC), β-catenin, E-cadherin, cyclin D1, diagnosis

Background and Objective: The genesis of esophageal squamous cell carcinoma (ESCC) is a multifactor and multistage process, in which Wnt signaling transduction pathway plays an important role in tumorigenesis and tumor progression. This study was to investigate the roles of four proteins in the Wnt pathway in tumorigenesis of ESCC, and their significances in the early diagnosis of ESCC.

Methods: The expression of adenomatous polyposis coli (APC), β-catenin, E-cadherin and cyclin D1 was detected by immunohistochemistry using tissue microarrays consisting of 199 specimens of ESCC, 164 specimens of normal mucosa, 34 specimens of basal cell hyperplasia and 30 specimens of dysplasia adjacent to cancer tissues. Results: The positive rates of APC and E-cadherin in ESCC were lower than those in the normal group (69.6% vs. 98.0%, p < 0.01; 19.6% vs. 96.3%, p < 0.01). The abnormal expression rates of β-catenin and cyclin D1 in ESCC were higher than those in the normal group (65.5% vs. 1.2%, p < 0.01; 70.9% vs. 0.8%, p < 0.01). In accordance with the following order, normal epithelia → basal cell hyperplasia → dysplasia → ESCC, hypoexpression of APC proteins occurred in ESCC, abnormalities of β-catenin and E-cadherin started to appear in dysplasia, and overexpression of Cyclin D1 emerged from basal cell hyperplasia. From well to poorly differentiated ESCC, the expression of APC, E-cadherin and cyclin D1 were gradually reduced, while β-catenin was increased. The expression of β-catenin was not correlated with APC (r = -0.10, p > 0.05), was negatively correlated with E-cadherin (r = -0.31, p < 0.01) and positively correlated with cyclin D1 (r = 0.49, p < 0.01).

Conclusion: APC, E-cadherin, β-catenin and cyclin D1 may play important roles in tumorigenesis of ESCC. Therefore, detection of E-cadherin, β-catenin and cyclin D1 proteins may be helpful for the early diagnosis of ESCC.

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant tumors in China, and little is known about the molecular mechanism of its tumorigenesis. Multistage processes and multifactors have been proposed for the evolution of ESCC, in which normal squamous epithelia undergo a series of histological and genetic progression towards basal cell hyperplasia, then dysplasia and finally ESCC. The Wnt signaling pathway is essential for the embryonic development in the early stage, and is likewise associated with carcinogenesis when abnormal oncogenes and antioncogenes in this pathway activates the downstream target genes. It is reported that overexpression of cyclin D1 activated by abnormal expression of adenomatous polyposis coli gene (APC) and β-catenin may be an early event of colorectal carcinoma. Although abnormality of the Wnt pathway has been implicated in a number of gastrointestinal adenocarcinomas, there are few reports on the relationship between the Wnt pathway and gastrointestinal squamous carcinoma. In this study, we examined the expression of four proteins, APC, β-catenin, E-cadherin and cyclin D1, which are closely correlated to the Wnt pathway, in ESCC and adjacent mucosa using tissue microarray (TMA)-based immunohistochemistry, and investigated the role of Wnt pathway in the incidence of ESCC, in order to identify valuable indices for early clinical diagnosis of the disease.

Materials and Methods

Materials. In total 199 esophageal cancer specimens used in this study were extractd from patients who had received biopsy without radiotherapy and chemotherapy before operation in the Fifth Affiliated Hospital of Medical School, Jinan University from January, 2001 to December, 2006. There were 125 males and 74 females, aged between 37 to 90 years, with a median age of 65 years. All the cases were diagnosed as squamous cell carcinoma by two pathologists independently. Among them, 34 were well differentiated, 129 were moderately differentiated and 36 were poorly differentiated ESCC.
Preparation of tissue chips. Before sample acquisition, the morphology of HE-stained slide on the paraffin block was observed under a microscope. The locations of typical characteristic morphology of ESCC and the surrounding tissues were circled. Samples were taken from the circled location in the paraffin block using the Beecher Instruments Tissue Arrayer (Silver Springs, MD). For each block, three 1mm cores (more than one was ESCC, the rest were adjacent mucosa) were punched from the circled regions in the donor block and arrayed on the recipient block to ensure the representation of the samples, and avoid missing information due to a loss of tissue cores. In total 199 specimens of ESCC, 164 specimens of the corresponding normal mucosa, 34 specimens of basal cell hyperplasia and 30 specimens of dysplasia were arrayed on the recipient block.

Immunohistochemistry and reagents. Reagents. Working solution of anti-mouse β-catenin monoclonal antibody was bought from Fuzhou Maixin Biotech. Concentrated anti-rabbit APC polyclonal antibody (working dilution 1:70), anti-mouse monoclonal E-cadherin monoclonal antibody (working dilution 1:70) and anti-mouse cyclin D1 monoclonal antibodies (working dilution 1:50), as well as the SP kit and DAB kit were bought from Beijing Zhongshan Biotech.

Immunohistochemistry. All TMA blocks were cut into 3μm-thick sections and seemingly sections were chosen for conventional staining using the HE method, followed by immunostaining of APC, β-catenin, E-cadherin, cyclin D1 using the streptavidin peroxidase (SP) method. All the sections were over heated for antigen retrieval. Internal controls were available on the TMA. For negative control, the primary antibody was replaced by PBS.

Assessment of immunostaining. Brown yellow granules in the cells were defined as positive staining. Results were semiquantitatively scored using the method proposed by Su et al.4 with slight modification. For β-catenin, APC, and E-cadherin, no positive cells or less than 2/3 cells with slight staining or less than 1/3 cells with moderate staining were defined as “-” (negative), while more than 2/3 cells with slight staining or more than 1/3 cells with moderate staining or strong staining were defined as “+” (positive). The staining of cyclin D1 was defined as follows: 0–10% positive cells were recorded as “-”; >10% positive cells were recorded as “+”. If there were more than one observational core in the specimen, the average scores was taken.

Statistical analysis. Data were loaded into EXCEL software and SPSS 13.0 statistical software was used for analysis. Intergroup positive rates were compared using the chi-square test, spearman relation test was used to analyze the relationship among the proteins. A p-value of < 0.05 was considered statistically significant for all procedures.

Table 1  Expression of APC, β-catenin, E-Cadherin and Cyclin D1 proteins in different esophageal tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>APC (cases (%) )</th>
<th>β-catenin (cases (%) )</th>
<th>E-cadherin (cases (%) )</th>
<th>cyclin D1 (cases (%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa</td>
<td>164</td>
<td>161 (98.0)</td>
<td>2 (1.2)</td>
<td>158 (96.3)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>34</td>
<td>34 (100)</td>
<td>0</td>
<td>32 (94.1)</td>
<td>3 (10.0)*</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>30</td>
<td>30 (100)</td>
<td>3 (8.7)b</td>
<td>19 (63.3)#</td>
<td>13 (41.7)#</td>
</tr>
<tr>
<td>ESCC</td>
<td>199</td>
<td>138 (69.6)*</td>
<td>130 (65.5)*</td>
<td>39 (19.6)*</td>
<td>141 (70.9)*</td>
</tr>
<tr>
<td>Well differentiation</td>
<td>34</td>
<td>26 (76.5)4</td>
<td>17 (50.0)</td>
<td>18 (52.9)4</td>
<td>29 (85.2)</td>
</tr>
<tr>
<td>Moderate differentiation</td>
<td>129</td>
<td>88 (68.3)</td>
<td>85 (66.1)</td>
<td>18 (14.0)</td>
<td>96 (74.4)</td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>36</td>
<td>24 (67.7)</td>
<td>28 (77.7)4</td>
<td>3 (8.3)</td>
<td>16 (45.2)4</td>
</tr>
</tbody>
</table>

ESCC: esophageal squamous cell carcinoma. *p < 0.05, vs. normal mucosa; #p < 0.01, vs. normal mucosa and basal cell hyperplasia; 4p < 0.05, vs. normal mucosa, basal cell hyperplasia and dysplasia; 4p < 0.01, vs. moderate differentiation.

Table 2  Correlations of β-catenin expression to APC, E-cadherin, cyclin D1 expression in esophageal squamous cell carcinoma

<table>
<thead>
<tr>
<th>β-catenin</th>
<th>APC (cases)</th>
<th>E-cadherin (cases)</th>
<th>cyclin D1 (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>86</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td>-</td>
<td>52</td>
<td>17</td>
<td>25</td>
</tr>
</tbody>
</table>

P >0.05 <0.01 <0.01

Results

Expressions of APC, β-catenin, E-cadherin and cyclin D1 proteins in different esophageal tissues. Representative immunohistochemical images of APC, β-catenin, E-cadherin and cyclin D1 proteins are shown in Figure 1. Positive expression of APC was located in the cytoplasm and nucleus (Fig. 1A1–A4). β-catenin was expressed in the cytoplasm and nucleus of ESCC, dysplasia, and partial normal and hyperplastic basal layers (Fig. 1B3 and B4). Most E-cadherin was expressed in the cell membrane, while a few were in the cytoplasm (Fig. 1C1 and C2). Cyclin D1 was positively expressed in the nucleus (Fig. 1D1–D4).

In relation to normal mucosa, APC and E-cadherin were hypoexpressed in ESCC, while β-catenin and cyclin D1 were overexpressed. In accordance with the following order, normal epithelia → basal cell hyperplasia → dysplasia → ESCC, abnormalities of APC proteins occurred at ESCC, β-catenin and E-cadherin started from dysplasia, and cyclin D1 emerged from basal cell hyperplasia. With the change of the differentiation degree of ESCC from high to low, expressions of APC, E-cadherin and cyclin D1 were reduced while β-catenin was increased. A summary of the expression of the four proteins in various histological types of esophageal lesions is listed in Table 1.

Relationship between expressions of APC, β-catenin, E-cadherin and cyclin D1 and clinicopathologic characteristics of ESCC. Correlations of sex, age, location and length of the tumor under endoscopy to clinicopathologic characteristics of ESCC were analyzed, and no significant differences were found.

Correlation of β-catenin to APC, E-cadherin, and cyclin D1 in ESCC. The expression of β-catenin was not correlated with APC, but was negatively correlated with E-cadherin and positively correlated with cyclin D1 (Table 2).
Discussion

In normal mature cells, the Wnt pathway regulates normal cellular activities. Most β-catenin within cells binds to E-cadherin on the cell membrane to form a complex-epidermal catenin and cadherin unit (ECCU). Free β-catenin is degraded and polyubiquitinated after binding to a protein complex consisting of molecules, such as APC, to regulate cellular proliferation, differentiation, adhesion and so on. Once the Wnt pathway is activated by abnormal expressions of oncogenes, antioncogenes and cellular adhesion molecule, β-catenin is accumulated in cytoplasm without degradation, and translocated into the nucleus, where it binds to Tcf/Lef and initiates transcription of its target genes such as Cyclin D1, resulting in cellular canceration.1

β-catenin is one of the most important proteins in the Wnt pathway. Accumulation of β-catenin in cytoplasm and nuclei is a signal to activate the Wnt signaling pathway. Overexpression of β-catenin is detected in many cancers.5-6 We found in the study that as the epithelium progressed from normal cells to hyperplasia, to dysplasia, and to ESCC, there was a graduate increase of β-catenin in the cytoplasm and nuclei, which suggests that an increase of
abnormal β-catenin expression is an early event in the tumorigenesis. Moreover, abnormal β-catenin expression is negatively correlated with ESCC differentiation, similar to the findings in colorectal cancers. Overexpression of cyclin D1 is an early event frequently occurs in ESCC, which appears to be an early event. Expression of E-cadherin is positively correlated with ESCC differentiation, similar to the findings in colorectal adenoma and colorectal cancer. Dai et al. reported that the positive rate of APC is significantly lower in colorectal adenoma and colorectal carcinoma than in colorectal adenoma and normal colorectal mucosa, which suggests that APC dysfunction may be a crucial cause to activate the Wnt pathway in colorectal cancers. Dai et al. reported that the positive rate of APC is significantly lower in colorectal adenoma and malignant colorectal adenoma than in colorectal adenoma and normal colorectal mucosa, which suggests that APC dysfunction may be an early event of canceration. However, we did not observe a decrease of APC protein in precancerous tissues, but found strong positive expression of APC in 70% of ESCC specimens, implying that APC may act differently between ESCC and other tumors. Similar results have been reported in oral squamous cell carcinoma. Studies on tumors besides adenocarcinomas of the digestive tract are rare. APC expression may differ among different histological types of digestive cancers.

Study on colorectal cancer progression has shown that reduced APC expression may be an “early” event and reduced E-cadherin expression may be a “late” event in the Wnt pathway. Our study showed a correlation between reduced E-cadherin and increased cytoplasmic and nuclear β-catenin expression, both of which were significantly different between normal and dysplasia tissues, and were associated with cancer differentiation. We speculate that E-cadherin expression may contribute to the development and progress of ESCC, which appears to be an early event.

Cyclin D1 is one of the target oncogenes of the Wnt pathway, whose transcription is activated by the β-catenin/TCF complex. Overexpression of cyclin D1 is an early event frequently occurs in digestive tumors. This is in line with our results, but the relationship between protein and differentiation is in discordance with the report on squamous cell cancer.

Currently, results for the detection of APC, β-catenin, E-cadherin and cyclin D1 are conflicting, owing to different experimental and specimen handing conditions. Therefore, the comparability of these results is questionable. TMA technology, which provides simultaneous analysis for the expressions of these proteins in ESCC specimens under uniform experimental conditions, can solve this problem commendably. We discovered a common point in the expression of APC, β-catenin, E-cadherin and cyclin D1 among ESCC and other carcinomas, which is that they all may possibly play important roles in the Wnt pathway. Additionally, we found that the expressions of the four proteins are different in ESCC and other solid tumors, especially in adenocarcinomas of the digestive tract. For example, APC protein is moderately and strongly expressed in over 70% of ESCC; E-cadherin protein emerges at the early stage of ESCC; reduced expression of cyclin D1 is positively correlated with the differentiation of ESCC, all of which differ from previous studies on adenocarcinomas of the digestive tract. Comparative studies on the effect of Wnt pathway in ESCC and other adenocarcinomas of the digestive tract or squamous cell carcinomas still require further investigation.

Acknowledgements

Grants: National High-Tech R & D Program (863) of China (No. 2006AA02A403)

References