GENETIC VARIATIONS OF THE CTNNA1 AND THE CTNNB1 GENES IN SPORADIC COLORECTAL CANCER IN POLISH POPULATION*

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Experimental as well as clinical observations have demonstrated that the E-cadherin/catenin complex is a powerful inhibitor of invasion. Abrogation of this pathway is implicated in the carcinogenesis of several malignancies, especially colorectal cancer.

The aim of the study was to determine the CTNNA1 and the CTNNB1 mutations and its relationship to clinical and pathological features of sporadic colorectal cancer (CRC) in Polish patients.

Material and methods. Paired tumor and normal tissue samples from 110 sporadic CRC patients undergoing resective surgery were prospectively studied for the alpha catenin (CTNNA1) gene and beta catenin (CTNNB1) gene mutations by PCR/single strand conformation polymorphism (SSCP).

Results. The CTNNA1 gene alteration in exon 7 were detected in 4 samples and in exon 3 of CTNNB1 gene were found in 3 samples. There was a trend at the limit of statistical significance associating younger age at diagnosis (<50) with CTNNA1 and the CTNNB1 mutations. The mutation of CTNNB1 seemed to occur more frequently in the proximal colon than distal. The CRC patients with CTNNA1 mutation had a significantly increased lymph node metastasis. On the other hand, there was no correlation between mutations and the other clinical variables (e.g. sex, grade and depth of invasion).

Conclusion. Although we found a low frequency of mutations in the CTNNA1 and the CTNNB1 genes, but the analysis the relationship with clinical and pathological features of CRC patients may indicated an association of these mutations with the risk and progression of CRC.

Key words: sporadic colorectal cancer, mutations, catenin gene, adhesion molecules

Epithelial cell-cell junctions, organized by adhesion proteins and the underlying actin cytoskeleton, are considered to be stable structures maintaining the structural integrity of tissues. The adherens junction complex is composed of a transmembrane protein, E-cadherin, and catenins. Alpha -catenin and beta-catenin link cadherins to the cytoskeleton at adherens junctions (1). Intercellular and cell-substratum interactions mediated by adhesion molecules are likely to play a part both in the structural morphology and functional differentiation of the tissue and therefore a loss in this control mechanism may well facilitate the neoplastic process (2, 3). Dysregulation or mislocalization of cell adhesion molecules and their regulators, usually correlates with loss of polarity, dedifferentiation, invasive tumor growth, and metastasis (4). Beta-catenin also associates with members of the T-cell factor (Tcf) family of transcription factors, and mutations in CTNNB1 lead to activation of Tcf-dependent transcription and increased cell growth. Hyperactivation of Wnt signaling, mostly by affecting beta-catenin functions, is a hallmark of colon cancer and of many other

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human cancers (5). The expression of alpha-catenin is often reduced during tumor progression, and several cancer-derived cell lines have mutations in the alpha-catenin gene (6, 7). Reintroduction of alpha-catenin into such lines reduces cell growth and attenuates tumor formation (8). Additionally it has been observed that alpha-catenin expression increases during adenoma formation (9). Although the tumor suppressor function of alpha-catenin is generally believed to result from its promotion of cell-cell adhesion, recent study suggest that alpha-catenin influences tumor progression by regulating beta-catenin signaling. It was demonstrated that alpha-catenin inhibits beta-catenin signaling in the nucleus by interfering with the formation of a beta-catenin Tcf DNA complex (10).

A combination of intact E-cadherin, alpha-catenin, and beta-catenin provides favorable prognostic value for patients with colorectal cancer, whereas a lack of any of these proteins is correlated with a poorer prognosis (11, 12). Moreover mutations in alpha catenin, beta-catenin, and E-cadherin genes have been found in some human cancers, and are often prognostic markers for poor clinical outcome in colon cancer (13, 14). In the present study, we investigated whether alpha-catenin (CTNNA1) and beta-catenin (CTNNB1) gene mutations were prone to occur in colorectal cancers in Polish population.

MATERIAL AND METHODS

Patients

Paired tumor and normal tissue specimens from 110 sporadic colorectal cancer patients were collected from the Department of General and Colorectal Surgery Medical University of Lodz. The excision of the primary tumor was histologically proven by examination of the resected margins. All tumors were histologically confirmed to be CRC including 99 cases tubular adenocarcinoma and 21 cases mucoid adenocarcinoma. There were 69 males and 51 females, the man age was 57 years (range: 37-85). The tumor was located in proximal colon (caecum, ascending and transverse colon) in 44 patients and distal (descending/sigmoid, rectum) in 76 cases. Tumor size was T1 in 4 patients, T2 in 46, T3 in 64 and T4 in 6 patients. In 46 of CRC patients were detected lymph node metastasis and in 5 patients were detected present of distant metastases. The histological grades of tumors were determined according to a three degrees' scale as follows; 23 grade I°, 79 grade II° and 18 grade III° tumors.

Detection of CTNNA1 and CTNNB1 genes mutation

DNA for genotyping was isolated from paired tumor and normal tissue specimens of CRC patients by used a commercial kit QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA (Qiagen). Detection of mutations exon 7 of the alpha-catenin gene (CTNNA1) and exon 3 of the beta-catenin (CTNNB1) gene was carried out by SSCP-PCR analysis. Mutation analysis of exon 7 of CTNNA1 gene was performed by amplification of the 295-bp fragment using the following primers: E7 forward: 5’-AAG AAG GGA ACA GAG ATG A -3’, E7 reverse:5’-TCC ATA AAT ATC TTA CTT CA-3’(15). Mutations in exon 3 of CTNNB1 gene were detected by amplification of 298-bp fragment using the following primers: E3 forward 5’-ACA AAC TGT TTT GAA AAT CCA–3’ and E3 reverse 5’- CGA GTC ATT GCA TAC TGT CC -3’ (16). PCR amplification was carried out as follows: 25 μl reaction containing 50 ng DNA, 10 mm Tris–HCl (pH 8.3), 3.5 mm MgCl₂, 0.2 mm dNTP, 10 pmol each primer and 1.5 U Taq polymerase (Qiagen). PCR amplification was performed under the following conditions: one cycle at 98°C for 120 seconds, 35 cycles (30 s at 94°C, 30 s at 55°C and 1.5 min at 72°C), followed by a final cycle of 5 minutes at 72°C. The PCR product was separated on a 1.5% agarose gel and visualized by ethidium bromide staining. Five μl PCR product was diluted in 5 μl denaturation buffer (95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol). The mixture was heated at 99°C for 2 min and after denaturation it was subsequently chilled on ice. Four μl of this mixture was run on a 7% polyacrylamide non-denaturing gel containing 6% glycerol at 40 W for 5 hours at room temperature. The DNA was visualized using modified silver staining.

All statistical analysis was performed using STATISTICA software, version 8.0 PL (StatSoft Inc.). The chi² test was used to determine
associations of the CTNNA1 and CTNNB1 mutational status with various clinicopathologic parameters. Statistical significance was set at p < 0.05.

RESULTS

The CTNNA1 gene alteration in exon 7 were detected in 4 samples and in exon 3 of CTNNB1 gene were found in 3 samples of 110 colorectal carcinomas. All of the CTNNA1 and the CTNNB1 mutations from cancer tissues were somatic mutations because DNA from the matched normal tissue was proven to be a wild type. The mutational analysis and clinicopathologic features are summarized in tab. 1. There was a trend at the limit of statistical significance associating younger age at diagnosis (<50) with CTNNA1 and the CTNNB1 mutations. There was no correlation with gender. The mutations of CTNNA1 and the CTNNB1 seemed to occur more frequently in the proximal colon than distal. Tumor grade was classified into well differentiated (grade I), moderately differentiated (grade II) and poorly differentiate adenocarcinomas (grade III). Nevertheless, no significant differences were observed in mutations frequencies and different tumors grade. Tumor stage was also classified according to the TNM staging. The CTNNA1 and the CTNNB1 mutations had no statistical significance association with the pathological assessment of the primary tumour (pT). Tumors with CTNNA1 mutations were more likely to have metastatic spread to the lymph nodes than those with wild-type. However, mutations in both genes were not correlated with distant colorectal cancer metastasis.

DISCUSSION

Alterations in the Wnt pathway are among the most common pathogenic events associated with colorectal carcinogenesis. The majority of CTNNB1 gene mutations have been reported at specific GSK-3β phosphorylation sites encoding by exon 3, resulting in decreased APC associated degradation, raised betacatenin protein levels, and increased TCF4 transcriptional activation (17). It was known that the CTNNB1 gene mutations were uncom-

Table 1. Correlation of the CTNNA1 and CTNNB1 mutational status with clinicopathologic features of CRC patients

<table>
<thead>
<tr>
<th>Clinicopathologic Features</th>
<th>Exon 7 CTNNA1</th>
<th>Exon 3 CTNNB1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mutation</td>
<td>wild type</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 50</td>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>≤ 50</td>
<td>1</td>
<td>74</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>female</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>Location tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>proximal</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>distal</td>
<td>1</td>
<td>75</td>
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<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>76</td>
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<tr>
<td>III</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Tumor invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1 pT2</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>pT3 pT4</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>Node invasion</td>
<td></td>
<td></td>
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<tr>
<td>N0</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>N1</td>
<td>4</td>
<td>42</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
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<tr>
<td>M0</td>
<td>4</td>
<td>111</td>
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<tr>
<td>M1</td>
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mon in sporadic colorectal carcinomas, ranging from 0% to 16% (13, 14, 17, 18, 19). Additionally, mutations in exon 3 of CTNNB1 occurred more frequently in tumor with high-frequency microsatellite instability (MSI-H) than in those with microsatellite-stable (MSS) (13, 18). Similarly with the previous results, in our study we detected CTNNB1 gene mutations only in 3 CRC patients, that is, only 2.7% subjects. Interestingly, in two of these patients we observed also the loss of hMSH2 gene expression (20). hMSH2 is involved in the correction of mispairing during replication and its mutation is associated with microsatellite instability. These suggest that the CTNNB1 mutations found in our cohort of sporadic colorectal tumors may be related with MSI. Unlike beta-catenin, however, the contribution of alpha-catenin to colorectal carcinogenesis remains unclear. Although loss of alpha-catenin expression is associated with the regulatory mechanisms leading to invasive phenotype and progressive disease in CRC, the CTNNA1 gene mutations were rarely detected in theses tumors (8). Also, in our study we observed changes in the exon 7 of CTNNA1 gene only in 4 cases of sporadic CRC (3.6%). The exon 7 CTNNA1 gene encoding the vinculin-, α-actinin- and formin-1-binding domains on alpha-catenin facilitate organization of the F-actin cytoskeleton and the regulation of actin dynamics during the formation of stable intercellular adhesions (3). Therefore mutations in this region may lead to loss of tumor suppression by alpha-catenin. The nonsense mutation was found in colonic cancer cells HCT-8 resulting in premature stop codon that truncates the protein (8).

Although we found a low frequency of mutations in both genes, but the analysis the relationship with clinical and pathological features of CRC patients may indicated an association of these mutations with the risk and progression of CRC. The CTNNA1 and the CTNNB1 mutations tend to be more frequently found in younger age at diagnosis (<50). These results may suggest that alteration in both of these genes gives an significant increased lifetime risk of developing CRC. All 3 of the CTNNB1 mutations were found in proximal colon cancers. The present observation that the CTNNB1 mutation were significantly more frequent in carcinomas located in the proximal colon than in the rectum is in accordance with previous findings, both in adenomas and carcinomas (13, 14). According to our data, CTNNB1 mutations, were often in the proximal colon tumors to. The higher frequency of CTNNB1 mutations among proximal colonic tumors could result from the preferential proximal location of sporadic MSI-H tumors. More recently, it has been also reported that the proximal and distal colon have distinct and specific gene expression profiles (21) and follow different tumorigenic pathways (22), which further supports the selection for specific WNT signaling levels in these two regions. The correlation between alpha and beta-catenin expression pattern and the TNM categories is a controversial issue. Some studies report that there is no correlation between alpha-catenin and beta-catenin these clinicopathological variables (23). On the other hand, it was demonstrated that reduced expression of alpha-catenin or/and beta-catenin was significantly correlated with the depth of invasion (24). Moreover, the frequency of lymph node metastases was significantly higher in those tumors with reduced alpha-catenin expression (12). Our data show that alteration only in CTNNA1 gene have a correlation with to increased lymph node involvement. This is in line with the previous reports in colon cancer cell lines, where the mutation in CTNNA1 gene has been associated with the transition from the non-invasive towards the invasive phenotype. Additionally, in a previous study of samples from colorectal carcinoma patients, many of the tumors examined had reduced expression of either E-cadherin (29%) or alpha-catenin (56%), but increased tumor cell invasion and metastasis correlated with reduced expression of alpha-catenin (24). This suggests that alpha-catenin may function in an adhesion-independent manner to regulate invasion and metastasis. Giannini et al showed that the alpha-catenin disrupted the interaction between the beta-catenin Tcf complex and DNA in vitro and repress transcription of genes involved in invasive phenotypes (10).

Taken together, the present study support that mutation in CTNNA1 and CTNNB1 gene are rare in Polish patients with nonfamilial colorectal carcinomas but occurrence of this mutation may be increased risk of CRC progression.
Cancer correlates with invasiveness, metastatic Level of alpha-catenin expression in colorectal Proc natl acad sci usa.

nin is essential in intestinal adenoma formation. 9.

α-catenin gene in human prostate cancer cells. Oncogene

sion-suppressor gene in human colon cancer cells. -.8.

- catenin inhibits beta-catenin signaling by preven


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