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Expression Level of Genes Coding for Cell Adhesion Molecules of Cadherin Group in Colorectal Cancer Patients

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Data Collection B
Statistical Analysis C
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Background: Colorectal Cancer (CRC) is one of the most frequently diagnosed neoplasms and also one of the main death causes. Cell adhesion molecules are taking part in specific junctions, contributing to tissue integrality. Lower expression of the cadherins may be correlated with poorer differentiation of the CRC, and its more aggressive phenotype. The aim of the study is to designate the cadherin genes potentially useful for the diagnostics, prognostics, and the treatment of CRC.

Material/Method: Specimens were collected from 28 persons (14 female and 14 male), who were operated for CRC. The molecular analysis was performed using oligonucleotide microarrays, mRNA used was collected from adenocarcinoma, and macroscopically healthy tissue. The results were validated using qRT-PCR technique.

Results: Agglomerative hierarchical clustering of normalized mRNA levels has shown 4 groups with statistically different gene expression. The control group was divided into 2 groups, the one was appropriate control (C1), the second (C2) had the genetic properties of the CRC, without pathological changes histologically and macroscopically. The other 2 groups were: LSC (Low stage cancer) and HSC (High stage cancer). Consolidated results of the fluorescency of all of the differential genes, designated two coding E-cadherin (CDH1) with the lower expression, and P-cadherin (CDH3) with higher expression in CRC tissue.

Conclusions: The levels of genes expression are different for several groups of cadherins, and are related with the stage of CRC, therefore could be potentially the useful marker of the stage of the disease, also applicable in treatment and diagnostics of CRC.

MeSH Keywords: **Cadherins • Cell Adhesion Molecules • Colorectal Neoplasms • Oligonucleotide Array Sequence Analysis**

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Background

Colorectal cancer (CRC) is a common and lethal disease. In 2008, Europe reported 436 000 cases of CRC, which constitutes 13.6% of the total number of cancers and makes it the most common malignant tumor on the continent. It is also the second most common cause of death from malignant tumors, after lung cancer (212 000, 12.3%). This means that in 2008, there were 1.7 million cancer deaths and 3.2 million new cases of cancer [1]. It will constitute approximately 9.7% of all new cases of malignant tumors. CRC is also one of the most commonly diagnosed diseases (incidence rate is 17.3/100 000 per year: 20.4 for men and 14.6 for women). On a positive note, despite growth in the number of new cases by 87 000 in 2004–2008, number of deaths from CRC increased only by 8000 per year [2]. This is certainly attributable to better diagnostics and therapies for colorectal cancer, including the development of molecular techniques.

Cadherins are well-studied cell adhesion molecules considered to be tumor suppressors. It should be emphasized that reduction of expression of cell adhesion molecules of E-cadherin group leads to promotion of tumor development [3].

Cadherins consist of 3 domains, and through their extracellular domain they link to a molecule of an adjacent cell. This bond is formed only in the presence of calcium ions. They participate in cell-cell adhesion to create adherens junctions (Zonulae adherens*)* by binding with their intracellular domain to the cytoskeleton of the cell via proteins of the catenin group (subsequently, beta and alfa), and thereby they are the condition for preservation of tissue integrity [4]. The E-cadherin/beta-catenin complex is frequently described as an important predictor; decreased expression may suggest that additional treatment such as radio- or chemotherapy may be required [5], particularly if there is a risk of distant metastasis [6].

Disruptions in expression of epithelial cadherin (E-cadherin coded by gene *CDH1*) will accompany the processes related to epithelial-mesenchymal transition (EMT), which is of key importance to tumor development and metastasis [4,7,8]. These are very complex and multivariate processes. The EMT process itself involves several signaling pathways such as Wnt/beta-catenin, TGF-beta, TNF-alpha, RAS, ILK (integrin-linked kinase), NF-kappa beta, HIF, AKT, or EGFR [4]. Attempts were made to take advantage of this phenomenon to hamper tumor development processes by targeting the CRC cell lines with potassium ionophore (nigericin), whose purpose was to slow down the whole process [8].

The impact of decreased E-cadherin expression in the context of various tumors has been described for neoplasms of the central nervous system such as meningiomas, gliomas, astrocytomas, and neuromas [9], laryngeal cancer [10], thyroid cancer [11], esophageal cancer [12], stomach cancer [5,13] and small intestine adenocarcinoma [14], and also small-cell lung carcinoma [15], hepatocellular carcinoma [16] and cholangiocarcinoma [17]. Decreased E-cadherin expression was also observed in breast cancer [5,7,18], ovarian cancer [19], cervical cancer [20], endometrial cancer [21], and prostate cancer [22]. The same is the case with CRC [23–27], which is also discussed in other publications.

In most cases CRC develops on the basis of changes in progenitor cells such as *Adenomatous Aberrant Crypt Foci* (ACF), of which there are 2 types: ACF involving mutation of ras proto-oncogene featuring hyperplastic polyps, and ACF involving mutation around the APC gene (found in 80% of sporadic CRC cases) featuring microadenomas. These changes are accompanied in the earliest stages by changes in expression of cell adhesion molecules of E-cadherin group, where inactivation of the APC/beta-catenin pathway was observed. Changes in expression of genes coding for cell adhesion molecules of the E-cadherin group will also accompany the processes related to progression of the mature tumor, where loss of adhesion properties of primary neoplasm cells condition its potential for metastases [28].

Another cell adhesion molecule of the cadherin group, whose expression is linked to the development of CRC, is the placental cadherin coded by gene *CDH3*. Its structure is typical of the structure of other molecules of the cadherin group and it also creates links between adjacent cells. However, in this case, because their extracellular domains are located differently with respect to each other, this link is significantly weaker than in cell adhesion molecules of the E-cadherin group. In this case, the bond is also formed in the presence of calcium, and the signal is conducted via proteins of the catenin group, which ultimately change the cytoskeleton conformation of the cell [29]. Unlike in cell adhesion molecules of the E-cadherin group, the expression of placental cadherin is significantly higher in tumor tissue than in healthy tissue. This applies to several tumors, including, inter alia, pancreatic cancer, testicular cancer, cholangiocarcinoma, lung cancer, stomach cancer, cervical cancer, and CRC. Due to their characteristics, these proteins are considered for cancer immunotherapy applications [30]. This has been recently confirmed by the newest findings presented by Yoshioka et al. concerning use of yttrium-labeled antibodies in mice against *CDH3*/P-cadherin (mAb-6) in CRC neoplasms and lung cancer [31]. Taking into account the already solid position of biologic targeted therapy, which at the present time is increasingly used in clinical practice and in CRC treatment, these findings are particularly interesting [32]. Even more interesting are the findings presented by van Marck et al., who report that expression of P-cadherin, like E-cadherin, increases the adhesion properties of tumor cells in CRC, although these properties are lost as the tumor develops [33]. There are also descriptions of epithelial-mesenchymal transition (EMT), which was observed during research on expression of E-cadherin, P-cadherin and beta-catenin [34].

The purpose of the present study was to identify genes coding for cell adhesion molecules of the cadherin group, with potential benefits related to early colorectal cancer detection and diagnostics.

Material and Methods

Tests were conducted on tissue samples obtained from patients who underwent standard surgical resections due to CRC. Transcriptomes of genes coding for cadherins in biopsy specimens obtained from the center of the lesion and from the surgical incision line (control) were designated by oligonucleotide microarrays. Next, variations in the profile of mRNA concentrations in intestinal biopsy specimens were evaluated, depending on the stage of disease progression. Validation of the microarray analysis was conducted with qRT-PCR technique.

Material for tests was obtained from a total of 64 patients (treated in the home institution – Clinical Department of General, Colorectal, and Trauma Surgery of the School of Health Sciences, Medical University of Silesia. These patients underwent standard resections of the large intestine due to CRC, which varied depending on location of lesions and progression of the disease. Tests were conducted with the consent of the Bioethics Commission of the Medical University of Silesia (KNW-6501-70/I/08), and they were performed on the condition that the patient had expressed informed consent. Molecular testing was conducted in the Molecular Biology Department of the School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec.

The criteria adopted for inclusion in and exclusion from tests are described below. Inclusion criteria were: CRC patients in all stages of cancer progression, patients treated through elective surgery (open abdominal surgery, abdominoperineal resection), excluding transanal endoscopic microsurgery and endoscopy procedures, and patient informed consent for participation in tests (on all stages of tests). Exclusion criteria were: patients on whom resurgery was performed due to primary illness, lack of histopathological confirmation for CRC, and patients with genetic diseases, either systemic or metabolic (excluding obesity as an isolated disease). After considering the foregoing criteria, 14 patients (6 women and 8 men) aged 39–86 years were qualified for further tests.

Material was obtained from the surgery which consisted in resection (performed in compliance with relevant standards) of the pertinent section of the large intestine. Material for tests included samples of healthy intestinal tissue and tumor tissue. Fragments of healthy intestine were extracted from the tissue, which did not show any macroscopic changes and was the most distant from the changed part of the intestine. Tumor tissue was obtained from the internal margin to avoid presence of dead tissue in the specimen. Tumor tissue was then divided into 2 parts. One part was sent to standard histopathological evaluation, while the other was sent to molecular analysis. Material was obtained immediately after extracting the resected fragment of the intestine, and processing of tumor tissue was limited as much as possible. Tissue was prepared only through classic surgical techniques. No electric or ultrasound instruments were used. Until molecular analysis, the material was kept in *RNAlater™ (QIAGEN)* stabilization reagent to prevent decay.

RNA extraction

After tissue homogenization, mRNA was extracted with use of *Trizol™* reagent *(Invitrogen™)* according to the manufacturer's protocol. After obtaining RNA, extracts were treated with DNase I in spin columns of *RNeasy Mini Kit (QIAGEN)* kit. Extracted RNA was tested quantitatively and qualitatively. Absorbance was measured with use of *GeneQuant II (Pharmacia BioTech)* spectrophotometer. Qualitative evaluation of RNA extracts was performed through electrophoresis in 0.8% agar gel stained with ethidium bromide.

Analysis with the technique of oligonucleotide microarrays

Analysis of the expression profile was performed with microarrays HG-U133A (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations. Obtained total cellular RNA was used for synthesis of double-stranded DNA (dsDNA) using *SuperScript™ Choice System (Invitrogen™)*. It was subsequently extracted in aqueous sample with phenol and chloroform using *Phase Lock Gel Light™ (Eppendorf)*. Qualitative evaluation was performed through agar gel electrophoresis. The dsDNA obtained was a substrate for obtaining the complementary biotinylated cRNA. This was done using the *BioArray™ HighYield™ RNA Transcript Labeling Kit (Enzo®)*. Qualitative evaluation was performed through electrophoresis according to the method described above. After fragmentation (using *GeneChip® Sample Cleanup Module (QIAGEN))*, qualitative evaluation was once again performed through electrophoresis. The obtained substrate was used as the basis for obtaining the hybridization mix using *GeneChip® Expression 3'-Amplification Reagents Hybridization Control Kit (Affymetrix®)* according to the *Gene Expression Analysis Technical Manual (Affymetrix®)*. In the next stage, hybridization with *GeneChip® Human Genome U133A (Affymetrix®)* microarray was performed. Staining with streptavidin phycoerythrin conjugate and rinsing was conducted

Table 1. Data of the starters used for amplification of fragments of genes CDH1, CDH3, GAPDH and β-actin.

Starter's name	Oligonucleotide sequence	Amplimer length	Gene	Location in the gene	Source of sequence
CDH1 F	5'-TGGGCCAGGAAATCACATCC-3'	140 bp	Cadherin 1	1627–1646	GeneBank NM004360
CDH1 R	5'-CTCAGCCCGAGTGGAAATGG-3'			1746–1765	
CDH3 F	5'-CCCCCAGAAGTACGAGGCCCA-3'	103 bp	Cadherin 3	2119–2139	GeneBank NM004360
CDH3 R	5'-ACGCCACGCTGGTGAGTTGG-3'			2202–2222	
βF	5'-TCACCCACACTgTgCCCATCTACgA-3'	295 bp	β-actin	2141–2165	GeneBank NM_001101
βP	5'-CAgCggAACCgCTCATTgCCAATgg-3'			2411–2435	
GAPDH F	5'-GAAGGTGAAGGTCGGAGTC-3'	226 bp	glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA	108–126 pz	GeneBank NM_002046
GAPDH R	5'-GAAGATGGTGATGGGATTTC-3'			333–314 pz	

according to the recommendations of the *Gene Expression Analysis Technical Manual (Affymetrix®)*. Fluorescence intensity was evaluated using *HP GeneArray Scanner G2500A (Agilent Technologies)*.

Validation of results with qRT-PCR technique

Validation was performed for CDH1 and CDH3 genes, which had been selected using appropriate statistical methods. It consisted in quantitative reverse transcriptase amplification using *Opticon® DNA Engine Sequence Detector (MJ Research®)*. Quantification of amplification products was performed using *QuantiTect™ SYBR® Green RT-PCR Kit (QIAGEN)*. The quantity of mRNA of CDH1 and CDH3 genes and endogenous control in the form of GAPDH was determined on the basis of kinetics of the RT-PCR reaction. Starters used in mRNA detection came from the Laboratory of DNA Sequencing and Oligonucleotide Synthesis of IBB PAN (Instytut Biochemii i Fizyki Polskiej Akademii Nauk, Poland) (Table1).

Specificity of qRT-PCR reaction was evaluated on the basis of electrophoresis in 6% polyacrylamide gel. An additional test involved designating the melting curve of DNA amplimer, which was designated after completing amplification with use of *SYBR® Green 1 (QIAGEN)* fluorochrome. Tests proved the synthesis of only the specific products of the reaction, which was reflected by the presence of 1 curve on amplimer dissociation curves.

Statistical analysis

Before beginning the statistical analysis proper, the results of mRNA fluorescence analysis of the tested genes were subjected to normalization using the *RMA Express (Ben Bolstad)* program. To allow additional comparison of the obtained results, the analysis was performed independently using 2 statistical programs: *Statistica v10 (StatSoft® Poland)* for full gene panel and *GeneSpring GX 11.0 (Agilent Technologies)* for genes coding for cadherins.

Results

After initial acceptance of transcriptomes for comparative analysis, according to the microarray manufacturer's (Affymetrix) guidelines, we conducted the analysis of consistency of biopsy specimens' clustering, which was based on the clinical and histopathological analysis and the molecular analysis.

The results showed that, although on the basis of clinical and histopathological analysis, the biopsy specimens were divided into 5 groups – the control group and 4 groups of adenocarcinoma (CSI-CSIV) – varying in stage of disease progression. Then, on the basis of the profile of mRNA concentrations, the biopsy specimens were divided into 4 groups – 2 control groups (C1 and C2) evaluated through histopathological analysis as specimens of healthy intestine, and 2 groups of adenocarcinoma in low stage of progression (LSC) (CS1) and high stage of progression (HSC) (CS2-CS4) (Figure 1).

In the next stage of the analysis, we designated the descriptive statistics parameters (median and interquartile range) which provide visualization of mRNA fluorescent signals in the indicated groups of transcriptomes (Figure 2).

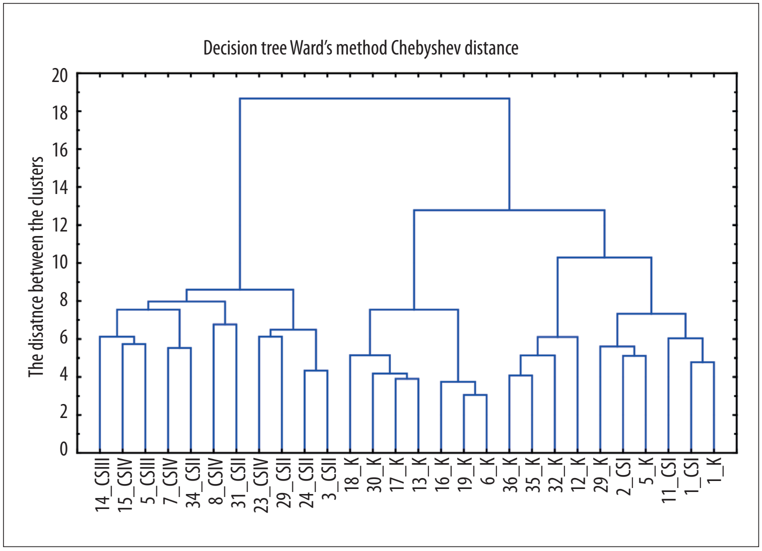

Figure 1. Agglomerative hierarchical clustering of the profiles of normalized levels of mRNA in transcriptomes using microarrays *GeneChip® Human Genome U133A (Affymetrix®)*. Vertical axis: The distance between the clusters. Horizontal axis: Probes.

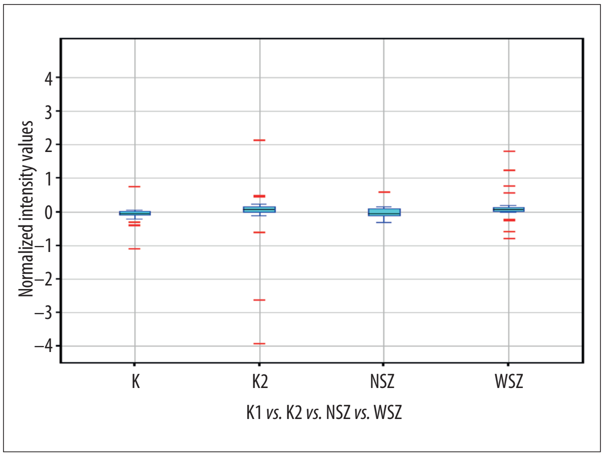

Figure 2. mRNA fluorescent signals in the indicated groups of transcriptomes.

The results show that the profile of 28 cadherin mRNA concentrations changes depending on the stage of disease progression. However, we still did not know whether the observed differences were statistically significant. Therefore, we used analysis of variance (ANOVA), which showed that for 28 cadherin ID mRNA concentrations, statistically significant differences were observed for 4 cadherin mRNA concentrations, assuming $p<0.05$ and FC parameter >2 (log2). To find out which groups of transcriptomes differentiate the indicated genes, we conducted the Tukey's HSD post hoc test to obtain the specific number of ID mRNA differentiating the analyzed groups (Table 2, Figure 3).

In addition, selection of differentiation genes was performed using the CLEAR-test algorithm [35], which features a method of analysis by combining inference for differential expression and variability of genes from individual groups. To identify statistically significant differences in gene expressions, we compared the individual groups obtained through hierarchical grouping of profiles of normalized mRNA concentrations (Table 3).

Among the analyzed genes coding for the cell adhesion molecules of the cadherin group, the most statistically significant differences in gene expression were observed in 2 homologous genes: CDH1 coding for E-cadherin (higher expression in healthy tissue) and CDH3 coding for P-cadherin (higher expression in adenocarcinoma tissue).

Validation of the results obtained by microarrays was conducted with QRT_PCR technique for genes CDH1 and CDH3, which had been selected as genes differentiating between the 2 independent statistical tests. The assessment of the profile of expression was performed with reference to endogenous control in the form of GAPDH. Differences in expression, which take into account the characteristics of the previously selected groups, are consistent with the previously observed regularities.

Discussion

Although surgery still plays the most important role in treatment of CRC, at the present time, especially in later stages of tumor progression, it is not practically used without any supplementary treatment. At this point, it is not just radiotherapy and chemotherapy, but also biologic targeted therapy, which is becoming increasingly popular. In the introduction, we only hinted at the possibility of practical application of BTC in CRC treatment; however, new effectors for such therapies and the possible ways of influencing them are being studied. Of course, the problem itself is much more complex and solving it requires deeper understanding of cell molecular pathways, which may affect the cell properties at the moment of transformation into

Table 2. Number of mRNA ID differentiating the analyzed groups.

A	p value	All IDmRNA	p<0.05	p<0.02	p<0.01	p<0.005	p<0.001
	Number of IDmRNA	28	4	0	0	0	0

B	Group of transcriptomes	C2	C1	LSC	HSC
	C2	4	1 CDH5	1 CDH5	1 CDH13
	C1	3	4	0	3 CDH1; CDH3; CDH13
	LSC	3	4	4	2 CDH3; CDH13
	HSC	3	1	2	4

A – number of genes expression changes according to p value; B – group of transcriptomes shows comparison between individual groups with gene names with statistically different gene expression; C1 – control group 1; C2 – control group 2; LSC – low stage cancer; HSC – high stage cancer.

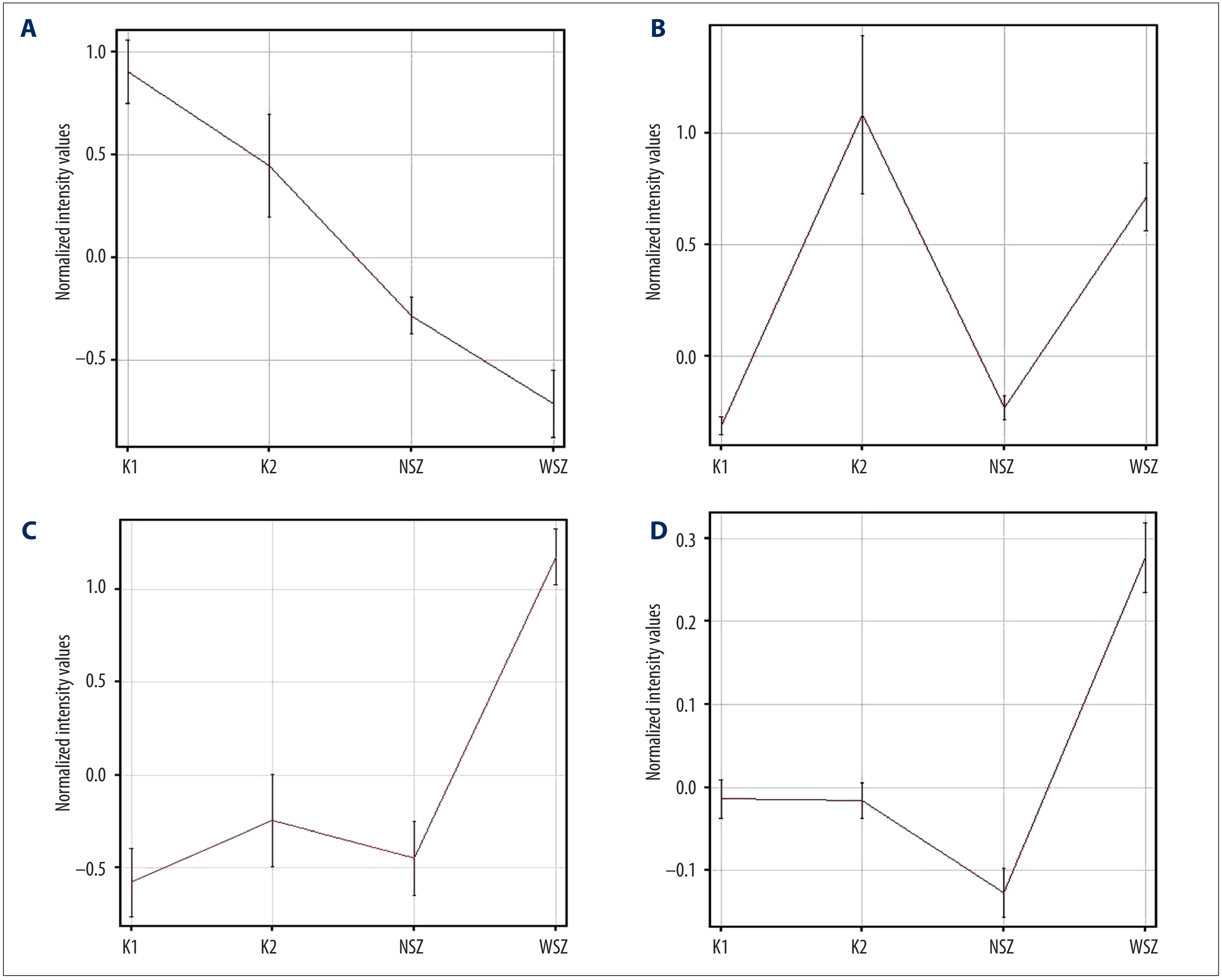

Figure 3. Variations in the profile of mRNA expression of cadherins selected as differentiating cadherins, designated by oligonucleotide microarrays. (**A**) CDH1, (**B**) CDH3, (**C**) CDH5, (**D**) CDH13.

Table 3. Change in the profile of expression of mRNA cadherins in individual analyzed groups for IDmRNAs that were selected as differentiating.

IDmRNA	C1 *vs.* LSC	C1 *vs.* HSC	C2 *vs.* LSC	C2 *vs.* HSC	LSC *vs.* HSC	C1 *vs.* C2
CDH1 201130_s_at	↑	↑	↑	↑		↑
CDH1 201131_s_at		↑		↑		↑
CDH3 203256_at	↓	↓		↓	↓	
CDH5 204677_at		↓	↑		↓	↓
CDH11 207172_s_at		↓		↓	↓	
CDH11 207173_x_at	↑	↓		↓	↓	
CDH13 204726_at		↓		↓	↓	
CDH17 209847_at		↑	↓		↑	↑
CDH19 206898_at			↑	↑		↓

Arrows indicate statistically significant changes; arrow direction indicates change of expression.

a tumor. The microarrays used in the study allow one to carry out a unique analysis of 22 283 mRNA of the analyzed genes and define dependencies in their expression.

However, one should realize the limitations of this method. The analyzed proteins will come from all cell compartments, and it is commonly known that a different location of the protein may result in different properties of the cell in the tissue, which has been shown by microarray tests supplemented by immunohistochemical tests [36].

The test group, although it was not large, had certain similarities to epidemiological data from large populations. Material came from tumors in higher stage of progression with significant and average differentiation, and in most of the cases diagnosis was made in the sixth decade of life. Tumors were most frequently located in the rectum and less frequently in the sigmoid colon, which required appropriate operative strategy in the surgery.

Results of clustering of profiles of normalized mRNA concentrations were of key importance to our observations. The data were clustered, yielding 4 heterogeneous groups (Figure 1). The C1 control group and the group of cancer tissue samples in high stage of progression (HSC) were beyond any dispute. However, the more interesting groups were those that included tissues that had been considered healthy in macroscopic and histopathological evaluation, but they had common characteristics of gene expression typical for tumor tissue. Other interesting findings were inferred from the analysis of the group of tumors in lower stages of cancer progression, in which 3 control samples were grouped together with it. This could be evidence of methodological error; however, this error was excluded by other tests of the degree of transcriptomes' differentiation (Figure 3). In the later part of the analysis it turned out that the data pertained to cancers in the first stage of progression on the UICC scale; however (and this could be statistically significant), they were the cancers with low and medium differentiation and were in the rectum (an organ whose vascularization and topography is very specific).

Selection of differentiation genes was carried out by comparing the previously specified groups of transcriptomes using 2 independent statistical programs: *Statistica v10 (StatSoft® Polska)* and *GeneSpring GX 11.0 (Agilent Technologies)*. This allowed us to define the group of genes coding for cell adhesion molecules of the cadherin group, whose differences in expression were statistically significant when comparing the groups obtained in the course of clustering. Thus, it was possible to ascertain that expression of CDH1 gene (homolog 201130_s_at) was significantly higher in healthy tissues when comparing all groups (except for LSC *vs.* HSC), which means that expression of E-cadherin is the highest in healthy tissue and decreases as the disease progresses. Consistent results were obtained for comparison of 201131_s_at homolog, and statistically significant differences in expression were observed only in comparisons C1 *vs.* HSC, C2 *vs.* LSC, C2 *vs.* HSC, and C1 *vs.* C2 (in case of the last one, both statistical methods were used, which seems to confirm heterogeneity of both groups). Validation of results by the qRT-PCR method also confirms the results obtained. These findings unambiguously indicate that E-cadherin is a marker that could potentially be used in defining the actual stage of tumor progression, independently of the still decisive histopathological evaluation, in tissues which (despite a healthy tissue phenotype) already have certain changes in gene expression typical for cancer tissue (e.g., polyps). Obviously, this could affect the therapeutic strategies applied with respect to the given patient. These observations have long been confirmed in studies conducted by many researchers, not only with regard to CRC [37]. In addition, Ngan et al. propose to use the research on expression of genes coding for E-cadherin as a predictor for liver metastases [38].

Similar tendencies towards reduced expression of the gene in the healthy tissue as compared to the CRC tissue were observed for genes CDH13; however, only for C1 *vs.* HSC, C2 *vs.* HSC, and LSC *vs.* HSC comparisons. Small deletions of CDH13 gene coding for cadherin were described in the context of stomach cancer and CRC; therefore, that gene was considered a suppressor gene with respect to several other tumors [39].

Gene CDH17 coding for LI-cadherin (liver-Intestine cadherin) is also a tumor suppressor gene. Our own results show that higher expression of CDH17 gene was observed in normal tissue (C1) *vs.* cancers in high stage of progression (HSC) and in cancers in low stage of progression (LSC) *vs.* cancers in high stage of progression (HSC), as well as the relevant control group C1 and heterogeneous group C2. This is yet further proof that this group is specific in the sense that the genotype looks suspicious but the results of histopathological evaluation are correct. These observations seem to confirm other authors' publications concerning many other tumors, including CRC. In the last case, in the research conducted by Kwak et al., the reduced expression of this gene was linked to lower differentiation of the tumor and overall lower survival of patients [40]. On the other hand, lower expression in C2 group *vs.* LSC group may be evidence of a small difference in profiles of gene expression between those groups.

Another gene, whose expression is lower in the tumor tissue, and therefore it can be considered tumor suppressor, is CDH19. According to our own results, higher expression of this gene was observed in healthy tissues as compared to tumor tissues for groups C2 *vs.* LSC and C2 *vs.* HSC. What is interesting is that an opposite expression was recorded for group C1 *vs.* C2. Since the results are not uniform and there are no publications on the subject in the context of CRC, final conclusions should be formulated after making additional tests.

Besides CDH1, another gene whose differences in expression were statistically significant was gene CDH3 coding for P-cadherin (placental cadherin). Unlike in homologous genes CDH1, increased expression of this gene was unequivocally evident in tumor tissues as compared to healthy tissues and tissues with lower progression of the disease. Such trends were observed when comparing all the groups selected in the course of clustering, except for C1 *vs.* C2 comparison; in this case, this seems understandable taking into account the not entirely explained profile of gene expression in C2. Also, in this case the research results were validated with the qRT-PCR technique. Thereby, as mentioned in the introduction, P-cadherin somewhat naturally becomes the potential target for BTC, and this was confirmed by the findings of other teams [29,31].

Another gene, whose expression patterns are similar to CDH3, is gene CDH2 coding for N-cadherin (neural cadherin). However, the results of our own research have shown that there was only 1 case of statistically significant difference in expression – expression was lower in C1 than in C2 – and it was additionally in contravention with the reports of other authors evaluating the expression of NCAM in the context of CRC [41], but this may confirm the findings of research on expression of this protein in the context of neuroblastoma, rhabdomyosarcoma and lung cancer, where it facilitates detachment of cells from the tumor to create metastases [42]. Since there are many controversies surrounding gene CDH2, its role as a target in biologic targeted therapy requires further research.

VE-cadherin (vascular endothelial cadherin), coded by gene CDH5, is another protein which has shown similar patterns of gene expression in the results of our own research. It has shown significantly higher expression in tumor tissue than in healthy tissue, although this was observed only in C1 *vs.* HSC, LSC *vs.* HSC and C1 *vs.* C2. The opposite expression trend was recorded when comparing suspected group C2 to LSC, and in this case expression was higher in C2. However, this certainly does not justify ultimate differentiation of both groups.

The last gene, whose expression was significantly higher in tumor tissue, is CDH11 (Table 3). In this case, the expression was also significantly higher in CRC tissue and it increased along with progression of the disease. Unfortunately, those results were not consistent – higher expression was observed in C1 group as compared to LSC. This gene codes for osteoblast cadherin (OB cadherin), which occurs mostly in musculoskeletal system tissues, and it participates in inflammatory processes related to rheumatic disorders [43]. Expression of this protein was also tied to tumors of other organs, such as osteosarcoma [44], salivary gland neoplasm [45], and prostate cancer, in this case in the aspect of bone metastasis [46]. Among the findings related to this cadherin, there were also many reports concerning CRC; in this case, the increased expression of OB-cadherin was observed in healthy tissue, which is in contrast with the obtained results [47].

The aforementioned findings may become an inspiration for development of practical applications. At present the process of determining the extent of cancer progression is still inaccurate, which may lead to incorrect assessment of the patient's actual condition and application of incorrect treatment [25]; therefore, it has been proposed to use determination of profiles of gene expression as a tool to define the actual progression and invasiveness of CRC. Other authors have pointed out that the problem with this method was that it is expensive [48], but if a correct approach is developed and more accessible and affordable methods are used, such tests will be justified [38]. Perhaps 1 of the methods could involve using serum-soluble E-cadherin, which could be helpful in prognostics of liver metastases [49].

Changes in expression of genes coding for cell adhesion molecules of the cadherin group can also provide much information on pathogenesis of various diseases. An example may include hypermethylation of E-cadherin gene by EBV and *H. pylori* in stomach cancer [50]. Despite the fact that we were unable to find studies proving a similar dependency in the context of CRC, it should be expected that a similar correlation also exists for this disease. Changes in mRNA expression of E-cadherins may also constitute evidence for the correlation between obesity and the increased risk of CRC, which would presumably take place through interaction of adipocytokines and glucocorticosteroids [51]. Intercellular adhesion processes may justify certain techniques used during surgeries to affect tumor properties. It was proven that increased pressure during insufflation hampers the processes associated with CRC metastases to liver [52]. Of course, research is also conducted on the potential effect of relevant substances on already known mechanisms, which is very important in the context of BTC. An example may be research conducted by Parafiniewicz et al., who found that administering Celecoxib caused reduction of soluble fraction of E-cadherin in cell lines, which was related to increased apoptosis of the cell and inhibition of angiogenesis [53]. There are also other known cases in which impact on other carcinogenesis-related mechanisms was observed. Tetraspanin works by affecting intercellular interactions and interactions between the cells and the extracellular matrix, which hinders tumor cell mobility and, consequently, reduces its capacity to create metastasis [54]. Other examples of similar substances include tunicamycin [26] and R-etodolac, which hinder the development of CRC tumors by increasing the expression of E-cadherin [27].

Of course, the test group that we analyzed is much too small to be able to draw any far-reaching conclusions as to specific practical applications of expression of the genes in question. In the course of our research we only selected genes that should be subject to further analyses in the future. In addition, in most of the cases our findings were not the first reports about the expression of the given gene in the context of CRC, of which genes coding for epithelial cadherin and placental cadherin are a good example. As mentioned at the beginning of this report, research devoted to them is much more advanced.

To summarize, we confirmed changes in profile of expression of CDH and CDH3 genes coding for cadherins in relation to colorectal cancer progression. They can potentially act as a diagnostic marker, which could be a useful tool in early cancer detection, before cancer can be detected through histopathological evaluation. In addition, potential suitability of gene CDH3 as a target in biologic targeted therapy was confirmed, and other genes coding for cadherins that could be useful in that respect were selected.

Conclusions

The levels of genes expression are different in several groups of cadherins, and are related with the stage of CRC; therefore, they could be potentially useful markers of the stage of the disease, as well as being applicable in treatment and diagnosis of CRC.

Conflict of interest

There are no actual or potential conflicts of interest, including any financial, personal, or other relationships with other people or organizations.

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