Differential survival trends of stage II colorectal cancer patients relate to promoter methylation status of \textit{PCDH10}, \textit{SPARC}, and \textit{UCHL1}

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Surgical excision of colorectal cancer at early clinical stages is highly effective, but 20–30% of patients relapse. Therefore, it is of clinical relevance to identify patients at high risk for recurrence, who would benefit from adjuvant chemotherapy. The objective of this study was to identify prognostic and/or predictive methylation markers in stage II colorectal cancer patients. Therefore, we selected six gene promoters (\textit{FZD9}, \textit{PCDH10} (protocadherin 10), \textit{SFRP2}, \textit{SPARC} (secreted protein acidic and rich in cysteine), \textit{UCHL1} (ubiquitin carboxyl-terminal hydrolase 1), and \textit{WIF1}) for methylation analysis in formalin-fixed, paraffin-embedded primary tumor samples of colorectal cancer patients ($n = 143$) who were enrolled in a prospective randomized phase III trial of the Austrian Breast and Colorectal cancer Study Group. Patients were randomized to adjuvant chemotherapy with 5-fluorouracil and leucovorin or surveillance only. Survival analyses revealed that combined evaluation of three promoters (\textit{PCDH10}, \textit{SPARC}, and \textit{UCHL1}) showed differential effects with regard to disease-free survival and overall survival in the two treatment groups (significance level 0.007). In the chemotherapy arm, a statistically insignificant trend for patients without methylation toward longer survival was observed ($P = 0.069$ for disease-free survival and $P = 0.139$ for overall survival). Contrary, patients in the surveillance arm without methylation in their gene promoters had shorter disease-free survival and overall survival ($P = 0.031$ for disease-free survival and $P = 0.003$ for overall survival), indicating a prognostic effect of methylation in this group (test for interaction, $P = 0.006$ for disease-free survival and $P = 0.018$ for overall survival). These results indicate that promoter methylation status of \textit{PCDH10}, \textit{SPARC}, and \textit{UCHL1} may be used both as prognostic and predictive molecular marker for colorectal cancer patients and, therefore, may facilitate treatment decisions for stage II colorectal cancer.

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Colorectal cancer is one of the most common forms of cancer causing 677 000 deaths worldwide per year (http://www.who.int/, July 2008). Although incidence is rising, the death rate from colorectal cancer has been going down for the past 15 years because of improved treatment and screening for early detection.\textsuperscript{1} The tumor is initially slow growing,
whereas it later often grows rapidly with the development of metastasis in regional and distal lymph node and other sites, including liver and lung.\textsuperscript{2} For patients with stage III colorectal cancer, adjuvant chemotherapy after surgical resection is currently recommended as a standard therapy as it has been shown to improve disease-free survival and overall survival.\textsuperscript{3} Significant controversy exists regarding the administering of chemotherapy to stage II patients.\textsuperscript{4} Surgical excision at early clinical stages is effective, although in about 20–30\% of patients the disease can recur. Therefore, determining whether a patient with stage II colorectal cancer should receive adjuvant therapy is a challenging decision due to the relative low probability of progression, and the fact that about two-thirds of patients would not benefit from chemotherapy and the costs would be tremendous.

A variety of genetic changes has been identified for colorectal cancer\textsuperscript{5} and some of them have been described as potentially useful biomarkers helping to better identify high-risk patients who would most likely benefit from adjuvant chemotherapy. These markers include, that is, microsatellite instability,\textsuperscript{6,7} loss of heterozygosity of 17p and 18q, expression of thymidylate synthase,\textsuperscript{8} or different gene expression signatures.\textsuperscript{9–11} In colorectal cancer, the commonly found KRAS mutations are considered as negative predictive marker for the use of anti-EGFR therapies; however, prognostic significance of KRAS mutations remains unclear.\textsuperscript{12}

In addition to genetic changes, aberrant DNA methylation represents a hallmark of cancer and has been extensively studied in colorectal cancers.\textsuperscript{13–17} Epigenetic signatures have been also shown to serve as potential diagnostic, prognostic, and predictive biomarkers (for a detailed review see Lao and Grady\textsuperscript{18} and Ogino et al\textsuperscript{19}). A recurrent methylation pattern referred to as CpG island hypermethylation was initially observed by Toyota et al\textsuperscript{20} that CpG island hypermethylation has gained a broad acceptance, and although there have been conflicting results about the prognostic or predictive role of CpG island hypermethylation,\textsuperscript{21,22} Ogino et al\textsuperscript{7} demonstrated that CpG island hypermethylation-high status is an independent predictor of cancer survival and seems to eliminate the adverse effect of BRAF mutation, which is associated with a high mortality. However, several studies identified a prognostic role for promoter methylation of genes not included in the CpG island hypermethylation panel such as MGMT, RASSF1A, or BAGE.\textsuperscript{23–25} Nevertheless, none of these markers has yet found its clinical application.

The aim of our study was to identify new prognostic and/or predictive markers in patients with stage II colorectal cancer who were enrolled in a prospective randomized phase III trial of the Austrian Breast and Colorectal cancer Study Group (Trial 91).\textsuperscript{26}

### Materials and methods

**Methyl-Profiler**

We analyzed the colorectal cancer cell lines SW480 and Caco-2 using two Methyl-Profiler DNA Methylation PCR Arrays (SA Biosciences, Qiagen, Hilden, Germany). The Human Colon Cancer Methyl-Profiler DNA Methylation PCR Array profiles the methylation status of 24 tumor suppressor genes frequently methylated in colon tumors. The Human WNT Signaling Pathway Methyl-Profiler PCR Array analyzes the promoter methylation status of a panel of 24 promoters of genes involved in WNT signaling during carcinogenesis and cellular differentiation. The assays were performed according to the manufacturer’s instructions (for details see Supplementary Material).

**Treatment of Colon Cancer Cell Lines with 5-Aza-2-Deoxycytidine**

Colorectal cancer cells Caco-2, HCT116, HT29, and SW480 were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured according to their recommendations. Cell lines were authenticated by DNA short-tandem repeat analysis by the Cell Culture Facility of the Center for Medical Research, Medical University Graz (Graz, Austria). To induce demethylation, cells were seeded at a density of 3–6 × 10\(^5\) cells and allowed to attach in the medium for 24 h. 5-Aza-2-deoxycytidine (5-Aza) (Sigma-Aldrich, St Louis, MO, USA) was added to a final concentration of 1 \(\mu\)M. Cells were incubated for 72 h with 5-Aza and the medium was renewed daily. Cells incubated under same conditions without 5-Aza treatment served as a control. After 72 h, cells were harvested and DNA and RNA were extracted for analysis. The experiment was performed in triplicates.

**DNA Extraction from Cultured Colon Cancer Cell Lines**

Cell number was measured with CASY (Roche, Penzberg, Germany). DNA was extracted from 5 × 10\(^6\) or 1 × 10\(^7\) cells using the Gentra Puregene Kit (Qiagen) according to the manufacturer’s instructions for cultured cells.

**RNA Extraction from Cultured Colon Cancer Cell Lines**

RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s recommendations. RNA was dissolved in 20–50 \(\mu\)l RNase free water and stored at \(-70^\circ\)C.

**Quantitative PCR**

A total of 16 genes were selected from Methyl-Profiler assays for expression analysis in the cell...
lines Caco-2, HCT116, HT29, and SW480 with and without 5-Aza treatment. cDNA from 2 μg extracted RNA of three replicates of each cell line was synthesized using Omniscript RT Kit (Qiagen). Expression analysis was performed by quantitative PCR using 25 or 50 ng of cDNA as a template and Fast SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA). Each sample was run in technical triplicates. RQ (relative quantification) or fold change was normalized to the lactate dehydrogenase A housekeeping gene using the 2-ΔΔCt method. Genes showing an RQ of >10-fold in at least one cell line after demethylation were selected and analyzed in patient samples. Primers were designed using Primer3Web 3.0.0 software. Sequences are listed in Supplementary Table 1.

Patients and Tissue Samples

All tumor specimens were obtained at the time of surgery before adjuvant therapy. Paraffin blocks were stored at room temperature and were identified only by an identification number. A hematoxylin/eosin-stained section of each tumor block was prepared and used for pathologic confirmation of present carcinoma.

We analyzed tumor samples from a total of 147 patients with stage II colorectal cancer according to the UICC (T3–T4, N0, M0) that were available from Austrian Breast and Colorectal cancer Study Group (Trial 91).26 All patients underwent a potentially curative resection without gross or microscopic evidence of residual disease and were recruited for a prospective randomized phase III trial of adjuvant chemotherapy with 5-fluorouracil and leucovorin. Patients were randomized to one of two postoperative treatment arms: (1) 5-fluorouracil/leucovorin and (2) surveillance only. Chemotherapy was administered as described previously.26

DNA Extraction of Formalin-Fixed, Paraffin-Embedded Tissues

DNA from formalin-fixed, paraffin-embedded tissues was extracted as published previously.27 Briefly, tissue samples were sliced into 5-μm-thick sections and manually dissected by a pathologist (SL) to reduce the proportion of non-tumor cells in the samples and scraped into an eppendorf tube. The minimum percentage of tumor cells was 40%. Slides were deparaffinized and tissues were lysed with lysis buffer (4 M urea, 200 mM Tris, 20 mM NaCl, 200 mM EDTA (pH 7.4)) containing proteinase K (20 mg/ml; Qiagen). DNA was precipitated with isopropanol at −20 °C overnight. The DNA pellet was washed, air dried, and dissolved in 20 μl TE buffer. For details see Supplementary Material.

 Bisulfite Conversion

Bisulfite modification of genomic DNA was conducted with the Epitect Plus Bisulfite Kit (Qiagen). Briefly, 1–2 μg of DNA was added to 85 μl bisulfite mix and 35 μl DNA Protect Buffer. Converted DNA was purified using the QIACube instrument (Qiagen).

Methylation Analysis

To determine the methylation status of six genes including FZD9, PCDH10 (protocadherin 10), SFRP2, SPARC (secreted protein acidic and rich in cysteine), UCHL1 (ubiquitin carboxyl-terminal hydrolase 1), and WIF1, we used quantitative, TaqMan-based real-time PCR MethyLight adapted from Weisenberger et al.28 Primers and probes, designed specifically for bisulfite-converted fully methylated DNA are listed in Supplementary Table 1. ALU primers and probes have been used as published previously20 and were used to normalize for the amount of input DNA. Converted CpGenome Universal Methylated DNA (Chemicon, Millipore, Billerica, MA, USA) was used as the 100% methylated control and whole genome amplified DNA (Repli-g Mini Kit; Qiagen) extracted from peripheral blood mononuclear cells of normal individuals was used as unmethylated control DNA. The 30 μl MethyLight reaction mixture contained 3 μl 10 × TaqMan Buffer A (Life Technologies, Carlsbad, CA, USA), 0.1 μl of AmpliTag Gold® DNA polymerase (Life Technologies), 200 μM dNTPs, 3.5 mM MgCl₂, 0.6 μM of each primer, 0.2 μM of each probe, and 4 μl of a bisulfite-converted DNA. All assays were able to detect 1% methylated DNA in a background of unmethylated DNA.

As DNA concentration is mostly overestimated by simple absorbance measurement and does not provide a comprehensive assessment of amplifiable DNA, we determined the amount of amplifiable DNA using ALU quantitative PCR and assigned the samples to five different categories. For details see Supplementary Material and Supplementary Table 2.

On each plate, a five-point standard curve for ALU (ranging from 5 to 0.0005 ng) was included in duplicate and used for normalizing the input DNA and converting the Ct values to mean values per copy numbers using the standard curve best-fit equation. Percentage of methylated reference was then calculated as follows: ((target mean value for sample)/(ALU mean value for sample))/(target mean value for 100%M)/(ALU mean value for 100%M)) × 100.

Statistical Analyses

Methylation data were generated in a blinded manner without the knowledge of any clinical data. For comparing means of two groups, the independent samples t-test was used. Baseline clinical data according to the methylation status were compared
in univariate analyses using the \(\chi^2\) test. The Mann–Whitney U-test was used for continuous variables. Survival time was defined as the period between the date of randomization and the date of death of any cause (overall survival) or the period between the date of randomization and date of first local/distant recurrence or last follow-up (disease-free survival). Patients who died before experiencing disease recurrence were censored at their date of death in the disease-free survival analysis. Survival rates were estimated by means of the Kaplan–Meier method. Differences between survival curves were analyzed by means of the log-rank test. The independent prognostic and/or predictive value of methylation status was studied with Cox models, which were adjusted for age, sex, tumor size, tumor grade, tumor localization, and treatment arm. Variables were coded as described in Table 1. These models were also applied to assess interactions between treatment and other covariates. All reported \(P\)-values are two-sided. For multiple comparisons, a Bonferroni-adjusted significance level of 0.007 was considered for multiple testing of seven promoters.

All analyses were performed with the use of IBM SPSS Statistics software, version 20.0

**Results**

**Selecting Candidate Genes for Methylation Analysis**

To identify aberrantly methylated promoters in colorectal cancer, we screened 46 promoters in two colorectal cancer cell lines (SW480 and Caco-2) using two Methyl-Profiler Assays (Qiagen) based on selective restriction digests of both methylated or unmethylated DNA, and subsequent quantitative polymerase chain reaction (PCR). Unmethylated promoters appear in green, intermediate methylation status is displayed in black, and highly methylated promoters are shown in red.

![Figure 1](image.png)

**Table 1** Patient and tumor characteristics

<table>
<thead>
<tr>
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<th>5-FU/LV, n (%)</th>
<th>Surveillance, n (%)</th>
<th>(P)-value</th>
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<tr>
<td>Number of patients (n)</td>
<td>71</td>
<td>72</td>
<td>0.945</td>
</tr>
<tr>
<td>Median age (years, range)</td>
<td>64.5 (30.4–78.0)</td>
<td>64.5 (35.4–78.0)</td>
<td>0.945</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>32 (45.1)</td>
<td>35 (48.6)</td>
<td>0.671</td>
</tr>
<tr>
<td>Male</td>
<td>39 (54.9)</td>
<td>37 (51.4)</td>
<td></td>
</tr>
<tr>
<td>T category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>62 (87.3)</td>
<td>59 (81.9)</td>
<td>0.373</td>
</tr>
<tr>
<td>T4</td>
<td>9 (12.7)</td>
<td>13 (18.1)</td>
<td></td>
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<tr>
<td>Grading</td>
<td></td>
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<tr>
<td>G1 and G2</td>
<td>58 (81.7)</td>
<td>59 (81.9)</td>
<td>0.969</td>
</tr>
<tr>
<td>G3 and G4</td>
<td>13 (18.3)</td>
<td>13 (18.1)</td>
<td></td>
</tr>
<tr>
<td>Tumor localization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum/Crypta ascendens</td>
<td>19 (26.8)</td>
<td>15 (20.8)</td>
<td>0.53</td>
</tr>
<tr>
<td>Flexur/Crypta transversum</td>
<td>13 (18.3)</td>
<td>18 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Sigma/Crypta descendens</td>
<td>39 (54.9)</td>
<td>39 (50.3)</td>
<td></td>
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<tr>
<td>Methylation</td>
<td></td>
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<tr>
<td>PCDH10</td>
<td>66 (93.0)</td>
<td>69 (95.8)</td>
<td>0.989</td>
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<tr>
<td>SPARC*</td>
<td>46 (68.7)</td>
<td>48 (68.6)</td>
<td></td>
</tr>
<tr>
<td>UCHL1</td>
<td>53 (74.6)</td>
<td>51 (70.8)</td>
<td></td>
</tr>
</tbody>
</table>

*The number of patients was 67 in the therapy arm and 70 in the surveillance group.*
Patients’ Characteristics

Tumor samples (n = 147) were derived from patients with stage II colorectal cancer available from a previous study conducted by the Austrian Breast and Colorectal cancer Study Group. Four patients had to be excluded owing to poor PCR amplification in ALU PCR. For SPARC six further samples had to be excluded owing to the same reason. Taken together, for a total number of 143 patients both data sets were available, methylation status of FZD9, PCDH10, SFRP2, UCHL1, and WIF1, and follow-up data. For SPARC only 137 data sets were used for statistical evaluation (Figure 3). Median follow-up of the study population was 11 years (95% confidence interval: 10–12). The samples analyzed were from 76 male and 67 female patients, of which 71 were previously assigned to the therapy arm and 72 to the surveillance group, respectively (Figure 3). Clinical data and characteristics were well balanced between both groups (Table 1). Median age of the two groups was 64 (range 30–78) for the therapy group and 65 (range 35–78) for the surveillance group, respectively. Median overall survival was 11 years in the treatment group and 12 years in the surveillance group, respectively.

Methylation Analysis of Patient Samples

As previous studies considered a percentage of methylated reference of ≥4 as biologically relevant because of a direct association with transcriptional silencing, we classified all samples below a percentage of methylated reference of 4 as unmethylated. All other samples were initially classified into four groups, including no, low, medium, and high methylation as illustrated in Table 2. FZD9 and PCDH10 were rarely unmethy-
with disease-free (disease free survival) and/or overall survival (overall survival). No statistically significant differences were detected between different methylation levels (no, low, medium, high) and survival except for SPARC \((P = 0.002)\) for patients with methylated promoters compared with those with no methylation. However, when patients were stratified by treatment assignment (5-fluorouracil/leucovorin or surveillance), survival analysis showed differential effects with regard to disease-free survival and overall survival (Figure 4). Disease-free survival tended to improve in patients of the surveillance group with methylated \(\text{SPARC}\) promoters \((P = 0.036)\), whereas overall survival was significantly improved in these patients \((P = 0.002)\). In contrast, methylation of \(\text{SPARC}\) was associated with shorter survival in patients who received therapy with 5-fluorouracil/leucovorin, although not statistically significant \((P = 0.132\) for disease-free survival and \(P = 0.250\) for overall survival) (Figure 4). The same trend toward both disease-free survival and overall survival was observed for patients in the surveillance group with methylation in \(\text{UCHL1}\) (disease-free survival \(P = 0.262\) and overall survival \(P = 0.244\)), whereas a nonsignificant relation between methylation and a shorter survival was observed in the chemotherapy group \((P = 0.248\) for disease-free survival and \(P = 0.199\) for overall survival). For \(\text{PCDH10}\) similar results were obtained; however, the number of events was too small to obtain accurate \(P\)-values. Combined evaluation of all three methylation markers showed an association between methylation and a shorter survival \((P = 0.069\) for disease-free survival and \(P = 0.139\) for overall survival) in the chemotherapy arm. However, patients in the surveillance arm had a significantly prolonged overall survival \((P = 0.031\) for disease-free survival and \(P = 0.003\) for overall survival) (Figure 4).

To determine a possible interaction between the combined methylation markers and treatment, an interaction term, the product of combined methylation markers and treatment, was incorporated into the Cox models. These analyses revealed a strong interaction between combined methylation markers and treatment (test for interaction, \(P = 0.006\) for disease-free survival and \(P = 0.018\) for overall survival).
Discussion

Our study indicates that promoter methylation status of three genes including PCDH10, SPARC, and UCHL1 may help to identify patients who might benefit from chemotherapy, and consequently facilitate treatment decisions for stage II colorectal cancer patients. All analyzed tumor samples were available from a previous study conducted by the Austrian Breast and Colorectal cancer Study Group, where the efficacy of adjuvant chemotherapy with 5-fluorouracil and leucovorin in stage II colon cancer was investigated. In the course of this study, patients were randomly stratified to either adjuvant chemotherapy with 5-fluorouracil/leucovorin or surveillance only. However, no statistically significant improvement on disease-free survival and/or overall survival could be achieved by the application of chemotherapy in this study. This highlights the need for additional prognostic markers that may prospectively identify patients who are likely to be cured with chemotherapy versus those patients who have a better prognosis after surgery only and would not benefit from chemotherapy.

Our study revealed that promoter methylation of UCHL1 and SPARC was correlated with a better clinical outcome in the surveillance group compared with patients without methylation at these promoters (Figure 4). In contrast, patients of the chemotherapy group, who were methylated at UCHL1 and SPARC promoter sites, seemed to have poorer overall survival and disease-free survival. A similar trend was observed for methylation of PCDH10 (Figure 4). A combined analysis of all three promoters was in concordance with the analysis of individual markers, with the trend for better survival or relapse time being even improved. This was also reflected in the Spearman’s correlation analysis, where the methylation status of almost all
analyzed promoters was significantly correlated, indicating that a combined analysis of more promoter regions could provide greater clinical reliability.

The involvement of PCDH10, SPARC, and UCHL1 in tumor formation and progression is well documented in the literature. PCDH10 is a member of the cadherin superfamily, which is known to be a cell–cell adhesion molecule. In the context of tumorigenesis, PCDH10 is found to have a role in growth control, tumor cell invasion, and metastasis, and has recently been proposed to be a tumor suppressor gene. PCDH10 methylation was frequently reported in several solid tumors and was closely associated with malignancy and negative outcome. In a recent study from Yu et al, PCDH10 promoter methylation was detected in 85% of primary colorectal tumors and all analyzed colorectal cancer cell lines. This is in concordance with our data where PCDH10 methylation was found in more than 90% of patients. However, in our study, poor prognosis due to methylation, as reported previously, was observed only for the chemotherapy group, although not being significant. For the surveillance group methylation of PCDH10 promoter showed a significantly better survival and relapse rate. In this context, it should be noted that only very few patients showed no PCDH10 methylation and the number of events was too small for reliable calculations of error probabilities; therefore, these data should be considered as preliminary and further investigations have to be conducted.

SPARC is described to be involved in endothelial permeability, regulation of endothelial barrier function, and cell migration and differentiation. Therefore, SPARC is predestined to have a role in tumor angiogenesis, proliferation, and cell migration. However, the role of SPARC as an oncogene or a tumor suppressor gene is still not fully elucidated. SPARC has been reported to act as a protumorigenic and antitumorigenic protein in different tumor entities.

For colorectal cancer also conflicting results have been reported. Expression of SPARC was shown to be significantly higher in tumor tissue than in normal colon or matched non-malignant counterparts. Therefore, SPARC was suggested as a potentially useful target candidate for cancer immunotherapy. Yoshimura et al reported a higher recurrence risk for colorectal cancer by an upregulated SPARC expression in adjacent tumor tissue. These data are consistent with our findings, where a decreased level of SPARC due to promoter methylation was associated with a better clinical outcome. This is also supported from animal models where SPARC deficiency strongly suppressed adenoma formation in the intestine in Sparc knockout mice intercrossed with Apc(Min/+) mice.

In contrast, a significant association of SPARC methylation and poorer prognosis was reported by Yang et al. Although the authors analyzed a large cohort of patients, they did not differentiate between tumor stages nor did they consider stratification by chemotherapy. In our study, SPARC promoter methylation tended to be associated with shortened overall survival and disease-free survival, only in patients who received chemotherapy. These results are supported by the fact that decreased SPARC expression has been observed in therapy-refractory tumors compared with those sensitive to chemotherapy, suggesting that upregulation of SPARC expression might enhance chemosensitivity. Hence, the slightly worse outcome for patients with SPARC methylation may be a result of decreased chemotherapy sensitivity in the absence of SPARC due to epigenetic silencing.

Similar to SPARC, the role of UCHL1 in tumor development is still controversially discussed. The gene product of UCHL1 is known to catalyze deubiquitination of proteins and is typically expressed in neurons. Mutations in UCHL1 are closely linked to the development of Parkinson disease, and are also found in neuronal tumors. However, recent studies indicate the involvement in other human non-neural cancers, such as breast, colorectal, and pancreatic cancer. Data from several in vitro studies and mouse models suggest that UCHL1 acts as an oncogene in cancer pathogenesis. Furthermore, high expression of UCHL1 in cancer-associated fibroblasts was shown as an independent, negative prognostic factor for overall and recurrence-free survival. Albo et al proposed that neurogenesis—in which UCHL1 is a key player—in colorectal cancer is involved in tumor progression and associated with poor outcome. In 2008, Mizukami et al proposed that hypomethylation resulting in re-expression of UCHL1 was significantly associated with lymph node metastasis in colorectal cancer. These data strongly support our findings where patients under surveillance tended to have a better outcome when UCHL1 promoter was methylated compared with patients who showed no methylation and therefore most likely had increased expression levels of UCHL1.

On the contrary, UCHL1 was also reported to act as a tumor suppressor and promoter methylation has been suggested as an independent prognostic factor in different cancers. In our data set, UCHL1 methylation was associated with a shortened overall survival and disease-free survival only in patients receiving chemotherapy. Whether the opposing results from the therapy group can be explained by decreased chemotherapy sensitivity, similar to SPARC, remains to be elucidated.

Although further studies are needed to clarify the exact mechanisms of PCDH10, SPARC, and UCHL1 in tumorigenesis and progression, and their interaction with the chemotherapy regimen, our data suggest that analysis of promoter methylation in tumor tissue may provide a suitable tool for determination of prognosis of stage II colorectal cancer patients. More importantly, methylation of PCDH10, SPARC, and UCHL1 may predict response...
to the adjuvant treatment. Therefore, our results could help to stratify early-stage colorectal cancer patients into a high-risk group promoter methylation of selected genes that would benefit from adjuvant chemotherapy.

To date, adjuvant chemotherapy for stage II colorectal cancer patients is still controversially discussed. Even if chemotherapeutic treatment has improved and the risk of toxicities has become more calculable, the guidelines from the American Society of Clinical Oncology (ASCO) from 2004 do not recommend a general treatment for patients in stage II.32

In conclusion, we could demonstrate that analyses of methylation in our selected genes might be used to determine patients with worse prognosis, as demonstrated in untreated patients, and that selection of these patients for adjuvant treatment may predict the benefit of chemotherapy. However, these arguments are based on a retrospective study with a relatively low number of cases. Furthermore, we cannot exclude confounding effects like BRAF mutations or resistance to 5-fluorouracil by microsatellite instability, which were previously shown to influence survival in colorectal cancer patients.33,34 However, owing to the lack of material and bad DNA quality, it was not possible to assess additional genetic markers in our study cohort. In conclusion, the identification and validation of new biomarker is challenging owing to several reasons (for a detailed review see Febbo et al35); therefore, our results should be evaluated together with other prognostic factors such as BRAF mutations prospectively in a larger clinical trial.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

References

Differential survival trends in colorectal cancer


Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)