Novel insights into transcriptional dysregulation in colorectal cancer


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Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Although CRC has been comprehensively characterized at the molecular level, the tumor heterogeneity hinders the identification of reliable diagnostic, prognostic and predictive biomarkers. Molecular stratification of CRC is based on prevalent gene mutations and transcription profiles but its significance for clinical practice remains obscure. Indeed, activating mutations in the genes KRAS, NRAS, and BRAF are the only predictive biomarkers for anti-EGFR antibody therapy routinely tested in the clinic for advanced stages of CRC. Gene expression signatures are important for clarifying the molecular mechanisms of CRC development and progression, but only two such tests for predicting recurrence risk are commercially available. The aim of our study was to propose a diagnostic approach based on mutation and gene expression analysis that can be routinely applied in the clinic for defining the most appropriate treatment strategy for each patient. We used qPCR to determine the presence of KRAS mutations and measure the transcription levels of a panel of 26 genes in 24 CRC patients. Statistical analyses were applied to check for associations between clinicopathological and molecular parameters. Our results reveal novel data concerning CRC carcinogenesis: almost universal downregulation of EGFR; differential role of the pro-inflammatory cytokines TNF-α and IL-6; overexpression of the vitamin B12 transporter transcobalamin 1; tumor-suppressor function of SETD2, CA7, and GUCA2B. The practical application of these findings has yet to be clarified.

Key words: CRC, gene expression, GUCA2B, KRAS

Colorectal cancer (CRC) is the third most frequent cause of cancer-related death worldwide [1, 2]. The improved surgical techniques and the introduction of more effective therapies has led to an incredible decline in CRC incidence and mortality among the population over 50 years of age in the last four decades [3]. Despite this positive trend, an alarming increase in incidence has been reported in younger people [4], necessitating the need for better prevention and early detection strategies. Important steps in this direction are the molecular characterization of CRC and the identification of suitable diagnostic, predictive and prognostic biomarkers. The first model of CRC tumorigenesis, describing it as a progressive accumulation of genetic and epigenetic events [5], was supplemented by more comprehensive description of the molecular events by the Cancer Genome Atlas Network in 2012 [6]. Thirty-two genes were found to be recurrently mutated with eight being most prevalent – APC, TP53, KRAS, PIK3CA, FBXW7, SMAD4, TCF7L2 and NRAS. In addition, copy-number alterations leading to amplification of ERBB2 and IGF2 were found to be common events. Tumors were divided into two groups, hypermutated and non-hypermutated with different genetic mutations predominating in the different groups. Thereafter, numerous attempts have been made towards molecular stratification of CRC based on genetic variations and transcriptional profiling [7–13] with the goal of improving personalized treatment approaches [14]. This resulted in the development of a classification system including four consensus molecular subtype (CMS) groups [15], which best represent the heterogeneity of CRC at the gene expression level [16]. While CMS1 is characterized by microsatellite instability, hypermutation and mutations in MSH6, BRAF and PTEN, the other three subtypes are chromosomally unstable and enriched in mutations in APC, KRAS, TP53, SMAD4 and PIK3CA. CMS2 is marked by upregulation of WNT and MYC downstream targets, overexpression of
EGFR, ERBB2, IGF2 etc., whereas activation of TGF-β and proteins involved in extracellular matrix remodeling is a key feature of CMS4. KRAS mutations are dominant in CMS3 together with metabolic deregulation [16]. The CMS system was challenged in a recent publication by Isella et al. (2017) due to the large contribution of the tumor stromal content towards the transcriptional profiles utilized for the creation of the CMS subgroups. The authors of the study propose CRIS (CRC intrinsic subtypes)-based stratification and provide strong evidence that it can be exploited together with clinical and pathological parameters for better prognostic assessment of CRC [17]. In spite of the accumulating data regarding the molecular mechanisms of CRC and the efforts made towards molecular stratification of this cancer, little of this knowledge has found application in the clinics. Various biomarkers have been suggested for diagnosis, prediction and prognosis (reviewed in [18]), as well as for early CRC detection (reviewed in [19]) but only few of them are actually used in routine practice. Microsatellite instability (MSI) is associated with better prognosis and considering its prevalence in hypermutated cancers, it can be considered that high mutation rate may indicate better prognosis [6]. As far as molecular target therapy is concerned, only three negative biomarkers exist for advanced colorectal cancer drug treatment. Mutations in KRAS, NRAS, BRAF predict resistance to anti-EGFR antibodies [20]. Indeed, patients wild type for KRAS, NRAS, BRAF and PIK3CA, who represent 30% of all cases, are most likely to benefit from this therapy [21] but acquired resistance due to EGFR mutations is a major problem [22–26]. No predictive biomarkers for anti-angiogenic treatment have been identified [16]. Gene expression profiling is indispensable for molecular subtyping of CRC but no established genomic signature has been useful in clinical practice [27]. Gene expression signatures have been proposed for early detection based on blood samples [28, 29]; for prognosis [30]; for estimating the risk of cancer occurrence [31]; for predicting response to chemotherapy in metastatic CRC [32] etc. Two such signatures, the Oncotype DX Colon Cancer Assay and the ColoPrint were validated as predictive markers for recurrence in stage II and III CRC but are not used everywhere. Isella et al. (2017) proposed reduction of the original CRIS 565 gene classifier to a set of 40 genes that can be used for clinical classification of individual patients, which would offer the greatest opportunity until now for personalized treatment. The aim of our study was to propose a diagnostic approach based on KRAS mutation and gene expression analysis that can be routinely applied in clinical practice for better evaluation of the patients and for appointing the most appropriate therapy. To this end, we measured the mRNA levels of an original constellation of 26 genes related to tumor growth, invasion and metastasis in CRC and correlated their expression to clinicopathological parameters, as well as to the KRAS mutation status of the patients. We did not find any significant associations between the expression levels of the chosen genes and tumor grade/stage or the presence of KRAS mutations. Interestingly, the only gene that shows any association to KRAS status is YKL40 [33]. Some of our results are novel and unexpected. First, SETD2, a histone methyltransferase, is ubiquitously downregulated in CRC, showing its significance as a tumor-suppressor gene also in this cancer type. Second, EGFR mRNA levels are decreased in a high proportion of cases, which can indicate reduced sensitivity to cetuximab even in KRAS wild type patients. Third, we show that TCN1, a vitamin B12 transporter, is highly overexpressed in CRC. Finally, two genes are universally lost in CRC – CA7 (carbonic anhydrase VII) and GUCA2B (uroguanylin), suggesting them as important tumor suppressor genes in CRC carcinogenesis.

**Patients and methods**

Patients and preparation of tissue samples. The study was approved by the Ethics Committee of the Medical University of Plovdiv, protocol # R-1838/15-07-2013. Patients signed an informed consent in agreement with the requirements of the WMA Declaration of Helsinki. Twenty-four patients who underwent surgery in the years 2013 or 2014 at the two University hospitals “Kaspela” and “St. George” were selected. Tumor tissue and normal colonic mucosa distal to the tumor site were isolated by intraoperative resection and were stored in RNA later (Qiagen, Netherlands) at −80°C. In addition, formalin-fixed tumor tissue was embedded in paraffin for histological evaluation and DNA isolation.

**Molecular analyses.** DNA was isolated with the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and the KRAS mutation status was determined with the Amoy Dx KRAS Seven Mutations Detection Kit (Amoy Diagnostics, Haicang, Xiamen, China). RNA from normal and tumor tissue samples was isolated with the RNeasy Mini Kit (Qiagen) and converted to cDNA with the RT2 First Strand Kit (Qiagen). Gene expression of YKL40, IL6, MAPK1, NFKB1, NR1P1, PIK3CG, and PTEN was measured by qPCR using the Maxima SYBR Green qPCR Master Mix (ThermoFisher Scientific, USA) and the primers listed in Table 1 (Integrated DNA Technologies, Leuven, Belgium) and Maxima SYBR Green/BOX qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The transcription levels of the other 19

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**Table 1. List of custom made primers used for qPCR.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer [5’ → 3’]</th>
<th>Reverse primer [5’ → 3’]</th>
</tr>
</thead>
<tbody>
<tr>
<td>YKL40 (CH3LI)</td>
<td>GACCAAGGAGGATGGACTGCCCT</td>
<td>TGTAAAGCCAGGATCCATCACGTGTT</td>
</tr>
<tr>
<td>IL6</td>
<td>AGGCCACAGGGTATGCTGGATT</td>
<td>ACAGGCAACAGCCGGTGAAGACC</td>
</tr>
<tr>
<td>MAPK1</td>
<td>TACTCTGCCACTAGTACTGCT</td>
<td>TCTCCTGTGTGCTGTTGATGTC</td>
</tr>
<tr>
<td>NFKB1</td>
<td>CCTCCATAAGGCAAGGAGTATACG</td>
<td>AGCTGAGTGGAGGAGGAGGAGGG</td>
</tr>
<tr>
<td>NR1P1</td>
<td>ACATGCAACAGGATGCCAGTCT</td>
<td>TGTATGACGCAGTGGAGGAGGAGG</td>
</tr>
<tr>
<td>PIK3CG</td>
<td>AGGTCTCGGGAGAGAGGAGGAGG</td>
<td>ACAAAGTGTCACACAGTGGCGCG</td>
</tr>
<tr>
<td>PTEN</td>
<td>TGTATGACGCAGTGGCGCGCGG</td>
<td>TGAAGATTGTCACACAGTGGCGCG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AGGTCCAGCATGACACTGCTG</td>
<td>AGCTGACAGGGAAACATCACCT</td>
</tr>
</tbody>
</table>
genes, AKT1, AXIN2, CA7, CD44, CTNNB1, EGFR, FGFR2, GPC1, GPC3, GUCA2B, KRAS, MACC1, MMP9, NOTUM, SETD2, SIRT3, TCN1, TNF and VEGF, were determined with customized RT² Profiler PCR Array (SA Biosciences, Qiagen). In all cases, samples were run in triplicates according to the MIQE guidelines. PCR reactions were carried out in Rotor Gene Q (Qiagen). The analysis was performed with the ΔΔCt method individually for each patient. Expression of the gene in the normal tissue served as the calibrator and GAPDH was used as a reference gene. GAPDH was found to be stably expressed in normal colonic mucosa and tumor tissue and thus proved suitable for normalization of the gene expression data (Suppl. Figure 1).

Statistics. The non-parametric Mann-Whitney U test was applied to compare the values of a continuous variable in two independent groups. To determine the presence of correlation between two continuous variables the non-parametric coefficient Kendall's tau-b was calculated. The Log-rank test (Mantel-Cox) was used to identify the statistical difference between the survival of patients belonging to two different groups. Boxplot diagrams were used for graphical visualization of the continuous variables as well as for distinguishing outliers in the data series. They were created in BoxPlotR (http://shiny.chemgrid.org/boxplotr/). All statistical analyses were performed with SPSS software, version 17.0.

Results

Demographic and clinical parameters of the patients. The clinical parameters of the patients included in the study are shown in Table 2. The majority of patients were male; the mean age at the time of diagnosis was 73.9 years with no significant difference between both genders. The majority of tumors were localized in the rectum (45.8%). In most patients, the tumors were staged as T2/T3 (70.8%) and graded as G2 (87.5%); lymph node engagement was observed in 25% of patients, whereas distant organ metastases were detected in 12.5%. Different postoperative therapies were assigned to 62.5% of the patients, including PCT (33.3%), adjuvant chemotherapy (16.7%) or chemotherapy combined with radiation or immunotherapy (12.5%). Within the timeframe of the study (2013–2017), 9 of the patients passed away and 15 are still under surveillance. The statistical analysis (Log-rank test) did not demonstrate any association between overall survival and T-stage (T1–4), age (≥<70 years) and gender of the patients. The presence of local (lymph nodes) or distant metastases also did not influence the survival of the patients.

KRAS mutation status. Mutations in the genes KRAS, NRAS and BRAF are the only predictive biomarkers in advanced CRC, which determine resistance to targeted therapy with anti-EGFR antibodies. With qPCR as the method of choice in the current study, we detected mutations in the gene KRAS in 8 of the 24 patients, which is in agreement with the reported average prevalence of these mutations in 30–40% of CRC patients. We did not observe any difference in the overall survival between the two groups – KRAS-mutant and KRAS-wild type.

Gene expression. We selected an original panel of 26 genes whose products participate in different signaling pathways or processes in CRC carcinogenesis (Figure 1). We used qPCR to measure the transcription levels of all genes and applied relative quantification individually for each patient using normal tissue as the calibrator. Despite the great heterogeneity among the studied samples, we grouped the genes in three different categories based on their differential expression in tumor versus normal tissue: (i) overexpressed genes (fold change >2, Figure 2A); (ii) genes with unchanged expression (fold change between 1 and 2, Figure 2B) and (iii) underexpressed genes (fold change <1, Figure 2C). The overexpressed genes were: NOTUM, TCN1, MACC1, YKL40, GPC3, AXIN2 and IL6 (Figure 2A, a1 insert). The most highly expressed gene in CRC tissue is NOTUM, a negative regulator of the Wnt signaling pathway, which was recently shown to be the only known extracellular deacylase that removes palmitoleate from Wnt proteins and inactivates them [34]. We recently demonstrated enhanced expression of NOTUM and deregulated expression of glypican (GPC1 and GPC3) in CRC tissue [35] and confirmed some of these findings here. Genes that showed on average unchanged expression between the tumor and the normal tissue were: GPC1, CTNNB1, VEGF, CD44 and AKTI.

Table 2. Clinical and demographic parameters of the patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>58.3</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤70 years</td>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>14</td>
<td>58.3</td>
</tr>
<tr>
<td>Mean age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women: 72.5±9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men: 74.9±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total: 73.9±7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localization of the tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caecum</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>4</td>
<td>16.7</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>Rectum</td>
<td>11</td>
<td>45.8</td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>2</td>
<td>8.3</td>
</tr>
<tr>
<td>T2</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>T3</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>Lymph nodes (N) and metastases (M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T(1–4)N(0–x)M0</td>
<td>15</td>
<td>62.5</td>
</tr>
<tr>
<td>T(1–4)N(1–x)M0</td>
<td>6</td>
<td>25.0</td>
</tr>
<tr>
<td>T(1–4)NM1</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>G1</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>G2</td>
<td>21</td>
<td>87.5</td>
</tr>
<tr>
<td>G3</td>
<td>2</td>
<td>8.3</td>
</tr>
</tbody>
</table>
different cellular processes. The transcription levels of the 26 genes are affected neither by the age or the sex of the patients, nor by the localization, T stage or the grade of the tumor. There are two possible explanations for this phenomenon: the heterogeneity of CRC and the small sample size which do not allow very definitive interpretation of the results.

The most extremely downregulated gene was GUCA2B. It was observed that the group with higher expression levels (≥ mean = 0.05) had a worse overall survival than the group with lower expression levels (< mean = 0.05) (Figure 3).

Despite the difficulty in extrapolating the results from such a small patient cohort and making general conclusions, some of the findings of our study are novel and deserve attention. First, in 46% of patients the two pro-inflammatory cytokines, IL-6 and TNF-α, were not differentially expressed in the tumor compared to the normal tissue. In 50% of the cases, IL6 displayed moderately elevated levels (2–6 fold), whereas TNF was underexpressed. Second, we did not observe upregulation of EGFR in any of the studied patients. On the contrary, the gene was underexpressed in 71% of the cases and showed normal regulation in the rest. The same is true for the gene KRAS. Third, we obtained information about the expression of genes that have not been previously discussed in the context of CRC. Thus, SETD2 encoding a histone H3K36 methyltransferase is downregulated in 67%

Figure 1. The panel of 26 genes whose transcription levels were measured in the study. The genes are roughly ordered according to the different signaling pathways they belong to or processes involved in CRC carcinogenesis. Receptor tyrosine kinases, e.g. EGFR and FGFR2 activate both MAPK/ERK and AKT/PKB signaling. The circle is filled in with a pattern pointing to the fact that the products of these genes are all part of a network regulating tumor growth, invasion and metastasis.
of our patients. TCN1, whose product transcobalamin is involved in vitamin B12 transport, is strongly overexpressed in the tumor tissue. Carbonic anhydrase VII (CA7) is a zinc metalloenzyme that catalyzes the reversible hydration of carbon dioxide and has multiple functions, among which is the regulation of the acid-base balance. Our results demonstrate extreme underexpression of the gene in 100% of the samples (Figure 2, c1 insert). Furthermore, a correlation was found between the expression levels of this gene and GUCA2B, the product of which (uroguanylin) is thought to participate in the regulation of salt and water homeostasis in the intestine and kidneys.

Discussion

The results of our study should be interpreted with caution due to the small number of patients included in it. Though, they reveal several interesting aspects of CRC carcinogenesis, which we hereby discuss in more detail.

**EGFR signaling.** Blocking of EGFR signaling with anti-EGFR monoclonal antibodies has emerged as a successful target therapy that has contributed to the increase in overall survival in CRC patients. Mutations in four genes are considered as negative predictive markers for this therapy – KRAS, NRAS, BRAF and PIK3CA, with quadruple wild type CRC patients benefiting most from cetuximab treatment [16]. EGFR overexpression is a hallmark of numerous human cancers, including CRC, and a few studies have looked into its effects on target therapy efficiency and prognosis. Some authors showed significant association between high EGFR expression and advanced tumor stage [36, 37], lymph node
metastasis [38] and poorer overall survival [39], whereas others did not find it to be a useful prognostic marker [37, 40]. Cetuximab was also shown to be more effective in patients with high protein expression of EGFR [41] or increased EGFR gene copy number [42]. Furthermore, mutations in the gene are associated with acquired resistance to anti-EGFR mAbs [26, 43]. Surprisingly, our results showed moderately decreased mRNA levels of EGFR in 71% of the patients in the study but this low expression did not show any association with clinicopathological features. We can only speculate that a combination of KRAS mutations and EGFR downregulation might be indicative of poor response to anti-EGFR therapy.

**Inflammation – IL-6 and TNF-α.** Despite the tight link between chronic inflammation and different types of human cancers [44], studies concerning the levels of pro-inflammatory cytokines in the CRC tumor environment are contradictory. Furthermore, the role of pro-inflammatory cytokines for CRC progression from adenoma has been disputed [45]. Tumor Necrosis Factor Alpha (TNF-α) activates the transcription factor NFκB, thus promoting proliferation and metastasis of tumor cells [44,46]. Several studies have demonstrated the link between increased TNF mRNA levels in the tumor tissue and advanced stages of CRC [47–49], suggesting that targeting TNF-α can be applied in treatment of CRC, especially when it has progressed from ulcerative colitis [50, 51]. However, some authors report suppression of this cytokine in tumor, attributed to promoter-methylation of the gene [52]. Data on TNF-α serum levels are not less contradictory. While some authors claim significantly higher levels of the cytokine in serum of CRC patients and link this to the tumor development and progression [53], others do not detect it at all [54, 55]. In contrast, studies investigating the role of IL-6 in CRC are much more definitive. The elevated levels of the cytokine in CRC tissue [56] and serum [57] have been shown to be significantly associated with higher risk of relapse and survival of the patients. A monoclonal antibody binding to the IL-6 receptor was shown effective in targeting colon cancer-stem like cells [58]. Despite the proposed downregulation of p53 by IL-6, which offers a link between inflammation and carcinogenesis [59], a meta-analysis by Zhou et al. (2014) did not find any significant association between IL-6 and the risk of CRC [60]. Our results show slightly increased mRNA level of IL6 and decreased level of TNF in 50% of the patients. We did not find any association between the levels of these two cytokines and tumor stage or patients’ age. The Kendal tau test showed significant correlation of IL6 expression with VEGF, NFκB1, PIK3CG, MAPK1, CTNNB1, AXIN2, GPC1, AKT1 and SIRT1, suggesting a possible role of this pro-inflammatory cytokine in activating/regulating a variety of signaling pathways.

**Histone modifications – SETD2.** Proper functioning of the epigenetic regulatory machinery is crucial for maintaining genome integrity and cellular function. SETD2 is a single gene responsible for trimethylation of H3K36, a histone modification implicated in transcription elongation, splicing and DNA repair [61]. It has been also proposed that this methyltransferase interacts with p53 and selectively regulates the transcription of the transcription factors’ target genes [62] but the significance of this interaction remains unknown. Inactivating mutations in the genes are most frequent in the clear cell renal cell cancer [61] but are also associated with high-grade gliomas [63]. Decreased mRNA levels of SETD2 have been associated with increasing tumor stage in breast cancer pointing to a tumor-suppressor role of this gene [64]. Our results of downregulated SETD2 expression in CRC tissue (67% of patients) lead to a similar conclusion, although we did not find a link between the expression of this gene and the clinicopathological parameters. Although not significant, an association between KRAS mutation status and SETD2 expression was noted, the functional importance of which remains to be elucidated.

**Vitamin B12 metabolism.** Transcobalamin 1 (TCN1) is a member of the vitamin B12-binding protein family. It is expressed in various tissues and facilitates the transport of cobalamin into cells. The role of vitamin B12 metabolism has been studied in gastric cancer [65, 66], breast phyllodes cancer [67] and prostate cancer [68]. A recent meta-analysis of gene expression data obtained by microarrays showed that TNC1 is overexpressed in CRC tissue thus acting as an oncogene [69]. Although the role of vitamins has been extensively studied in gastrointestinal diseases (reviewed in [70]), the function of genetic variants and mRNA levels of TCN1 in CRC development and progression have not been thoroughly addressed. Our results demonstrate highly an increased transcription level of the gene in tumor tissue in 67% of CRC patients, in agreement with the results obtained from Chu et al. (2014).

**Carbonic anhydrase VII and CRC.** The meta-analysis of Chu et al. (2014) showed that CA7 and GUCA2B are also among the top eight differential genes in CRC, which act as tumor suppressors. Our results corroborated this view by showing decreased mRNA levels of both genes in 100% of the patients. Actually, these two genes were the most prominently downregulated genes within the gene panel. Carbonic anhydrase VII (CA7) belongs to a group of metalloenzymes that catalyze the reversible hydration of carbon dioxide and are involved in many physiological and pathological processes. Inhibiting CAs represents a potential novel treatment for obesity, infections and cancer [71]. Expression of CA IX is highly elevated in many tumors as a consequence of hypoxia [71] and is linked to worse prognosis in CRC [72, 73]. Only few studies address the role of CA VII in cancer. Upregulation of the gene is associated with poor prognosis in astrocytoma patients [74], whereas decreased CA7 mRNA levels significantly correlated with poor differentiation, positive lymph node metastasis, advanced TNM stage and increased death rate in CRC patients [75]. Despite accumulating data, the differential role of CA isoenzymes in CRC remains to be elucidated.
Guanylyl cyclase C signaling in CRC carcinogenesis.

The gene GUCA2B codes for the hormone uroguanylin, one of the two ligands that activate guanylyl cyclase C (GUCY2C) signaling axis controlling fluid and electrolyte homeostasis [76]. Loss of uroguanylin (GUCA2B) and guanylin (GUCA2A) abrogates GUCY2C signaling, which is a universal feature of CRC [77, 78], strongly suggesting a role of GUCY2C as a tumor suppressor. In addition, GUCY2C signaling is downregulated in inflammatory bowel disease (IBD) [79,80] providing a novel link between chronic inflammation and CRC carcinogenesis and suggesting a target for prevention of CRC by hormone replacement therapy [76]. The extreme downregulation of GUCA2B in all our patients conforms to previous studies underscoring the important role of uroguanylin-mediated signaling in CRC. Although ubiquitously lost, the levels of the gene are lower in patients with longer survival time. Also, GUCA2B expression showed no significant correlation with the expression of IL6 and TNF, which questions a direct link between GUCY2C signaling and inflammation. We have to note, however, that we did not study the expression of the other GUCY2C ligand, guanylin, and thus cannot make definitive conclusions since both hormones might have redundancy in function. In general, our results suggest that uroguanylin, as a secreted protein, can be indeed used as a biomarker for early detection of CRC [81], but hormone replacement therapy (linacotide) should be applied rather for protection/prevention of inflammation-induced CRC than for treatment of advanced CRC.

Our study provides novel data on the transcriptional activity of a panel of 26 genes related to the tumor growth, invasion and metastasis in CRC. It offers new insights into a variety of signaling pathways involved in colorectal carcinogenesis, some of which are still waiting for their clinical implications.

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