Polymorphisms in microRNA genes as predictors of clinical outcomes in colorectal cancer patients

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Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies worldwide. It is routinely cured by a 5-fluorouracil (5-FU)-based chemotherapy which improves outcomes in patients. We investigated the effect of single nucleotide polymorphisms (SNPs) in miRNA-related genes that have been previously reported as important in patients with stage III CRC and treated with 5-FU-based chemotherapy. Two SNPs (rs4919510 in mir-608 and rs232120 in mir-219-1) were genotyped in 1083 CRC patients recruited in the Czech Republic to evaluate their effect on clinical outcomes. Carriers of the variant T allele in rs232120 and receiving 5-FU chemotherapy were associated with a significantly worse survival [hazard ratio (HR) = 2.18; 95% confidence interval (CI): 1.20–3.98; adjusted P = 0.01] and an increased risk of relapse (HR = 1.94; 95% CI: 1.6–2.5; adjusted P = 0.01). After further stratification for tumor grading, stage III patients carrying the G allele of rs4919510 and undergoing adjuvant chemotherapy were at decreased risk of relapse (HR = 0.44; 95% CI: 0.2–0.9; adjusted P = 0.03). The present study confirms that variations in miRNA-encoding genes may be an important factor for modulating CRC prognosis and predicting therapy response.

Introduction

Colorectal cancer (CRC) is one of the most frequent malignancies in Western countries with one of the highest rates of morbidity and mortality worldwide (1). Chemotherapy is the most widespread treatment for CRC, with 5-fluorouracil (5-FU) frequently used as the main chemotherapeutic agent (2,3). High-risk stages II, III or IV are usually recommended for 5-FU-based chemotherapy. However, there is a substantial proportion of CRC patients that do not benefit from 5-FU-based regimen (either in the adjuvant or palliative settings) (2–4). To improve the response to CRC treatment, one strategy could be a selection of appropriate subgroups of patients with predicted favorable prognosis after 5-FU chemotherapy. At present, effective biomarkers to be used as predictors of response to chemotherapy, survival and progression have not been clearly identified (5,6).

MicroRNAs (miRNAs) are small, evolutionarily conserved, non-coding RNA molecules (21–25 nucleotides in length) that have an important function in post-transcriptional gene regulation (7). They negatively regulate gene expression by inhibiting translation and causing degradation of target messenger RNA (mRNA). miRNAs work to fine tune translation through specific miRNA binding. The interest in miRNAs has grown up with the discovery of their implications as key regulators in many diseases including cancer (8,9). The aberrant expression or alteration of miRNAs characterize many different types of cancer, including CRC. Emerging data indicate that these patterns could be used for cancer classification improvements and to ameliorate cancer treatment response prediction and prognosis (10).

Various studies linked miRNA aberrant expression and genetic variations in both miRNA-encoding genes and in their binding sites in target genes to CRC risk, prognosis and drug response (11–14). We have recently reported the first evidence of the clinical impact of single nucleotide polymorphisms (SNPs) in 3' untranslated region affecting miRNA binding in genes of specific DNA repair pathways in CRC (13,15,16). So far, many pharmacogenetic studies have been carried out under the assumption that genetic variants lead to static effects on protein expression and function (6). However, the presence of SNPs directly in miRNA-encoding genes may have an even higher impact on CRC. These genetic variations may affect miRNAs biogenesis, maturation and/or target site binding. For instance, a single base pair change can affect binding and recognition of the processing machinery or, alternatively, a SNP in the mature sequence can alter target site interactions, ultimately influencing the binding to the target site and the regulation of target gene expression (17). Finally, genetic variations in the seed sequence can significantly transform the nature of miRNA itself and therefore change its target library (18). The aforementioned examples impressively enforce the need of increasing elucidation of the significance of the interactions of genetic variants, epigenetic modulation and drug response. Importantly, the frequency of SNPs in miRNAs is significantly lower compared with the rest of the genome, indicating the importance of their evolutionary conservation (19).

Several SNPs in miRNA-related genes have been previously associated with the risk of CRC (20) and a polymorphism in mir-26a-1 was associated with response to irinotecan-based chemotherapy in CRC patients (21). More recently, Lin et al. (22) found that a polymorphism in miR-608 was associated with increased risk for both recurrence and death and a polymorphism in miR-219-1 was associated with increased risk for death in a mixed population of stage III CRC patients from USA undergoing 5-FU-based chemotherapy. In the present study, we took advantage of these interesting results to investigate whether these two SNPs were also related to clinical outcome in a set of CRC patients from the Czech Republic with complete follow-up information that were previously genotyped for SNPs in miRNA binding sites (13,15).

Material and methods

Study population and data collection

Blood samples from 1083 subjects were collected among patients with histologically confirmed CRC, recruited between September 2003 and October 2010 from several oncological departments in the Czech Republic (Prague, Benešov, Brno, Liberec, Pilsen, Příbram, Ústí nad Labem and Zlín). A detailed description of the CRC population has been reported in reference (13).
All subjects were informed and provided written consent to participate in the study and to approve the use of their biological samples for genetic analyses, according to the Helsinki declaration. The design of the study was approved by the local Ethics Committee. Study subjects provided information on their lifestyle habits, body mass index, diabetes and family/personal history of cancer, using a structured questionnaire to determine demographic characteristics and potential risk factors for CRC.

Follow-up of the patients

Eight hundred and sixty-six of the CRC cases were initially monitored, with follow-up until 31 August 2011. A second group, consisting of 232 CRC patients was recruited later on and followed up until 31 March 2013. For all subjects, clinical data at the time of diagnosis, including location of the tumor, UICC (International Union Against Cancer) tumor-node-metastasis (TNM) stage system, pathological tumor stage and adjuvant chemotherapy treatment were collected, along with information about distant metastasis, relapse and date of death. Fifteen patients were excluded from the analyses because they appeared (after recruitment) to have Lynch syndrome or any other familial CRC form. Four hundred and eleven CRC cases received a 5-FU-based adjuvant regimen as first-line postoperative therapy. The therapy consisted of either a Mayo regimen, delivered as a bolus infusion of 5-FU (425 mg/m²) and leucovorin (10 mg/m²) for 5 days every 4 weeks six times or a simplified DeGrammont regimen which consisted of a 2 h intravenous infusion of leucovorin (200 mg/m²), then a 5-FU intravenous bolus (400 mg/m²) followed by a 46h 5-FU continuous intravenous infusion (2400–3000 mg/m²). Four hundred and forty subjects did not receive any adjuvant chemotherapy after surgery.

In this study, the outcome variables measured were 5-FU-based chemotherapy, overall survival (OS, time from diagnosis until death or censorship) and event-free survival (EFS, time of surgery or end of chemotherapy until date of relapse, death or censorship).

Selection of candidate genes/polymorphisms and SNP genotyping

This study is a replication of significant associations observed in a different CRC population recently reported in reference (22). In particular, the above work of Lin et al., describes two polymorphisms in two miRNA genes (rs213210 in mir219-1 and rs4919510 in miR608) associated with prognosis in patients with stage III CRC treated with fluoropyrimidine-based chemotherapy.

Genomic DNA was isolated from peripheral blood lymphocytes as described in reference (23). The two selected SNPs were genotyped using the KASPTR genotyping assay by LGC genomics (Hoddesdon, Herts, UK) (protocol available from LGC Genomics http://www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/kasp-technical-resources/). For quality control purposes, duplicate samples (5% of the total numbers of samples) were repeated for each SNP, no template controls were included in each plate and Hardy–Weinberg equilibrium test was performed.

Statistical analyses

Pearson’s chi-squared test (1 degree of freedom), with a type-I error threshold set at α = 0.05, was used to verify whether the genotypes were in Hardy–Weinberg equilibrium.

For all the genotypes, regression coefficients for additive hazard regression model were estimated.

OS in patients was evaluated using the date of death (regardless of the cause) or the date of follow-up termination as the end point. EFS was defined as the time from surgery/end of therapy to the occurrence of distant metastasis, recurrence or death, whichever came first. The survival curves for OS and EFS were derived by the Kaplan–Meier method and statistical significance was determined using the log-rank test (R version 2.14-2, Survival package). The relative risk of death was estimated as hazard ratio (HR) using Cox regression (R version 2.14-2, Survival package). Multivariate survival analyses were adjusted for age, gender, T, N, M status and chemotherapy for the whole group of patients. After stratification for treatment and grading only age and gender were kept as covariates in the model.

Results

In total we performed a survival analysis on 1083 CRC patients. Characteristics of the study population are reported in Table I. For both SNPs, the genotype distribution was in Hardy–Weinberg equilibrium (for rs213210: χ² = 0.32 and P = 0.85; for rs4919510: χ² = 1.15 and P = 0.56).

The mean (median) OS and EFS for patients were 86.5 (80.5) and 72.6 (62.4) months, respectively. In the preliminary univariate assessment of covariates gender, age, T, N, M status, chemotherapy treatment, CRC stage and pathological tumor stage were associated with OS and EFS (Table I). Advanced age, male gender and current smoking status were related to a shorter OS. In particular, men showed also a higher risk of relapse or metastasis [OS: HR = 1.54, 95% confidence interval (CI) = 1.23–1.92, P = 0.0011; EFS: HR = 1.35, 95% CI: 1.09–1.68, P = 0.006]. Five established prognostic factors (T, N, M status, tumor grade and pathological stage) were associated with decreased patients’ survival and increased risk of recurrence. Moreover, adjuvant chemotherapy was also associated with survival (Table I).

Both genotyped polymorphisms were not significantly associated with survival and recurrence in the whole group of cases (for rs213210, OS: HR = 1.06, 95% CI: 0.72–1.57, P = 0.75; EFS: HR = 1.12, 95% CI: 0.77–1.62, P = 0.56; for rs4919510, OS: HR = 1.01, 95% CI: 0.79–1.29, P = 0.91; EFS: HR = 0.91, 95% CI: 0.72–1.16, P = 0.46).

After stratification for 5-FU-based chemotherapy, we observed that patients undergoing this treatment and carrying the variant T allele of rs213210 had a shorter OS (HR = 2.18; 95% CI: 1.20–3.98; P = 0.01) and EFS (HR = 1.94; 95% CI: 1.16–3.25; P = 0.01) than those with the wild-type genotype (Table II). These patients also showed a similar significant trend across levels in the Kaplan–Meier curves but only for EFS (log-rank test: P = 0.02, median survival time for CC carriers not reached, median survival time for T allele carriers = 52.9 months; Figure 1A). No association was found in the group of CRC patients not treated with 5-FU-based chemotherapy.

After further stratification for cancer stage among cases following a 5-FU-based chemotherapy, rs4919510 was associated with a decreased risk of recurrence only in patients with stage III cancer (Table II). In particular, carriers of the variant G allele were at significantly decreased risk of recurrence when compared with CC genotype carriers (HR = 0.44, 95% CI: 0.20–0.94, P = 0.03; log-rank test: P = 0.06, median survival time for CC carriers and for G allele carriers not reached; Figure 1B).

Discussion

In the present work, two SNPs in miRNA-encoding genes resulted associated with CRC survival and recurrence in concomitance with adjuvant chemotherapy. In particular, among patients undergoing 5-FU treatment carriers of the variant T allele of rs213210 (in mir-219-1) were associated with an increased risk of recurrence and death. On the other hand, carriers of the variant G allele of rs4919510 (in miR-608) resulted at decreased risk of recurrence when compared with wild-type genotype carriers only among stage III CRC patients undergoing 5-FU chemotherapy.

This study partially confirms the observations of Lin et al. (22) and further highlights the importance of the presence of SNPs in miRNA-related genes as determinants of clinical outcomes. In fact, in the previous investigation, based on a similar design of the study, the authors observed for the same SNPs the significant associations only for the subset of stage III patients undergoing 5-FU treatment. Moreover, variant alleles for both SNPs were associated with worse survival while for only rs4919510 also with an increased risk of recurrence. In the present study, an association with worse survival and increased risk of relapse was observed only for rs213210 and considering the group of all CRC patients undergoing 5-FU-based chemotherapy, independently of the staging. Interestingly, rs4919510 was inversely associated with risk of recurrence.

Notably, both studies were similar regarding the number of patients and the age and sex distributions; however, there was a substantial difference in the proportion of patients with stages III and IV (in Lin et al. study there were 331 stage III (30.2%) and 392 stage IV CRC (35.8%) patients; in our study, 244 stage III (28.3%) and 177 stage IV (20.5%) patients). As a consequence, also the proportion of patients undergoing fluoropyrimidine-based chemotherapy was different [in Lin et al. 808 cases (73.8%) versus 287 not undergoing (26.2%); in our study 411 cases (48.3%) versus 440 not undergoing (51.7%)]. This last issue may partially explain observed differences in the results. On the other hand, it is particularly interesting that for both...
Table I. Clinical and anamnestic characteristics significantly affecting OS and EFS of the CRC patients with complete follow-up (Cox regression)

<table>
<thead>
<tr>
<th></th>
<th>OS (95% CI)</th>
<th>EFS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>427</td>
<td>Ref</td>
</tr>
<tr>
<td>Males</td>
<td>656</td>
<td>1.54 (1.23–1.92)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<tr>
<td>≤55</td>
<td>293</td>
<td>Ref</td>
</tr>
<tr>
<td>56–62</td>
<td>248</td>
<td>1.43 (1.05–1.95)</td>
</tr>
<tr>
<td>63–70</td>
<td>294</td>
<td>1.39 (1.04–1.88)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>248</td>
<td>2.02 (1.50–2.72)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>No</td>
<td>533</td>
</tr>
<tr>
<td>Yes</td>
<td>493</td>
<td>1.26 (1.02–1.56)</td>
</tr>
<tr>
<td>pT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>Ref</td>
</tr>
<tr>
<td>2</td>
<td>166</td>
<td>2.64 (0.94–7.40)</td>
</tr>
<tr>
<td>3</td>
<td>535</td>
<td>5.84 (2.17–15.71)</td>
</tr>
<tr>
<td>4</td>
<td>136</td>
<td>9.21 (3.36–25.26)</td>
</tr>
<tr>
<td>pN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>498</td>
<td>Ref</td>
</tr>
<tr>
<td>2</td>
<td>260</td>
<td>2.17 (1.69–2.79)</td>
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<tr>
<td>3</td>
<td>136</td>
<td>3.40 (2.35–4.91)</td>
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<tr>
<td>pM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>725</td>
<td>Ref</td>
</tr>
<tr>
<td>2</td>
<td>177</td>
<td>4.80 (3.83–6.02)</td>
</tr>
<tr>
<td>3–4</td>
<td>190</td>
<td>2.35 (1.57–3.53)</td>
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<tr>
<td>5-FU-based chemotherapy</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>411</td>
<td>Ref</td>
</tr>
<tr>
<td>No</td>
<td>440</td>
<td>1.42 (1.13–1.790)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>149</td>
<td>Ref</td>
</tr>
<tr>
<td>2</td>
<td>293</td>
<td>2.14 (1.32–3.48)</td>
</tr>
<tr>
<td>3</td>
<td>244</td>
<td>3.75 (2.33–6.03)</td>
</tr>
<tr>
<td>4</td>
<td>177</td>
<td>11.87 (7.44–18.95)</td>
</tr>
<tr>
<td>Pathological tumor stage</td>
<td>1</td>
<td>Ref</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>Ref</td>
</tr>
<tr>
<td>3</td>
<td>466</td>
<td>1.84 (1.26–2.69)</td>
</tr>
<tr>
<td>3–4</td>
<td>199</td>
<td>2.35 (1.57–3.53)</td>
</tr>
</tbody>
</table>

Significant results in bold. *Numbers may not add up to 100% of available subjects because of missing information. bEx-smokers included into non-smokers.

Table II. SNPs associated with outcomes in (A) patients receiving 5-FU-based chemotherapy and in (B) stage III CRC patients receiving 5-FU-based chemotherapy

<table>
<thead>
<tr>
<th>(A) Gene</th>
<th>SNP</th>
<th>OS Event/no event</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>EFS Event/no event</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-219-1</td>
<td>rs213210</td>
<td>CC</td>
<td>98/242</td>
<td>Ref</td>
<td>213/242</td>
<td>1.28 (0.93–1.75)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT+TT</td>
<td>14/18</td>
<td>Ref</td>
<td>19/13</td>
<td>2.18 (1.20–3.98)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC+GG</td>
<td>79/191</td>
<td>Ref</td>
<td>110/156</td>
<td>1.00 (0.62–1.61)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT+TT</td>
<td>31/66</td>
<td>Ref</td>
<td>38/58</td>
<td>1.00 (0.62–1.61)</td>
<td>0.99</td>
</tr>
<tr>
<td>miR-608</td>
<td>rs4919510</td>
<td>CC</td>
<td>53/105</td>
<td>Ref</td>
<td>58/99</td>
<td>1.48 (0.52–4.25)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT+TT</td>
<td>41/84</td>
<td>Ref</td>
<td>52/72</td>
<td>1.48 (0.52–4.25)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC+GG</td>
<td>14/28</td>
<td>Ref</td>
<td>11/31</td>
<td>1.00 (0.50–2.01)</td>
<td>0.99</td>
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</table>

<table>
<thead>
<tr>
<th>(B) Gene</th>
<th>SNP</th>
<th>OS Event/no event</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>EFS Event/no event</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-219-1</td>
<td>rs213210</td>
<td>CC</td>
<td>53/105</td>
<td>Ref</td>
<td>58/99</td>
<td>1.48 (0.52–4.25)</td>
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<td></td>
<td></td>
<td>CT+TT</td>
<td>41/84</td>
<td>Ref</td>
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<td>1.48 (0.52–4.25)</td>
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<tr>
<td></td>
<td></td>
<td>GC+GG</td>
<td>14/28</td>
<td>Ref</td>
<td>11/31</td>
<td>1.00 (0.50–2.01)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Significant results in bold. aAdjusted for gender, age and pathological tumor stage.

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studies the individual genetic background is relevant in association with 5-FU-based cancer treatment.

Above observed discrepancies for rs4919510 may also be due to significant differences in ethnicities of the two investigated populations. CRC patients recruited in the study of Lin et al. were Americans and, as reported by the authors, there was a substantial proportion of Afro-Americans (9.1%) and others ethnicities (10.3%) that were included in the study population. In the present study, CRC patients were all of Caucasian origin and from the Czech Republic, a small country in Central Europe that is characterized by a homogeneous population (Czechs 96%, others 4%; ref. 24). This condition may help in interpreting the observed discordances and similarities among the two studies. In another study conducted by Ryan et al. (18), rs4919510 was tested in association with CRC risk and clinical outcome in a mixed American population (60% Caucasians and 40% African-Americans) consisting of 245 CRC cases. Specifically, the GG genotype was associated with an increased risk of death in Caucasians and with a non-significant reduced risk of death in African-Americans. Authors suggested that the observed relationship might be due perhaps to a reduced power, given the smaller number of African-American individuals (n = 94), more than a missed epidemiological relevance (18). An additional study found similar results on CRC survival in a Chinese population that were in agreement with the findings of Ryan et al. in Caucasians (25). All these studies reinforce the hypothesis of the relevance of rs4919510 on CRC prognosis, with different trends of the associations, most likely due to ethnicity.

For what concerns rs213210, only few studies investigated the role of this polymorphism in the risk of cancer (esophageal squamous cell carcinoma, ref. 26), and in the cancer recurrence (non-small cell lung cancer, ref. 27 and CRC, ref. 22) with no significant association.
Polymorphisms in microRNA genes

Emerging. Notably, also in these studies it is not possible to compare the results since there are different ethnicities involved.

In view of a potential application of these SNPs as prognostic markers in CRC therapy, it seems necessary to clarify their impact in various populations. As reported by Adeyemo and Rotimi (28,29), most genetic-risk models derived from genome-wide association studies results in European Americans may generate spurious findings when applied to other populations. Conversely, certain diseases and conditions may show less marked disparity in incidence, but the underlying genetic-risk factors could be quite informative. Personalized medicine permits to optimize drug selection, dose and treatment duration, while averting adverse drug reactions of the individual patient. In this sense, genetic variants may be important instruments for personalized medicine as they can be useful biomarkers for the optimization of specific drugs. However, our understanding of the distribution of human pharmacogenomic variation remains limited and a poor representation of ethnically diverse samples from various parts of the world persists. For example, a considerable part of the studies based on American populations, are not accurately discriminating the distinct effect of Europeans and non-Europeans ancestry. These limitations result in insufficient evidence for genomic medicine in diverse populations. Therefore, the need for more genomic research in diverse populations is obvious.

A single miRNA can target hundreds of mRNAs, and it has been estimated that mature miRNAs regulate the expression of ~30% of all human genes (30). SNPs in miRNAs may alter the transcription of the primary miRNA transcript, may alter pre- and pri-miRNA maturation and processing or may affect miRNA::mRNA interaction with a common consequence of an alteration of their expression and/or maturation (17). Moreover, ~50% of miRNA genes are located in cancer-related chromosomal regions (31). Some established polymorphisms in miRNAs have been already associated with cancer (17,32); however, results are still inconclusive due to the high heterogeneity of cancer subtypes, the limited sample sizes of the studies and differences in the ethnicity of patients among the studies. A recent meta-analysis indicated that rs2910164 in miR-146a is associated with a decreased CRC risk (32). Recent studies suggest the importance of miRNAs in cancer drug resistance, particularly in cancer chemosensitivity and chemoresistance (33). In particular, Ma et al. (34) have recently reviewed the available studies that investigated miRNA functions in the development of drug resistance in cancer treatments, with few miRNAs indicated as crucial in chemosensitivity to fluoropyrimidine-based drugs in colon cancer.

rs4919510 is located in the mature hsa-miR-608 sequence. There are several predicted target sites for this miRNA as identified by MREditor tool (35), and 17 of them have been validated [as derived by querying miRWalk tool (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/)]. After a gene enrichment analysis for those targets, few pathways result significantly associated: RNA processing (GO:0006396; adjusted \( P = 0.004 \)), RNA metabolic process (GO:0016070; adjusted \( P = 0.004 \)), regulation of translation (GO:0006417; adjusted \( P = 0.008 \)) and negative regulation of translation (GO:0017148; adjusted \( P = 0.02 \)) (Supplementary Table 1, available at Carcinogenesis Online). Moreover, the presence among the predicted targets of five genes involved in the 5-FU metabolism (as extracted from the Pharmacogenomics Knowledge Database, www.pharmgkb.org, ‘fluorouracil pharmacogenomics’ and ‘fluoropyrimidine pharmacodynamics’) such as CDA, XRCC3, MTHFR, MTR and TP53 may be a further possible explanation of the observed association only in stage III and 5-FU-based postoperative chemotherapy-treated patients.

In contrast, for hsa-miR-219-1 which contains rs213210, 726 predicted targets have been found by MREditor tool, but none of them resulted as validated when investigated by miRWalk tool. After a gene enrichment analysis for those targets, few pathways were significantly represented: regulation of transcription (GO:0006355 and GO:0045449; \( P = 0.0068 \) and \( P = 0.01 \), respectively), regulation of RNA metabolic process (GO:0051252; \( P = 0.001 \)), transcription from RNA polymerase II promoter (GO:0006366; \( P = 0.003 \)) and regulation of gene expression (GO:0010468; \( P = 0.009 \)) (Supplementary Table 2, available at Carcinogenesis Online). When crossing the list of predicted target genes for miR-219-1 with the genes involved in the 5-FU metabolism (‘fluorouracil pharmacogenomics’ and ‘fluoropyrimidine pharmacodynamics’ as extracted from the Pharmacogenomics Knowledge Database, www.pharmgkb.org), ABCG2 and TP53 seem to be in common in the two lists. Interestingly, those genes, together with BCL2 are also in common with another list of 68 genes that are cited by the literature to be in connection with 5-FU, as extracted from GLAD4U database (Gene List Automatically Derived For You; bioinfo.vanderbilt.edu/gl4d4u).

The influence of genetic variants on drug response is becoming increasingly understood, as indicated by the study of Lin et al. and replicated by our present findings. In the recent years, there has been a rapid increase regarding genes that are regulated by epigenetic processes such as miRNA-related post-transcriptional processes (6). It is conceivable that SNPs in miRNA genes might alter miRNA processing and ultimately change the expression levels of the miRNA itself. Although the improvements in molecular biology technologies are helping to give novel insight into clinical outcome and drug response, the consequences of interactions of genetic variants with binding of miRNAs to genes are poorly understood so far. In fact, a functional assay that could test the effective functionality of SNPs in miRNA genes is not yet available. In order to fill this gap, we extracted data of miRNA and RNA sequencing in normal and tumor tissues of CRC patients from the TCGA database (36), with two main aims: (i) to test correlation between miRNAs of interest and their relative predicted/validated (when available) targets; (ii) to compare miRNAs expression profiles in normal and tumor tissues (data generated from two datasets: colon adenocarcinoma and rectal adenocarcinoma; http://tcga-data.nci.nih.gov/docs/publications/coadread_2012/). When we tested the relationships between expression data of miR-219-1 and its predicted targets, for some of them a significant correlation

Fig. 1. Kaplan–Meier EFS curves stratified (A) for rs213210 in all CRC patients undergoing 5-FU-based chemotherapy, and (B) for rs4919510 in stage III CRC patients undergoing 5-FU-based chemotherapy. MST, median survival time.
(negative for some target genes and positive for others) was observed 
(Supplementary Table 3, available at Carcinogenesis Online). These 
results may justify further analyses, since such differential directions
(negative/positive correlations) may be explained by the presence of 
genetic variations. In this context, unfortunately, we could not per-
form genotype analyses since the data are not publicly available.
Additionally, we evaluated the expression levels of miR-219-1 among
normal (n = 11) and CRC tumor tissues (n = 559) in the TCGA data-
set. We observed a substantial differential expression of miR-219-1 by
comparing either normal versus matched tumor tissues (P = 0.0031)
or normal versus the whole set of tumor tissues (P = 0.0045). We
could not equally test miR-608 because no expression data for this
miRNA are currently available in the TCGA database.

Further characterization of miRNA SNPs would improve our under-
standing of miRNA biogenesis and the potential contribution of
these SNPs to disease development and prognosis, which may also
favor therapeutic interventions.

Supplementary material

Supplementary Tables 1–3 can be found at http://carcin.oxfordjour-
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References

3. Hoff,P.M. et al. (2012) Literature review and practical aspects on the man-
gagement of oxaliplatin-associated toxicity. Clin. Colorectal Cancer, 11,
93–100.
genetics on drug response: challenges of pharmacoeigenomics.
Pharmacogenomics, 14, 1807–1809.
Oncol., 13, e249–e258.
8. Calif,G.A. et al. (2002) Frequent deletions and down-regulation of micro-
RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia.
347–355.
prognosis and therapeutic outcome in colon adenocarcinoma. JAMA, 299,
425–436.
12. Landi,D. et al. (2008) Polymorphisms within micro-RNA-binding sites and
13. Pardini,B. et al. (2013) Variation within 3’-UTRs of base excision
repair genes and response to therapy in colorectal cancer patients: a
6044–6056.
solid tumors defines cancer gene targets. Proc. Natl Acad. Sci. USA, 103,
2257–2261.
15. Naccarati,A. et al. (2012) Polymorphisms in miRNA-binding sites of
nucleotide excision repair genes and colorectal cancer risk. Carcinogenesis,
33, 1346–1351.
16. Vyometalkova,V. et al. (2014) Variations in mismatch repair genes and
18. Ryan,B.M. et al. (2012) rs4919510 in hsa-mir-608 is associated with out-
come but not risk of colorectal cancer. PLoS One, 7, e36306.
microRNA target sites are potentially associated with human cancers.
20. Goel,A. et al. (2010) Recent insights into the pathogenesis of colorectal
21. Boni,V. et al. (2011) Role of primary miRNA polymorphic variants in
metastatic colon cancer patients treated with 5-fluorouracil and irinotecan.
Pharmacogenomics J., 11, 429–436.
as predictors of clinical outcomes in colorectal adenocarcinoma patients.
23. Pardini,B. et al. (2008) DNA repair genetic polymorphisms and risk of
25. Xing,J. et al. (2012) Genetic polymorphisms in pre-microRNA genes as
prognostic markers of colorectal cancer. Cancer Epidemiol. Biomarkers
smoke exposure as risk factors for oesophageal squamous cell carcinoma.
polymorphisms on the recurrence of patients with completely resected non-
diseases show wide variation across multiple populations. Public Health
Genomics, 13, 72–79.
32. Ma,X.P. et al. (2013) Association between microRNA polymorphisms and
cancer risk based on the findings of 66 case-control studies. PLoS One, 8,
e79584.
33. Blower,P.E. et al. (2007) MicroRNA expression profiles for the NCI-60
523–531.
model that accounts for microRNA accessibility and Pumilio binding accu-

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