Published in final edited form as:

Clin Cancer Res. 2012 July 15; 18(14): 3982–3991. doi:10.1158/1078-0432.CCR-11-2951.

Genetic polymorphisms in microRNA-related genes as predictors of clinical outcomes in colorectal adenocarcinoma patients

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Abstract

Purpose—To evaluate the effects of single-nucleotide polymorphisms (SNPs) in microRNA-related genes on clinical outcomes in colorectal cancer (CRC) patients receiving first-line fluoropyrimidine-based chemotherapy.

Experimental Design—Forty-one SNPs in 26 microRNA-related genes were genotyped in 1,097 CRC patients recruited at the University of Texas MD Anderson Cancer Center. Patients were enrolled between 1990 and 2008 and last follow-up was in 2010. The associations between genotypes and recurrence-free survival (RFS), progression-free survival (PFS), and overall survival (OS) stratified by clinical stage were analyzed in 741 newly diagnosed patients (diagnosed within 1 year) and replicated the findings in additional 356 patients.

Results—In patients with stage III disease, *mir608:rs4919510* was associated with increased risk for both recurrence (HR=2.72; 95%CI, 1.38 to 5.33) and death (HR=3.53; 95%CI, 1.42 to 8.73).

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The associations were confirmed in the replication set and the combined HR for training and replication sets was 1.94 (95% CI, 1.31 to 2.86) for recurrence and 2.35 (95% CI, 1.40 to 3.93) for death, respectively. The mir219-1:rs213210 showed consistent association with death in the training set (HR=3.86; 95% CI, 1.33 to 11.22), the replication set (HR = 3.33; 95% CI, 1.39 to 7.98) and combined dataset (HR = 3.53; 95% CI, 1.80 to 6.95). In combined analysis of these two SNPs, patients carrying the variant genotypes at both sites exhibited a 5.6 fold increased risk of death.

Conclusion—Genetic polymorphisms in the microRNA pathway may predict prognosis in stage III CRC patients treated with fluoropyrimidine-based chemotherapy.

Keywords

colorectal cancer; polymorphism; microRNA; chemotherapy; recurrence; survival

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second-leading cause of cancer-related deaths in Western countries (1). Stage-I disease usually can be cured by surgery alone, but patients with high-risk stage-II, -III, or -IV disease are recommended for chemotherapy in the adjuvant or palliative setting (1-3). 5-Fluorouracil (5-FU) is the most frequently used chemotherapeutic agent for treating CRC patients. However, a substantial portion of CRC patients do not benefit from 5-FU based treatment regimens, whereas treatment-related toxicity may be severe and dose-limiting (4, 5). One strategy to improve the efficacy and reduce toxicity is to determine subgroups of patients who would have a poor or favorable prognosis after 5-FU-based chemotherapy, which may allow personalized treatment of CRC patients.

MicroRNA (miRNA) is a growing family of 21- to 23-nucleotide, endogenous, single-stranded non-coding RNAs that post-transcriptionally regulate the expression of target mRNA transcripts. A series of biogenesis steps yield mature miRNAs from pri-miRNA to pre-miRNA (6) (7) (8). Given that impaired miRNA processing can result in a substantial decrease in mature miRNA levels(9) and many miRNAs target mRNA transcripts involved in cell proliferation, differentiation, and apoptosis (10), it is conceivable that single-nucleotide polymorphisms (SNPs) in genes of miRNA and of the miRNA biogenesis pathway may have effects on cancer risk, prognosis, and treatment response. SNPs are inherited genetic variations, can be easily assayed by high-throughput and reliable technology, is capable of affecting gene expression or protein function, and therefore, are alternative or complementary markers to tissue based biomarkers. Several genome-wide association studies (GWAS) have identified at least 16 CRC susceptibility SNPs (11-14). However, due to the large sample size requirement and the heterogeneity of tumors and treatment, GWAS of clinical outcomes of CRC or any other cancers has not been successfully conducted yet. Candidate gene approach is still the main approach in association studies of clinical outcomes. Several SNPs in the miRNA-related genes have been associated with the risk of cancers(15-17). A pre-miRNA SNP (rs11614913) in miR-196a2 was found to be associated with poor survival in individuals with non-small-cell lung cancer (18). A SNP in miR26a-1 was associated with disease response to irinotecan-based chemotherapy in patients with metastatic CRC (19). However, another study in

Korean patients with surgically resected CRC did not observe any association between the polymorphisms in microRNA biogenesis-pathway and clinical outcomes (20). To further dissect these issues, we have performed a comprehensive study with regard to genetic variations in microRNA-related genes and clinical outcomes of CRC patients.

Materials and Methods

Study Population and Data Collection

1097 histologically confirmed colorectal adenocarcinoma patients were recruited at the University of Texas MD Anderson Cancer Center between January 1990 and June 2008, with follow-up to January 2010. Among them, there were 741 newly diagnosed patients (diagnosed within 1 year), which was analyzed as a training set in this study. The remaining 356 patients had a long history (diagnosed >1 year) of CRC prior to recruitment and was used as a replication set. A self-administered questionnaire was used for all patients to collect epidemiological data including demographical characteristics, tobacco use, family history of cancer and medical history. We reviewed patients' medical records to collect clinical information including date of diagnosis, performance status, clinical stage, tumor location, histological grade, pathological stage, treatment, and tumor recurrence/progression. Information on vital status was obtained from the medical records and Social Security Death Index (SSDI). Each patient donated 10 to 20-mL blood sample. All study participants signed an informed consent form, and the study was approved by the Institutional Review Board of MD Anderson Cancer Center.

SNP Selection and Genotyping

The selection of genes and SNPs has been described previously (16). Briefly, after an extensive mining of the International HapMap Project database (<http://www.hapmap.org>) (21), dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>) (22) and miRBase registry (<http://microrna.sanger.ac.uk/>) (23) for SNPs in microRNA pathway genes, we identified 41 potential functional polymorphisms: 24 SNPs in 11 genes related to miRNA biogenesis, 7 SNPs in 7 pre-miRNAs or mature miRNAs, and 10 SNPs in 8 pri-miRNAs. DNA was isolated from the peripheral blood samples using a QIAampDNA extraction kit (Qiagen, Valencia, California). SNP genotyping was done using the SNPLEX assay, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Internal quality controls and negative controls were used to ensure genotyping accuracy, and 5% of all samples were randomly selected and genotyped in duplicate, with 100% concordance. The average call rate for the SNPs was 98.8%. Two SNPs in pri-miRNAs (rs107822 and rs10425222) were excluded from further analysis due to the call rate <95%.

Statistical Analysis

The study endpoints were overall survival (OS), progression-free survival (PFS), and recurrence-free survival (RFS). OS was calculated from pathologic diagnosis to death regardless of the cause. PFS was defined as the time from pathologic diagnosis until disease progression or death, whichever occurred first. RFS was defined as the time from the date of pathologic diagnosis until first recurrence or death from any cause. Statistical analyses were performed using STATA software (version 10, STATA Corporation, College Station,

TX). Cox's proportional hazards model was used to estimate hazard ratios (HRs) and their 95% confidence intervals (CIs) for the multivariate survival analyses in the training set and the replication set. The analyses were adjusted for age, gender, ethnicity, clinical stage, histologic grade, and if necessary, treatment modality and chemotherapy regimen. The Benjamini-Hochberg False Discovery Rate (FDR) method was used to control for multiple comparisons with a threshold of $q=0.05$ (24). The Cochran Q statistics test was used for the assessment of heterogeneity. If the result of the Q test was not significant, the fixed-effects model was chosen. Otherwise, the pooled HR was estimated using the random effects model. Associations between genotypes and OS, PFS, and RFS were estimated using the Kaplan-Meier method, and statistical significance was determined using the log-rank test. We also evaluated the combined effects of the SNPs by the number of genotypes identified from the main effects analysis of single SNPs. All P-values were two-sided and a $P \leq 0.05$ was considered statistically significant.

Results

Patient Characteristics

Table 1 shows selected demographic, tumor, and treatment characteristics in 1097 CRC patients included in this study. Of the 741 patients in training set, the median patient age was 58 years (range, 19–97 years). The mean age (year $\pm$ SD) for stage I, II, III, and IV patients was 58.5 ± 1.5 , 59.1 ± 1.0 , 58.5 ± 0.84 , and 55.2 ± 0.77 , respectively. The median follow-up time was 32.5 months. There were 41 recurrences (22.7%) and 31 deaths (17.1%) among 181 patients with stage II disease, and 60 recurrences (27.8%) and 41 deaths (19.0%) among 216 patients with stage III disease, but median RFS and OS had not been reached during the follow-up time. Among 277 patients with stage IV disease, 148 patients (53.4%) had progression and 147 (53.1%) were dead, with median PFS of 18.2 months and median OS of 36.1 months. Age ($P=0.03$) and clinical stage ($P=2 \times 10^{-17}$) were significantly associated with RFS and PFS, while clinical stage ($P=2 \times 10^{-25}$) and histological grade ($P=0.0006$) correlated with OS. In addition, those who underwent curative surgery or received fluoropyrimidine-based chemotherapy had better clinical outcome.

There were 356 patients included in replication set. The mean age for stage I, II, III, and IV patients was 62.3 ± 2.0 , 59.1 ± 1.0 , 58.5 ± 0.84 , and 55.2 ± 0.77 , respectively. The median OS for the four stages was 63.2, 79.6, 71.8, and 36.6 months, respectively. These patients were diagnosed outside of MD Anderson Cancer Center over a year ago and came to MD Anderson Cancer Center for treatment due to potential tumor recurrence or progression; therefore, the recurrence, progression and death rates were higher than newly diagnosed patients (Table 1).

Training Set

We assessed the association of each individual SNP with OS, PFS, and RFS using a multivariate Cox model, adjusting for age, gender, ethnicity, smoking status, tumor site and histologic grade, and stratified by stages (Table 2). Among them, GEMIN4:rs7813 and rs910925 were in strong linkage disequilibrium ($r^2=0.99$), thus we only included rs7813 in the subsequent analysis.

Due to the excellent prognosis and rare events of recurrence and death, we did not carry out analysis of stage I patients. Among 117 patients with stage II disease who received 5-FU based chemotherapy, the most significant association with recurrence was conferred by the variant allele of RAN:rs14035 in a dose-dependent manner (per allele HR=2.32; 95% CI, 1.28 to 4.21; P=0.005, q=0.06). The homozygous variant genotype of AGO2:rs4961280 was significantly associated with an increased risk of death (HR=5.49; 95% CI, 1.39 to 21.74; P=0.02, q=0.18). Among 179 patients with stage III disease who received 5-FU based chemotherapy, the variant-containing genotypes of *mir608*:rs4919510 exhibited increased risks of both recurrence (HR=2.72; 95% CI, 1.38 to 5.33; P=0.004; q=0.03) and death (HR=3.53; 95% CI, 1.42 to 8.73; P=0.006; q=0.05). Moreover, these patients had a significantly shorter RFS (104.3 versus >117.8 months, log-rank P = 0.01; Figure 1A) and OS (125.5 vs. >117.8 months, log-rank P = 0.01) compared with those with the wild-type genotype. The GEMIN3: rs197412 and XPO5:rs11077 had highly significant associations with recurrence (HR=2.07, 95% CI, 1.31 to 3.27; P=0.002; q=0.03; and HR = 0.38, 95% CI, 0.19 to 0.78; P=0.002; q=0.03, respectively), while *mir604*:rs2368392 and *mir219-1*:rs213210 showed highly significant associations with death (HR=5.20; 95% CI, 1.55 to 17.50; P=0.007; q=0.05; and HR=3.86; 95% CI, 1.33 to 11.22; P=0.01; q=0.06, respectively). This increase in risk of death resulted in a decreased OS for those with the variant-containing genotypes of *mir219-1*:rs213210 compared to patients carrying the homozygous wild type genotype (64.8 vs. >117.8 months, log-rank P = 0.003; Figure 2A). Among 218 patients with stage IV disease, the effects of the variant-containing genotypes of *let-7f-2*:rs17276588 and *mir30c-1*:rs16827546 on progression were significant after adjusting for multiple comparison (HR=3.12; 95% CI, 1.61 to 6.06; P=0.0008, q=0.008; and HR=2.12; 95% CI, 1.22 to 3.67; P=0.007; q=0.04, respectively). Two SNPs, *Drosha*:rs13078 (HR = 1.66; 95% CI, 1.09 to 2.52; P=0.02) and *TRBP*:rs784567 (HR = 1.59; 95% CI, 1.03 to 2.43; P=0.04), were associated with the risk of death, but lost significance after adjusting for multiple comparison.

Replication Set

Genotype data was analyzed in 53 stage II, 108 stage III, and 110 stage IV patients. As shown in Table 3, the significant associations between *mir608*:rs4919510 and risk of both recurrence and death and the association between *mir219-1*:rs213210 and risk of death were confirmed in stage III patients in the replication set. Patients with the variant allele-containing genotypes of the *mir608*:rs4919510 showed increased risks of recurrence (HR=1.64; 95% CI, 1.02 to 2.63; P=0.04) and death (HR=1.93; 95% CI, 1.03 to 3.62; P=0.04) in the replication set. The pooled analysis HR was 1.94 (95% CI, 1.31 to 2.86; P=0.0008) for recurrence and 2.35 (95% CI, 1.40 to 3.93; P=0.001) for death. Furthermore, compared with patients carrying the wild-type genotype, those with the variant-containing genotypes had a decrease in RFS in replication set (16.0 vs. 23.7 months, log-rank P = 0.02; Figure 1B) and combined dataset (35.0 vs. 57.6 months, log-rank P = 0.0001; Figure 1C), respectively. The increase in risk of death also resulted in a decrease in OS in combined dataset (74.8 vs. 103.7 months, log-rank P = 0.0009, data not shown).

The variant-containing genotypes of the *mir219-1*:rs213210 showed consistent association with death in replication set (HR = 3.33; 95% CI, 1.39 to 7.98; P=0.007) and combined

dataset (HR = 3.53; 95% CI, 1.80 to 6.95; P=0.0003). These patients also had a significantly shorter OS than those with the wild-type genotype in replication set (50.4 vs. 76.0 months, log-rank P = 0.03; Figure 2B) and combined dataset (60.7 vs. 83.1 months, log-rank P = 0.003; Figure 2C).

Cumulative Effect

Since the variant genotypes of *mir608:rs4919510* and *mir219-1:rs213210* were confirmed in stage III patients receiving adjuvant fluoropyrimidine-based therapy, we next performed a joint analysis of these two SNPs. There was a significant trend for the increased risk of death with the increasing number of variant genotypes in training set (P for trend = 0.0001) and replication set (P for trend = 0.003). In the combined dataset, compared with patients without any variant genotype, patients carrying one variant genotype had a 2.51-fold increased risk of death (95% CI, 1.47 to 4.28; P=0.001), and the risk further increased to 5.6-fold (95% CI, 1.91 to 16.45; P= 0.002) for patients carrying two variant genotypes (P for trend = 5.25×10^{-5}). Similar cumulative effect was also observed for recurrence in the training (P for trend=0.0006), replication (P for trend =0.01), and combined sets (P for trend =0.001). In the combined dataset, carrying one or two variant genotypes resulted in 1.67-fold (95% CI, 1.12-2.49; P=0.01) and 3.53-fold (95% CI, 1.51-8.26; P=0.004) increased risk of recurrence, respectively.

Discussion

Our results reveal that *mir608:rs4919510* and *mir219-1:rs213210*, alone or in combination, showed consistent association with clinical outcome for patients with stage III disease in the training and replication set. To our knowledge, this is the largest study to date to investigate the role of genetic variations in miRNA pathway in clinical outcomes for CRC patients. Currently, tumor-node-metastasis (TNM) system remains the most commonly used criterion to predict prognosis and define the need for chemotherapy in CRC patients (25). Fluoropyrimidine-based chemotherapy was routinely used in stage III disease, but recurrence rates remained high.(26) Risk stratification based on germline genotype status would provide information for individual chemotherapy. In the present study, *mir608:rs4919510* was consistently associated with both recurrence and survival. A previous study has demonstrated that the *mir608:rs4919510* variant is associated with recurrence in patients with renal cell carcinoma (27). The SNP *rs4919510* is located in the mature *mir608* sequence and was predicted to bind to *mir608* target sites within the ACDC (adiponectin and collagen domain containing), CD4 (CD4 antigen), GHR (growth hormone receptor), RXRB (retinoic X receptor beta), and TP53 (tumor protein p53) genes with lower free energies than the wild type allele (28). These targets could affect CRC clinical outcome, for example, previous investigations reported that stage III CRC patients with tumors carrying TP53 mutation benefited from 5-FU based postoperative chemotherapy (29) (30). Therefore, it is conceivable that the variant *miR-608* could elicit different biological activities of these target molecules that could affect CRC clinical outcome. It has been reported that the variant allele of *mir219-1:rs213210* was associated with susceptibility to developing esophageal cancer (31). We found that CRC patients carrying the variant genotypes of *rs213210* had a worse survival, possibly through a mechanism of affecting the expression of mature

mir219-1. The expression of mir219-1 was highly up-regulated in metastatic hepatocellular carcinoma and was also capable of predicting patient survival and recurrence (32). A previous study found that miR-219 is a target of the CLOCK and BMAL1 complex and exhibits robust circadian rhythms of expression (33), and another study showed that circadian clock protein BMAL1 coordinates *in vivo* tumor growth, S-phase progression, thymidylate synthase, and 5-FU therapeutic index (34). It is tempting to speculate that circadian clock related mechanism may partially explain the link between miR-219 and recurrence in stage III CRC patients receiving 5-FU based therapy. The functional consequence of mir219-1:rs213210 is not clear. Further studies are warranted to evaluate whether this SNP affects mir219-1 expression and processing, and the biological mechanisms underlying the association of this SNP and recurrence.

We observed a clear and significant trend towards increased recurrence and death risk as the number of variant genotypes of *mir608*:rs4919510 and *mir219-1*:rs213210 and patients carrying two variant genotypes had a 5.6-fold increased risk of death. These results suggest that the cumulative influence of multiple SNPs was able to further enhance the separation between the patients with stage III disease who would have unfavorable prognosis following 5-FU-based adjuvant chemotherapy.

The effect of *GEMIN3*:rs197412, *XPO5*:rs11077 and *mir604*:rs2368392 was also highly significant in stage III disease, though the results were not confirmed in the replication set. The variant allele of *GEMIN3*:rs197412 has been shown to be associated with susceptibility to developing premalignant oral lesions (35). *XPO5*:rs11077 was recently identified as a predictor of disease control rate for colon cancer patients treated with 5-fluorouracil and irinotecan (19). Polymorphisms affecting the expression of proteins involved in general miRNA biogenesis, such as *GEMIN3* and *XOP5*, could alter global miRNA homeostasis and tissue-specific miRNA alterations (36-38). The global repression of miRNA maturation has been demonstrated to promote cellular transformation and tumorigenesis (9). The functional consequence of SNPs in pre-miRNA sequences, such as *mir604*:rs2368392, have already been suggested to affect the processing and levels of the mature miRNA(18)., Given that variability in expression levels of miRNA may influence tumor progression and sensitivity to a fluoropyrimidine agent (39) (40), these potential functional SNPs could emerge as potential predictors of prognosis and the efficacy of fluoropyrimidine.

For stage II diseases, *RAN*: rs14035 was associated with OS with high significance in patients receiving surgery and adjuvant fluoropyrimidine treatment. The key element in the nuclear export of pre-miRNAs is *XPO5*-*RAN* GTP-pre-miRNA heteroternary complex. The disruption in the pre-miRNA nucleo-cytoplasmic transport would impair the production of mature miRNAs in cancer cells (41). *RAN* is overexpressed in most cancer cell lines including colon cancer, suggesting its role in tumor transformation (42). Moreover, it was reported that *RAN* suppressed the activation of JNK and inhibited apoptosis induced by an anticancer drug (43). In patients with stage IV CRC, *let7f-2*:rs17276588 had significant predictive effect on progression in patients receiving fluoropyrimidine-based chemotherapy. This SNP remained significant after adjusting for multiple comparison in the training set and also showed the same direction in the replication set, although did not reach statistical significance. The let-7 family of miRNAs was found to regulate Ras activity by binding to

the 3'-UTR of the human Ras gene (44). K-ras mutations have been associated with increased risk of relapse and death in CRC patients by the multi-center Colorectal Cancer Collaborative Group (RASCAL) study (45) (46). Nakajima et al. demonstrated an association between members of the let-7 family and responsiveness to the oral fluoropyrimidine S-1 (47). Therefore, it is biologically plausible that SNPs in let7 family of miRNAs affect CRC prognosis and treatment response.

Since the training and replication set split as we presented here may not be optimal due to the over-representation of recurrence and progression patients in the replication set, we also randomly split the entire dataset into a training and a replication set and re-performed all the analyses (Supplementary Table 1 and 2) and the results were comparable to the current split. For example, for survival, the mir608 SNP ($P=0.019$) and mir219-1 SNP ($P=0.036$) ranked the top two in P-values in the new training set. In the new validation set, mir219-1 SNP was the most significant SNP and mir608 SNP was borderline. In pooled analysis, the mir219-1 SNP ranked the first and the mir608 SNP ranked the third. Because we believe the original training set was cleaner and more optimal for initial screening, we presented the original results. Since our study is the first to comprehensively evaluate SNPs in miRNA pathways as prognostic factors for CRC patients receiving 5-FU based chemotherapy, the data of pooled analysis of all SNPs would provide valuable information to investigators in the field who may perform independent validation.

The major strength of our study is the large population size that allowed us to perform stage-stratified analyses in training and replication sets, which limited the confounding of tumor and treatment heterogeneity. The significant results, particularly mir608:rs4919510 and mir219-1:rs213210, remained significant after adjusting for multiple comparison and also were replicated in the replication set; therefore, these SNPs are not likely to be false positives. An additional strength is the comprehensive clinical data for each of these patients. The main limitation of this study is that the replication set was not newly diagnosed patients and those patients came to MD Anderson mainly due to potential recurrence or progression. Therefore, the relative small and heterozygous population in the replication set may result in false negatives.

In conclusion, we identified several highly plausible candidate SNPs in the miRNA pathway that are associated with clinical outcome in CRC patients and also observed significant cumulative effect of multiple SNPs in predicting prognosis in CRC patients receiving 5-FU-based chemotherapy. Further studies are needed to replicate these SNPs in independent populations, to functionally characterize the significant genetic variants, and to find the biological mechanisms underlying the associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This work was supported by a Multidisciplinary Research Program (MRP) grant on colorectal cancer from The University of Texas MD Anderson Cancer Center

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Translational Relevance

MicroRNA plays important roles in cellular proliferation, differentiation, and apoptosis. This study comprehensively evaluated the associations between single nucleotide polymorphisms (SNPs) in microRNA-related genes and clinical outcomes in colorectal cancer (CRC) patients receiving first-line fluoropyrimidine-based chemotherapy, and identified several biologically plausible individual predictors of recurrence and survival as well as cumulative effect in predicting patient outcomes. The validation and incorporation of the identified SNPs and interactions with the clinical variables may help identify CRC patients with distinct prognosis to allow for personalized cancer therapy.

Figure 1. Kaplan-Meier recurrence-free survival curves of patients with stage III disease receiving fluoropyrimidine-based chemotherapy by genotypes of *mir608*:rs4919510. (A) training set, (B) replication set, and (C) combined set.

Figure 2. Kaplan-Meier overall survival curves of patients with stage III disease receiving fluoropyrimidine-based chemotherapy by genotypes of mir219-1:rs213210. (A) training set, (B) replication set, and (C) combined set

Table 1

Selected demographic and clinical characteristics of CRC patients

Variables	Training set (N=741)					Replication set (N=356)						
	Recurrence /progression	No recurrence /progression	P Value	Dead	Alive	P value	Recurrence /progression	No recurrence /progression	P value	Dead	Alive	P value
Age, mean (SD)	55.9(13.2)	58.1(12.7)	0.03	57.5(13.9)	57.3(12.6)	0.86	56.2(12.5)	53.8(13.5)	0.25	56.7(125)	54.75(12.7)	0.16
Gender, N (%)												
Male	161(63.4)	297(61.1)		147(66.5)	312(60.0)		188(59.7)	17(42.5)		128(58.7)	78(56.5)	
Female	93(36.6)	189(38.9)	0.55	74(33.5)	208(40.0)	0.09	127(40.3)	23(57.5)	0.04	90(41.3)	60(43.5)	0.68
Race, N (%)												
Caucasian	204(80.6)	407(83.9)		182(82.7)	429(82.7)		263(83.5)	31(77.5)		179(82.1)	116(84.1)	
African-American	23(9.1)	39(8.0)		20(9.1)	42(8.1)		19(6.0)	3(7.5)		15(6.9)	7(5.1)	
Others	26(10.3)	39(8.0)	0.50	18(8.2)	48(9.2)	0.83	33(10.5)	6(15.0)	0.63	24(11.0)	15(10.9)	0.78
Tumor location, N (%)												
Proximal	66(26.4)	156(32.2)		61(28.0)	161(31.1)		88(28.9)	12(30.0)		63(30.3)	37(27.0)	
Distal	75(30.0)	126(26.0)		72(33.0)	129(25.0)		111(36.5)	14(35.0)		71(34.1)	55(40.1)	
Rectal	109(43.6)	202(41.7)	0.23	85(39.0)	227(43.9)	0.08	105(34.5)	14(35.0)	0.98	74(35.6)	45(32.8)	0.52
Stage, N (%)												
Stage I	5(2.0)	61(12.6)		2(0.9)	64(12.3)		37(11.7)	1(2.5)		23(10.6)	15(10.9)	
Stage II	41(16.1)	140(28.8)		31(14.0)	150(28.8)		78(24.8)	9(22.5)		43(19.7)	45(32.6)	
Stage III	60(23.6)	156(32.1)		41(18.6)	175(33.7)		108(34.3)	7(17.5)		70(32.1)	45(32.6)	
Stage IV	148(58.3)	129(26.5)	2×10 ⁻¹⁷	147(66.5)	131(25.2)	1×10 ⁻²⁵	92(29.2)	23(57.5)	0.002	82(37.6)	33(23.9)	0.01
Histology grade, N (%)												
Well-differentiated	9(3.6)	25(5.2)		6(2.8)	28(5.5)		10(3.3)	0(0.0)		6(2.8)	4(2.9)	
Moderate-differentiated	192(76.5)	377(78.7)		157(72.0)	413(80.5)		237(77.2)	33(84.6)		158(74.9)	113(83.1)	
Poorly-differentiated	50(19.9)	77(16.1)	0.29	55(25.2)	72(14.0)	0.0006	60(19.5)	6(15.4)	0.40	47(22.3)	19(14.0)	0.16
Primary tumor curative surgery												
Yes	158(62.2)	388(79.8)		113(51.1)	433(83.3)		272(86.3)	22(55.0)		176(80.7)	119(86.2)	
No	96(37.8)	98(20.2)	2×10 ⁻⁷	108(48.9)	87(16.7)	1×10 ⁻¹⁹	43(13.7)	18(45.0)	7×10 ⁻⁷	42(19.3)	19(13.8)	0.18
Fluoropyrimidine-based chemotherapy												
Yes	214(84.3)	318(65.4)		174(78.7)	359(69.0)		239(75.9)	37(92.5)		176(80.7)	101(73.2)	
No	40(15.7)	168(34.6)	6×10 ⁻⁸	47(21.3)	161(31.0)	0.007	76(24.1)	3(7.5)	0.02	42(19.3)	37(26.8)	0.10

Table 2

SNPs associated with outcomes of CRC patients receiving 5-FU based chemotherapy stratified by stages

Gene	SNP	Model [#]	Training set			Replication set			Pooled analysis		
			HR(95%CI)	P value [*]	q value	HR(95%CI)	P value [*]	HR(95%CI)	P value	P for Hetero. [†]	MAF ^{‡‡}
Recurrence:											
Stage II											
RAN	rs14035	add	2.32(1.28-4.21)	0.005	0.06	1.00(0.56-1.78)	1.00	1.52(0.67-3.46)	0.32	0.05	12%
mir373	rs12983273	add	2.48(1.19-5.14)	0.02	0.07	1.22(0.63-2.37)	0.55	1.68(1.03-2.74)	0.04	0.16	13%
Drosha	rs6877842	dom	0.29(0.09-0.92)	0.04	0.16	1.05(0.52-2.10)	0.89	0.75(0.41-1.36)	0.34	0.06	18%
Stage III											
mir608	rs4919510	dom	2.72(1.38-5.33)	0.004	0.04	1.64(1.02-2.63)	0.04	1.94(1.31-2.86)	0.0008	0.22	17%
GEMIN3	rs197412	add	2.07(1.31-3.27)	0.002	0.03	1.07(0.72-1.57)	0.74	1.47(0.72-2.81)	0.24	0.03	10%
XPO5	rs11077	dom	0.38(0.19-0.78)	0.008	0.04	1.29(0.79-2.10)	0.31	0.72(0.22-2.38)	0.59	0.005	40%
GEMIN4	rs2740348	dom	2.56(1.25-5.24)	0.01	0.04	0.86(0.50-1.50)	0.61	1.45(0.50-4.21)	0.50	0.02	18%
GEMIN3	rs197388	add	1.82(1.08-3.05)	0.02	0.07	0.99(0.57-1.70)	0.96	1.36(0.94-1.99)	0.11	0.11	29%
GEMIN4	rs7813	add	1.62(1.06-2.49)	0.03	0.07	1.25(0.84-1.87)	0.27	1.41(1.05-1.89)	0.02	0.39	14%
Progression											
Stage IV											
let7f-2	rs17276588	dom	3.12(1.61-6.06)	0.0008	0.008	1.69(0.72-4.01)	0.23	2.48(1.47-4.19)	0.0007	0.28	2%
mir30c-1	rs16827546	dom	2.12(1.22-3.67)	0.007	0.04	1.12(0.50-2.51)	0.78	1.73(1.10-2.73)	0.02	0.20	4%
Drosha	rs6877842	dom	1.61(1.07-2.43)	0.02	0.08	0.97(0.58-1.62)	0.89	1.32(0.96-1.82)	0.09	0.13	18%
DICER	rs13078	dom	1.51(1.01-2.24)	0.04	0.12	0.74(0.45-1.21)	0.23	1.07(0.53-2.16)	0.41	0.03	14%
Survival:											
Stage II											
AGO2	rs4961280	rec	5.49(1.39-21.74)	0.02	0.18	4.61(0.73-29.16)	0.10	5.16(1.71-15.53)	0.004	0.84	13%
Stage III											
mir608	rs4919510	dom	3.53(1.42-8.73)	0.006	0.05	1.93(1.03-3.62)	0.04	2.35(1.40-3.93)	0.001	0.28	17%
mir219-1	rs213210	dom	3.86(1.33-11.22)	0.01	0.06	3.33(1.39-7.98)	0.007	3.53(1.80-6.95)	0.0003	0.83	6%
mir604	rs2368392	rec	5.20(1.55-17.50)	0.007	0.05	0.51(0.17-1.50)	0.22	1.62(0.16-15.95)	0.68	0.005	25%
Stage IV											

Gene	SNP	Model [#]	Training set			Replication set			Pooled analysis		
			HR(95%CI)	P value	q value	HR(95%CI)	P value	*	HR(95%CI)	P value	MAF ^{††}
DICER	rs13078	dom	1.66(1.09-2.52)	0.02	0.21	0.87(0.51-1.48)	0.61		1.30(0.93-1.80)	0.12	14%
TRBP	rs784567	rec	1.59(1.03-2.43)	0.04	0.31	0.76(0.42-1.38)	0.37		1.13(0.65-2.31)	0.05	4%

* Adjusted for age, gender, ethnicity, smoking status, tumor site, histologic grade, and treatment.

[#] Genetic model of inheritance: dom- dominant model, rec- recessive model, add- additive model.

[†] Cochran's Q statistic to test for heterogeneity between studies.

^{††} MAF: minor allele frequency

Table 3

Association of mir608:rs4919510 and mir219-1:rs213210 with recurrence and survival in stage III patients receiving fluoropyrimidine-based chemotherapy

Genotype	Training set				Replication set				Pooled analysis			
	Event/No event	HR (95%CI)	P*	MST†	Log rank p	Event/No event	HR (95%CI)	P*	MST†	Log rank P	HR (95%CI)	P*
Recurrence:												
mir608:rs4919510												
CC	25/91	Reference		>117.8		62/4	Reference		24.5		Reference	87.8
CG+GG	19/44	2.72 (1.38-5.33)	.004	104.3	.01	39/2	1.64 (1.02-2.63)	.04	15.4	.04	1.65 (1.13- 2.41)	.01
Survival:												
mir608:rs4919510												
CC	16/100	Reference		>117.8		41/25	Reference		79.7		Reference	103.7
CG+GG	12/51	3.53 (1.42-8.73)	.006	125.5	.01	24/17	1.93 (1.03-3.62)	.04	62.7	.08	1.96 (1.19- 3.21)	.008
mir219-1:rs213210												
CC	22/133	Reference		>117.8		55/36	Reference		76.4		Reference	101.8
CT+TT	6/14	3.86 (1.33-11.22)	.01	64.8	.003	8/4	3.33 (1.39-7.98)	.007	50.4	.03	3.22 (1.70- 6.10)	.0003

* Adjusted for age, gender, ethnicity, smoking status, tumor site, histologic grade, and treatment

† MST, median event-free survival times in months

Table 4

Cumulative analysis of mir608:rs4919510 and mir219-1:rs213210 in stage III patients receiving fluoropyrimidine-based chemotherapy

No. of Variant Genotypes	No recurrence, n (%)	Recurrence, n (%)	HR(95%CI)	P value *
Training set				
0	78(80.41)	19(19.59)	1(reference)	
1	52(71.23)	21(28.77)	2.97(1.40-6.28)	0.004
2	3(60.00)	2(40.00)	10.48(2.02-54.47)	0.005
p for trend				0.0006
Replication set				
0	3(5.36)	53(94.64)	1(reference)	
1	1(2.50)	39(97.50)	1.63(0.98-2.70)	0.06
2	1(16.67)	5(83.33)	3.41(1.21-9.58)	0.02
P for trend				0.01
Combined set				
0	81(52.94)	72(47.06)	1(reference)	
1	53(46.90)	60(53.10)	1.67(1.12-2.49)	0.01
2	4(36.36)	7(63.64)	3.53(1.51-8.26)	0.004
P for trend				0.001
	Alive, n (%)	Dead, n (%)		
Training set				
0	86(88.66)	11(11.34)	1(reference)	
1	57(78.08)	16(21.92)	7.49(2.38-23.56)	0.0006
2	4(80.00)	1(20.00)	14.98(1.36-164.94)	0.03
P for trend				0.0001
Replication set				
0	21(37.50)	35(62.50)	1(reference)	
1	17(42.50)	23(57.50)	2.04(1.04-4.01)	0.04
2	2(33.33)	4(66.67)	7.34(2.03-26.52)	0.002
P for trend				0.003
Combined set				
0	107(69.93)	46(30.07)	1(reference)	
1	74(65.49)	39(34.51)	2.51(1.47-4.28)	0.001
2	6(54.55)	5(45.45)	5.60(1.91-16.45)	0.002
P for trend				5.25×10⁻⁵

* Adjusted for age, gender, ethnicity, smoking status, tumor site, histologic grade, and treatment