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Genetic polymorphisms in pre-microRNA genes as prognostic markers of colorectal cancer

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Abstract

Background—Cumulative data has shown that microRNAs (miRNAs) are involved in the etiology and prognosis of colorectal cancer (CRC). Genetic polymorphisms in pre-miRNA genes may influence the biogenesis and functions of their host miRNAs. However, whether these polymorphisms are associated with CRC prognosis remains unknown.

Methods—We analyzed the effects of seven single nucleotide polymorphisms (SNPs) in pre-miRNA genes on the prognosis of a Chinese population with 408 CRC patients with surgically-resected adenocarcinoma.

Results—Two SNPs were identified to be significantly associated with recurrence-free survival and overall survival of the patients. The most significant SNP was rs6505162 in *pre-miR-423*. Compared to the homozygous wild-type genotype, the variant-containing genotypes of this SNP were significantly associated with both the overall survival (HR=2.12, 95% CI1.34–3.34, P=0.001) and the recurrence-free survival (HR=1.59, 95% CI1.08–2.36, P=0.019). Another SNP, rs4919510 in *pre-miR-608*, was also associated with altered recurrence-free survival (HR=0.61, 95% CI 0.41–0.92, P=0.017). These effects were evident only in patients receiving chemotherapy but not in those without chemotherapy. In addition, the combined analysis of the two SNPs conferred a 2.84-fold (95% CI 1.50–5.37, P=0.001) increased risk of recurrence and/or death. Similarly, this effect was only prominent in those receiving chemotherapy (P<0.001) but not in those without chemotherapy (P=0.999).

Conclusions—Our data suggest that genetic polymorphisms in pre-miRNA genes may impact CRC prognosis especially in patients receiving chemotherapy, a finding that warrants further independent validation.

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Impact—This is one of the first studies showing a prognostic role of pre-miRNA gene SNPs in CRC.

Keywords

Polymorphism; microRNA; colorectal cancer

INTRODUCTION

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with more than1.2 million new cases and 600,000 deaths annually (1). The highest incidence rates of CRC have been observed in regions of developed countries such as Western Europe, North America, and Oceania. In recent decades, the incidence rates are rapidly increasing in several regions that previously had low CRC risk, including countries within Eastern Asia and Eastern Europe (1, 2). Especially, trends of increased CRC incidence and mortality have been observed in China (2). Moreover, CRC is also ranked as the third most common cause of cancer death among both men and women in the United States (3). Although CRC is a disease that is largely influenced by lifestyle and dietary factors (4), recent studies have suggested that inter-individual genetic variations such as single nucleotide polymorphisms (SNPs) may affect risk for CRC (5–7). In addition, emerging evidence has shown that SNPs may be used as surrogate biomarkers of the genetic background of CRC patients to predict therapeutic response and prognosis (8–11).

MicroRNAs (miRNAs) are a group of endogenous single-stranded small non-coding RNAs that have emerged as key regulators of fundamental biological processes through regulating the expression of more than 30% of human genes (12, 13). MiRNAs are initially transcribed as primary miRNAs (pri-miRNA) with several hundred nucleotides, which are further processed into hairpin-structured precursor miRNAs (pre-miRNA) that have approximately 70 nucleotides (14, 15). Pre-miRNAs are the direct precursors of mature miRNAs that have 18–25 nucleotides in length. It has been reported that SNPs in the pri-miRNAs and pre-miRNAs could affect the processing and subsequent maturation of miRNAs, leading to altered mature miRNA expression levels (16, 17). In addition, Hu et al. screened ~400 human pre-miRNAs and their surrounding regions and found that pre-miRNA genes had a significantly lower number of common SNPs than the surrounding regions, suggesting that pre-miRNAs are highly conserved and may be functionally important (18). Consistently, various subsequent studies have reported that pre-miRNA SNPs might confer altered risk of many solid tumors (18–22). However, the role of these SNPs on cancer clinical outcome remains to be evaluated.

Abnormal expression of miRNAs has been well documented as biomarkers or functional regulators in the tumorigenesis and prognosis of CRC (2, 23–26). Because SNPs in miRNA-related genes may impact mature miRNA expression, it follows that these SNPs may also be implicated in cancer development and clinical outcome. Consistently, several recent studies have suggested that SNPs in miRNA biogenesis genes, pri-miRNAs, and miRNA binding sites were associated with altered CRC risk and outcomes (27–29). Nonetheless, to the best of our knowledge, no studies have been reported on the role of SNPs in pre-miRNA regions in CRC prognosis. In the current study, we sought to assess the association of pre-miRNA SNPs and the overall survival and recurrence-free survival in a homogeneous population of Chinese CRC patients.

MATERIALS AND METHODS

Study population

The population used in this study has been described previously (11). Briefly, newly diagnosed and histologically confirmed CRC patients were enrolled in the Xijing Hospital and Tangdu Hospital affiliated with the Fourth Military Medical University (FMMU) in Xi'an, China. There were no restrictions on age, gender, cancer stage or other demographic variables on enrollment. The patient enrollment began from February 2006. As of April 2010, a total of 496 eligible CRC patients were recruited. The rate of recruitment among all eligible patients is 90%. All patients included in this study had histopathologically CRC tumors only and no other cancers before or at the time of diagnosis. For this study, we excluded 88 of the 496 patients, which comprised of 26 patients who did not undergo surgery or only received palliative operation, 48 patients who had incomplete clinical and/or follow-up data, 6 patients who died within one month of surgery, and 8 patients who had poor quality and/or quantity DNA samples. Finally, we included a total of 408 CRC patients with completely validated demographic, clinical, and follow-up data. All patients were Han Chinese with adenocarcinoma and received surgery after diagnosis. Some patients received adjuvant chemotherapy after surgery. No patients received neo-adjuvant or radiation therapy. Informed consent was obtained from each enrolled patient. This study was approved by the local research ethics committees of the participating institutes.

Demographic and clinical data collection

Demographic data were collected through in-person interview at the time of initial visit or follow-up in the clinics, medical chart review, or consultation with the treating physicians by trained clinical research specialists. For data acquired from multiple sources, the research staff compared and validated that these data were consistent. If discrepancies were identified, the patients, family members, and/or treating physicians were further contacted for verification. Individual who smoked more than 100 cigarettes in their lifetime were defined as ever smokers, otherwise as never smokers. Never drinkers were defined as those who consumed less than or equal to one drink per month. One drink was defined as one bottle or can of beer, one medium glass of wine, or one mixed drink. Detailed clinical information was collected by medical chart review and consultation with treating physicians. The follow-up information on recurrence and death was updated at 6-month intervals through onsite interview, direct calling, or medical chart review by trained clinical specialists. The latest follow-up data in this study were obtained in March 2011. For patients enrolled after August 2008, 5-ml of blood was obtained for genomic DNA extraction. For patients enrolled before August 2008, genomic DNA was extracted from approximately 100 mg of adjacent normal tissues obtained by a pathologist after surgery.

SNP selection and genotyping

The candidate SNPs were selected based on a literature review of pre-miRNA epidemiological studies (20, 21, 30–33). Altogether, we genotyped 10 SNPs, including rs895819 in *pre-miR-27a*, rs2910164 in *pre-miR-146a*, rs2292832 in *pre-miR-149*, rs6505162 in *pre-miR-423*, rs2289030 in *pre-miR-492*, rs3746444 in *pre-miR-499*, rs2368392 in *pre-miR-604*, rs2043556 in *pre-miR-605*, rs4919510 in *pre-miR-608*, and rs17759989 in *pre-miR-633*. Genotyping was done using the Sequenom iPLEX platform (Sequenom Inc., CA). Laboratory personnel conducting genotyping were blinded to patient information. Strict quality control measures were implemented during genotyping with over 99% concordance between samples genotyped in duplicate.

Statistical and data analysis

Two major endpoints were evaluated in this study: overall survival and recurrence-free survival. Overall survival time was defined as the time from initial surgery to death from any cause. Recurrence-free survival time was defined as the time from initial surgery to local recurrence, distant metastasis, death from any cause, or to the date of last follow-up. Recurrence is defined as, after the treatment of the primary tumor by surgery and/or chemotherapy, the re-growth of tumor in the original organ (local-regional recurrence) or a different organ (distant metastasis). All recurrent tumors in this study were confirmed as having the same histopathological characteristics as the primary tumor. Second primary tumors with different histopathological characteristics were excluded. All patients without recurrence or lost during follow-up were censored for analysis. Recurrence was confirmed through the combined evaluations of imaging findings (ultrasound, computed tomography, positron emission tomography, magnetic resonance imaging) and laboratory results (mainly carcinoembryonic antigen test). The Hardy-Weinberg equilibrium (HWE) of each SNP was tested using a goodness-of-fit chi-square test. Hazard ratios (HR) and 95% confidence interval (CI) were estimated by multivariate Cox proportional hazards model, adjusting for age, gender, education level, body mass index (BMI), smoking status, drinking status, chemotherapy, tumor position, tumor differentiation, and tumor stage, where appropriate. Three genetic models (dominant, recessive, and additive) were tested and the best fitting model was selected for all downstream analyses. The tests for interactions between significant SNPs and demographic and clinical variables were conducted by including a cross-product term into the Cox proportional hazards model. Kaplan-Meier curves and a log-rank test were used to assess the differences in overall survival and recurrence-free survival. STATA software package (version 8, STATA Corp., College Station, TX) was used for these analyses. All P values were two-sided. $P \leq 0.05$ was considered the threshold of statistical significance.

RESULTS

Characteristics of the study subjects

The distribution of patients' demographic and clinicopathologic characteristics are summarized in Table 1. A total of 408 CRC patients were included in this study. The average age at diagnosis was 59.4 (range, 22–90), and average BMI was 22.7 (range, 15.8–32.9). All patients received surgery within two months after diagnosis. All patients were histologically confirmed by pathological examination as having adenocarcinoma. There were 230 (56.4%) male patients and 178 (43.6%) female patients. The majority of patients were never smokers (70.8%) and never drinkers (89.5%). There were approximately equal numbers of patients with colon cancer (47.1%) and rectal cancer (52.9%), which is consistent with previous reports that the incidence rates of colon and rectal cancers are generally of the same magnitude in countries with low CRC risk such as China (34). A total of 192 (47.1%) patients had stage 2 tumor, while stage 0, 1, 3, and 4 tumors were presented in 2.0%, 14.2%, 27.2% and 9.6% of patients, respectively. The majority of patients (66.4%) had moderately differentiated tumors. No patient received neo-adjuvant chemotherapy, radiotherapy or targeted therapy, but most patients (78.2%) received adjuvant chemotherapy after surgery. Among the 319 patients receiving chemotherapy, 266 (83.4%) were treated using the FOLFOX regimen, including folinic acid (FOL), fluorouracil (F) and Oxaliplatin (OX). During follow-up, there were 93 (22.8%) patients who developed recurrences, 94 (23.0%) patients who died, and 134 (32.8%) patients who had at least one event (recurrence and/or death). The median overall survival time was 23.0 months and the median recurrence-free survival time was 19.9 months.

Main effect analyses of individual SNPs

In the 10 genotyped SNPs, three SNPs (rs2292832, rs17759989, and rs3746444) were excluded from downstream analyses because of failing to pass quality control of genotyping. The average call rate of the remaining seven SNPs was 96.8% (range, 90.3%–100%). The detailed genotyping results of the seven SNPs are listed in Table 2. Except for rs2043556 whose HWE *P* value was 0.001, the HWE *P* value for all other SNPs were non-significant, ranging from 0.217 to 1.000. The HWE *P* value was 0.490 for rs6505162 and 0.228 for rs4919510. We did not identify any genotyping error after carefully checking the genotyping results of rs2043556. The significant deviation from HWE for this SNP could potentially result from the differences between our CRC patient population and the general population of cancer-free individuals, and the results related to this SNP need to be interpreted with caution. Overall, two SNPs, rs6505162 in *pre-miR-423* and rs4919510 in *pre-miR-608*, exhibited a significant association with the overall survival and the recurrence-free survival of the CRC patients. For both SNPs, the results of the dominant genetic model analysis were more significant for both the overall and the recurrence-free survivals compared to the recessive and additive genetic models, except for the overall survival analysis for rs4919510, in which the result of the additive model (*P*=0.063) was slightly more significant than that of the dominant model (*P*=0.067). We therefore used the dominant model as the best fitting model in this study. Compared to the homozygous wild-type (WW) genotype, the variant-containing (WV + VV) genotypes of this SNP were significantly associated with unfavorable overall and recurrence-free survival with an HR of 2.12 (95% CI, 1.34–3.34; *P*=0.001) and 1.59 (95% CI, 1.08–2.36; *P*=0.019), respectively. Another significant SNP was rs4919510. Under a dominant genetic model, the variant-containing genotypes of this SNP were associated with favorable overall and recurrence-free survival with an HR of 0.64 (95% CI, 0.40–1.03; *P*=0.067) and 0.61 (95% CI, 0.41–0.92; *P*=0.017). There are 139 (34.1%) subjects whose DNA samples were obtained from tissues and 269 (65.9%) from blood. We conducted a test for interaction between both significant SNPs and DNA source and did not identify any significant interaction (*P* for interaction for overall survival and recurrence-free survival: 0.371 and 0.646 respectively for rs6505162, and 0.926 and 0.918 respectively for rs4919510).

Stratified analysis of rs6505162 and rs4919510 by host variables

We focused on the recurrence-free survival in all the downstream analyses because this endpoint reflects both recurrence and death. We conducted stratified and interaction analyses of rs6505162 and rs4919510 with the major demographic and clinical variables. As shown in Table 3, four borderline significant ($0.05 < P < 0.1$) interactions were identified, including the interactions between rs6505162 and BMI (*P* for interaction = 0.096) and tumor stage (*P* for interaction = 0.091), as well as the interactions between rs4919510 and gender (*P* for interaction = 0.092) and chemotherapy (*P* for interaction = 0.072). The significant association between recurrence-free survival and rs6505162 remained significant in patients with higher BMI (HR=2.60, 95% CI 1.47–4.58, *P*=0.001) and higher tumor stage (HR=1.87, 95% CI 0.99–3.53, *P*=0.053), compared to those with lower BMI (HR=0.83, 95% CI 0.43–1.63, *P*=0.593) and lower tumor stage (HR=1.09, 95% CI 0.62–1.92, *P*=0.772), respectively. The significant effects conferred by rs4919510 was more evident in male patients (HR=0.47, 95% CI 0.27–0.81, *P*=0.006) and patients receiving chemotherapy (HR=0.48, 95% CI 0.30–0.75, *P*=0.001), compared to the female patients (HR=0.94, 95% CI 0.50–1.79, *P*=0.856) and patients without chemotherapy (HR=1.57, 95% CI 0.48–5.07, *P*=0.454), respectively. Kaplan-Meier curves indicated at least a borderline significant difference in term of time to recurrence or death, between the wild-type and variant-containing genotypes of both rs6505162 (Log rank *P*=0.093) (Figure 1A, left panel) and rs4919510 (Log rank *P*= 0.059) (Figure 1B, left panel). For both SNPs, the significant effect was more evident in patients receiving chemotherapy (Log rank *P*, 0.085 and 0.041 for rs6505162 and rs4919510,

respectively, Figures 1A and 1B, middle panel), but not in those without chemotherapy (Log rank *P*, 0.706 and 0.979 for rs6505162 and rs4919510, respectively, Figures 1A and 1B, right panel). We also generated recurrence-free survival curves that were adjusted by the demographic and clinical variables listed on Table 1 from the Cox regression, to determine the differences in recurrence-free survival time between the different genotypes of rs6515062 and rs4919510. The adjusted curves were similar to their corresponding Kaplan-Meier curves and consistent with the results of the Cox proportional hazards analyses. That is, for both SNPs, significant differences in recurrence-free survival time were observed in all patients as well as patients receiving chemotherapy, but not in those patients without chemotherapy (Supplementary Figure 1).

Effects of chemotherapy on patient outcome by pre-miRNA SNPs

Because both rs6505162 and rs4919510 conferred significantly altered risk of recurrence-free survival in patients receiving chemotherapy but not in those without chemotherapy, we sought to evaluate whether the effect of chemotherapy on patient outcome was also influenced by these SNPs. As showed in Table 4, patients receiving chemotherapy exhibited a better recurrence-free survival compared to those without chemotherapy (HR=0.61, 95% CI 0.37–1.00, *P*=0.048). Interestingly, this effect remained at least borderline significant in patients with the low-risk genotypes of either SNPs (WW in rs6505162 or WV + VV in rs4919510). That is, the effect of chemotherapy on recurrence-free survival remained borderline significant in patients with the homozygous wild-type genotype of rs6505162 (HR=0.52, 95% CI 0.27–1.01, *P*=0.052) and significant in patients with the variant-containing genotypes of rs4919510 (HR=0.41, 95% CI 0.23–0.73, *P*=0.003). In comparison, no significant effect by chemotherapy on recurrence-free survival was observed in patients with the high-risk genotypes of both SNPs (*P* value, 0.248 for rs6505162 and 0.329 for rs4919510).

Joint effects of rs6505162 and rs4919510 on CRC risk

Finally, we conducted joint analysis to assess the cumulative effect of the two significant SNPs on CRC recurrence-free survival. Using the patients with the low-risk genotype in both SNPs as reference group, patients with the high-risk genotype of both SNPs exhibited a 2.84-fold (95% CI, 1.50–5.37, P=0.001) increase in risk of recurrence and/or death (Table 5). Consistently, this effect was more prominent in patients receiving chemotherapy (HR=3.81, 95% CI 1.87–7.78, *P*<0.001) but not in those without chemotherapy (HR=1.00, 95% CI 0.10–10.24, *P*=0.999). Kaplan-Meier curves showed a borderline significance in term of recurrence-free survival time in the joint effect analysis (Log rank *P*=0.062, Figure 2. left panel). Consistent with the Cox analysis, this effect was more evident in those receiving chemotherapy (Log rank *P*=0.043, Figure 2. middle panel), than those without chemotherapy (Log rank *P*=0.936, Figure 2. right panel).

DISCUSSION

In this study, we evaluated the effects of SNPs in pre-miRNA on the clinical outcomes of a Chinese CRC population. We found that two SNPs, rs6505162 in *pre-miR-423* and rs4919510 in *pre-miR-608,* were significantly associated with altered overall survival and recurrence-free survival of the patients. In addition, joint effects of the two SNPs on CRC outcome were observed, especially in patients treated with chemotherapy.

Genetic polymorphisms in pre-miRNA genomic regions have been associated with the risk and clinical outcomes of various solid malignancies such as cancers of breast, prostate, lung, bladder, esophagus and kidney (18, 21, 31, 33, 35–37). In addition, a few studies also reported the implications of pre-miRNA SNPs in the risk of CRC (38, 39). However, it

remains to be determined whether these SNPs affect the clinical outcome of CRC patients. The only previously reported epidemiological study on miRNA-related SNPs was conducted by Lee et al, who did not identify any significant effect between miRNA-related SNPs and CRC survival on a multivariate basis in a population of 420 Korean CRC patients (40). In another small study with 61 patients, Boni et al. found that SNPs in the primary precursor region (pri-miRNAs) of *pri-miR-26a1* and *pri-miR-100* were associated with the clinical response of metastatic colon cancer patients treated with 5-fluorouracil and irinotecan (29). In the current study, we found that rs6505162 in *pre-miR-423* and rs4919510 in *pre-miR-608* were significantly associated with altered overall and recurrence-free survivals of Chinese CRC patients. Both SNPs have been evaluated in previous studies with mixed results. For instance, it has been reported that rs6505162 was associated with a significantly increased risk of ovarian cancer (41), contrary to another three studies showing that it conferred a reduced risk of esophageal cancer, and recurrence or survival of renal cell carcinoma and prostate cancer (37, 42, 43). The expression level of miR-423 has been reported to be significantly increased in breast cancer tissues compared to normal tissues (44). However, whether rs6505162 modulates the expression of miR-423 is not clear. The rs4919510 variant has been associated with an increased risk of recurrence in patients with renal cell carcinoma (42). Different alleles of this SNP has been predicted by *in silico* algorithms to exhibit differential capacities to bind to the potential target genes of miR-608 such as insulin receptor *INSR* and tumor suppressor *TP53* (45), suggesting that the variant allele of rs4919510 could have different biological functions in relation to its various targets. It has been reported that miR-608 was down-regulated in ovarian cancer (46), but whether rs4919510 has an effect on the expression of miR-608 remains unknown. The conflicting results of these two SNPs among different cancers may be accounted for by differences in study design, sample size, and populations, or biological functions specific to different cancer types. Additional studies with well-powered homogeneous study populations, together with functional characterizations, are warranted to provide additional definitive evidence.

In stratified analyses, we found that the effects of both rs6505162 and rs4919510 on recurrence-free survival were only evident in patients receiving chemotherapy (Table 3). A test for interaction revealed a borderline significant interaction effect between chemotherapy and rs4919510. The finding of this potential interaction is in accordance with the observation that the hazard ratios of this SNP in the stratified analysis were opposite in patients with chemotherapy (HR=0.48) and those without chemotherapy (HR=1.57) (Table 3). We did not identify a significant interaction between chemotherapy and rs6505162, which is consistent with the observation that for this SNP, both of the patients with and without chemotherapy had elevated risk of recurrence and/or death (HR, 1.58 and 1.96, respectively). Nonetheless, given the modest samples size of our study population, the interaction analysis may not be adequately powered. Therefore, the results are not definitive at this point and future studies with larger patient populations are needed to validate these findings. When we conducted the joint effect analysis by combining the low-risk genotypes of both SNPs as the reference group, we found those patients with the high-risk genotypes of both SNPs had a significantly increased risk of death and/or recurrence (P=0.001). In accordance, this effect was more prominent in patients receiving chemotherapy (HR=3.81, P<0.001) than those without chemotherapy (HR=1.00, P=0.999) (Table 5). MicroRNAs have been extensively implicated in affecting the response to chemotherapy in patients with various cancers including CRC (23, 47–49). However, it remains to be assessed whether SNPs in miRNA gene regions influence CRC chemo-response by affecting the expression and function of the host miRNAs. Our data in this study suggest that the two significant SNPs identified in this study may possibly modulate the effects of chemotherapy on CRC outcome individually and jointly. If validated, they have the potential to be incorporated

with other prognostic factors to select high-risk patients that are more likely to benefit from the therapeutic benefits of chemotherapy.

Our study has several strengths. First, the patients were enrolled from Xi'an and adjacent area. This region is highly attractive in conducting population-based research because of the geographical stability with low mobility rate. Second, the patients analyzed in this study were highly homogenous, in that all the patients had adenocarcinoma and were surgically treated to remove the primary tumor. Additionally, all chemotherapy treatments were started within two months of surgery and 83.4% of the 319 chemotherapy-treated patients received the same first-line adjuvant FOLFOX chemotherapy. The highly homogenous patient characteristics and treatments, as well as low rate of patient loss to follow-up, greatly reduced the confounding effects of the heterogeneous therapeutics modalities identified in most biomarker studies of cancer clinical outcome. Our study also has limitations. First, our study was restricted to Han Chinese and whether the findings can be generalized to other ethnic groups needs further evaluations. In addition, we could not rule out the possibility of chance findings in our study due to the relatively short follow-up time (median follow-up time, 23.7 months) and modest sample size. The enrollment of this study is still ongoing with a low rate of patient loss, which will further enable us to obtain higher statistical power for in-depth analyses. Another interesting observation in this study was that the HR was stronger for overall survival than recurrence-free survival for rs6505162. This could be explained by the possibility that the variant allele of this SNP conferred a higher risk of dying of primary cancer or its sequelae. However, the lack of complete data on sequelae in this patient population prevented us from further analyzing the patient outcome based on different causes of death.

In conclusion, our data showed for the first time that polymorphisms in *pre-miRNAs* were significantly associated with CRC clinical outcomes in a Chinese population, especially in patients receiving chemotherapy. Further validations of these findings are needed using independent populations and functional characterizations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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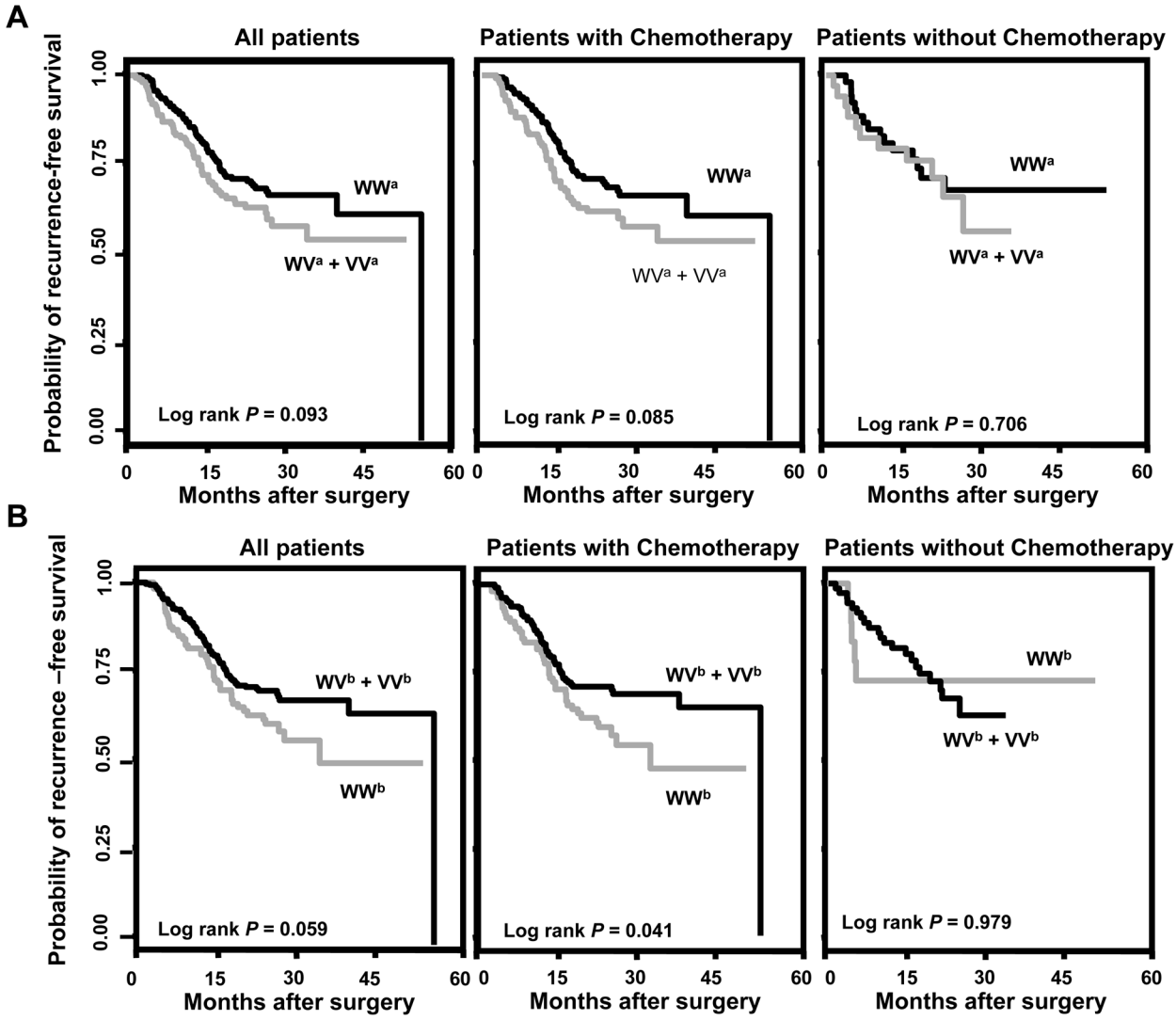

Figure 1.

Kaplan-Meier recurrence-free survival curves of CRC in all patients, in patients with chemotherapy and in patients without chemotherapy. A, rs6505162, dominant model; B, rs4919510, dominant model. WW^a, WV^a and VV^a indicate the homozygous wild-type, heterozygous and homozygous variant genotypes of rs6505162, respectively. WW^b, WV^b and VV^b indicate the homozygous, heterozygous and homozygous variant genotypes of rs4919510, respectively.

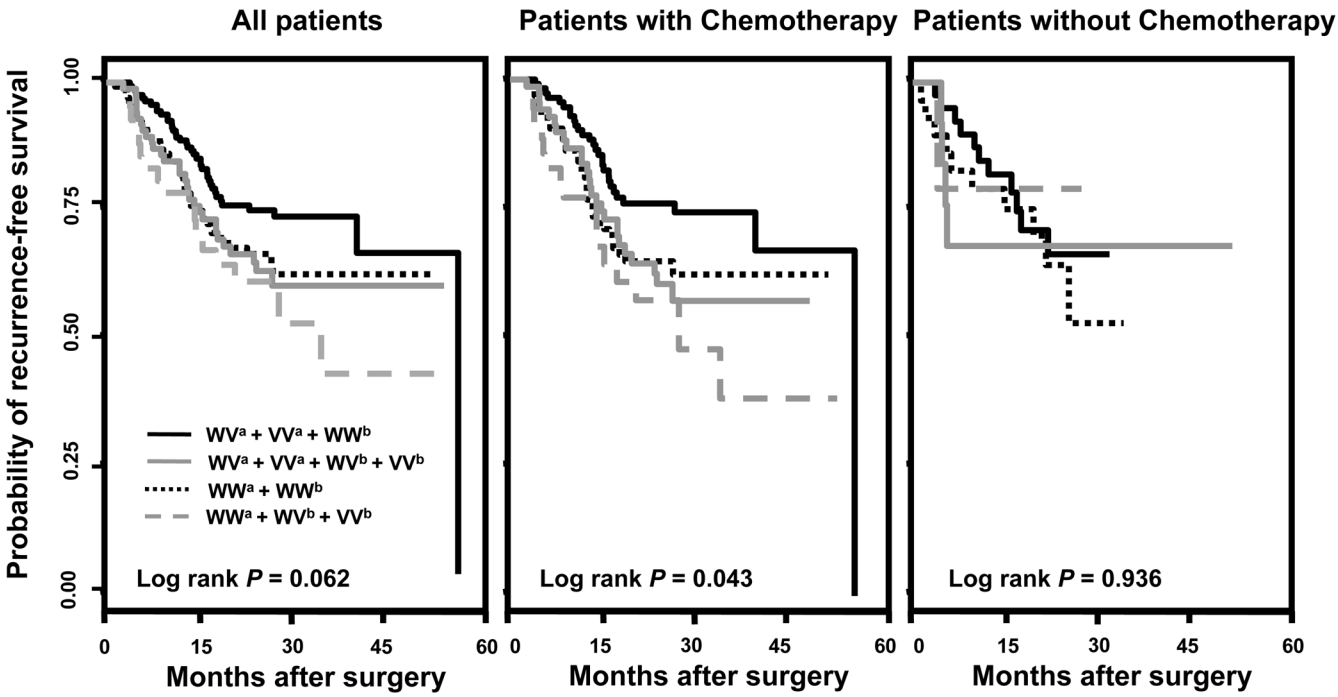

Figure 2.

Kaplan-Meier recurrence-free survival curves of joint effects between rs6505162 and rs4919510 under a dominant model. $WW^a + WV^b + VV^b$ indicate the combination of the low-risk genotypes of both rs6505162 and rs4919510; $WV^a + VV^a + WV^b + VV^b$ indicate the combination of the high-risk genotypes of rs6505162 and the low-risk genotypes of rs4919510; $WW^a + WW^b$ indicate the combination of the low-risk genotypes of rs6505162 and the high-risk genotypes of rs4919510; $WV^a + VV^a + WW^b$ indicate the combination of the high-risk genotypes of both rs6505162 and rs4919510.

Table 1

Demographic and clinicopathological characteristics of 408 Chinese CRC patients

Variables	Number of patients (%), n=408
Age, mean (range) (in years)	59.4 (22–90)
Body mass index, mean (range)	22.7(15.8–32.9)
Gender	
Male	230 (56.4)
Female	178(43.6)
Smoking status	
Ever	119 (29.2)
Never	289 (70.8)
Drinking status	
Ever	43 (10.5)
Never	365 (89.5)
Education	
Up to high school	178(43.6)
College degree or higher	171(41.9)
Unknown	59 (14.5)
Tumor position	
Colon	192 (47.1)
Rectum	216 (52.9)
Tumor stage	
0	8 (2.0)
1	58 (14.2)
2	192 (47.1)
3	111 (27.2)
4	39 (9.6)
Tumor differentiation	
Poor	37 (9.1)
Moderate	271 (66.4)
Well	100 (24.5)
Chemotherapy	
Yes	319 (78.2)
No	89 (21.8)
Recurrence	
Yes	93 (22.8)
No	315 (77.2)
Death	
Yes	94 (23.0)
No	314 (77.0)
Event (recurrence and/or death)	
Yes	134 (32.8)

Variables	Number of patients (%), n=408
No	274 (67.2)

Table 2

Association of pre-miRNA SNPs with overall and recurrence-free survival of CRC patients.

		Overall survival			Recurrence-free survival		
Gene and SNP	**Genotype[a]**	**Death/total**	**HR (95% CI)[b]**	***P* value**	**Event/total[c]**	**HR (95% CI)**	***P* value**
pre-miR-27a	WW	48/213	1(reference)		69/213	1(reference)	
rs895819	WV	39/167	0.88(0.55– 1.41)	0.599	55/167	0.75(0.50– 1.13)	0.168
HWE *P* 0.375	VV	6/25	0.66(0.26– 1.70)	0.393	9/25	0.76(0.35– 1.64)	0.478
	Dom		0.85(0.54– 1.34)	0.476		0.75(0.51– 1.11)	0.153
	Rec		0.71(0.28– 1.76)	0.456		0.87(0.41– 1.85)	0.721
	Add		0.85(0.59– 1.22)	0.374		0.81(0.59– 1.11)	0.195
pre-miR-146a	WW	24/118	1(reference)		34/118	1(reference)	
rs2910164	WV	50/200	1.13(0.68– 1.89)	0.632	69/200	1.10(0.72– 1.69)	0.663
HWE *P* 1.000	VV	19/85	0.95(0.49– 1.83)	0.877	30/85	0.94(0.54– 1.66)	0.840
	Dom		1.08(0.66– 1.75)	0.761		1.06(0.70– 1.59)	0.797
	Rec		0.88(0.50– 1.56)	0.659		0.89(0.54– 1.46)	0.646
	Add		0.99(0.73– 1.36)	0.959		0.99(0.76– 1.29)	0.932
pre-miR-423	WW	47/242	1(reference)		74/242	1(reference)	
rs6505162	WV	40/141	**2.18(1.36– 3.51)**	**0.001**	52/141	**1.73(1.15– 2.59)**	**0.008**
HWE *P* 0.490	VV	7/25	1.79(0.75– 4.28)	0.192	8/25	1.02(0.44– 2.36)	0.960
	Dom		**2.12(1.34– 3.34)**	**0.001**		**1.59(1.08– 2.36)**	**0.019**
	Rec		1.29(0.55– 2.99)	0.557		0.82(0.36– 1.87)	0.642
	Add		**1.61(1.15– 2.25)**	**0.005**		1.28(0.95– 1.73)	0.100
pre-miR-492	WW	54/233	1(reference)		76/233	1(reference)	
rs2289030	WV	31/136	0.72(0.43– 1.18)	0.194	46/136	0.83(0.54– 1.26)	0.379
HWE *P* 0.217	VV	7/28	1.05(0.46– 2.40)	0.910	9/28	0.95(0.44– 2.03)	0.885
	Dom		0.77(0.48– 1.23)	0.274		0.85(0.57– 1.26)	0.411
	Rec		1.19(0.53– 2.68)	0.669		1.02(0.48– 2.15)	0.956
	Add		0.88(0.60– 1.28)	0.498		0.90(0.65– 1.24)	0.530
pre-miR-604	WW	46/199	1(reference)		67/199	1(reference)	
rs2368392	WV	35/162	1.17(0.73– 1.88)	0.524	50/162	0.97(0.64– 1.47)	0.883
HWE *P* 0.292	VV	11/42	1.46(0.69– 3.11)	0.327	15/42	1.47(0.79– 2.77)	0.227

		Overall survival			Recurrence-free survival		
Gene and SNP	**Genotype[a]**	**Death/total**	**HR (95% CI)[b]**	***P* value**	**Event/total[c]**	**HR (95% CI)**	***P* value**
	Dom		1.22(0.78– 1.91)	0.382		1.06(0.72– 1.55)	0.780
	Rec		1.37(0.66– 2.84)	0.397		1.49(0.82– 2.74)	0.194
	Add		1.19(0.85– 1.67)	0.299		1.12(0.84– 1.51)	0.436
pre-miR-605	WW	45/184	1(reference)		66/184	1(reference)	
rs2043556	WV	42/200	1.01(0.63– 1.61)	0.962	60/200	0.86(0.58– 1.28)	0.460
HWE *P* 0.001	VV	7/23	1.47(0.60– 3.62)	0.401	8/23	0.93(0.41– 2.11)	0.856
	Dom		1.06(0.67– 1.66)	0.808		0.87(0.59– 1.27)	0.471
	Rec		1.46(0.61– 3.50)	0.392		1.00(0.45– 2.23)	0.999
	Add		1.11(0.76– 1.61)	0.596		0.91(0.66– 1.25)	0.549
pre-miR-608	WW	30/122	1(reference)		47/122	1(reference)	
rs4919510	WV	47/213	0.67(0.41– 1.10)	0.112	63/213	**0.62(0.41– 0.95)**	**0.027**
HWE *P* 0.228	VV	16/72	0.54(0.25– 1.16)	0.112	23/72	0.58(0.31– 1.08)	0.086
	Dom		0.64(0.40– 1.03)	0.067		**0.61(0.41– 0.92)**	**0.017**
	Rec		0.69(0.34– 1.40)	0.306		0.78(0.43-– 1.38)	0.389
	Add		0.71(0.50– 1.02)	0.063		**0.72(0.53– 0.97)**	**0.032**

Note: The significant *P* values (≤0.05) are in bold.

[a] WW, homozygous wild-type genotype; WV heterozygous genotype; VV, homozygous variant genotype; Dom, dominant model; Rec, recessive model; Add, additive model

[b] Adjusted for age, gender, education level, BMI, smoking status, drinking status, chemotherapy, tumor position, tumor differentiation, tumor stage.

[c] Event: recurrence and/or death.

Table 3

Association of rs6505162 and rs4919510 with recurrence-free survival stratified by host characteristics

Variables	Stratum	Genotype[a]	rs6505162			rs4919510		
			Event/total[c]	HR (95% CI)[b]	*P* value	Event/total	HR (95% CI)	*P* value
Overall		WW	74/242	1(reference)		47/122	1(reference)	
		WV + VV	60/166	**1.59(1.08–2.36)**	**0.019**	86/285	**0.61(0.41–0.92)**	**0.017**
Age								
	Younger (<61)	WW	42/117	1(reference)		26/55	1(reference)	
		WV + VV	33/83	1.33(0.76–2.32)	0.319	49/145	**0.44(0.25–0.79)**	**0.005**
	Older(≥ 61)	WW	32/125	1(reference)		21/67	1(reference)	
		WV + VV	27/83	**2.05(1.07–3.93)**	**0.031**	37/140	0.77(0.41–1.46)	0.427
				P for interaction	0.387		*P* for interaction	0.361
Gender								
	Male	WW	38/131	1(reference)		28/65	1(reference)	
		WV + VV	38/99	**2.17(1.26–3.74)**	**0.005**	48/165	**0.47(0.27–0.81)**	**0.006**
	Female	WW	36/111	1(reference)		19/57	1(reference)	
		WV + VV	22/67	1.12(0.60–2.13)	0.717	38/120	0.94(0.50–1.79)	0.856
				P for interaction	0.401		*P* for interaction	0.092
Educational level								
	Up to high school	WW	27/114	1(reference)		17/49	1(reference)	
		WV + VV	27/64	**2.06(1.14–3.74)**	**0.017**	36/128	0.66(0.35–1.24)	0.194
	College or higher	WW	36/98	1(reference)		25/55	1(reference)	
		WV + VV	27/73	1.51(0.87–2.60)	0.144	38/116	**0.52(0.30–0.91)**	**0.022**
				P for interaction	0.469		*P* for interaction	0.302
Body mass index (BMI)								
	BMI <22.7	WW	36/123	1(reference)		23/64	1(reference)	
		WV + VV	24/77	0.83(0.43–1.63)	0.593	36/135	**0.49(0.25–0.97)**	**0.041**
	BMI ≥ 22.7	WW	38/117	1(reference)		24/56	1(reference)	
		WV + VV	34/83	**2.60(1.47–4.58)**	**0.001**	48/144	0.61(0.35–1.06)	0.078
				P for interaction	0.096		*P* for interaction	0.741
Smoking status								

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Variables	Stratum	Genotype[a]	rs6505162			rs4919510		
			Event/total[c]	HR (95% CI)[b]	*P* value	Event/total	HR (95% CI)	*P* value
	Never smoker	WW	55/170	1(reference)		32/91	1(reference)	
		WV + VV	43/119	1.44(0.92–2.27)	0.113	65/197	0.69(0.43–1.11)	0.125
	Ever smoker	WW	19/72	1(reference)		15/31	1(reference)	
		WV + VV	17/47	**2.70(1.15–6.31)**	**0.022**	21/88	**0.34(0.13–0.88)**	**0.027**
				P for interaction	0.401		*P* for interaction	0.206
Drinking status								
	Never drinker	WW	65/218	1(reference)		40/112	1(reference)	
		WV + VV	50/147	1.50(0.98–2.30)	0.064	74/252	0.64(0.41–1.00)	0.051
	Ever drinker	WW	9/24	1(reference)		7/10	1(reference)	
		WV + VV	10/19	**7.88(1.24–50.07)**	**0.029**	12/33	0.46(0.11–1.90)	0.283
				P for interaction	0.591		*P* for interaction	0.636
Chemotherapy								
	No	WW	15/54	1(reference)		5/18	1(reference)	
		WV + VV	11/35	1.96(0.79–4.83)	0.144	21/71	1.57(0.48–5.07)	0.454
	Yes	WW	59/188	1(reference)		42/104	1(reference)	
		WV + VV	49/131	**1.58(1.01–2.49)**	**0.046**	65/214	**0.48(0.30–0.75)**	**0.001**
				P for interaction	0.852		*P* for interaction	0.072
Tumor position								
	Colon	WW	34/116	1(reference)		24/60	1(reference)	
		WV + VV	30/76	1.09(0.59–2.00)	0.785	40/132	0.60(0.32–1.12)	0.108
	Rectum	WW	40/126	1(reference)		23/62	1(reference)	
		WV + VV	30/90	**1.91(1.06–3.42)**	**0.030**	46/153	0.66(0.37–1.19)	0.169
				P for interaction	0.254		*P* for interaction	0.538
Tumor differentiation								
	Poor and Moderate	WW	57/184	1(reference)		41/94	1(reference)	
		WV + VV	50/124	**1.81(1.16–2.82)**	**0.009**	65/213	**0.54(0.35–0.85)**	**0.008**
	Well	WW	17/58	1(reference)		6/28	1(reference)	
		WV + VV	10/42	0.88(0.32–2.44)	0.808	21/72	1.65(0.46–5.91)	0.440
				P for interaction	0.253		*P* for interaction	0.218
Tumor stage								

Variables	Stratum	Genotype[a]	rs6505162			rs4919510		
			Event/total[c]	HR (95% CI)[b]	*P* value	Event/total	HR (95% CI)	*P* value
	Stage 0–2	WW	32/148	1(reference)		23/76	1(reference)	
		WV + VV	24/110	1.09(0.62–1.92)	0.772	32/181	**0.51(0.28–0.92)**	**0.026**
	Stage 3–4	WW	42/94	1(reference)		24/46	1(reference)	
		WV + VV	36/56	1.87(0.99–3.53)	0.053	54/104	0.65(0.35–1.22)	0.183
				P for interaction	0.091		*P* for interaction	0.476

Note: The significant *P* values (≤0.05) are in bold.

[a] WW, homozygous wild-type genotype; WV heterozygous genotype; VV, homozygous variant genotype; Dom, dominant model

[b] Adjusted for age, gender, education level, BMI, smoking status, drinking status, chemotherapy, tumor position, tumor differentiation, tumor stage, where appropriate.

[c] Event: recurrence and/or death.

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Table 4

Effects of chemotherapy on CRC recurrence-free survival stratified by rs6505162 or rs4919510

SNP and Variables[a]	Event/total[c]	HR (95% CI)[b]	*P* value
In all patients			
No chemotherapy	26/89	1(reference)	
Chemotherapy	108/319	**0.61(0.37–1.00)**	**0.048**
In patients with WW genotype of rs6505162			
No chemotherapy	15/54	1(reference)	
Chemotherapy	59/188	0.52(0.27–1.01)	0.052
In patients with WV + VV genotype of rs6505162			
No chemotherapy	11/35	1(reference)	
Chemotherapy	49/131	0.62(0.27–1.40)	0.248
In patients with WW genotype of rs4919510			
No chemotherapy	5/18	1(reference)	
Chemotherapy	42/104	1.75(0.57–5.41)	0.329
In patients with WV + VV genotype of rs4919510			
No chemotherapy	21/71	1(reference)	
Chemotherapy	65/214	**0.41(0.23–0.73)**	**0.003**

Note: The significant *P* values (≤0.05) are in bold.

[a] WW, homozygous wild-type genotype; WV heterozygous genotype; VV, homozygous variant genotype; Dom, dominant model

[b] Adjusted for age, gender, education level, BMI, smoking status, drinking status, chemotherapy, tumor position, tumor differentiation, tumor stage, where appropriate.

[c] Event: recurrence and/or death.

Table 5

Joint effects of rs6505162 and rs4919510 on CRC recurrence-free survival.

Genotype[a]		All patients			Patients with chemotherapy [d]			Patients without chemotherapy		
		Event/total[c]	HR (95% CI)[b]	*P* value	Event/total	HR (95% CI)	*P* value	Event/total	HR (95% CI)	*P* value
rs6505162	rs4919510									
WW	WV + VV	43/159	1(reference)		32/118	1(reference)		11/41	1(reference)	
WV + VV	WV + VV	43/126	**1.81(1.11–2.94)**	**0.017**	33/96	**1.85(1.03–3.31)**	**0.040**	10/30	2.09(0.74–5.89)	0.164
WW	WW	30/82	**1.84(1.09–3.13)**	**0.024**	26/69	**2.34(1.27–4.29)**	**0.006**	4/13	0.92(0.21–3.98)	0.908
WV + VV	WW	17/40	**2.84(1.50–5.37)**	**0.001**	16/35	**3.81(1.87–7.78)**	**<0.001**	1/5	1.00(0.10–10.24)	0.999

Note: The significant *P* values (≤0.05) are in bold.

[a] WW, homozygous wild-type genotype; WV heterozygous genotype; VV, homozygous variant genotype.

[b] Adjusted for age, gender, education level, BMI, smoking status, drinking status, chemotherapy, tumor position, tumor differentiation, tumor stage, where appropriate.

[c] Event: recurrence and/or death.