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Genetic Variations in Stem Cell-Related Genes and Colorectal Cancer Prognosis

Hushan Yang,

Division of Population Science, Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, 1025 Walnut Street, Room 1015, Philadelphia, PA 19107, USA

Falin Qu,

Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710032, China

Ronald E. Myers,

Division of Population Science, Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, 1025 Walnut Street, Room 1015, Philadelphia, PA 19107, USA

Guoqiang Bao,

Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710032, China

Terry Hyslop,

Department of Experimental Therapeutics, Department of Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA

Guang Hu,

Stem Cell Biology Group, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Science, Research Triangle Park, NC 27009, USA

Fei Fei, and

State Key Laboratory of Cancer Biology, Cell Engineering Research Centre & Department of Cell Biology, Fourth Military Medical University, Xi'an 710032, China

Jinliang Xing

State Key Laboratory of Cancer Biology, Cell Engineering Research Centre & Department of Cell Biology, Fourth Military Medical University, Xi'an 710032, China

Hushan Yang: hushan.yang@jefferson.edu

Abstract

Background—Many properties of cancer cells are reminiscent of those in normal stem cells. Genes important to stem cell development have been significantly implicated in the etiology and clinical outcome of colorectal cancer (CRC). However, the associations of genetic variations in these genes with CRC prognosis have not yet been elucidated.

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Correspondence to: Hushan Yang, hushan.yang@jefferson.edu.

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Conflict of interest None.

Methods—We analyzed the effects of eight potentially functional single nucleotide polymorphisms (SNPs) in six stem cell-related genes on the prognosis of a well-characterized population of 380 Chinese CRC patients diagnosed from February 2006 to January 2010.

Results—The most significant finding was related to rs879882, a variant in the 5' region of *POU5F1* gene which encodes a protein essential for embryonic stem cell self-renewal and pluripotency, and induced pluripotent stem cell reprogramming. The variant-containing genotypes of rs879882 were associated with an increased risk of recurrence (hazard ratio [HR]=2.10, 95 % confidence interval [CI] 1.17–3.76, $P=0.01$). In chemotherapy-stratified analysis, the association remained borderline significant in patients receiving chemotherapy (HR=1.97, 95 % CI 0.89–4.34, $P=0.09$). In addition, a nonsynonymous SNP of *APC* gene was also significantly associated with recurrence risk in chemotherapy-treated patients (HR=2.63, 95 % CI 1.14–6.06 $P=0.02$). Further analyses showed a combined effect of the two SNPs in predicting CRC recurrence in patients receiving chemotherapy ($P=0.04$) but not in those without chemotherapy ($P=0.43$). Moreover, an exploratory multivariate assessment model indicated that these two variants enhanced the power to predict recurrence after chemotherapy.

Conclusion—We presented one of the first epidemiologic studies showing that stem cell-related genetic variants may impact CRC clinical outcomes, especially in chemotherapy-treated patients.

Keywords

Stem cell; Polymorphism; Colorectal cancer

Background

Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide [1]. More than 40 % of CRC patients have recurrence or succumb to the disease after initial treatments [2–4]. To reduce CRC mortality, it is critical to identify those patients who are most likely to recur and progress for more intensive and targeted modalities.

Emerging evidence has shown that single nucleotide polymorphisms (SNPs) may be used as surrogates of patients' genetic backgrounds to predict therapeutic response and prognosis [5, 6]. For example, a number of genome-wide association studies identified several robust SNPs associated with clinical outcomes of hepatitis C virus infection and leukemia [7–10]. Several pharmacogenetic tests using SNPs to predict the response of cancer patients to specific therapies have been recognized by the Food and Drug Administration [11]. In CRC, SNPs of *UGT1A1* gene are currently used to predict the toxicity and efficacy of irinotecan, a major chemotherapy agent treating advanced CRC [11]. These findings highlight the potential importance of integrating the background genetic profiles of cancer patients in therapy selection and prognosis prediction.

Recently, remarkable progress has been made in research on the potential of stem cells in treating many previously incurable degenerative diseases and cancers. Unlike many traditional treatment strategies, the unique therapeutic property of stem cells is the ability of self-renewal and differentiation. A stem cell can generate an exact copy of itself through self-renewal and produce another progeny cell capable of differentiating into a wide variety of more specialized adult cells [12]. These capacities make the use of stem cells a promising therapy for many common human diseases. However, the self-renewal capacity of stem cells, if dysregulated, may turn normal stem cells into cells that acquire neoplastic transformation capacity and perpetuate to become cancer stem cells (CSCs). CSCs and stem cell-like progenitor cells have been identified in a many cancers, including CRC [13–20]. It is hypothesized that many malignancies develop from a small proportion of CSCs within the bulk of primary tumors [21]. Mounting evidence also suggests that CSCs play a pivotal role

in cancer clinical outcomes [22]. This is because CSCs are highly resistant to cytotoxic systemic therapies, thus leading to increased recurrence and metastasis, and reduced survival [23, 24]. Recent research has started to elucidate the molecular mechanisms underlying CSC transformation and resistance to systemic therapies [25]. Several signaling pathways have been implicated in stem cell development and CSC transformation. Defects in genes of these pathways are frequently identified in CRC tumors [26–28]. In addition, tumor-derived expression signatures of these genes are also significantly associated with CRC prognosis [29, 30]. It is unclear, however, whether genetic variants in stem cell-related genes impact the clinical outcomes of CRC as well as other cancers.

In this study, to test the hypothesis that sequence variants in stem cell-related genes affect CRC prognosis, we evaluated the effects of eight potentially functional SNPs in six important stem cell-related genes on the recurrence and death of 380 CRC patients. To our knowledge, this is one of the first studies to employ a pathway-based approach to investigate the effects of stem cell-related gene variants on cancer prognosis.

Methods

Population, Demographic, and Clinical Data

This is a longitudinal cohort study with a retrospective inception date. The study included a population of incident CRC patients enrolled in the Xijing Hospital and the Tangdu Hospital affiliated to the Fourth Military Medical University (FMMU) in Xi'an, China. All patients are Han Chinese with newly diagnosed and histologically confirmed CRC. There were no restrictions on age, gender, and cancer stage on enrollment. Patients with previous diagnosis of other cancers were ineligible for this study. This study was initiated in August 2008. However, we retrospectively identified eligible patients to be included in this study back to February 2006. As of January 2010, 465 CRC patients have been enrolled with complete demographic and clinical information. The data in this study were collected through in-person interview, medical chart review, or consultation with the treating physician. Data acquired from multiple sources were compared and validated. If discrepancies were identified, patients or relatives were further contacted for verification. If the data from different sources of a patient were inconsistent and could not be validated, the patient was excluded from this study. Finally, a total of 85 of the 465 patients did not have validated clinical outcome data and therefore were excluded from this study. The enrollment is currently ongoing at a rate of about 30 patients per month. Informed consent was obtained for each patient. The research has been approved by local research ethics committees of participating institutes. For patient enrolled after August 2008, 5 ml of blood is available for genomic DNA extraction. For patients enrolled before August 2008, genomic DNAs were extracted from approximately 100 mg of adjacent normal tissues obtained by a pathologist after surgery. Tumor and adjacent normal tissues are available for 71 % of all the patients in this study. Approximately 98 % of patients in this study received surgery and 51 % received adjuvant chemotherapy after surgery. No patients received neoadjuvant chemotherapy before surgery, radiation therapy, or targeted therapy.

Gene/Polymorphism Selection and Genotyping

The candidate stem cell-related genes and polymorphisms were selected based on literature review and database interrogation. The detailed selection procedure is described in Supplementary Materials and Methods. Overall, 11 potentially functional SNPs in 6 important stem cell-related genes were genotyped in the 380 patients, using the Sequenom iPLEX genotyping system, according to the manufacturer's instructions (Sequenom Inc., CA, USA). Laboratory personnel conducting genotyping were blinded to patient information. The 11 SNPs are summarized in Supplementary Table 1.

Statistical Analysis

The primary endpoint for this study is the association between stem cell-related variations and patient recurrence. The time to recurrence was defined as the time from initial treatment to local recurrence, distant metastasis, or the date of last follow-up. Recurrence was established through the results of positive findings in imaging and/or laboratory tests. In our hospitals, serum carcinoembryonic antigen (CEA) is the most commonly used marker to monitor CRC recurrence. There are different methods to measure CEA with varied normal reference ranges. Among them, the chemiluminescence immunoassay approach with 0–5 ng/ml as the normal reference range is mostly used in our hospitals. Patients who exhibited abnormally higher CEA values in a sustained period of time were suspected for CRC recurrence and sent for further imaging tests. The secondary endpoint is overall patient survival, which was defined as the time between initial treatment to death from any cause. In addition, event-free survival time was also assessed, which was defined as the time from initial treatment to local recurrence, distant metastasis, death from any cause, or the date of last follow-up. Kaplan–Meier curve and log rank test were used to assess the differences in time to recurrence, death, or event. Hazard ratios (HRs) were estimated from a multivariate Cox proportional hazards model. SAS (version 9.1, SAS Institute Inc, Cary, NC, USA) and STATA software packages (version 8, STATA Corp., College Station, TX, USA) were used for the above analyses. We also conducted the risk genotype analysis of rs879882 and rs459552, the two SNPs showing at least a borderline significant association with recurrence in patients receiving chemotherapy. The risk genotype was defined as the variant-containing genotype of either SNP. Using the subject group without any risk genotype as the reference group, we calculated the HRs and 95 % CIs for the subjects with at least one risk genotype using Cox proportional hazards model. A receiver operating characteristic (ROC) curve was constructed and the area under the curve (AUC) was calculated to evaluate the specificity and sensitivity of predicting recurrence by different combinations of clinical, epidemiological, and stem cell-related genetic variables. All *P* values were two-sided. *P* 0.05 was considered the threshold of statistical significance.

Results

Characteristics of the Study Population

The distribution of demographic and clinical characteristics of the 380 CRC patients is summarized in Supplementary Table 2. Almost all (97.9 %) patients underwent surgery after diagnosis, whereas half (50.5 %) of them received chemotherapy after surgery. Among the 192 patients receiving chemotherapy, 185 (96.4 %) received the FOLFOX regimen including folinic acid (FOL), 5′ fluorouracil (F), and oxaliplatin (OX). The percentage of chemotherapy-treated patients in stages 0/1, 2, 3, and 4 was 43.1, 45.2, 59.6, and 55.8 %, respectively. Consultation with the treating physicians suggested that factors other than medical conditions also affected the decisions of patients to receive chemotherapy, such as patients' socioeconomic status and willingness to receive chemotherapy, etc. Among the 43 stage 4 patients, 38 patients were surgically treated to remove both the primary and the metastatic tumors.

Main Effects and Stratified Analyses by Individual Polymorphisms

For the 11 SNPs genotyped, the average call rate is 99.5 % (98.7–100 %). Three SNPs (rs7775, rs288326, and rs12255372) were excluded from further analyses due to small minor allele frequencies (<2 %). The associations of the remaining eight SNPs with CRC recurrence, death, and event were listed in Table 1. Only the dominant genetic model was tested to obtain the optimum statistical power. Among the eight SNPs, rs879882, a SNP in the 5′ region of *POU5F1*, exhibited a statistically significant association with CRC recurrence. Compared to the homozygous wild-type (WW) genotype, the variant-containing

(WV+VV) genotypes were associated with a 2.10-fold (95 % CI 1.17–3.76, $P=0.01$) increased recurrence risk (Table 1). This SNP was not associated with patient death (HR0 1.05, 95 % CI 0.56–1.98, $P=0.87$) but showed a borderline significant association with event (HR=1.49, 95 % CI 0.94–2.36, $P=0.09$) (Table 1), mostly driven by the association with recurrence. Kaplan–Meier curves were constructed for rs879882 on time to recurrence (log rank $P=0.04$) and event (log rank $P=0.20$) (Fig. 1). In stratified analyses of rs879882 (Table 2), the recurrence risk remained at least borderline significant in old subjects ($P=0.06$), females ($P=0.01$), never smokers ($P=0.004$), patients with rectal cancer ($P=0.01$), adenocarcinoma ($P=0.01$), and moderate tumor differentiation ($P=0.02$), and patients receiving chemotherapy ($P=0.09$) (Table 2). The risk remained significant in patients with both non-metastatic (stages 0–3) ($P=0.008$) and metastatic (stage 4) ($P=0.05$) disease (Table 2).

Stem Cell-Related SNPs and Recurrence in CRC Patients Receiving Chemotherapy

Since chemotherapy is the mainstream CRC treatment, we evaluated the associations of the eight SNPs with recurrence in those patients receiving chemotherapy (Table 3). In this analysis, the association of rs879882 remained borderline significant (HR=1.97, 95 % CI 0.89–4.34, $P=0.09$). In addition, rs459552, a nonsynonymous SNP (nsSNP) in *APC* gene, was also significantly associated with recurrence (HR=2.63, 95 % CI 1.14–6.06, $P=0.02$) (Table 3). Kaplan–Meier curves were constructed for rs879882 (log rank $P=0.17$) and rs459552 (log rank $P=0.03$) on time to recurrence in patients receiving chemotherapy (Fig. 2). We also restricted this analysis within non-metastatic patients only and the results did not show substantial changes (data not shown). However, when the analysis was restricted to patients with only stage 2 or 3 disease, the association was not significant (HR=1.80, 95 % CI 0.72–1.47, $P=0.20$, log rank $P=0.15$, data not shown), likely due to the small number of patients meeting these criteria.

Combined Effect of the Risk Genotypes on Recurrence in Patients Receiving Chemotherapy

We further evaluated the combined effect between rs879882 and rs459552 on CRC recurrence in chemotherapy-treated patients. Compared to subjects without any risk genotype, those subjects with at least one risk genotype exhibited a 2.53-fold (95 % CI 1.06–6.05, $P=0.04$, log rank $P=0.07$) increased recurrence risk (Supplementary Table 3). Further analysis indicated that this combined effect was not evident in CRC patients without chemotherapy (HR01.42 95 % CI 0.60–3.40, $P=0.43$, log rank $P=0.60$), suggesting that these two SNPs may be predictive markers specific to chemotherapy, instead of a general marker of CRC prognosis.

Multivariate Recurrence Assessment Model

We then attempted to build a multivariate assessment model of recurrence risk in chemotherapy-treated CRC patients under different scenarios of predictor combinations. We calculated the AUC by sequentially adding established prognostic clinical variables (tumor position, pathology, stage, differentiation), epidemiological variables (age, gender, smoking status), and the two genetic variants, rs879882 and rs459552 (Fig. 3). The AUC increased from 0.70 (clinical and epidemiologic variables) to 0.77 (clinical and epidemiological variables, plus the two stem cell-related SNPs). This increase in AUC demonstrates the potential of improving clinical outcome assessment by the addition of genetic variables over using only clinical and epidemiological information.

Discussion

Currently, there are a few CRC population studies evaluating a limited number of stem cell-related variants [31–39]. Most of these studies were focused on CRC etiology only, although extensive laboratory studies have provided compelling evidence that stem cell-related genes also influence CRC prognosis [40–42]. To our knowledge, our study is one of the first applications using a pathway-based approach to evaluate stem cell-related variants in cancer prognosis. We found significant associations between SNPs in *POU5F1* and *APC* genes that conferred altered recurrence risk, especially in patients receiving chemotherapy. We also demonstrated the combined effects of the two SNPs, in conjunction with clinical and epidemiological variables, on enhancing the power to predict recurrence.

These findings are biologically plausible in that both *POU5F1* and *APC* have been significantly implicated in CRC etiology and clinical outcomes. *POU5F1* (*OCT3/OCT4*) encodes a transcriptional factor important to the control of self-renewal and pluripotency in both human and mouse ESCs [43, 44]. Recently, several elegant studies found that co-transfection of *POU5F1* and *SOX2* with *KLF4* and *c-MYC* or *LIN28* and *NANOG* are sufficient to generate induced pluripotent stem (iPS) cells from differentiated somatic cells in mouse and human [45–47]. It was then proven that *POU5F1* alone is sufficient to reprogram human and mouse neural stem cells into iPS cells [48, 49], further highlighting the indispensability of *POU5F1* in stem cell maintenance and iPS cell generation. Moreover, various independent studies have shown that *POU5F1* is highly overexpressed in CRC tissues, and the high expression levels of *POU5F1*, *SOX2*, and *PROM1* (a stem cell and CSC surface marker) are correlated with the recurrence and death of chemotherapy-treated CRC patients [50–53]. The rs879882 SNP is located at about 1.1 kb upstream of the transcription start site of *POU5F1*. Although the promoter sequence of human *POU5F1* has not been well-characterized experimentally, a comparative analysis of human, mouse, and bovine *POU5F1* promoter region indicated that the promoter may encompass as large as 2.5 kb upstream of the transcription start site [54]. We conducted bioinformatics analyses to search for potential transcription factor binding sites within the sequence harboring rs879882 using three algorithms: TRANSFAC/Match, JASPAR/MatScan, and ORegAnno [55] (data not shown). No transcription factor binding site was identified, hindering that the observed association may be due to an indirect effect from another causal variant in linkage disequilibrium. Future tagging SNP-based approaches, as well as functional characterizations such as luciferase and gel shift assays, are warranted to pinpoint the functional locus that confers the association between rs879882 and CRC recurrence.

Because genes related to normal and cancer stem cells have been consistently reported to influence response to chemotherapy, we conducted a chemotherapy-stratified analysis to identify SNPs influencing chemotherapy outcome. In addition to rs879882, we also found a nsSNP, rs459552, in *APC* gene that was associated with a significantly elevated risk of recurrence in chemotherapy-treated CRC patients. *APC* encodes a pivotal tumor suppressor protein that interacts with *GSK3B* and *AXIN* to form a destruction complex that degrades beta-catenin, thus antagonizing the WNT signaling transduction cascade and prevents CRC initiation and progression [37]. A wide spectrum of *APC* germline mutations leads to familial adenomatous polyposis, a hereditary CRC-prone syndrome [56]. In addition, several studies have reported that *APC* mutations and dysfunctions modulate the response of CRC patients to oxaliplatin and 5-fluorouracil, the two key drugs included in the FOLFOX chemotherapy regimen used in this study [57–60], which is consistent with the findings of our study. The significant SNP, rs459552, is a nsSNP leading to a valine to aspartic acid substitution at codon 1822. This SNP has been assessed in multiple CRC case-control studies with mixed results, possibly due to the interactions with demographic and lifestyle factors in CRC risk modulation [35, 61–63]. Our study is the first to report an association of

this SNP with CRC prognosis. The position of this missense variant is close to the fifth cysteine-rich beta-catenin binding repeat region, underscoring the possibility that it may be the functional locus that regulates beta-catenin destruction, although as yet no in vitro study has been conducted to confirm this hypothesis.

We also tested the combined effects of risk genotypes between rs879882 and rs459552 on recurrence in patients receiving chemotherapy. Furthermore, when we incorporated these two SNPs into a multivariate outcome assessment model, we obtained a significant improvement of discriminatory ability (Fig. 3). It is commonly recognized that cancer treatment response is affected by complex interactions between genetic and environmental factors and between host and tumor characteristics. The observed improvement in the discriminatory ability underscores the importance of incorporating genetic biomarkers in the prediction of clinical outcomes. However, given the small sample size and the post hoc data-mining nature, our model is exploratory and needs further validation before clinical applications. Nevertheless, it is clear from our results that gene–gene and gene–environment interactions play an important role in determining CRC prognosis.

Our study has several strengths. First, the patient population is enrolled from Xi'an and adjacent areas. This region is highly attractive in conducting population-based research due to the geographical stability with low mobility rate. Second, the patients had relatively homogenous treatments. Approximately 98 % of patients received surgery to remove the primary tumor. In around half of the patients who received chemotherapy after surgery, over 96 % received the same first-line FOLFOX regimen. These homogenous patient characteristics and treatments greatly reduced the confounding effects of the heterogeneous therapeutic modalities in most cancer clinical outcome studies.

Our study also has limitations. First, our sample size may not be large enough to detect minimal associations and interactions. Also, given the exploratory nature of our study and the small number of analyzed SNPs, we did not conduct multiple comparison corrections. However, with the current sample size, we have the power to detect a reasonable degree of modest associations. The homogeneous patient characteristics and treatments in our population also minimize confounding effects and enhance our statistical power. Second, our study has a relatively short follow-up time and a large portion of low-stage patients receiving chemotherapy. However, our population has already presented with a considerable percentage of events. The continuing growth of our population with a low rate of patient loss during follow-up will further enable us to obtain adequate power for more in-depth interaction analyses. Third, in this study, we only included selected pathways and genes we considered most relevant based on current knowledge. Also, the sample size and multiple comparison issue would not allow us to evaluate a more comprehensive gene panel. In the future, along with the further rapid growth of our study population and the knowledge on stem cells, the test of a more comprehensive panel of stem cell-related genes using a haplotype-tagging SNP-based genotyping approach would be desired to provide additional insights on the associations between stem cell gene variations and CRC prognosis.

Overall, we presented one of the first epidemiologic studies supporting a role of genetic polymorphisms of stem cell-related pathway genes in cancer prognosis. Our results suggest that individual as well as combined genotypes of stem cell-related variants may be used to assess the clinical outcome of colorectal cancer. Our next steps include the validation of these findings using independent populations, high-density mapping based on a tagging SNP approach, and functional characterizations through in vitro and in vivo studies. If validated, our findings could form the basis for future larger molecular epidemiological studies of the stem cell pathway gene variants. Results from these studies could provide a translational

bridge between basic and clinical researches on human stem cells, as well as contribute to the development of therapeutic strategies to improve the prognosis of CRC patients.

Conclusion

We presented one of the first epidemiologic studies showing that stem cell-related genetic variants may impact CRC clinical outcomes, especially in chemotherapy-treated patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Kaplan-Meier (a) recurrence-free curve and (b) event-free curve by *POU5F1* rs879882

Fig. 2. Kaplan–Meier recurrence-free curve by (a) *POU5F1* rs879882 and (b) *APC* rs459552 in colorectal cancer patients treated with chemotherapy

Fig. 3. ROC curves from different models showing the improvement of assessment ability. Clinical variables include tumor position, pathology, stage, differentiation, and surgery. Epidemiological variables include age, gender, and smoking status. Genetic variables include rs879882 and rs459552

Table 1
Association of stem cell-related polymorphisms with clinical outcome of colorectal cancer patients

SNP	Gene	Genotype ^a	Recurrence/total	HR (95 % CI) ^b	P value	Death/total	HR (95 % CI) ^b	P value	Event/no event ^c	HR (95 % CI) ^b	P value
rs459552	APC	WW	41/319	1	0.49	38/315	1	0.70	68/319	1	0.76
		WV+VV	10/61	1.30 (0.62–2.71)		9/61	1.26 (0.53–2.55)		15/61	1.10 (0.60–2.00)	
rs2229992	APC	WW	23/152	1	0.45	17/152	1	0.87	34/152	1	0.69
		WV+VV	28/225	0.81 (0.46–1.42)		30/221	1.05 (0.56–1.99)		49/225	0.91 (0.58–1.44)	
rs7903146	TCF7L2	WW	49/343	1	0.65	44/339	1	0.85	78/343	1	0.83
		WV+VV	2/37	1.14 (0.64–2.02)		3/37	1.12 (0.33–3.75)		5/37	0.90 (0.36–2.26)	
rs3811463	LIN28	WW	39/260	1	0.22	33/256	1	0.82	59/260	1	0.52
		WV+VV	12/115	0.65 (0.33–1.29)		14/115	0.93 (0.47–1.82)		24/115	0.85 (0.51–1.40)	
rs6697410	LIN28	WW	42/314	1	0.71	42/311	1	0.38	70/314	1	0.89
		WV+VV	9/63	1.15 (0.55–2.43)		5/62	0.65 (0.25–1.70)		13/63	1.04 (0.57–1.92)	
rs12747426	LIN28	WW	40/264	1	0.11	33/260	1	0.42	61/264	1	0.18
		WV+VV	11/115	0.57 (0.28–1.14)		14/115	0.76 (0.39–1.42)		22/115	0.70 (0.42–1.18)	
rs879882	POU5F1	WW	26/244	1	0.01	31/240	1	0.87	49/244	1	0.09
		WV+VV	24/134	2.10 (1.17–3.76)		16/134	1.05 (0.56–1.98)		33/134	1.49 (0.94–2.36)	
rs11915160	SOX2	WW	46/358	1	0.67	45/354	1	0.22	77/358	1	0.83
		WV+VV	5/22	2.23 (0.81–4.75)		2/22	0.38 (0.08–1.77)		6/22	1.07 (0.43–2.66)	

^a WW homozygous wild-type genotype, WV heterozygous genotype, VV homozygous variant genotype

^b Adjusted for age, gender, smoking status, tumor position, tumor pathology, tumor differentiation, tumor stage, surgery, and chemotherapy

^c Event: recurrence and/or death

Table 2

Association of rs879882 in *POU5F1* gene with recurrence of colorectal cancer patients stratified by host characteristics

Variables	Stratum ^a	Genotype ^b	Recurrence/total	HR (95 % CI) ^c	P value
Overall		WW	26/244	1	0.01
		WV+VV	24/134	2.10 (1.17–3.76)	
Age	Young (≤ 61)	WW	15/134	1	0.27
		WV+VV	11/61	1.63 (0.69–3.90)	
	Old (>61)	WW	11/110	1	0.06
		WV+VV	13/73	2.29 (0.95–5.48)	
Gender	Male	WW	18/141	1	0.39
		WV+VV	13/71	1.39 (0.66–2.92)	
	Female	WW	8/103	1	0.01
		WV+VV	11/63	3.85 (1.33–11.20)	
Smoking status	Never smoker	WW	20/181	1	0.004
		WV+VV	22/105	2.61 (1.36–5.00)	
	Ever smoker	WW	6/63	1	0.87
		WV+VV	2/29	0.86 (0.16–4.82)	
Tumor position	Colon	WW	11/121	1	0.28
		WV+VV	9/65	1.42 (0.56–3.60)	
	Rectum	WW	15/123	1	0.01
		WV+VV	15/69	2.80 (1.63–2.34)	
Tumor pathology	Adenocarcinoma	WW	24/230	1	0.01
		WV+VV	24/131	2.07 (1.15–3.71)	
Tumor differentiation	Moderate	WW	17/166	1	0.02
		WV+VV	17/81	2.30 (1.15–4.62)	
Tumor stage	Non-metastatic (stages 0–3)	WW	20/208	1	0.008
		WV+VV	22/124	2.40 (1.26–4.58)	
	Metastatic (stage 4)	WW	6/33	1	0.05
		WV+VV	2/10	0.07 (0.01–1.01)	
Chemotherapy	Yes	WW	15/120	1	0.09
		WV+VV	13/70	1.97 (0.89–4.34)	

Variables	Stratum ^a	Genotype ^b	Recurrence/total	HR (95 % CI) ^c	P value
	No	WW	11/124	1	0.10
		WV+VV	11/63	2.05 (0.86–4.89)	

^aThere are limited number of subjects in the strata other than “adenocarcinoma” in “tumor pathology” and “moderate” in “tumor differentiation”. Young and old patients were separated by the median value of age in the patients

^bWW/homozygous wild-type genotype, WV/heterozygous genotype, VV/homozygous variant genotype

^cAdjusted for age, gender, smoking status, tumor position, tumor pathology, tumor differentiation, tumor stage, surgery, and chemotherapy, where appropriate

Table 3

Association of stem cell-related polymorphisms with recurrence of colorectal cancer patients stratified by chemotherapy

SNP	Gene	Genotype ^a	Chemotherapy			No chemotherapy		
			Recurrence/total	HR (95 % CI) ^b	P value	Recurrence/total	HR (95 % CI) ^b	P value
rs459552	APC	WW	19/157	1	0.02	22/161	1	NA
		WV+VV	10/35	2.63 (1.14–6.06)		0/26	NA	
rs2229992	APC	WW	10/71	1	0.70	13/80	1	0.28
		WV+VV	19/119	1.17 (0.52–2.62)		9/106	0.62 (0.25–1.49)	
rs7903146	TCF7L2	WW	28/174	1	0.50	21/168	1	0.67
		WV+VV	1/18	0.50 (0.07–3.78)		1/19	0.64 (0.08–5.03)	
rs3811463	LIN28	WW	23/136	1	0.28	16/124	1	0.76
		WV+VV	6/57	0.60 (0.23–1.52)		6/59	0.86 (0.32–2.32)	
rs6697410	LIN28	WW	24/152	1	0.97	18/161	1	0.80
		WV+VV	5/38	0.98 (0.33–2.89)		4/25	1.17 (0.35–3.86)	
rs12747426	LIN28	WW	23/149	1	0.41	17/115	1	0.35
		WV+VV	6/42	0.67 (0.26–1.72)		5/72	0.61 (0.21–1.73)	
rs879882	POU5F1	WW	15/120	1	0.09	11/124	1	0.10
		WV+VV	13/70	1.97 (0.89–4.34)		11/63	2.05 (0.86–4.89)	
rs11915160	SOX2	WW	27/184	1	0.88	19/173	1	0.06
		WV+VV	2/8	0.88 (0.19–4.23)		3/14	3.47 (0.96–12.58)	

^a WW/homozygous wild-type genotype, WV/heterozygous genotype, VV/homozygous variant genotype

^b Adjusted for age, gender, smoking status, tumor position, tumor pathology, tumor differentiation, tumor stage, and surgery