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Original Article

Functional STR within *PTPN11*: a novel potential risk factor for colorectal cancer

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Abstract: *PTPN11* was previously regarded as a proto-oncogene, but recent reports have found that it acts as a tumor repressor in hepatocellular carcinogenesis and a prognostic predictor for colorectal carcinoma (CRC), although, its role in colorectal carcinogenesis is still unclear. This hospital-based case-control study with 830 CRC cases and 878 controls was carried out to determine the effect of the short tandem repeat (STR) polymorphism, located in the 3'UTR, on CRC risk in the study population of Chinese adults. Distribution of the genotypic frequency between CRC cases and controls in the Xuzhou study center revealed that the risk of CRC decreased as the repeat numbers increased. Compared with the 11/12 genotype, those with the 13/14 genotype were conferred reduced risk of CRC (OR=0.74, 95% CI=0.59-0.95, *P*=0.02), while carriers with the 15/16 genotype showed a marked reduction in CRC risk (OR=0.50, 95% CI=0.34-0.74, *P*=0.0004). A similar trend in genotype and allelic frequency was also observed in the Suining study center as well as in the pooled results. Using RT-qPCR analysis, longer alleles were found to upregulate the expression of *PTPN11* in both tumor tissues and adjacent non-tumor tissues, with the expression of *PTPN11* in non-CRC tissues observed to be 2.5-fold higher than those of CRC tissues. In the gain-of-function *in vitro* studies, it was found that constructs with allele 14 had the highest luciferase expression, while the allele 12 constructs had much lower expression, indicating that the STR polymorphism could influence the transcriptional activity and therefore was able to modulate *PTPN11* expression. In conclusion, these findings indicate that the STR polymorphism located in *PTPN11* modulates colorectal carcinogenesis probably through a motif change in the 3'UTR. Further studies with more study centers and the inclusion of other ethnic Chinese populations would have to be carried in the future so as to substantiate this observation.

Keywords: *PTPN11*, STR, colorectal carcinoma, risk

Introduction

Colorectal carcinoma (CRC), which is one of the most common malignant tumors, is an issue of global Public Health concern [1]. The past decade have witnessed growing numbers of CRC cases in the world, especially in the developed urban areas of China [2]. Although good progress has been made in understanding the carcinogenesis of CRC, the molecular mechanisms are yet to be elucidated. The use of genome-wide association studies (GWAS) in recent years has implicated the role of genetic variations in tumorigenesis [3-6]. However, as a commercial platform, GWAS has its limitations such as, low replication support, no complete cover in genetic variations recruitment [7]. Thus, a good CRC related genetic variation identification mechanism is needed that would help to elucidate the molecular mechanisms of CRC carcinogenesis and risk prediction.

The activation of the Ras/Erk pathway is a known classical stimulator for cell proliferation in CRC [8], while its upstream enhancer, Shp2, encoded by *PTPN11* is reported to be involved

Table 1. The main characteristics of the subjects included in the study

Characteristics	Cases N (%)	Controls N (%)	*P*-value
Xuzhou group	423	457	
Age (Mean ± SD)	59.1±9.4	58.4±10.2	0.29
Gender			
Male	304 (71.9)	344 (75.3)	
Female	119 (28.1)	113 (24.7)	0.25
Tumor site			
Colon	195 (46.1)	-	
Rectum	228 (53.9)	-	
Tumor stage			
I	37 (8.8)	-	
II	122 (28.8)	-	
III	157 (37.1)	-	
IV	107 (25.3)	-	
Suining group	407	421	
Age (Mean ± SD)	59.5±9.9	58.7±9.8	0.26
Gender			
Male	295 (72.5)	312 (74.1)	
Female	112 (27.5)	109 (25.9)	0.59
Tumor site			
Colon	188 (46.2)	-	
Rectum	219 (53.8)	-	
Tumor stage			
I	38 (9.3)	-	
II	123 (30.2)	-	
III	154 (37.8)	-	
IV	92 (22.7)	-	

in the carcinogenesis of a number of cancers including leukemia [9], hepatocellular cancer (HCC) [10] and CRC [11]. Somatic *PTPN11* mutations lead to aberrant expression of Shp2, which influences a variety of cellular functions such as cancer metastasis, apoptosis and survival [12-14]. Originally identified as a proto-oncogene, *PTPN11* has recently been demonstrated to have a tumor suppressor role in HCC, indicating its dual role in carcinogenesis [10].

Zhao and colleagues in a recent study found that one functional short tandem repeat (STR) was involved in HCC carcinogenesis through the modulation of PTPN11 expression [15]. Thus, given that PTPN11/Shp2 act as tumor suppressor for CRC [16], we postulated that probably the functional STR rs199618935, located within *PTPN11* might regulate its expression so as to affect CRC initiation. Here, we conducted a hospital-based case-control study, complemented by *in vitro* functional studies, so as to determine the association and/or effect of the STR on CRC susceptibility in a given ethnic Chinese population.

Materials and methods

Study populations

All recruited subjects were local residents within the study area and are of the Han Chinese ethnicity. A total of 423 patients with histopathologically diagnosed primary CRC and 457 cancer-free subjects were recruited from the Affiliated Hospital of Xuzhou Medical University between January 2012 and February 2015. Similarly, 407 patients with histopathologically diagnosed primary CRC and 421 cancer-free subjects from the Shehong Hospital of Traditional Chinese Medicine were recruited during the same period. The cancer-free individuals (control subjects) were from a community nutritional survey that was conducted in the same study area during the same period as the recruitment of the cancer patients. The selection criteria for controls included no family history of any cancer and matched to cases on age and sex. Peripheral blood was sampled from the subjects before receiving any medication. Tumor tissues from a total of 89 patients diagnosed with CRC were collected from the available frozen stored tissues of CRC resections from 2012 to 2014. The 89 CRC cases were confirmed by pathologic diagnosis and none of these patients had ever received preoperative chemotherapy or radiotherapy. These 89 patients were part of the recruited 830 cases. Tumor stages were determined according to a modified American Joint Committee on Cancer and International Union against Cancer standard. After surgical resection, fresh tissues were immediately stored at -80°C until DNA/RNA isolation. The study was approved by the Ethics Committees of the Affiliated Hospital of Xuzhou Medical University and the Shehong Hospital of Traditional Chinese Medicine. Written informed consent was obtained from all participants.

DNA extraction and genotyping

Genomic DNA of peripheral blood samples were isolated using genomic DNA extraction kit (Qiagen), and DNA fragments with the locus were amplified with a pair of primers (forward primer: 5'-GTGTCCCTTCTACTTCCCTCT-3', reverse primer: 5'-GCTGGGCTTGTGACTTGT-TT-3'). All PCR products were detected by 7%

Table 2. Genotypic and allelic distribution of *PTPN11* STR in enrolled groups

Group	Repeat number	Genotypic frequency		OR (95% C.I.)[a]	*P*	Allelic frequency		OR (95% C.I.)[a]	*P*
		Cases (%)	Controls (%)			Cases (%)	Controls (%)		
Xuzhou	11/12	396 (93.6)	380 (83.2)	1.00 (reference)	-	619 (73.2)	565 (61.8)	1.00 (reference)	
	13/14	168 (39.7)	217 (47.5)	0.74 (0.59-0.95)	0.02	183 (21.6)	256 (28.0)	0.65 (0.52-0.81)	0.02
	15/16	46 (10.9)	88 (19.3)	0.50 (0.34-0.74)	0.0004	44 (5.2)	93 (10.2)	0.43 (0.30-0.63)	0.0004
Suining	11/12	382 (93.9)	348 (82.7)	1.00 (reference)	-	593 (72.9)	515 (61.2)	1.00 (reference)	
	13/14	158 (38.8)	203 (48.2)	0.71 (0.54-0.91)	0.008	174 (21.4)	240 (28.5)	0.63 (0.50-0.79)	0.008
	15/16	45 (11.1)	82 (19.5)	0.49 (0.33-0.73)	0.0005	47 (5.8)	87 (10.3)	0.47 (0.32-0.68)	0.0005
Pooled	11/12	778 (93.7)	728 (82.9)	1.00 (reference)	-	1212 (73.0)	1080 (61.5)	1.00 (reference)	
	13/14	326 (39.3)	420 (47.8)	0.73 (0.61-0.86)	0.0004	357 (21.5)	496 (28.2)	0.64 (0.55-0.74)	<0.00001
	15/16	91 (11.0)	170 (19.4)	0.50 (0.38-0.66)	<0.00001	91 (5.5)	180 (10.3)	0.45 (0.35-0.59)	<0.00001

[a]Adjusted for sex, age, tumor site and tumor stage.

native-PAGE and observed by silver staining method. Six types of bands were found in the PCR products (allele 11, allele 12, allele 13, allele 14, allele 15 and allele 16), and each band was sequenced. The STR genotypes were determined by DNAMAN software and N-BLAST by two independent researchers (Huiping Wang and Xue Yang).

RT-qPCR analysis

Total RNA was isolated from tissue samples using RNA isolation kit (Qiagen). cDNA was generated using random primers and Superscript II reverse transcriptase (Invitrogen). A SYBR Green qPCR was performed using Roche Light Cycler 480 to quantify relative PTPN11 expression in these samples. GAPDH was chosen as the internal control. Primer sequences used for PTPN11 and GAPDH were as follows: PTPN11-F: 5'-TCAGCACAGAAATAGATGT-G-3', PTPN11-R: 5'-TGCTTATCAAAAGGTAGTCA-3', GAPDH-F: 5'-CTCTCTGCTCC TCCTG-TTCGAC-3', GAPDH-R: 5'-TGAGCGATGTGGCTCGGCT-3'. The 25 μl total volume final reaction mixture consisted of 1 μM of each primer, 12.5 μl of Master Mix (Applied Biosystems), and 50-100 ng of cDNA. The negative control experiments were performed with distilled H_2O as template. The expression levels of target genes were normalized with GAPDH using the $2^{-\Delta\Delta CT}$ method [17]. In addition, the melting curve analysis was performed for the PCR products to check the specificity of the primers.

Plasmids construction and Luciferase assay

The HT-29 and SW480 colorectal cancer cell lines were obtained directly from Shanghai Cell Bank of Chinese Academy of Sciences. Cells were cultured in RPMI1640 (Hyclone) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 incubator. The partial structures (about 570 bp) of human PTPN11-3'UTR containing allele 12, 14 and 15 of rs199618935 were amplified with forward primer 5'-GATCT-CTAGACCCCAACTGTTAGTCAATCTGAGC-3' and reverse primer 5'-CATGGAT-CCTTGTCCCGCTA-CTGTAAGCAGC-3' from three homozygous human genomic DNA samples. The PCR products were separated on agarose gel and extracted, purified, and cloned with TA cloning Kit (Promega). The repeat numbers of different alleles were confirmed by sequencing. Finally, the 3'UTR of Renilla luciferase in the vector pRL-SV40 (Promega) was replaced with the cloned 3'UTR of PTPN11 by restriction enzymes Xbal and BamHI. The resulting constructs were verified by sequencing. Cell from the two cell lines were seeded at 1×10^5 cells per well in 24-well plates (BD Biosciences). 24 hours after the plating, cells were transfected by Lipofectamine 2000 according to the manufacturer's manual. In each well, 500 ng constructed pRL-SV40 vector and 50 ng pGL3 control vector were cotransfected. Six replicates were performed for each group and each experiment was repeated at least three times. After 24 hours of transfection, cells were harvested by the addition of 100 ml passive lysis buffer. Renilla luciferase activities in cell lysate were measured with the Dual Luciferase assay system (Promega) in TD-20/20 luminometer (Turner Biosystems) and were normalized with the firefly luciferase activities.

Statistical analysis

Genotype distribution was analyzed by Hardy-Weinberg equilibrium with χ^2 test. The classifi-

Table 3. Stratified analysis on the association between *STR* and CRC risk based on tumor stage in enrolled CRC cases

Group	Repeat number	Tumor stage		OR (95% C.I.)[a]	*P*
		I+II	III+IV		
Xuzhou	11/12	71	157	1.00 (reference)	-
	13/14	61	80	0.59 (0.37-0.91)	0.02
	15/16	27	27	0.45 (0.26-0.84)	0.01
Suining	11/12	74	148	1.00 (reference)	-
	13/14	58	68	0.58 (0.34-0.92)	0.02
	15/16	29	30	0.52 (0.29-0.93)	0.03
Pooled	11/12	145	305	1.00 (reference)	-
	13/14	119	148	0.59 (0.44-0.80)	0.001
	15/16	56	57	0.48 (0.32-0.73)	0.0009

[a]Adjusted for sex, age, tumor site and tumor stage.

cation method for this multi-allelic polymorphism was assigned according to a previous report, which investigated STR [15]. A comparison of genotypic and allelic frequency between CRC cases and controls was by χ^2 test. Unconditional Logistic regression analysis was used to evaluate the correlation between the STR and CRC risk after adjusting for gender, age, tumor site and tumor stage. Tumor stage was of importance to survival, thus stratified analysis by tumor stage (I+II, III+IV) for enrolled CRC cases was carried out using binary logistic regression. One-way ANOVA was used to analyze the difference in Luciferase reporter gene expression. The difference in normalized expression of PTPN11 in the tissues collected was compared using Student's *t* test. The statistical analyses were performed using the statistical software package SPSS 18.0 (SPSS Inc.). All *p* values less than 0.05 were considered statistically significant.

Results

Baseline characters of subjects

The main characteristics of enrolled participants are displayed in **Table 1**. Distribution of sex and age between CRC cases and cancer-free controls showed no obvious difference in both centers (*P*>0.05), indicating that there was adequately matching. Distribution of tumor site and tumor stage had similar trend in both study centers.

The STR polymorphism and CRC risk

Genotypic and allelic frequency distribution and the correlation of rs199618935 with CRC risk is shown in **Table 2**. A correlation between rs199618935 and CRC risk was found in the Xuzhou center, where, the risk of CRC attenuated while the repeat numbers increased. Compared with the 11/12 genotype, carriers with 13/14 genotype were conferred reduced risk of CRC (OR=0.74, 95% CI=0.59-0.95, *P*=0.02), while carriers with 15/16 genotype showed marked reduction in CRC risk (OR=0.50, 95% CI=0.34-0.74, *P*=0.0004). Allelic distribution also displayed similar pattern as genotypic distribution, which was confirmed by the trend of data from the Suining study center.

The STR influenced CRC tumor staging

Given that clinicopathological factors may influence CRC risk, we did stratified analysis on tumor site and tumor stage of enrolled CRC cases in **Table 3**. After stratification, we found that cases in the Xuzhou study center with higher tumor stages (III+IV) had a reduced trend in odds ratio that was inversely related with dose independent effect. This relationship was substantiated by the data from the Suining study center and the pooled analysis.

The STR polymorphism increased PTPN11 expression in CRC tissue

In order to determine the effect of the target STR on its gene expression, we first performed an RT-qPCR to check the level of expression between different genotyped CRC tissue samples and adjacent non-cancer tissues. As shown in **Figure 1**, we found that the target STR polymorphism correlated with *PTPN11* expression in genotyped groups. PTPN11 expression in the 14-14/14-15 genotype showed marked upregulation when compared with reference (those genotyped with 12-12), with the fold change prominent in both cancer tissues and adjacent normal tissues (2.53-fold and 2.23-fold respectively, *P*<0.01). Without taking the genotypes into consideration (**Figure 2**), we found that PTPN11 expression in adjacent normal tissues was much higher than those in CRC tissues (approximately 2.5-fold).

Gain-of-function analysis

After confirmation a genotype-phenotype correlation in the qPCR analysis, we went on to do a gain-of-function analysis so as to determine the effect of current target STR polymorphism. Different plasmids were constructed with allele

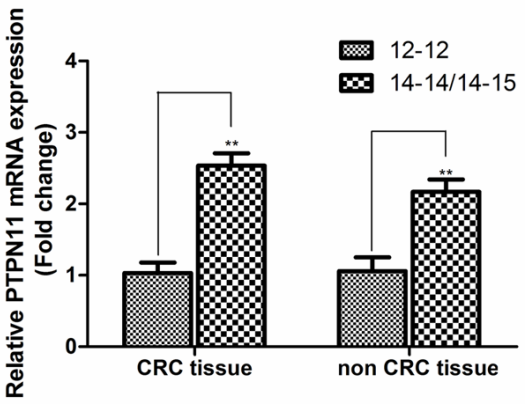

Figure 1. Expression of *PTPN11* in CRC tissues and adjacent non tumor tissues with different genotypes. PTPN11 mRNA expression (Mean ± SEM) by rs199618935 genotype. 70 cases for genotype 12-12, 11 for genotype 14-14, 8 for genotype 14-15. **Indicated *P*<0.01 compared with genotype 12-12 in the same tissue group.

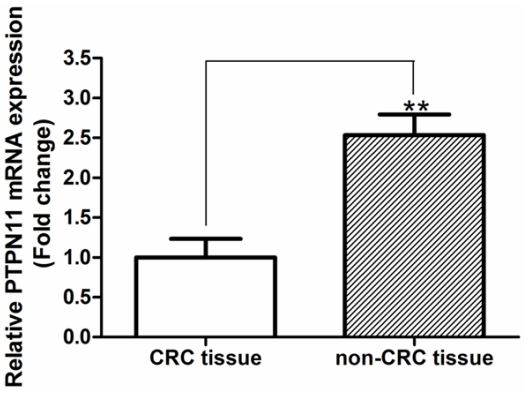

Figure 2. Expression of *PTPN11* in CRC tissues and adjacent non tumor tissues. **Indicated *P*<0.01 compared with CRC counterparts.

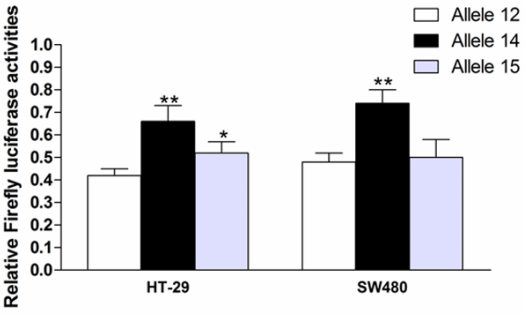

Figure 3. Influence of STR rs199618935 on PTPN11 transcription activity based on Luciferase reporter assay. *Indicated *P*<0.01 compared with constructs with allele 12 in the same cell line group; **Indicated *P*<0.01 compared with constructs with allele 12 in the same cell line group.

12, allele 14 and allele 15, and then transient transfections were performed in two common CRC cell lines (HT29 and SW480). As shown in **Figure 3**, luciferase reporter gene assay showed that constructs with allele 14 and allele 15 had an increased expression, with the allele 14 construct displaying a marked upregulation (*P*<0.01). While constructs with allele 15 had a statistically significant increase in expression in HT29 cells (*P*<0.01), this was not obvious in SW480 cells (*P*<0.01) (data not shown).

Discussion

CRC mechanism study had rapid progress in recent years, while the application of new diagnostic methods such as colonoscopy and MRI has helped in early diagnosis and therefore reduced the mortality of people diagnosed with CRC cases [18], there is still an apparent increase in the incidence rate of CRC globally in the last decade, especially in China [2]. Epidemiological investigations of CRC cases have found that genetic variations were involved in colorectal carcinogenesis. Previous CRC studies have mainly focused on single nucleotide polymorphisms [19], ignoring other types of genetic variations. STR was first used for paternity testing in forensic science [20], however, recent studies have shown functional STRs to be associated with some important clinical conditions [21]. Thus, the identification of an association between functional STRs with CRC would give us a better insight into CRC pathogenesis. In the current study, we report that the STR, rs199618935, could modulate CRC risk in the Han Ethnic Chinese population. Further, *in vitro* studies showed that this STR influenced the transcriptional activity and therefore led to the downregulation of PTPN11 expression in CRC tissues.

PTPN11 had previously been identified as a proto-oncogene, but reports from HCC studies have also found it to have anticancer role [10, 16], although its role in this is still unknown. Similarly, *PTPN11* is thought to have a promising role as a marker for CRC prognosis and/or prediction, yet its role in CRC carcinogenesis and genetic variation function within PTPN11 is still not clear. In this current study, we found that the functional STR rs199618935 could modulate CRC risk by exerting an influence on the transcriptional activity of PTPN11.

Furthermore, while the repeat number of STR increased, the expression of *PTPN11* was upregulated. As in the case of HCC, we observed in our *in vitro* studies that *PTPN11* expression might play an anticancer role in CRC carcinogenesis.

Our data on the distribution of the allelic frequency between CRC cases and controls showed a trend which seems to suggest that the trinucleotide copy could attenuate CRC risk, however, this needs to be substantiated in future studies. The qPCR analysis indicated that the STR polymorphism could influence *PTPN11* expression, an observed correlation which was further collaborated by the *in vitro* gain-of-function studies. Interestingly, it was the allele 14 but not allele 15 that demonstrated the highest expression using the Luciferase reporter gene assay. Since polymorphism within the functional region could affect adjacent gene expression, and given that the STR is located in the 3'UTR region of *PTPN11*, we postulated that probably the STR may disrupt the surrounding region, which binds to some miRNAs, therefore, enhancing the expression [22], while the trinucleotide difference between allele 15 and allele 14 probably being the motif disrupter. However, this hypothesis needs further functional studies to ascertain. Similarly, while the association of the STR rs199618935 with CRC susceptibility in Ethnic Han Chinese was investigated and observed in two centers in China, more study centers must be used as well as the inclusion of other ethnic Chinese populations so as to generate more conclusive data.

In conclusion, we demonstrated that the functional trinucleotide repeats located in the 3'UTR of *PTPN11* could modulate CRC risk by affecting its transcriptional activity, therefore, *PTPN11* is a potential biomarker for CRC risk and a novel promising target in CRC therapy.

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Disclosure of conflict of interest

None.

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References

[1] Siegel R, Desantis C and Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin 2014; 64: 104-117.

[2] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[3] Jia WH, Zhang B, Matsuo K, Shin A, Xiang YB, Jee SH, Kim DH, Ren Z, Cai Q, Long J, Shi J, Wen W, Yang G, Delahanty RJ, Ji BT, Pan ZZ, Matsuda F, Gao YT, Oh JH, Ahn YO, Park EJ, Li HL, Park JW, Jo J, Jeong JY, Hosono S, Casey G, Peters U, Shu XO, Zeng YX and Zheng W. Genome-wide association analyses in East Asians identify new susceptibility loci for colorectal cancer. Nat Genet 2013; 45: 191-196.

[4] Lemire M, Qu C, Loo LW, Zaidi SH, Wang H, Berndt SI, Bezieau S, Brenner H, Campbell PT, Chan AT, Chang-Claude J, Du M, Edlund CK, Gallinger S, Haile RW, Harrison TA, Hoffmeister M, Hopper JL, Hou L, Hsu L, Jacobs EJ, Jenkins MA, Jeon J, Kury S, Li L, Lindor NM, Newcomb PA, Potter JD, Rennert G, Rudolph A, Schoen RE, Schumacher FR, Seminara D, Severi G, Slattery ML, White E, Woods MO, Cotterchio M, Le Marchand L, Casey G, Gruber SB, Peters U and Hudson TJ. A genome-wide association study for colorectal cancer identifies a risk locus in 14q23.1. Hum Genet 2015; 134: 1249-1262.

[5] Wang H, Burnett T, Kono S, Haiman CA, Iwasaki M, Wilkens LR, Loo LW, Van Den Berg D, Kolonel LN, Henderson BE, Keku TO, Sandler RS, Signorello LB, Blot WJ, Newcomb PA, Pande M, Amos CI, West DW, Bezieau S, Berndt SI, Zanke BW, Hsu L, Lindor NM, Haile RW, Hopper JL, Jenkins MA, Gallinger S, Casey G, Stenzel SL, Schumacher FR, Peters U, Gruber SB, Tsugane S, Stram DO and Le Marchand L. Trans-ethnic genome-wide association study of colorectal cancer identifies a new susceptibility locus in VTI1A. Nat Commun 2014; 5: 4613.

[6] Whiffin N, Hosking FJ, Farrington SM, Palles C, Dobbins SE, Zgaga L, Lloyd A, Kinnersley B, Gorman M, Tenesa A, Broderick P, Wang Y, Barclay E, Hayward C, Martin L, Buchanan DD, Win AK, Hopper J, Jenkins M, Lindor NM, Newcomb PA, Gallinger S, Conti D, Schumacher F, Casey G, Liu T, Campbell H, Lindblom A, Houlston RS,

Tomlinson IP and Dunlop MG. Identification of susceptibility loci for colorectal cancer in a genome-wide meta-analysis. Hum Mol Genet 2014; 23: 4729-4737.

[7] Le Marchand L. Genome-wide association studies and colorectal cancer. Surg Oncol Clin N Am 2009; 18: 663-668.

[8] Lu Y, Yang H, Yuan L, Liu G, Zhang C, Hong M, Liu Y, Zhou M, Chen F and Li X. Overexpression of miR-335 confers cell proliferation and tumour growth to colorectal carcinoma cells. Mol Cell Biochem 2016; 412: 235-245.

[9] Tartaglia M and Gelb BD. Germ-line and somatic PTPN11 mutations in human disease. Eur J Med Genet 2005; 48: 81-96.

[10] Bard-Chapeau EA, Li S, Ding J, Zhang SS, Zhu HH, Princen F, Fang DD, Han T, Bailly-Maitre B, Poli V, Varki NM, Wang H and Feng GS. Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. Cancer Cell 2011; 19: 629-639.

[11] Chang W, Gao X, Han Y, Du Y, Liu Q, Wang L, Tan X, Zhang Q, Liu Y, Zhu Y, Yu Y, Fan X, Zhang H, Zhou W, Wang J, Fu C and Cao G. Gene expression profiling-derived immunohistochemistry signature with high prognostic value in colorectal carcinoma. Gut 2014; 63: 1457-1467.

[12] Hartman ZR, Schaller MD, Agazie YM. The tyrosine phosphatase SHP2 regulates focal adhesion kinase to promote EGF-induced lamellipodia persistence and cell migration. Mol Cancer Res 2013; 11: 651-664.

[13] Nabinger SC and Chan RJ. Shp2 function in hematopoietic stem cell biology and leukemogenesis. Curr Opin Hematol 2012; 19: 273-279.

[14] Yang Z, Li Y, Yin F and Chan RJ. Activating PTPN11 mutants promote hematopoietic progenitor cell-cycle progression and survival. Exp Hematol 2008; 36: 1285-1296.

[15] Zhao X, Hu S, Wang L, Zhang Q, Zhu X, Zhao H, Wang C, Tao R, Guo S, Wang J, Xu J, He Y and Gao Y. Functional short tandem repeat polymorphism of PTPN11 and susceptibility to hepatocellular carcinoma in Chinese populations. PLoS One 2014; 9: e106841.

[16] Cai P, Guo W, Yuan H, Li Q, Wang W, Sun Y, Li X and Gu Y. Expression and clinical significance of tyrosine phosphatase SHP-2 in colon cancer. Biomed Pharmacother 2014; 68: 285-290.

[17] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-408.

[18] Hoffman A, Teubner D and Kiesslich R. Competition in colon cancer screening? What is the role of colonoscopy? Viszeralmedizin 2014; 30: 18-25.

[19] Mimori K, Tanaka F, Shibata K and Mori M. Review: single nucleotide polymorphisms associated with the oncogenesis of colorectal cancer. Surg Today 2012; 42: 215-219.

[20] Alford RL, Hammond HA, Coto I and Caskey CT. Rapid and efficient resolution of parentage by amplification of short tandem repeats. Am J Hum Genet 1994; 55: 190-195.

[21] Gymrek M, Willems T, Guilmatre A, Zeng H, Markus B, Georgiev S, Daly MJ, Price AL, Pritchard JK, Sharp AJ and Erlich Y. Abundant contribution of short tandem repeats to gene expression variation in humans. Nat Genet 2016; 48: 22-29.

[22] Farazi TA, Spitzer JI, Morozov P and Tuschl T. miRNAs in human cancer. J Pathol 2011; 223: 102-115.