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Original Article

DNA-methyltransferase 3B 39179 G > T polymorphism and risk of sporadic colorectal cancer in a subset of Iranian population

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Abstract

BACKGROUND: Epigenetic event is a biological regulation that influences the expression of various genes involved in cancer. DNA methylation is established by DNA methyltransferases, particularly DNAmethyltransferase 3B (DNMT3B). It seems to play an oncogenic role in the creation of abnormal methylation during tumorigenesis. The polymorphisms of the DNMT3B gene may influence DNMT3B activity in DNA methylation and increase the susceptibility to several cancers. These genetic polymorphisms have been studied in several cancers in different populations.

METHODS: In this study, we performed a case–control study with 125 colorectal cancer patients and 135 cancer-free controls to evaluate the association between DNMT3B G39179T polymorphism (rs1569686) in the promoter region and the risk of sporadic colorectal cancer. Up to now, few studies have investigated the role of this gene variant in sporadic colorectal cancer with no familial history. The genotypes of DNMT3B G39179T polymorphism was analyzed by PCR-RFLP.

RESULTS: We found that compared with G allele carriers, statistically the DNMT3B TT genotype (%34) was signif cantly associated with increased risk of colorectal cancer (adjusted OR, 3.993, 95% CI, 1.726-9.238, P = 0.001). Compared with DNMT3B TT genotype, the GT and GG genotypes had lower risk of developing sporadic colorectal cancer (OR = 0.848, 95% CI = 0.436-1.650).

CONCLUSIONS: Our findings were consistent with that of previously reported case–control studies with colorectal cancer. These results suggest that the DNMT3B G39179T polymorphism influences DNMT3B expression, thus contributing to the genetic susceptibility to colorectal cancer. Further mechanistic studies are needed to unravel the causal molecular mechanisms.

KEYWORDS: DNMT3B Polymorphism, DNA Methylation, Epigenetic, Sporadic Colorectal Cancer.

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Colorectal cancer (CRC) is defined as a cancer emerging in the colon or rectum which can be divided into sporadic, familial and hereditary cases.[1] In most cases, 75% of colorectal cancers occur sporadically and 25% are associated with family history.[2] The cause of sporadic colorectal cancer includes different genetic and environmental factors that interact together to raise the risk of this cancer.[3] One of the genetic factors is epigenetic alteration in the genome. Epigenetic process is a reversible or heritable biological change in gene expression that takes place without an alteration in DNA sequence. The events implicated during this process, includes DNA methylation and histone modifications.[4] These mechanisms are often deregulated in many cancers where they affect expression of genes involved in different stages of cancer. Opposing to genetic changes, these epigenetic modifications are reversible.[5] DNA methylation is catalyzed by DNA methyltransferases, including DNMT3A and DNMT3B, which are responsible for forming DNA methylation arrangements during some biological processes such as gametogenesis and early embryogenesis.[6] Numerous studies have revealed that overexpression of DNMT3B which is involved

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in de novo methylation, plays a key role in the carcinogenesis.[7] Similar to the many cancers, epigenetic changes also occur in different oncogenes and tumor suppressor genes present in colorectal cancer.[8-11] Several SNPs of DNMT3B in numerous studies are shown to be effective on the DNMT3B activity in DNA methylation which alter susceptibility to numerous types of tumors.[12-15] Most studies work on DNMT3B 149C>T (rs2424913) and DNMT3B 579G>T (rs2424909) in the promoter region to investigate their relation with some cancers such as lung, breast, colon, and prostate cancers.[15-17] However, the DNMT3B gene also has a single G39179T SNP in the promoter region, and this possibly affects the gene function and is associated with both high promoter activity of DNMT3B and high susceptibility to colorectal cancer.[18] In some studies, it is described that DNMT3B TT genotype is meaningfully associated with this cancer.[19-21] Because both environmental and genetic factors are involved in rise of the sporadic colorectal cancer, it is suggested that these two interact with each other to contribute to the increases of cancer susceptibility in individuals.[22-26] In the present study, in order to determine whether G39179T polymorphism in DNMT3B promoter in Iranian population is significantly linked with cancer susceptibility, we evaluated the allele frequency of G39179>T. In this work, we also explored the relation between some factors such as smoking, INSAID use, BMI, and physical activity with this polymorphism. Therefore, studies on genetic mechanisms implicated in epigenetic process have become a field of great interest in the detection and treatment of cancers.

Methods

Population study

The study was conducted on Iranian population, including 125 patients with colorectal cancer and 135 control subjects without cancer. All the cases and controls were selected from individuals referred to various hospitals in Isfahan, a city located in the central region of Iran between 2007 and 2010. We selected the patients who were grouped in sporadic colorectal cancer with no familial history. The healthy subjects had no history of familial colorectal cancer or other related cancers referred for occasional colonoscopy examination during the same period of time. All demographic and disease related information were recorded for further analysis. The study was confirmed by the university ethical committee and informed written consent was obtained from all patients and controls.

DNA extraction

Peripheral blood samples from each subject were drawn in vacuum tubes containing EDTA and stored at 4C°. Genomic DNA was extracted from peripheral blood lymphocytes by using standard procedures according to the manufacturer's instructions.

DNMT3B genotyping

The genotyping of the G39179T polymorphism was performed by PCR-RFLP. The PCR mixture contained 150 ng of genomic DNA and 2.5 µL of 10X PCR buffer, 1 U of Taq-DNA-polymerase, 200 µmol/L of dNTPs and 400 nmol/L of primer forward :5'GGGGGCCTGGAGGTCTCATTAT3' and reverse: 5'ACGGATGGGTTGGCAGGC TATA3') in total volume of 25 µL. PCR thermal cycling was consisted of an initial denaturation at 94°C for 10 min, followed by 32 cycles of 94°C for 50 s, 57°C for 50 s, 72°C for 50 s, and a final extension of 72°C for 10 min. Subsequently, the 343 bp PCR product was digested for 3 h with 5 units of PvuII (*Fermentas*) at 37°C and resolved on 2% agarose gel and stained with ethidium bromide for visualization under UV light. The wild-type homozygous G allele was remained undigested but the homozygous T allele produced two bands (241 and 102 bp) and the G/T heterozygote produced three bands (Figure 1). RFLP bands were visualized under UV light with ethidium bromide staining. The genotyping by PCR-RFLP assay was further confirmed by subjecting around 20% of randomly selected PCR products for sequence

analysis. The results of PCR-RFLP genotyping and sequencing were 100% concordant.

Statistical analysis

Statistical analyses were performed using the SPSS 16 software package (SPSS company, Chicago, IL, USA). The Fisher's exact chi-square test was first used to compare the frequency distribution of age, gender, smoking status and BMI between cases and controls. Hardy-Weinberg equilibrium assumption was performed to compare the observed and expected genotype frequencies using $\chi 2$ test, to evaluate the association between DNMT3B G39179T polymorphism and colorectal cancer risk. The odds ratio (OR) and 95% confidence interval (CI) was calculated using an unconditional logistic regression model and adjusted by age and gender accordingly. P<0.05 was considered statistically significant.

Results

In our population study, there were no major variances in the mean age and gender distribution between patients and controls, suggesting that the matching found on these two variables was sufficient (Table 1). There was no proof of a deviation from Hardy-Weinberg equilibrium among the cases or controls. The mean age of the patient and control subjects were 58 years (58.38 ± 11.644) and 58 years (58.27 ± 10.416), respectively. Compared with the controls, the cases had lower NSAID use (*P* value = 0.033) and activity (*P* value = .0.001) but had higher BMI (*P* value = 0.031). There was no significant relation for smoking (*P* value = 0.489, Table 2).

However, all these variables were further adjusted for any residue confounding effect in later multivariate logistic regression analyses. The genotype of DNMT3B polymorphism in all patients and controls were successfully genotyped (Figure 1). The genotyping by PCR-RFLP assay was completely con□rmed by sequence analysis of PCR products. The genotype distribution in CRC patients and healthy controls was consistent with that expected by Hardy-Weinberg equilibrium. The frequency of DNMT3B T/T, G/T and G/G genotypes in sporadic CRC patients was 34.4% , 49.6% and 16%, respectively and the frequency of T and G alleles was 59.2 % and 48.8%, respectively.

When the DNMT3B G39179T genotype was used as the reference group, the GT genotype was not associated with any risk (adjusted OR = 0.848, 95%CI 0.436-1.650; *P* value = 0.375), but the TT genotype was associated with signi□cantly increased risk for CRC (adjusted OR = 3.993, 95% CI = 1.726-9.238; *P* value = 0.001). The colorectal cancer risk related to the DNMT3B G39179T genotypes are shown in Table 3. No differences were found when the results were stratified by gender in both patients and controls (*P* value = 0.556) with respect to the genotype distribution of DNMT3B G39179T in the male (GG = 18.5%, GT = 62.2% and TT = 19.3%) and female (GG = 16.8%, GT = 58.4% and TT = 24.8%). Because of low frequency of homozygous wild-type genotype, we combined this genotype with heterozygous genotype into one group and compared it with the reference group. When compared with the reference group, the combined GG and GT genotype in both males and females (male: adjusted OR 0.123, 95% CI 0.040-0.382; females: adjusted OR 0.323, 95% CI 0.137-0.762) and the group under and over 59 years old (under 59: adjusted OR 0.142, 95% CI 0.050-0.405; over 59: adjusted OR 0.316, 95% CI 0.131-0.759) were associated with significant decreased risk of sporadic colorectal cancer (Table 4).

Table 1. Characteristics of the study population

Variables	Patients (n = 125)	Control (n = 135)	P value
Age	58.38±11.64	58.27±10.416	0.932
Gender			
Male	66	69	0.441
Female	59	66	

Table 2. Frequency distributions of selected variables in CRC cases and cancer-free controls

Variables	Cases(n = 125)	Controls(n = 135)	P value
BMI (mean ± SD) (kg/m^2)	26.403±4.475	25.29±3.78	0.031
NSAID use			
Regular	19	36	0.033
Irregular/none	106	99	
Physical activity			
Few	76	51	
Moderate	35	54	0.001
High	14	30	
Smoking			
Ever	21	18	0.489
Never	104	117	

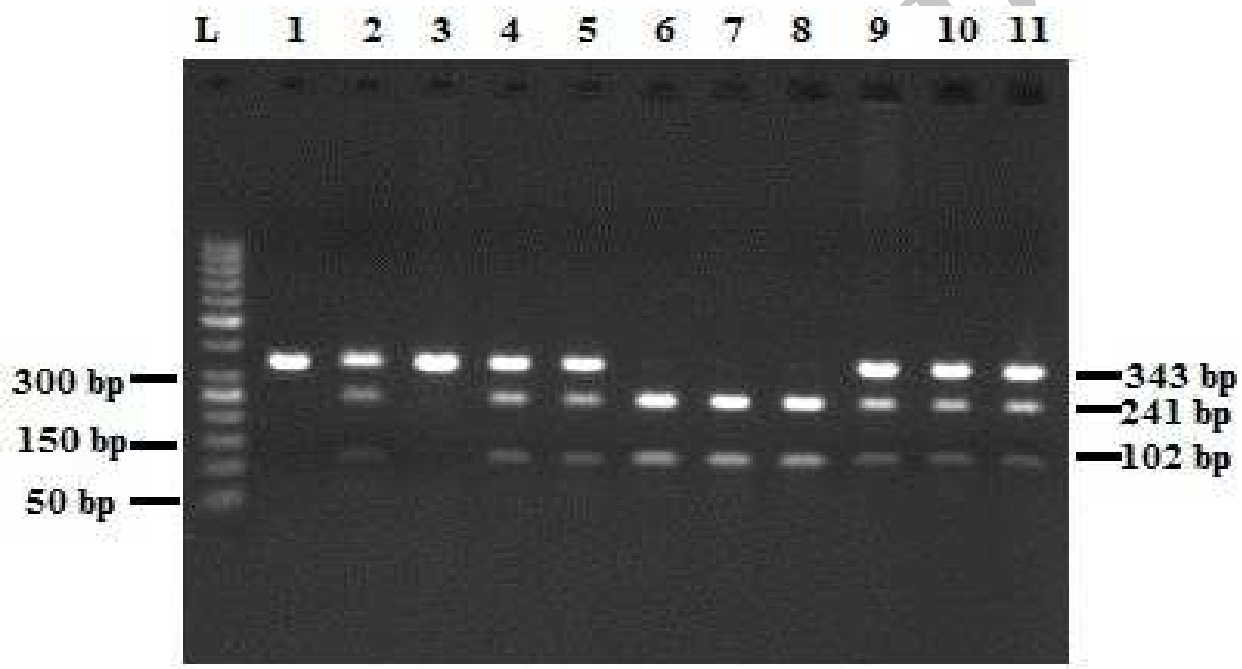

Figure 1. Genotyping of DNMT3BG39179T polymorphism by PCR-RFLP
Lane L: 50 bp DNA ladder; lane 1: PCR product (undigested); lane 3 Homozygous GG lane 2,4,5,9,10,11 Heterozygous GT genotype; lanes 6 to 8: Homozygous TT genotype

Table 3. Allele and genotype frequencies for DNMT3B G39179T polymorphism

	Colorectal cancer		Control			
	n	%	n	%	OR[1] (%95 CI)	*P* value
Allele frequency						
G	102	40.8%	123	45.6%	1.734 (1.225-2.445)	0.002
T	148	59.2%	147	54.4%		
Genotype frequency						
GG	20	16%	26	19.3%	0.848 (0.436-1.650)	
GT	62	49.6%	95	70.4%		0.001
TT	43	34.4%	14	10.3%	3.993 (1.726-9.238)	

[1](95% CI), confidence interval

Table 4. Stratification analysis of DNMT3B G 39179 T genotype frequency of colon cancer and control group

Variable	TT Genotype	GT+GG Genotype	Odds ratio of GT+GG genotype	
	Control/case	Control/Case	Crude	Adjusted
Gender				
male	4/22	65/44	0.123 (0.040-0.382)[a]	0.123 (0.040-0.382)[a]
female	10/21	56/38	0.323 (0.137-0.762)[b]	0.323 (0.137-0.762)[b]
Age				
<59	5/22	57/42	0.142 (0.050-0.405)[a]	0.142 (0.050-0.405)[a]
≥59	9/21	64/40	0.316 (0.131-0.759)[b]	0.316 (0.131-0.759)[b]

[a] $P = 0.0001$, [b] $P = 0.007$

Discussion

The most common variations in human DNA are single nucleotide polymorphisms that may affect susceptibility to specific cancers in individuals.[17] Several studies have proposed that some polymorphisms in the promoter areas of genes related to cancers may affect the expression and function of its proteins and therefore, may be significantly associated with cancer risk.[9-12,17] A number of investigations have suggested that abnormal DNA methylation at cytosine nucleotide may play a significant role in carcinogenesis.[5-8] It is assumed that DNMT3B has an important role in cancer owing to its ability to catalyze de novo DNA methylation, which in turn might cause silencing of genes involved in cancer through promoter hyper methylation.[10] Here, we evaluated the association between the colorectal cancer and DNMT3B G39179T polymorphism among Iranian patients. Our finding is indicative of G39179T polymorphism in promoter of DNMT3B and is associated with the risk of colorectal cancer. We found that the TT variant genotype was associated with a significantly increased risk of sporadic colorectal cancer (genotype frequency = 34.4% OR = 3.993, 95% CI = 1.726-9.238; *P* value = 0.001). Compared with healthy control, the allele frequency of T in cases was 59.2% and the allele frequency of G was 40.8%, implying that this SNP would be beneficial for the susceptibility to colorectal cancer. Although the analyses were stratified by age and gender of patients, only the TT genotype was associated with an increased risk of sporadic CRC. In addition, this study revealed that the combined genotypes (GG+GT) of the polymorphism were linked with reduced risk of cancer development compared to the TT genotype. In concordance with our results, two studies have shown that the G39179T polymorphism was associated with colorectal cancer.[14,15] This result proposes that the 39179G→T variant can be used as an indicator of genetic susceptibility to colorectal cancer. Similar to our work, some studies have reported an increased risk for lung, breast, head and neck cancers in those individual who are carriers the T allele.[16,21,27] Furthermore, our results were consistent with a previous study, which showed that carriers of the G allele have a lower risk for development of the lung and breast cancers.[16,27] These observations propose that the DNMT3B polymorphism might act in a histologically specific way. In the current study, unlike the previous two studies,[14,15] we observed that the association between the DNMT3B39179G>T polymorphism and risk of sporadic colorectal cancer was equal in both males and females and the group under and over 59 years old subjects. These findings suggest that genetic susceptibility to sporadic colorectal cancer is different from familial colorectal cancer [14,15] We guessed that the additional reason for this disconcordance was the fact that Chinese and Korean might have dissimilar genotype distribution of DNMT3B with Iranian population, because both of them are different from the Iranian ethnic population. Comparing to the study performed in the Korean and Chinese populations,[14,15] in our study, the distribution

and frequency of the G/T and GG genotypes were higher and T/T genotype were lower than that of Korean and Chinese populations. In this study, we analyzed relation between genotypes of DNMT3B G39179T and selected variables in Table 2, but there was no significant association. Since in our study, this variant of DNMT3B was evaluated in sporadic colorectal cancer and may have interaction with some kind of environmental factors,[28-34] it is essential to investigate the complex interactions of DNMT3B G39179T polymorphism with several environmental factors in different populations. Also, other studies are needed in order to clarify the role of DNMT3B variant in the expression level of DNMT3B in colorectal cancer patients and the function of the DNA methylation. The genotype of DNMT3B SNP in different ethnic populations remains to be evaluated.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

All the authors have carried out the study, participated in the design of the study and acquisition of data performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

References

1. Cappell MS. Pathophysiology, clinical presentation, and management of colon cancer. Gastroenterol Clin North Am. 2008; 37(1):1-24, v.
2. Fahy B, Bold RJ. Epidemiology and molecular genetics of colorectal cancer. Surg Oncol. 1998; 7(3-4):115-23.
3. Morson BC. Symposium on colorectal cancer. 1. Pathology of colorectal cancer. Can J Surg. 1978; 21(3):206-8.
4. Esteller M. Epigenetics in cancer. N Engl J Med. 2008; 358(11):1148-59.
5. Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, et al. DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature. 2002; 416(6880):552-6.
6. Chen CL, Yan X, Gao YN, Liao QP. [Expression of DNA methyltransferase 1, 3A and 3B mRNA in the epithelial ovarian carcinoma]. Zhonghua Fu Chan Ke Za Zhi. 2005; 40(11):770-4.
7. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999; 99(3):247-57.
8. Bestor T, Laudano A, Mattaliano R, Ingram V. Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. J Mol Biol. 1988; 20;203(4):971-83.
9. Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet. 2000; 16(4):168-74.
10. Linhart HG, Lin H, Yamada Y, Moran E, Steine EJ, Gokhale S, et al. Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing. Genes Dev. 2007; 21(23):3110-22.
11. Khatami F, Noorinayer B, Ghiasi S, Mohebi R, Hashemi M, Zali MR. Single Nucleotide Polymorphisms of DNA Methyltransferase 1 Gene and Gastric Cancer in Iranian Patients: a Case Control Study. . Iranian Journal of Cancer Prevention 2008; 111-118.
12. Fan H, Liu D, Qiu X, Qiao F, Wu Q, Su X, et al. A functional polymorphism in the DNA methyltransferase-3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma. BMC Med. 2010; 8:12.
13. Kawakami K, Ruszkiewicz A, Bennett G, Moore J, Grieu F, Watanabe G, et al. DNA hypermethylation in the normal colonic mucosa of patients with colorectal cancer. Br J Cancer. 2006; 94(4):593-8.
14. Guo X, Zhang L, Wu M, Wang N, Liu Y, Er L, et al. Association of the DNMT3B polymorphism with colorectal adenomatous polyps and adenocarcinoma. Mol Biol Rep. 2010; 37(1):219-25.
15. Hong YS, Lee HJ, You CH, Roh MS, Kwak JY, Lee MJ, et al. DNMT3b 39179GT polymorphism and the risk of adenocarcinoma of the colon in Koreans. Biochem Genet. 2007; 45(3-4):155-63.
16. Montgomery KG, Liu MC, Eccles DM, Campbell IG. The DNMT3B C-->T promoter polymorphism and risk of breast cancer in a British population: a case-control study. Breast Cancer Res. 2004; 6(4):R390-4.

17. Abuli A, Bessa X, Gonzalez JR, Ruiz-Ponte C, Caceres A, Munoz J, et al. Susceptibility genetic variants associated with colorectal cancer risk correlate with cancer phenotype. Gastroenterology. 2010; 139(3):788-96, 96 e1-6.
18. Iacopetta B, Heyworth J, Girschik J, Grieu F, Clayforth C, Fritschi L. The MTHFR C677T and DeltaDNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. Int J Cancer. 2009; 125(1):84-90.
19. Wang YM, Wang R, Wen DG, Li Y, Guo W, Wang N, et al. Single nucleotide polymorphism in DNA methyltransferase 3B promoter and its association with gastric cardiac adenocarcinoma in North China. World J Gastroenterol. 2005; 11(23):3623-7.
20. Fan H, Zhang F, Hu J, Liu D, Zhao Z. Promoter polymorphisms of DNMT3B and the risk of colorectal cancer in Chinese: a case-control study. J Exp Clin Cancer Res. 2008;27:24.
21. Liu Z, Wang L, Wang LE, Sturgis EM, Wei Q. Polymorphisms of the DNMT3B gene and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Lett. 2008; 268(1):158-65.
22. Karpinski P, Myszka A, Ramsey D, Misiak B, Gil J, Laczmanska I, et al. Polymorphisms in methyl-group metabolism genes and risk of sporadic colorectal cancer with relation to the CpG island methylator phenotype. Cancer Epidemiol. 2010;34(3):338-44.
23. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology. 2008; 135(4):1079-99.
24. Casillas MA, Jr., Lopatina N, Andrews LG, Tollefsbol TO. Transcriptional control of the DNA methyltransferases is altered in aging and neoplastically-transformed human fibroblasts. Mol Cell Biochem. 2003; 252(1-2):33-43.
25. Kondo Y, Issa JP. Epigenetic changes in colorectal cancer. Cancer Metastasis Rev. 2004; 23(1-2):29-39.
26. Lee SJ, Jeon HS, Jang JS, Park SH, Lee GY, Lee BH, et al. DNMT3B polymorphisms and risk of primary lung cancer. Carcinogenesis. 2005; 26(2):403-9.
27. Shen H, Wang L, Spitz MR, Hong WK, Mao L, Wei Q. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. Cancer Res. 2002; 62(17):4992-5.
28. Fan H, Liu DS, Zhang SH, Hu JB, Zhang F, Zhao ZJ. DNMT3B 579 G>T promoter polymorphism and risk of esophagus carcinoma in Chinese. World J Gastroenterol. 2008; 14;14(14):2230-4.
29. Jones JS, Amos CI, Pande M, Gu X, Chen J, Campos IM, et al. DNMT3b polymorphism and hereditary nonpolyposis colorectal cancer age of onset. Cancer Epidemiol Biomarkers Prev. 2006; 15(5):886-91.
30. Zhao H, Du W, Gu D, Wang D, Xue F, Ge J, et al. DNMT3B 579G>T promoter polymorphism and the risk for idiopathic thrombocytopenic purpura in a Chinese population. Acta Haematol. 2009; 122(1):31-5.
31. Nystrom M, Mutanen M. Diet and epigenetics in colon cancer. World J Gastroenterol. 2009; 21; 15(3):257-63.
32. Pellegrini ML, Argibay P, Gomez DE. Dietary factors, genetic and epigenetic influences in colorectal cancer (Review). Experimental and Therapeutic Medicine. 2010;1:241-50.
33. Lopatina N, Haskell JF, Andrews LG, Poole JC, Saldanha S, Tollefsbol T. Differential maintenance and de novo methylating activity by three DNA methyltransferases in aging and immortalized fibroblasts. J Cell Biochem. 2002; 84(2):324-34.
34. Weisenberger DJ, Velicescu M, Cheng JC, Gonzales FA, Liang G, Jones PA. Role of the DNA methyltransferase variant DNMT3b3 in DNA methylation. Mol Cancer Res. 2004; 2(1):62-72.