

TP53 codon 72 Polymorphism and P53 Protein Expression in Colorectal Cancer Specimens in Isfahan

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Abstract- The TP53 tumor suppressor gene plays important roles in genomic stability. A common polymorphism at codon 72 of TP53 gene has been associated with increased risk for many human cancers. The p53 protein is expressed in colorectal cancer, but the reported prevalence of its expression varies widely. In the present study, the p53 protein expression in different genotypes of its codon 72, was investigated. We undertook a case-control study on 250 controls and 250 paraffin block specimens of sporadic colorectal adenocarcinomas from the city of Isfahan. PCR amplification of TP53 codon 72 polymorphism: TP53 codon 72 genotypes were detected by PCR using specific primer pairs for amplifying the proline or the arginine Alleles. The PCR reaction was done separately for each of the two polymorphic variants. The amplified products were subjected to electrophoresis on 1% agarose gel in 1× TBE buffer and visualized on a transilluminator using ethidium bromide. Immunohistochemical Staining: We evaluated the expression patterns of p53 protein, as potential prognostic marker in colorectal cancer specimens by immunohistochemical staining. Statistical analyses: The χ^2 -test was used to assess the significance of any difference in the prevalence of TP53 codon 72 polymorphism between colorectal cancer patients and controls. The odds ratio and 95% CI (confidence intervals) was used as a measure of the strength of the association. Statistical significance level was set to $P \leq 0.05$. In control samples, the genotype distribution for TP53 polymorphism showed 30.4%, 45.2% and 24.4% for the arginine/arginine, arginine/proline and proline/proline genotypes, respectively. Allelic frequencies corresponded to 0.663 for the arginine allele and 0.338 for the proline allele. In the cancer group 38.8% of the cases were arginine/arginine, 40.4% were arginine/proline and 20.8% were proline/proline. The corresponding frequencies were 0.590 for the arginine allele and 0.410 for the proline allele. A significant difference between cases and controls was found for the arginine/arginine genotype compared with (grouped) arginine/proline and proline/proline genotypes (Odds Ratio = 1.451 (1.002-2.103), $P=0.048$). Overexpression of p53 was observed in 50.8 percent of cancer specimens which most of them were arginine/arginine genotype ($P < 0.001$). TP53 polymorphism and arginine/arginine genotype may be correlated with overexpression of p53 and increased risk for colorectal cancer in city of Isfahan.

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Introduction

Colorectal carcinogenesis is a complex multistage process that show a high frequency of TP53 alterations and the large majority of these cancers are adenocarcinomas (1,2). TP53 is the most important tumor suppressor gene that is involved in many pathways such as apoptosis, cellular transcriptional regulation, and cell cycle control (3,4). The p53 protein has important role in cell cycle control, being involved

in G1-phase arrest for DNA repairs or activation of the cell death machinery (5). Accumulation of the protein in the cytoplasm being done following DNA damage, then the protein translocates to the nucleus and activates gene transcription machinery for cell cycle arrest, to allow repair of damaged DNA (6). Also p53 protein, in response to an excessive DNA damage, would activate programmed cell death pathway, through transcriptional control of several genes (7,8). TP53, located on chromosome 17p13, is one of the most mutated genes

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affecting many types of human cancers (9,10). In addition to mutations, several polymorphisms in the wild-type TP53 gene locus have been detected which could alter its function (11,12). Among the 14 polymorphisms identified in the TP53 gene, the most commonly polymorphism in the general population which associated with cancer development is the codon 72 Arg (arginine) to Pro (proline) substitution (13). The TP53 Arg72Pro, located in exon 4 at codon 72, involving a guanine to cytosine nucleotide exchange, which leads to nonconservative change of an Arg to Pro. Because of functional differences between the two polymorphic variants of TP53, genotype at codon 72 may affects susceptibility to colorectal cancer development. Also it was proposed that the p53 codon 72 polymorphism influences the expression of p53 (14), and it is logical that this polymorphism may play a role in p53 protein expression in colorectal cancer. The p53 protein is expressed in colorectal cancer, but the reported prevalence of its expression varies widely (15-17). So, in the present study, the p53 codon 72 polymorphism and p53 protein expression were characterized for a group of patients from city of Isfahan in order to explore a possible association between colorectal cancer with this polymorphism and to determine if there is a correlation between this polymorphism and p53 protein expression.

Patients and Methods

Study population and samples

We performed a case-control study on 250 paraffin blocks of sporadic colorectal adenocarcinomas and 250 healthy controls, in order to examine possible associations between the Arg72Pro alleles and the risk of cancer. Incident colorectal cancer cases (histologically confirmed) attending the Alzahra Hospital (Isfahan) over the period 2002-2006 made up the case group. Proximal tumors were defined as occurring in the cecum through to the transverse colon; tumors in the splenic flexure, descending and sigmoid colon were defined as being distal. Other disorders of colorectal region such as HNPCC, familial adenomatous polyposis, Inflammatory Bowel Disease (IBD), Hamartoma, simultaneous occurrence of adenomas, previous or synchronous adenocarcinomas were excluded from this study. As control group, we used peripheral blood from 250 healthy age and sex matched persons. Controls were noncancer persons who already underwent colonoscopy.

DNA isolation from colorectal tissue and blood samples

Genomic DNA from the tumors and blood samples was prepared using High pure PCR Template preparation DNA isolation kit (Roche, Germany) for tissue and whole blood, according to manufacturer's instructions.

PCR amplification of TP53 codon 72 polymorphism

The TP53 codon 72 Pro allele were detected by PCR using the primer pair p53Pro+/ p53Pro- (p53Pro+: 5'-GCCAGAGGCTGCTCCCC; and P53Pro-: 5'-CGTGCAAGTCACAGACTT) and the p53 codon 72 Arg allele by the primer pair p53Arg+/p53Arg- (p53Arg+: 5'-TCCCCCTTGCCGTCCCAA and p53Arg-: 5'-CTGGTGCAGGGGCCACGC) as previously described¹³. Between 100 to 300 nanograms DNA was used as template in a 25 µl PCR reaction mixture containing 1.5 µmol MgCl₂, 1 U Taq polymerase (Sinagen, Co. Ltd., Tehran, Iran) and 2 µmol either of the primer pairs.

PCR cycling conditions were carried out with an initial denaturation step for 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 60°C (for Arg) or 54 °C (for Pro) and 30 s at 72 °C. A final extension step was performed at 72 °C for 5 min. The PCR reaction was done separately for each of the two polymorphic variants. The amplified products were subjected to electrophoresis on 1% agarose gel in 1× TBE buffer and visualized on a transilluminator using ethidium bromide.

Immunohistochemical staining

Five-micron sections containing tumor tissue and normal colonic mucosa as internal positive control were cut slides. After routine deparaffinization and rehydration including an endogenous peroxide block with methanol-peroxide for 30 minutes, the sections were microwaved for nonenzymatic epitope retrieval at 800W for 5 minutes. Fifty milliliters H₂O were replenished, and an additional microwaving step followed at 800W for 5 minutes. The slides were let cool in the buffer for 20 minutes. The immunostainings were performed by using the avidin-biotin-peroxidase amplification system. After a blocking in normal serum for 20 minutes, the slides were incubated with the primary antibodies overnight at room temperature. The biotinylated secondary antibody was applied for 30 minutes, and is followed by 3 applications of DAB/0.02% hydrogen peroxide. The slides were washed with phosphate-buffered saline (PBS) between the

incubations. A Harris hematoxylin counterstain was used.

Statistical analyses

The χ^2 -test was used to assess the significance of any difference in the prevalence of TP53 codon 72 polymorphism between colorectal cancer patients and controls. The odds ratio and 95% CI (Confidence Intervals) was used as a measure of the strength of the association. Also associations between qualitative variables were evaluated using the χ^2 -test. Statistical significance level was set to $P \leq 0.05$.

Results

This analysis included 250 adenocarcinomas and 250 cancer-free control subjects. The general and clinicopathological characteristics of the cases are shown in table 1. The age of 250 patients (104 women and 146 men) ranged from 32 to 93 years (mean age 65.16 ± 12.34 years in men, 62.14 ± 15.78 years in women).

To analyze the codon 72 polymorphism, we used a PCR-based assay that specifically, amplify either TP53 Pro or TP53 Arg allele and give a PCR product by using specific primers for Pro allele (Figure 1) and/or Arg allele (Figure 2) respectively. Detection of TP53 codon 72 polymorphism by allele specific PCR was successfully conducted in all cases and controls. The distribution of the three different genotypes of codon 72 in exon 4 of TP53 in our cases and controls is shown in table 2. In control samples, the genotype distribution for p53 polymorphism showed 30.4%, 45.2% and 24.4% for the Arg/Arg, Arg/Pro and Pro/Pro genotypes, respectively. Allelic frequencies corresponded to 0.663 for the Arg allele and 0.338 for the Pro allele (Table 3). In the cancer group 38.8% of the cases were Arg/Arg, 40.4% were Arg/Pro and 20.8% were Pro/Pro (Table 2). The corresponding frequencies in this group were 0.590 for the Arg allele and 0.410 for the Pro allele (Table 3). A significant difference between cases and controls was

found for the Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes (Odds Ratio = 1.451 (1.002-2.103), $P=0.048$).

Table 1. General and clinicopathologic data of patients with colorectal Cancer

Factor	N (%)
Sex	
Male	146 (58.4 %)
Female	104 (41.6 %)
Age	
≤ 59	82 (32.8 %)
≥ 60	168 (67.2 %)
Localization	
Proximal	153 (61.2 %)
Distal	97 (38.8 %)
Dukes stage	
A- B	78 (31.2 %)
C-D	172 (68.8 %)
TNM staging	
I	62 (24.8 %)
II	74 (29.6 %)
III	82 (32.8 %)
IV	32 (12.8 %)

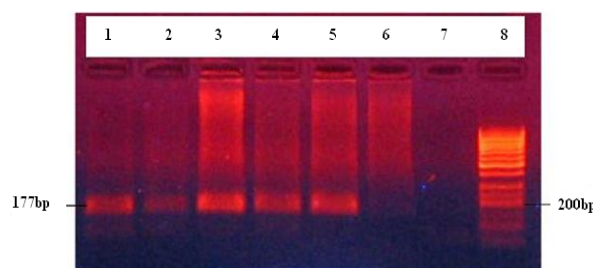


Figure 1. PCR amplification of the TP53 codon 72 (electrophoresis in 1% agarose gel) in 6 colorectal adenocarcinoma specimens.

lane 1-5: positive for Pro allele (177bp)

lane 6: negative for Pro allele

lane 7: negative control

lane 8: DNA marker

Table 2. Distribution of TP53 codon 72 polymorphism genotypes among colorectal cancer cases and controls in Isfahan

Genotype	Cases (n=250)		Controls (n=250)		Odds Ratio (95% CI)
	n	%	n	%	
A/A	97	38.8%	76	30.4%	1.451*
A/P	101	40.4%	113	45.2%	(1.002-2.103)
P/P	52	20.8%	61	24.4%	

A/A: Arg/Arg genotype;

P/P: Pro/Pro genotype;

CI : Confidence Intervals

A/P: Arg/Pro genotype;

n: number

* : χ^2 test, $P=0.048$ (Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes)

Table 3. Allelic frequencies of TP53 codon 72 among colorectal cancer cases and controls in Isfahan

Allele	Patients	Controls	Odds Ratio (95% CI)
Arg	0.590	0.663	0.733*
Pro	0.410	0.338	(0.558-0.964)

CI: Confidence Intervals

* : χ^2 test, $P=0.026$

Table 4. P53 Expression in colorectal cancer specimens

Genotypes of tumor specimens	N	P53 Expression	
		Positive n(%)	Negative n(%)
A/A	97	69 (71.1)	28 (28.9)
A/P	101	48 (47.5)	53 (52.5)
P/P	52	10 (19.2)	42 (80.8)
Total	250	127 (50.8)	123 (49.2)

$P < 0.001$

A/A: Arg/Arg genotype;

A/P: Arg/Pro genotype;

P/P: Pro/Pro genotype;

n: number

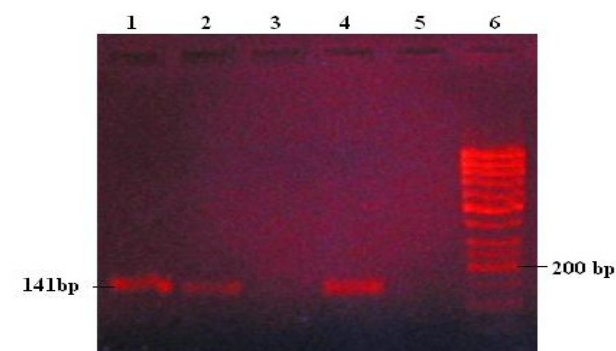


Figure 2. PCR amplification of the TP53 codon 72 (electrophoresis in 1% agarose gel) in 6 colorectal adenocarcinoma specimens.

lane 1, 2, 4 : positive for Arg allele (141bp)

lane 3: negative for Arg allele

lane 5: negative control

lane 6: DNA marker

The Arg allele was found more often in patients than in controls (Odds Ratio = 0.733 (0.558-0.964), $P=0.026$). Overexpression of P53 was seen in 50.8 percent of colorectal specimens and the most of them were Arginine/Arginine genotype ($P < 0.001$) (Table 4).

Discussion

Polymorphism in p53 codon 72 produces two different p53 proteins because of a single base change altering CGC to CCC in the fourth exon of the p53 gene, altering amino acid residue 72 from Arg to Pro. Of the Arg/Arg, Arg/Pro and Pro/Pro genotypes, Arg/Arg induces apoptosis with faster kinetics and suppresses transformation more efficiently than Pro/Pro (18,19). An association of the TP53 codon 72 polymorphism with several cancers susceptibilities has been reported (20-30). In particular, both Arg and Pro alleles have been shown to be associated with a high risk of malignancy. The role of the Arg/Pro polymorphism in colorectal cancer susceptibility was examined in a few studies (31-42), which reported controversial results. We investigated the genotype frequencies of TP53 codon 72 in 250 sporadic colorectal adenocarcinomas and 250 healthy individuals from Isfahan. We found a significant difference between cases and controls for the Arg/Arg genotype compared with (grouped) Arg/Pro and Pro/Pro genotypes (Odds Ratio = 1.451 (1.002-2.103), $P=0.048$). The Arg allele was found more often in patients than in controls (Odds Ratio = 0.733 (0.558-0.964), $P=0.026$). These findings are in agreement with the original study of Storey *et al.* on cervical cancer (43). They showed that p53Arg72 protein is more susceptible to degradation by the HPV E6 proteins, and degradation of p53 protein by HPV E6 is correlated with increased risk for HPV-associated cancers. In this study we did not consider HPV infections in accordance to detection of p53 genotypes and it is an important issue for future studies. Our finding also seems to be consistent with the results reported by Perez *et al.* (31) which support an appreciable association between the Arg allele and colorectal cancer. However there are contradictory findings about the mechanisms which lead to the increase of the Arg allele in human cancers (44-46) implicate that the involvement of TP53 polymorphism in human cancer demands further study.

Although the exact effect of the p53 codon 72 polymorphism on the function of p53 protein remains unknown, it is proposed that this polymorphism

influences the expression of the p53 gene (14). As such, we suspected that the p53 polymorphism may play a role in p53 protein expression in colorectal cancer. The p53 overexpression detected by immunohistochemistry is based on the accumulation of p53 protein in cells. In colorectal carcinomas the correlation between p53 gene status and p53 immunostaining was estimated in over 70% of the cases (47). The p53 overexpression was detected in our study in 127 cases (50.8%) which most of them were Arg/Arg genotype ($P < 0.001$). So we demonstrated that p53 overexpression was associated with the p53 codon 72 polymorphism and tended to be more frequent in the colorectal carcinomas with a Arg/Arg genotype. In previously reported studies, p53 overexpression was observed in 60.6% (15); 67.3% (16) and 30% of the cases (17).

In conclusion the findings of the present study indicated that TP53 codon 72 polymorphism may be a genetic predisposing factor for colorectal adenocarcinomas and p53Arg72 protein may be correlated with p53 overexpression and increased risk for colorectal cancer in Isfahan.

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References

1. Calvert PM, Frucht H. The genetics of colorectal cancer. *Ann Intern Med* 2002;137(7):603-12.
2. Gazelle GS, McMahon PM, Scholz FJ. Screening for colorectal cancer. *Radiology* 2000;215(2):327-35.
3. Dulić V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper JW, Elledge SJ, Reed SI. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell* 1994;76(6):1013-23.
4. Woods DB, Vousden KH. Regulation of p53 function. *Exp Cell Res* 2001;264(1):56-66.
5. Sherr CJ. Principles of tumor suppression. *Cell* 2004;116(2):235-46.
6. Kaelin WG Jr. The p53 gene family. *Oncogene* 1999;18(53):7701-5.
7. Robles AI, Harris CC. p53-mediated apoptosis and genomic instability diseases. *Acta Oncol* 2001;40(6):696-701.
8. Prives C, Hall PA. The p53 pathway. *J Pathol* 1999;187(1):112-26.
9. Khan SA, Thomas HC, Toledano MB, Cox IJ, Taylor-Robinson SD. p53 Mutations in human cholangiocarcinoma: a review. *Liver Int* 2005;25(4):704-16.
10. Borresen-Dale AL. TP53 and breast cancer. *Hum Mutat* 2003;21(3):292-300.
11. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat* 2002;19(6):607-14.
12. Soussi T, Bérout C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001;1(3):233-40.
13. Sreeja L, Syamala V, Raveendran PB, Santhi S, Madhavan J, Ankathil R. p53 Arg72Pro polymorphism predicts survival outcome in lung cancer patients in Indian population. *Cancer Invest* 2008;26(1):41-6.
14. Gottlieb TM, Oren M. p53 in growth control and neoplasia. *Biochim Biophys Acta* 1996;1287(2-3):77-102.
15. Yamagiwa H, Onishi N, Onishi T, Onishi H, Watanabe K, Kadowaki Y, Nishii M, Kato Y. Immunohistochemical study of PCNA, EGFR, c-erbB-2 and p53 in carcinomas of large intestine. *Rinsho Byori* 1995;43(7):703-7.
16. Saleh HA, Jackson H, Banerjee M. Immunohistochemical expression of bcl-2 and p53 oncoproteins: correlation with Ki67 proliferation index and prognostic histopathologic parameters in colorectal neoplasia. *Appl Immunohistochem Mol Morphol* 2000;8(3):175-82.
17. Zhang Hong. Evaluation of four antibodies in detecting p53 protein for predicting clinicopathological and prognostic significance in colorectal adenocarcinoma. *Clin Cancer Res* 1999;5(12):4126-32.
18. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 1999;19(2):1092-100.
19. Dumont P, Leu JI, Della Pietra AC 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 2003;33(3):357-65.
20. Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, Wiencke JK, Christiani DC. The p53 codon 72 polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000;9(10):1037-42.
21. Lee JM, Lee YC, Yang SY, Shi WL, Lee CJ, Luh SP, Chen CJ, Hsieh CY, Wu MT. Genetic polymorphisms of p53 and GSTP1, but not NAT2, are associated with susceptibility to squamous-cell carcinoma of the esophagus. *Int J Cancer* 2000;89(5):458-64.

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22. Zehbe I, Voglino G, Wilander E, Genta F, Tommasino M. Codon 72 polymorphism of p53 and its association with cervical cancer. *Lancet* 1999;354(9174):218-9.
23. Soultz N, Sourvinos G, Dokianakis DN, Spandidos DA. p53 codon 72 polymorphism and its association with bladder cancer. *Cancer Lett* 2002;179(2):175-83.
24. Kalemi TG, Lambropoulos AF, Gueorguiev M, Chrisafi S, Papazisis KT, Kotsis A. The association of p53 mutations and p53 codon 72, Her 2 codon 655 and MTHFR C677T polymorphisms with breast cancer in Northern Greece. *Cancer Lett* 2005;222(1):57-65.
25. Langerod A, Bukholm IR, Bregard A, Lønning PE, Andersen TI, Rognum TO, Meling GI, Lothe RA, Børresen-Dale AL. The TP53 codon 72 polymorphism may affect the function of TP53 mutations in breast carcinomas but not in colorectal carcinomas. *Cancer Epidemiol Biomarkers Prev* 2002;11(12):1684-8.
26. Mahasneh AA, Abdel-Hafiz SS. Polymorphism of p53 gene in Jordanian population and possible associations with breast cancer and lung adenocarcinoma. *Saudi Med J* 2004;25(11):1568-73.
27. Shen H, Zheng Y, Sturgis EM, Spitz MR, Wei Q. P53 codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck: a case-control study. *Cancer Lett* 2002;183(2):123-30.
28. Dong M, Nio Y, Yamasawa K, Toga T, Yue L, Harada T. p53 alteration is not an independent prognostic indicator, but affects the efficacy of adjuvant chemotherapy in human pancreatic cancer. *J Surg Oncol* 2003;82(2):111-20.
29. Tsai MH, Lin CD, Hsieh YY, Chang FC, Tsai FJ, Chen WC, Tsai CH. Prognostic significance of the proline form of p53 codon 72 polymorphism in nasopharyngeal carcinoma. *Laryngoscope* 2002;112(1):116-9.
30. Yu MW, Yang SY, Chiu YH, Chiang YC, Liaw YF, Chen CJ. A p53 genetic polymorphism as a modulator of hepatocellular carcinoma risk in relation to chronic liver disease, familial tendency, and cigarette smoking in hepatitis B carriers. *Hepatology* 1999;29(3):697-702.
31. Pérez LO, Abba MC, Dulout FN, Golijow CD. Evaluation of p53 codon 72 polymorphism in adenocarcinomas of the colon and rectum in La Plata, Argentina. *World J Gastroenterol* 2006;12(9):1426-9.
32. Lung FW, Lee TM, Shu BC, Chang FH. p53 codon 72 polymorphism and susceptibility malignancy of colorectal cancer in Taiwan. *J Cancer Res Clin Oncol* 2004;130(12):728-32. Epub 2004 Sep 7.
33. Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutiérrez-Enríquez S, Hall J, Guino E, Peinado MA, Capella G, Canzian F. A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. *Oncogene* 2004;23(10):1954-6.
34. Zhu ZZ, Wang AZ, Jia HR, Jin XX, He XL, Hou LF, Zhu G. Association of the TP53 codon 72 polymorphism with colorectal cancer in a Chinese population. *Jpn J Clin Oncol* 2007;37(5):385-90.
35. Kawajiri K, Nakachi K, Imai K, Watanabe J, Hayashi S. Germ line polymorphisms of p53 and CYP1A1 genes involved in human lung cancer. *Carcinogenesis* 1993;14(6):1085-9.
36. Murata M, Tagawa M, Kimura M, Kimura H, Watanabe S, Saisho H. Analysis of a germ line polymorphism of the p53 gene in lung cancer patients; discrete results with smoking history. *Carcinogenesis* 1996;17(2):261-4.
37. Hamajima N, Matsuo K, Suzuki T, Nakamura T, Matsuura A, Hatooka S, Shinoda M, Kodera Y, Yamamura Y, Hirai T, Kato T, Tajima K. No associations of p73 G4C14-to-A4T14 at exon 2 and p53 Arg72Pro polymorphisms with the risk of digestive tract cancers in Japanese. *Cancer Lett* 2002;181(1):81-5.
38. Sayhan N, Yazici H, Budak M, Bitisik O, Dalay N. P53 codon 72 genotypes in colon cancer. Association with human papillomavirus infection. *Res Commun Mol Pathol Pharmacol* 2001;109(1-2):25-34.
39. Schneider-Stock R, Boltze C, Peters B, Szibor R, Landt O, Meyer F, Roessner A. Selective loss of codon 72 proline p53 and frequent mutational inactivation of the retained arginine allele in colorectal cancer. *Neoplasia* 2004;6(5):529-35.
40. Olschwang S, Laurent-Puig P, Vassal A, Salmon RJ, Thomas G. Characterization of a frequent polymorphism in the coding sequence of the Tp53 gene in colonic cancer patients and a control population. *Hum Genet* 1991;86(4):369-70.
41. Sjölander A, Birgander R, Athlin L, Stenling R, Rutegård J, Beckman L, Beckman G. P53 germ line haplotypes associated with increased risk for colorectal cancer. *Carcinogenesis* 1995;16(7):1461-4.
42. Koushik A, Tranah GJ, Ma J, Stampfer MJ, Sesso HD, Fuchs CS, Giovannucci EL, Hunter DJ. p53 Arg72Pro polymorphism and risk of colorectal adenoma and cancer. *Int J Cancer* 2006;119(8):1863-8.
43. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* 1998;393(6682):229-34.
44. Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA, James N, McGregor JM, Harwood CA, Yulug IG, Vousden KH, Allday MJ, Gusterson B, Ikawa Sh, Hinds PhW W, Tim Crook T, Kaelin Jr WG. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nat Genet* 2000;25(1):47-54.

45. Kaelin WG Jr. The emerging p53 gene family. *J Natl Cancer Inst* 1999;91(7):594-8.
46. Monti P, Campomenosi P, Ciribilli Y, Iannone R, Aprile A, Inga A, Tada M, Menichini P, Abbondandolo A, Fronza G. Characterization of the p53 mutants ability to inhibit p73 beta transactivation using a yeast-based functional assay. *Oncogene* 2003;22(34):5252-60.
47. Graziano F, Cascinu S. Prognostic molecular markers for planning adjuvant chemotherapy trials in Dukes' B colorectal cancer patients: how much evidence is enough? *Ann Oncol* 2003;14(7):1026-38.