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Original Research Article

The Significance of *TP53* Gene Polymorphisms as A Risk Factor For Non-Hodgkin's Lymphoma in Iraqi Patients

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Abstract

Genetic factors including single nucleotide polymorphisms have been implicated as predisposing factors for large numbers of malignancies. When genetic disorders occur in a tumor suppressor gene, like *TP53* gene, the results are expected to have devastating effects. The current study aimed to assess the effect of certain polymorphisms in *TP53* genein the individual's susceptibility to non- Hodgkin's lymphomas among Iraqi patients. A total of 62 patients with these malignancies and 34 apparently healthy individuals were enrolled for this study. DNA was extracted from blood samples and fragment in *TP53* corresponding for *TP*53 p.Arg72Pro, *TP*53 p.Pro47Ser and r.13494 G>A polymorphisms were amplified using specific primers. Genotyping was performed with restriction fragment length polymorphisms. The results revealed significant association of *TP*53 p.Arg72Pro polymorphism in both heterozygous and mutant homozygous genotypes with incidence of NHLs, while both *TP*53 p.Pro47Ser and r.13494 G>A polymorphisms had no such association. These results strongly indicate the importance of proline allele of *TP*53 p.Arg72Pro as a predisposing factor for NHLs.

Key Words: Lymphoma, *TP53* gene, Single nucleotide polymorphism.

أهمية التغايرات الجينية في جين *TP53* في حدوث الاورام اللمفاوية اللاهوجكينية

الخلاصة

تمثل العوامل الجينية بما فيها التغايرات الجينية عوامل مؤهبة للاصابة بعدد كبير من السرطانات ، وعندما يحصل الخلل الجيني في الجينات المثبطة للورم كجين *TP53*، فمن المتوقع ان تكون لنتائج ذلك تأثيرا مدمرا . هدفت هذه الدراسة الى تقييم تأثير عدد محدد من تعدد التغايرات الجينية في جين *TP53* على مدى استعداد المرضى العراقيين للاصابة بالأورام اللمفاوية اللاهودجكينية . شملت الدراسة 62 مريضا بهذه الاورام و34 شخصا سليما ظاهريا . استخلص الDNA من عينات الدم وتمت مضاعفة قطع جين *TP53* المتضمنة للتغايراتp.Arg72Pro *TP*53 وp.Pro47Ser *TP*53 و r.13494 G>Aوذلك باستخدام بادئات خاصة . اجري التنميط الجيني بطريقة تعدد الاشكال لطول الشظايا المقيدة . كشفت النتائج عن علاقة معنوية بين التغاير p.Arg72Pro *TP*53 في شكليه (متغاير الزيجة ومتماثل الزيجة الطافر) مع حدوث الاورام اللمفاوية اللاهوجكينية ، في لم يظهر اي من التغايرين p.Pro47Ser *TP*53 وr.13494 G>A مثل هذه العلاقة . تشير هذه النتائج الى أهمية الاليل المشفر للبرولين في التغايرالجيني p.Arg72Pro *TP*53 كعامل خطورة للاصابة بالأورام اللمفاوية اللاهوجكينية.

الكلمات المفتاحية: الاورام اللمفاوية اللاهوجكينية ، جين *TP53* ، التغايرات الجينية احادية القاعدة.

Introduction

Non-Hodgkin's Lymphomas (NHLs) are a group of closely related illnesses involving a malignant transformation of lymphoid cells. However, these cells have discriminative, immuno-phenotypic, genetic, morphologic and clinical features [1]. A wide range of genetic and non-genetic factorsare implicated to be influence the occurrence of NHLs, while the precisecauses are not known [2].

The protein p53, encoded by *TP53* gene (OMIM191170), has a crucial role in several vital body functions. On one hand, it is essential for stress response which preserve the stability of the whole genome when the body exposes to various injuries such as DNA damage, metabolite stress, hypoxia and activation of oncogenes [3]. On the other hand, this protein is one of the most known tumor suppressors. In this regard, it achieves huge numbers of activities among which are the induction of arrest in cell cycle, senescence, and of programmed cell death [4]. Thus, it is reasonable to say that disorders in this gene may influence the occurrence and progression of NHLs.

As many as 547 single nucleotide polymorphisms (SNPs) in *TP53* gene have been recorded (https://www.ncbi.nlm.nih.gov/gene) making this gene one of the most polymorphic region in the human genome. Of course, the vast majority of these SNPs are not functional. However, few of them were found to be associated with some malignancies [5].

One of the most extensively investigated SNP is p.Arg72Pro (rs1042522). It is a non-synonymous SNP which involves the substitution of arginine in the codon 72 by proline. Several meta-analysis studies have linked this polymorphism with different malignancies including lung [6], gastric [7] and breast cancers [8,9].

The second functionally important SNP is p.Pro47Ser (rs1800371). Similar to the previous variant, it is a non-synonymous polymorphism, but the proline is substituted by serine at codon 47. Only limited number of studies have been conducted on this variant may be due to its low frequency with a minor allele frequency of less than 5% in African population[3,10]. However, Singamsetty et al.[11] reported significant increase in colorectal cancer among South Indian population carrying Ser47 allele, while Al-Awadi et al. [12] and Al-Qasem et al. [13] did not found such association in in Kawati and Saudi women with breast cancer.

The presence of function SNPs is not only restricted to the coding motifs of the gene but also to the noncoding regions (introns). r.13494G>A (rs1625895) is located in intron 6, and was previously reported as a risk factor for colon and breast[14], and ovarian cancers[15].

In Iraq, there are only few studies pointing the association of genetic polymorphisms with NHLs [16]. Therefore, this study aimed to assess the role of these SNPs as a risk factors for NHLs.

Materials and Methods

The Study Population

A prospected case-control study comprised of 62 confirmed NHLs patients during theperiod from January 2015 to June 2015 from Hematology Center/ Al-Mustansiriya University and Teaching Hospital of Pediatric, Baghdad, Iraq. Another family unrelated, 34healthyindividuals were recruited to becontrolgroup. Mean ages of patients and controls were 52.17 and 47.22 years respectively. All subjects were informed and supplied with written consent to take part in this investigation and to use their samples in genetic analysis. After agreement, a direct interview with each participant was made and an information including age, sex, smoking (current and previous), dwelling, body mass index (BMI) first relative history of NHLs, and diabetes mellitus was obtained.

Samples, DNA Extraction and Genotyping

About 3 ml of peripheral blood was obtained from each participant in EDTA tube. The nucleic acid (DNA)was extracted from leukocytes using ready kit (g SYNCTM DNA Mini/ Geneaid/ Korea) following the protocol supplied with the kit. Primer set and restriction enzymes are shown in table (1).

Table 1: Primers sets and restriction enzymes for different SNPs

Polymorphism	Primers (5'→3')	Product (bp)	Enzyme
*TP*53 p.Arg72Pro rs1042522	F: TTTCACCCATCTACAGTCCC R:ACCTAGGCTCAGGGCAACTAGCCG	Arg/Arg: 318 Pro/Pro: 182, 136 Arg/Pro: 318,182,136	BstUI
*TP*53 p.Pro47Ser rs1800371	F:CTGGTAAGGACAAGGGTTGG R: TCATCTGGACCTGGGTCTTC	Pro/Pro:201 Ser/Ser:156, 45 Pro/Ser:201,156,45	MspI
r.13494 G>A rs1625895	F:TATGAGCCGCCTGAGGTCTGG R:TACAGGCATGAGCCACTGCGC	G/G: 240 AA: 164, 76 G/A: 240, 164, 76	MspI

The PCR conditions for the three segments involves an initial denaturation for 5 min at 95^{o}C, followed by 35 cycles of denaturation for 30 secat 94^{o}C, annealing for 30 sec at 59^{o}C and elongation for 45 secat 72^{o}C. Finally the mixture was subjected to 72^{o}C for 10 min as final extension step.

A master mix with 50 µl capacity (Bioneer/Korea) was used for amplification.From Five µL of template DNA from each sample and 2µl from each primer were dispensed to master mix tube. After mixing, the mastermix tubes were placedinto previously programmed (Hybaid thermal cycler/ UK).Amplicons'size were detected through comparison with a commercial 50bp ladder (Bioneer/Korea). Ten percent of PCR product of each of *TP*53 p.Arg72Proand (*TP*53 p.Pro47Ser and r.13494 G>A) were mixed with 5□l 10X NEB buffer and 1□l of *BstUI* or *MspI* (10U) restriction enzyme (Biolabs Inc./USA) in an eppendorf tube. The volume was adjusted to 50 □l using deionized sterile H_2O. The tube was then incubated at 37^{o}C for 2 and 3 hours for the first and second enzyme respectively. Digests were separated on a 3% gel stained with ethidium bromide and analyzed U.Vtransilluminator with camera.Genotyping was determined depending on the resulted base pair after digestion.

Statistical Analysis

Statistical Package for the Social (SPSS) Version 16.0 was used for data analysis. Data are reported as mean± standard deviation (SD). Genotype frequencies were examined for deviation from Hardy-Weinberg Equilibrium (HWE) using Chi-square test. This test was also employed to collate the distributions of allele frequencies in NHLs patients and controls. The risk associated with individual alleles or genotypes was calculated by estimating the odds ratio (OR) through binary logistic regression test.

Results

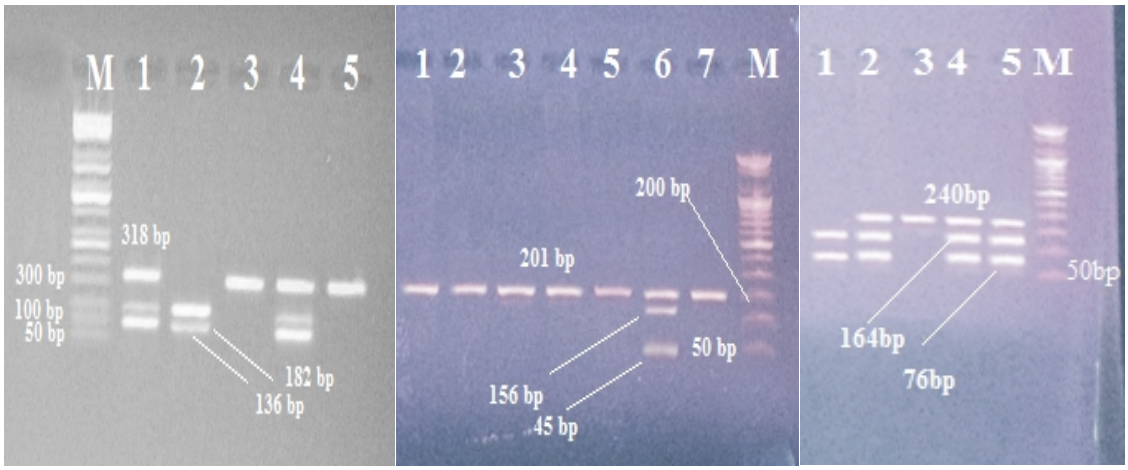
The study population characteristics are shown in Table-2.Of the studied risk factors, only BMI had significant effect on the susceptibility to NHLs.

Table 2: The study population characteristics

Variable	Patients N=62	Controls N=34	*P*-value
Mean age in years (SD)	52.17 (9.22)	47.22 (7.91)	0.294
Gender			0.206
Male	41(66.13%)	26(62.5%)	
Female	21(33.87%)	8(37.5%)	
Family history			0.217
No	56(90.32%)	33 (97.06%)	
Yes	6 (9.68%)	1(2.94%)	
Mean BMI (SD)	27.14 (4.27)	23.71 (2.02)	0.028
Smoking			0.075
Never	35 (56.45%)	25 (73.53%)	
Smoker (ex/current)	27 (43.55%)	9(26.47%)	
Dwelling			0.592
Urban	49(79.03%)	27(79.41%)	
Rural	13(20.97%)	7(20.59%)	
Diabetes Mellitus			0.97
Non-diabetic	55(88.71%)	31(91.18%)	
Diabetic	7 (11.29%)	3(8.82%)	

BMI: body mass index, N: number, SD: standard deviation

Figure 1 shows the gel electrophoresis of *TP53* polymorphisms after digestion with the restriction enzymes.



(A) **(B)** **(C)**

Figure 1: Agarose gel electrophoresis of *TP53* polymorphisms after digestion with restriction enzymes and staining with ethidium bromide. (A) Different genotype patterns of *TP53* p.Arg72Pro polymorphism. M: 50 bp DNA marker, lanes 1 and 3: Arg/Pro genotypes, lane 2: Pro/Pro genotype, lanes 2 and 5: Arg/Arg genotypes.(B) Two genotype patterns of *TP53* p.Pro47Ser polymorphism. Lanes 1-5 and 7: Pro/Pro genotypes, lane 6: Pro/Ser genotype, M: 50 bp DNA marker. (C) Different genotype patterns of r.13494G>Apolymorphism. Lane1: A/A genotype, lanes 2,4,5G/A genotypes, lane 3: G/G genotype, M: 50 bp DNA marker.

Genotype frequencies for the three selected TP53 SNPs were in agreement with HWE. A remarkable result to emerge from the data is that there were significant differences in the frequencies of different genotype of *TP53* p.Arg72Pro poly-morphism between NHLs patients and controls. Among NHLs patients, the frequencies of Arg/Arg,Arg/Pro and Pro/Pro were 22.58%, 37.1% and 40.32% respectively compared to 44.12%, 35.29% and 20.59% respectively among controls (OR=3.136, 95%CI=1.126-8.738, P=0.029 and OR=5.844, 95%CI=1.898-17.997, *P*=0.002 for Arg/Arg and Pro/Pro genotypes respectively) as shown in Table 3. Analysis of allele frequency confirms these results as the frequencies of Arg and Pro alleles among patients were 41.13% and 58.87% respectively compared to 61.76% and 38.24% respectively among controls with highly significant differences (OR= 2.213, 95%CI=1.261-4.238, *P*=0.007).

TP53 p.Pro47Ser polymorphism appeared as momoallelic phenotype in controls (Pro allel), and only two NHLs patients had heterozygous Pro/Ser genotype (Table3). As there was only one genotype in controls, the statistical analysis is nonsense.

Although the heterozygous genotype of r.13494G>Apolymorphism (GA) in controls (35.29%) was higher than that of patients (30.65%) the different was insignificant (OR=0.748, 95%CI=0.297-1.1885, *P*= 0.538).Similarly, the frequency of the mutant homozygous genotype (AA) was higher in controls(14.7%) than controls (11.29%), but also the difference was insignificant difference (OR=0.661, 95%CI=0.183-2.288, *P*=0.528). These results are confirmed though allelic levels in that the difference in the frequency of allele A between patients and controls was not significant (OR=1.319, 95%CI=0.692-2.515, *P*=0.409).

Table 3: Frequency of genotypes and allele of *TP*53 gene polymorphisms in NHLs patients and controls

Genotypes and Alleles	Patients N=62	Control N=34	*P*-value	OR(95%CI)
***TP53* p.Arg72Pro Genotypes**				
Arg/Arg	14(22.58%)	15(44.12%)	0.061	1.0 (Reference)
Arg/Pro	23 (37.1%)	12(35.29%)	0.029	3.136 (1.126-8.738)
Pro/Pro	25(40.32%)	7 (20.59%)	0.002	5.844(1.898-17.997)
Alleles			0.007	
Arg	51 (41.13%)	42(61.76%)		1.0 (Reference)
Pro	73 (58.87%)	26(38.24%)		2.213 (1.261-4.238)
***TP53* p.Pro47Ser Genotypes**				
Pro/Pro	60(96.77%)	34 (62%)	0.119	1.0 (Reference)
Pro/Ser	2(3.23%)	0(0%)	0.280	-------
Ser/Ser	0(0%)	0(0 %)	0.841	-------
Alleles			-------	
Pro	122(98.39%)	64(100%)		1.0 (Reference)
Ser	2(1.61%)	0(0%)		---------
r.13494G>AGenotypes				
GG	36 (58.6%)	17(50%)	0.738	1.0 (Reference)
GA	19 (30.65%)	12(35.29%)	0.538	0.748(0.297-1.1885)
AA	7(11.29%)	5(14.7%)	0.528	0.661 (0.183-2.288)
Alleles			0.409	
G	91(73.39%)	46 (67.65%)		1.0 (Reference)
A	33(26.61%)	22(32.35%)		1.319 (0.692-2.515)

N: number; OR: odds ratio; CI: confidence interval

Discussion

The *TP53*gene is a 20 kb gene on 17p13.1 comprising 11 exon and 10 introns [17]. The protein encoded by this gene (p53) has very important part in cell division and programmed cell death. Mutations and polymorphism in the gene can affect the protein function and eventually predispose for some malignancy. The current study revealed high association of the allele encoding for proline in the *TP53* p.Arg72Pro with the NHLs (OR=2.213, 95%CI= 1.261-4.238, *P*=0.007). Similar results have been obtained previously in breast, gastric, lung and urinary bladder cancers [6,7,8,9,18]. However, many authors reported null results with different cancers [19-22].

The supposed more common G nucleotide of the codon CGC which encodes for arginine in this polymorphism was found to be associated with a protein that has 15-fold capacity to induce apoptosis more than that associated with the C nucleotide of the codon CCC which encodes proline[23]. Evidence from these observations was further supported by the observation that patients with different cancers who carry Arg/Arg genotype response more favorably to radiation and chemotherapy [24]. Taking into account that most chemotherapies of NHLs (such as resveratrol) depend on apoptosis induction[25], it is easy to explain the highly association between Pro72variant and the susceptibility to NHLs.

TP53 p.Pro47Ser appeared biallelic on in two patients with NHLs and there was no homozygous mutant genotype. This low prevalence of this SNP is in agreement with what recorded in some neighboring countries like Kuwait and Saudi Arabia where the SNP appeared monoallelic in healthy controls in diallelic in very small percentage of women with breast cancer [12,13]. However, very few studies reported Ser47 variant as a risk factor for certain cancers [11]. This variant was recorded to have up 5-fold decrease in the resultant protein for induction of apoptosis in comparison with the variant Pro47[26].

The current study revealed no significant association between r.13494G>A and the susceptibility to NHLs neither at genotypes levels nor at allele levels. Though it is not underwent intensive investigation, similar results have been reported in Brazilian Barretts esophagus patients [27] and Indian cervical cancer patients [28]. In contrast, significant effect of this SNP was documented on the developing of breast [14], ovarian [15], colon[14] lung [29,30], and prostrate [31] cancers.This effect was attributed to two factors. The first factor is referred to the role that the non-coding pieces of *TP53* might have a roleas a regulator of gene expression, while the second factor is the reduced standard of apoptosis and increased survival rate after DNA insulting exhibited by r.13494A variant [32].

Taken together, these findings strongly suggest the role of Pro72 variant of *TP53*p.Arg72Pro SNPas a predisposing factor for NHLs. However, further study with larger sample size involving different types of NHLs are needed to draw a solid conclusion.

References

1.Schuetz JM, Daley D, Graham J, et al. Genetic variation in cell death genes and risk of non-hodgkin lymphoma.PLoS One, 2012;7: e31560.

2.Hartge P, Smith MT. Environmental and behavioral factors and the risk of non-Hodgkin's lymphoma. Cancer Epidemiol. Biomarkers Prev, 2007; 16:367-368.

3.Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer, 2009;9: 95-107.

4.Aylon Y, Oren M. Living with p53, dying of p53. Cell,2007;130: 597–600.

5.Tian X, Dai S, Sun J et al. Association between *TP53* Arg72Pro polymorphism and leukemia risk: a meta-analysis of 14 case-control studies. Scientific Reports, 2016;6:2409, DOI: 10.1038/srep24097.

6. Dai S, Mao C, Jiang L. P53 polymorphism and lung cancer susceptibility: a pooled analysis of 32 case-control studies. Hum Genet,2009;125:. 633-638.
7. Zhou Y, Li N, Zhuang W, et al. P53 codon 72 polymorphism and gastric cancer: a meta-analysis of the literature. Int J Cancer, 2007; 121, 1481-1486.
8. Zhang Z, Wang M, Wu D, et al. P53 codon 72 polymorphism contributes to breast cancer risk: a meta-analysis based on 39 case-control studies. Breast Cancer Res Treat. 2010; 120: 509-517.
9. Firoozabadi SR, Shiryazdi SM, Keshavarz F et al. Frequency of *TP53* gene codon 72 polymorphisms in women with breast cancer in Iran. Int J Medical Lab. 2016; 3(2):86-91.
10. Murphy ME. Polymorphic variants in the p53 pathway. Cell Death Differ, 2006; 13: 916-920.
11. Singamsetty GK, Malempati S, Bhogadhi S, et al.*TP53* alterations and colorectal cancer predisposition in south Indian population: a case-control study. TumourBiol, 2014; 35:2303-11.
12. Alawadi S, Ghabreau L, Alsaleh M, et al. P53 gene polymorphisms and breast cancer risk in Arab women. Med Oncol, 2011; 28: 709-715.
13. Al-Qasem A, Toulimat M, Tulbah A, et al. The p53 codon 72 polymorphism is associated with risk and early onset of breast cancer among Saudi women. OncolLett, 2012; 3: 875-878.
14. Peller S, Kopilova Y, Slutzki S, et al. A novel polymorphism in intron 6 of the human p53 gene: a possible association with cancer predisposition and susceptibility. DNA Cell Biol, 1995;14:983-990.
15. Wang-Gohrke S, Rebbeck TR, Besenfelder W, et al. p53 germline polymorphisms are associated with an increased risk for breast cancer in German women. Anticancer Res, 1998; 18: 2095-2099.
16. Ali MA, Naif HM, Al-Mayah QS. The impact of genetic variants in IL-10 and IL-12p40 on the susceptibility to non-Hodgkin lymphoma.Diyala J Med. 2016;10(1):46-53.
17. Lamb P, Crawford L. Characterization of the human p53 gene. Mol Cellular Biol, 1986;6(5):1379-1385.
18. Zhang R, Chen W, Zhang W et al. Genetic polymorphisms of p53 codon 72 and bladder cancer susceptibility: a hospital-based case-control study. Genetic Testing Mol Biomar, 2010;15(5):337-341.
19. Yi K, Yang L, Lan Z et al. The association between p53 codon polymorphism and endometrial cancer risk: a systemic review and meta-analysis. Int Gynecol Cancer, 2016; 26(6):1121-1128.
20. Benhessou M, Assoumou SZ, Boumba LMA et al. The p53 codon 72 polymorphism in Moroccan women and risk of ovarian cancer. Br Microbiol Res J, 2016;12(1):1-5.
21. Mojtahedi Z, Hashemi SB, Khademi B et al. P53 codon 72 polymorphism association with head and neck squamous cell carcinoma. Braz J Otorhinolaryngo, 2010;76(3):316-320.
22. Berrada N, Amzazi S, El-Hassani RA et al. No evidence of correlation between p53 codon polymorphism and risk of bladder cancer in Morrocan patients. Clin Cancer Investigation J, 2013;2(4):3012-306.
23. Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer,2000; 108: 196–199.
24. Dumont P, Leu JI, Della Pietra AC et al. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet, 2003;33: 357–365.
25. Frazzi R, Tigano M. The multiple mechanisms of cell death triggered by resveratrol in lymphoma and leukemia. Int J Mol Sci, 2014;15:4977-4993.
26. Li X, Dumont P, Della Pietra A, et al. The codon 47 polymorphism in p53 is functionally significant. J Biol Chem,2005; 280, 24245-24251.
27. Pilger DA, Lopez PL, Segal F, et al. Analysis of R213R and 13494 g>a polymorphisms of the p53 in individuals with esophagitis, intestinal metaplasia of the cardia and Barrett's Esophagus compared with a control group. Genomic Med,2007; 1, 57-63.
28. Mitra S, Misra C, Singh RK et al.Association of specific genotype and haplotype of p53 gene with cervical cancer in India. J Clin Pathol,2005; 58, 26-31.
29. Wang W, Spitz MR, Yang H, et al. Genetic variants in cell cycle control pathway confer

susceptibility to lung cancer. Clin Cancer Res,2007 13, 5974-5981.

30. Cherdyntseva NV, Gervas PA, Litvyakov NV, et al. Agerelated Function of tumor suppressor gene *TP53*: Contribution to cancer risk and progression. ExpOncol,2010 32, 205-208.
31. Mittal RD, George GP, Mishra J et al.Role of functional polymorphisms of P53 and P73 genes with the risk of prostate cancer in a case-control study from Northern India. Arch Med Res, 2011; 42, 122-127.
32. Lehman TA, Haffty BG, Carbone CJ, et al. Elevated frequency and functional activity of a specific germline p53 intron mutation in familial breast cancer. Cancer Res,2000;60, 1062-1069.