Cellular and Molecular Biology ™ **54**, OL1025-OL1031
DOI 10.1170/116

ISSN 1165-158X
2008 Cell. Mol. Biol.™

EVALUATION OF THE POLYMORPHISMS IN THE EXONS 2 TO 4 OF THE TP53 IN CERVICAL CARCINOMA PATIENTS FROM A BRAZILIAN POPULATION

T.A.A.M. FERNANDES[1], G.L.F. LIMA[1], F.C.G. DE SOUZA[1], J.V. FERNANDES[2], R.V. MEISSNER[1,2,]

[1] Universidade Federal do Rio Grande do Norte, Centro de Biociências, Programa de Pós-Graduação em Genética e Biologia Molecular, Av. Salgado Filho, S/N, Campus Universitário, Lagoa Nova, CEP:59072-970, Natal, RN, Brazil.

[2,] Universidade Federal do Rio Grande do Norte, Centro de Biociências, Departamento de Microbiologia e Parasitologia, Av. Salgado Filho, S/N, Campus Universitário, Lagoa Nova, CEP:59072-970, Natal, RN, Brazil, Fax +55-84-32119210; E-mail: vmrosely@yahoo.com.br

Received March 10th, 2007; Accepted November 11th, 2007; Published June 1st, 2008

Abstract – The majority of *TP53* polymorphisms and cervical cancer association studies have only analyzed codon 72 polymorphism. Eight polymorphisms were reported in the region encompassing exon 2 to 4 of *TP53* that codify the aminoterminal p53 region containing domains involved in the transcription transactivation and apoptosis induction. We investigated if the polymorphisms present in this region were associated with cervical cancer risk. A total of 140 samples (83 from Brazilian patients with cervical carcinoma and 57 from Brazilian healthy women) were analyzed by PCR and DNA sequencing. Only three from the eight *TP53* polymorphisms described in the analyzed region were polymorphic within our samples: the 11827 base from intron 2, the 16bp duplication in the intron3 and the codon 72 (Arg>Pro) from exon 4. No statistically significant association was observed between polymorphisms from intron 2 and the 16bp duplication from intron 3 with cervical cancer. No statistically significant difference in the frequency of homozygotes for Arg in relation to other genotypes was found when comparing patient and healthy groups (OR=0.70; 95% CI= 0.31-1.56; p= 0.222). However, Arg/Pro heterozygotes were more frequent within HPV positive cancer patients than in healthy women (p=0.023; OR (Arg/Pro:Pro/Pro)= 5.82; 95% CI: 1.22-30.78; p=0.024).

Key words: cervical cancer, *TP53* gene, polymorphisms

INTRODUCTION

Mutation of the *TP53* gene has been reported rarely in cervical carcinogenesis (25) and the abrogation of p53 function by the E6 human papillomavirus (HPV) protein is considered to be one of the major events in this kind of cancer (37). Infection by high-risk HPV types, predominantly HPV16 and HPV18, has been recognized as the main cause of cervical cancer and its precursor lesions (12,36). The high risk HPVs encode two major oncoproteins, E6 and E7, which bind to and degrade or inactivate the host human tumor suppressor proteins, p53 and RB, respectively (38). Because mutation of the *TP53* gene has been reported rarely in cervical carcinogenesis (25), abrogation of p53 function by the E6 protein is considered to be one of the major events in this kind of cancer (37). However, only a subset of women infected with high-risk HPV, with or without cervical lesions, will eventually develop cervical cancer. Therefore, additional and yet unknown factors are likely to contribute to neoplastic transformation of HPV- infected cervical epithelial cells. Genetic susceptibility is likely to be an important factor.

The possible contribution of inherited polymorhisms in the *TP53* gene to the development of cervical cancer was proposed by Storey *et al.*(31). They suggested that the potential of the E6 protein in degrading p53 was dependent on the polymorphism of the codon 72 (Arg>Pro) in *TP53* tumor suppressor gene. Individuals bearing Arginine (Arg) homozygous genotype were more likely to develop cervical

Abbreviations: Arg, Arginine; **bp,** Base pairs; **CI,** Confidence interval; **HPV,** Human Papillomavirus; OR**, Odds Ratio;** Pro**, Proline**

cancer than heterozygous individuals because arginine p53 variant were more susceptible to degradation by E6 from high-risk HPV (31). Subsequent functional studies suggested that p53 variants could be differentiated by their abilities to bind to components from transcriptional machinery and to activate transcription, to repress the transformation of primary cells (32) and to induce apoptosis (32, 5). Conflicting results have however been obtained from worldwide group studies analyzing the role of the codon 72 polymorphism *TP53* on cervical carcinogenesis (16, 23).

The region encompassing exon 2 to 4 of the *TP53* codify the aminoterminal p53 region, which contain domains involved in the transcription transactivation (33) and proline-rich regions which acts as an apoptosis inductor (27). Eight polymorphisms [11827 nucleotide in the intron 2; 11992 and 12015 nucleotides and the 16bp duplication in the intron3; and the following nucleotides in the exon 4: 12026 nucleotide (at codon 34), 12032 nucleotide (at codon 36), 12063 nucleotide (at codon 47), 12139 (at codon 72)] were reported within these region (26).

The majority of *TP53* polymorphisms and cervical cancer association studies in the literature have analyzed codon 72 polymorphism (16). Some studies of the 16bp duplication polymorphism at the intron 3 in cervical cancer cases were carried out in Korea (14), USA (4) and India (23) and only one analyzed the intron 6 MspI restriction fragment length polymorphism at nucleotide 13494 in this cancer (23). None of them found an association of such polymorphisms with neoplasia development. Since there are a limited number of reports of other polymorphisms both in populations and disease association studies and considering that the most frequently studied *TP53* polymorphisms have shown a significant variation among populations from distinct regions and/or ethnical groups, we studied the influence of the polymorphisms present in the region encompassing exon 2 to 4 of the *TP53* gene on cervical cancer development in patients from Rio Grande do Norte, a state in the northeast region of Brazil.

MATERIALS AND METHODS

Sample Population

Frozen tumor samples were obtained from 83 women with a histological diagnosis of cervical carcinoma (57% of the patients presented *in situ* squamous carcinoma cells, 35% invasive squamous carcinoma cells, 8% adenocarcinoma) at the Hospital Dr. Luiz Antônio, Natal, Rio Grande de Norte, northeast of Brazil. Fifty-seven peripheral blood samples were collected from healthy women. Controls were volunteers women without cervical cancer and the majority of them were students from Universidade Federal do Rio Grande do Norte. All were born in Rio Grande do Norte.Women were classified as African-mixed descendants or as European descendants based mainly on an interview about ethnic origins of their ascendants and, in case of doubts, in their visual appearance (skin colour, hair and other physical traits). Women with an African origin (at least, one of the parents and/or grandparents) were classified as African-mixed descendants, since it is difficult to find African-Brazilians without any European origin in Rio Grande do Norte.This study was approved by the Ethics Committee at the *Universidade Federal do Rio Grande do Norte*.

DNA isolation

Tumor DNA was isolated from frozen tissue fragments (0.5cm3) after maceration with liquid nitrogen and incubation at 55ºC overnight in 2 mL digestion buffer (0.5 mg Proteinase K, 0.01 M Tris HCl; 0.02 M EDTA; 0.1M NaCl; 0.5% SDS; pH 8.0) and phenol clorophorm treatment, according to standard protocols (24). Blood DNA was isolated from peripheral blood according to a previous procedure described (17), with modifications (Tween 20 was replaced by Triton X-100).

TP53 intron 3 polymorphism (16 bp duplication) (TP53PIN3) genotyping

The sequence encompassing TP53PIN3 was amplified by PCR using the following primers: F3R+ (5'TCATGGGACTGACTTTCTGCT3') and RS3-4- (5´CGTGCATTCTGGGAGCTTCA3´) (18). PCR conditions were: 1U Taq DNA polymerase, 20mM Tris.HCL (pH8.4), 50mM KCl, 1.5 mM $MgCl_2$, 0.1mM dNTP and 1 µM of each primer in a 25 µL final volume reaction. The PCR cycles were: 95°C for 5 min, followed by 40 cycles at 95°C for 1 min, 64°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 10 min. PCR products were analyzed by silver staining after electrophoresis in a acrylamide gel (28) and were of 252bp (A1) or 268bp(A2) sizes, depending on the presence of the 16bp duplication.

PCR and nucleic acid sequencing for detection of the polymorphisms comprising the region between exons 2 and 4

Three different PCR reactions were performed using the primers pairs 2IN+ (5'AGTGTCTCATGCTGGATC3') / RS3-4- (5´CGTGCATTCTGGGAGCTTCA3´) (18), PEX3+ (5'ACTTCCTGAAAACAACGTTCTG3') / P53R- (5'GACCGTGCAAGTCACAGACTT3') or P53+ (5'TCCCCCTTGCCGTCCCAA3') (2)/ ROS- (5'CCAAGG AATACACGTGGATGA3') providing products of 409 (bases 11652 to 12126), 395 (bases 11906 to 12302) and 556bp (bases 12021 to 12576) size fragments, respectively, allowing us to sequence and analyze all the region between exons 2 and 4 and a small portion of intron 4 (Figure 1). Each PCR reaction was performed in a 50µL mixture containing 1.0 U Taq DNA polymerase, 20mM Tris.HCL (pH8.4), 50mM KCl, 1.5 mM $MgCl_2$, 0.10 mM dNTP 0.25 µM each primer and 0.5 µL of DNA solution. The PCR cycling conditions comprised a first step at 95°C for 5 min,

followed by 40 cycles at 95°C for 1 min, 59°C for 1 min and 72°C for 1 min with a final extension step at 72°C for 10 min. The PCR products were analyzed and quantified by electrophoresis in 2% agarose gel using "*Low Mass DNA Ladder*" (Invitrogen). Each PCR product was sequenced, and for DNA sequencing about 160 ng of PCR products were concentrated in a 96-well plate using the post-reaction clean-up protocol from *DYEnamic™ ET dye terminator kit for MegaBace DNA analysis Systems* (Amersham Bioscience, São Paulo, Brazil), and digested in 1U of shrimp alkaline phosphatase and 5U of Exonuclease I. Each PCR sequencing reaction was performed in a 10 µL volume containing 0.25 µM of primer (the same used in PCRs) and 4 µL of DYEnamic ET terminator premix . The cycling program for 35 cycles was: 95ºC/20 s, 50ºC/ 15 s and 60ºC/ 1 min. Sequencing reactions were run in MegaBACE 1000 Sequencing System (Amersham Pharmacia, Brazil). The sequences were joined using the software CAP (11) and compared with the normal human TP53 sequence (U94788) obtained from GeneBank database. The polymorphisms were determined by visual inspection of the electropherogram generated by the software Chromas Demo version 2.24 (10). Sixty-three of our patients were sequenced with three different primer pairs and 16 samples that were heterozygous for the 16bp duplication (intron 3) were sequenced only using the p53+/ROS- primer pair.

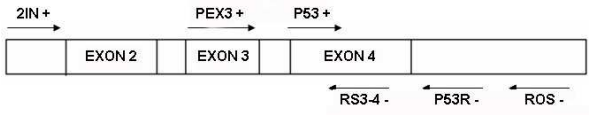

Figure 1. Localization of primers used in PCR and DNA sequencing reactions in the region encompassing exon 2 to 4 of *TP53* (out of scale).

Detection of HPV DNA sequences

Tumor DNA samples were submitted to PCR using the generic primers MY09/MY11, that allowed to amplify a 450-bp fragment of the *L1* gene from genital HPV types (22). Subsequent HPV typing was performed in Dr. Luisa Villa's laboratory at Ludwig Institute for Cancer Reseach, São Paulo, using dot blot hybridization (2) (36).

Statistics

Allele frequencies were determined by the direct count of the alleles. Genotypic distributions were examined for significant departure from Hardy-Weinberg equilibrium by goodness of fit χ^2 test.The significance among differences in allelic and genotypic frequencies observed between the groups were evaluated by Pearson's χ^2 test or Fisher's exact test (when the number of individuals within a class was lower than five). The software WINPEPI (1) was used for both statistical tests, as well as to calculate the odds ratio (OR). A $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

All the DNA samples that had the region encompassing exons 2 to 4 of TP53 amplified by PCR were also sequenced. Since the sequences showed some variation in size, extension and legibility, the number of samples analyzed for each polymorphism was slightly variable. Although DNA was extracted from the frozen tumor samples in the patients group, they should contain at least 5% of stroma cells (34). Therefore, the fraction of normal cells present in the samples should ensure correct *TP53* genotyping (34).

The 12026 (at codon 34) and 12063 (at codon 47) nucleotides were monomorphic for the most common described alleles in all samples. The healthy women samples were monomorphic for the polymorphism at 11992; 12015 and 12032 (at codon 36) nucleotides. Two samples in patient group were heterozygous for the polymorphism at 11992 nucleotide (1.2% allelic frequency considering all analyzed individuals for this region, patients and healthy women; n= 104), other two patients were heterozygous for 12015 nucleotide (1.2% allelic frequency considering all analyzed individuals) and one sample in the patient group was heterozygous for polymorphism at 12032 nucleotide (at codon 36) (0.6% allelic frequency considering all analyzed individuals). In the case of the polymorphism at codon 47, the polymorphism can be population-specific or the frequency of the mutant allele can be low. Felley-Bosco *et al*. (7) reported no allele variant in Caucasians (n=69) and 4.7% in African-Americans (n=32) at this codon. *TP53* polymorphism IARC database reported a allele variant frequency ranging from 0.6 to 6.7% at codon 47 (26). A similar situation is also true for the mutant allele at codon 36, which is present in 0.6% (26) or 4% (6) of the populations already studied. The frequence of variant allele at 12015 nucleotide in our population was 1.2 % (considering all analyzed individuals) and it was similar to others already reported (0.5 to 2.2% (26)). On the other hand, the *TP53* polymorphism at codon 34 was observed in a high frequency (20%) in South Africa (35) and the described frequences of allelic variant of polymorphism at 11992 nucleotide from different studied populations ranged from 3.2 to 22.9%, being highest in African and Southest Pacific descendants than European origin ones (26). Since there are no reports of codon 47 polymorphism in other populations, and considering that the most frequently studied *TP53* polymorphisms (16 base pair duplication at intron3, codon 72 (Arg/Pro) and intron 6 MspI restriction fragment length polymorphism at nucleotide 13494) have shown a significant

variation among populations from distinct regions and/or ethnical groups, it might be possible that the codon 34 polymorphism is absent in our population. Otherwise, if present, its frequency should be low, taking into account that 88 women were analyzed (including tumoral and peripheral blood samples) and none of them presented such polymorphism.

The other *loci* herein analyzed (11827 nucleotide from intron 2, the intron 3 16bp duplication and 12139 nucleotide at codon 72) were polymorphic and their genotypic frequencies were distributed according to Hardy-Weinberg equilibrium expectations within cancer patients as well as within healthy women, in both ethnical groups (Table 1).

Table 1. Genotypic and allelic frequencies of *TP53* gene polymorphisms

Polymorphism and studied group		N	Genotype (%)			A1	p (G:A)	*H-W* (p)
			A1-A1	A1-A2	A2-A2			
Intron 2 (base 11827)								
Cancer	European	19	63.2	31.6	5.2	0.789	0.410:	1.000
	African mixed	32	71.9	28.1	0.0	0.859	0.360	1.000
	Total	51	68.6	29.4	2.0	0.833	-	1.000
Healthy	European	14	71.4	21.4	7.2	0.821	0.880:	1.000
	African-mixed	23	60.9	21.7	17.4	0.717	0.312	0.378
	Total	37	64.9	21.6	13.5	0.757	-	0.213
16bp duplication at intron 3								
Cancer	European	31	67.7	29.0	3.3	0.823	0.115:	1.000
	African-mixed	52	84.6	15.4	0.0	0.923	0.049*	0.811
	Total	83	78.3	20.5	1.2	0.886	-----	1.000
Healthy	European	25	72.0	28.0	0.0	0.860	0.332:	1.000
	African-mixed	32	81.3	15.6	3.1	0.891	0.621	1.000
	Total	57	77.2	21.1	1.7	0.877	-----	1.000
Codon 36								
Cancer	European	20	100.0	0.0	0.0	1.000	1.000:	----
	African-mixed	43	97.7	2.3	0.0	0.988	1.000	1.000
	Total	63	98.4	1.6	0.0	0.992	-----	1.000
Healthy	European	16	100.0	0.0	0.0	1.000	-----	-----
	African-mixed	25	100.0	0.0	0.0	1.000		-----
	Total	41	100.0	0.0	0.0	1.000	-----	-----
Codon 72								
Cancer	European	28	7.2	46.4	46.4	0,304	0.794:	1.000
	African-mixed	45	4.5	42.2	53.3	0,256	0.527	0.890
	Total	73	5.5	43.8	50.7	0,274	-----	0.864
Healthy	European	18	16.7	22.2	61.1	0,278	1.000:	0.339
	African-mixed	29	17.3	24.1	58.6	0,293	0.873	0.273
	Total	47	17.0	23.4	59.6	0,287	-----	0.149

N = Number of individuals; p (G:A) = Significance of differences in genotypic (G) (Fisher's exact test) and allelic (A) (X^2) frequencies between ethnical classes; H-W (p) = Significance of differences among observed and expected genotypic frequencies within population, according to Hardy-Weinberg equilibrium (Fisher's exact test); A1: Frequence of Allele A1; * Statistically significant ($p \leq 0.05$)

Previous population studies of the 16bp duplication polymorphism at the intron 3 of the *TP53* showed that African origin groups presented higher frequency of A2 allele (+16bp) than Caucasian origin ones (8, 30, 37). The A2 frequencies of our control groups were 14% (European descendants) and 10.9% (African-mixed), being the A2 allele frequency of the African-mixed group lower than those already reported to other African origin groups. There were no differences in the allelic and genotypic frequencies between ethnical groups of healthy women, neither when each ethnical patients group was compared with the correspondent group of healthy women. However, differences in allele frequencies between ethnical groups within cancer patients were observed for the 16bp duplication polymorphism at intron 3 (χ^2 = 3.870; p = 0.049). A lower frequency of the allele A2 (presence of 16bp duplication) was detected in the African-mixed patients when compared to the European descendants patients (7.7% *vs.* 17.7%; p=0.049). These results suggest that the differences observed between both ethnical groups in the cancer samples could be a consequence of the small number of European descendants patients in our sample. Studies of this polymorphism in cervical cancer cases were carried out only in Korea (A2 (control group)=2,7%)(14), USA (A2 (control group, caucasians)=13%)(4) and India (A2 (control group)=18.8%) (23) and none of them found an association of such polymorphism with cervical neoplasia development.

In regards to the polymorphism of 11827 nucleotide from intron 2, Ge *et al.*(9) reported that there are significant differences in the genotypic distribution of this polymorphism among patients with lung carcinoma and healthy individuals. In our study, statistically significant differences were not found in the genotypic and allelic frequencies between ethnical groups for the polymorphisms at intron 2 and codon 72 within both the patient and the healthy women groups. Based on these results, data from both ethnical groups were analyzed as a set. Differences in the distribution of genotypic and allelic frequencies (p=0.124, Fisher's exact test and p = 0.209; χ^2=1.579; DF = 1, respectively) were also absent when comparing cancer and healthy groups in relation to the polymorphism at position 11827 from intron 2.

Conflicting results have been obtained by several groups worldwide when analyzing the influence of the polymorphism at codon 72 on cervical carcinogenesis. The discrepancies among the results have been attributed to several factors, including the occurrence of E6 variants with distinct characteristics according to the geographic region, ethnical variation within studied populations, number of analyzed samples, Hardy-Weinberg equilibrium status in the populations and the methodologies employed for the polymorphism detection (13, 16, 20, 21). In statistical analyses, different authors have used different genotypes or genotypic combinations as reference categories when calculating ORs to estimate risks. A meta-regression analysis identified that the most important factor contributing to heterogeneity among results for invasive lesions was deviation from Hardy-Weimberg equilibrium in the control group (16). In the present study, the distribution of alleles fit the Hardy-Weimberg equilibrium in all groups.

In the northeast of Brazil, results obtained from a survey with patients from the João Pessoa city (20) suggested that arginine homozygous would confer a higher susceptibility for the development of HPV-associated cervical tumors than other genotypes. HPV DNA was identified in 53/ 73 (72,6 %) of patients. Although the occurrence of HPV-negative cervical cancers is controversial and this level of detection is low, it is compatible with the detection method used (3, 36). High risk HPV was detected in 45/53 (84.9%) HPV-positive patients. The most prevalent HPV type was HPV 16 (64%). The others HPV types detected were: 18,31,33,45,52,53,58, 66; all in low frequencies. In 2 patients, the HPV type was not identified and in 7 HPV positive patients, the HPV type was not determined. Three of the women were infected simultaneously by two types of HPV. The HPV status of our controls has not been determined in the present study but we have previously detected 24.5% HPV infection in women without cervical lesions from this population. The low incidence of HPV 18 have already been described in Recife, Brazil (19). When comparing the healthy women group with only the patients whose HPV DNA was identified in the tumor, there was a lack of a statistically significant cancer risk of arginine homozygous over other genotypes (A2/A2: A1/A1+ A1/A2; OR=1.05; CI 95%= 0.43-2.55, p=1.000). The differences in the allelic frequencies between patients with cancer and healthy women were not statistically significant (χ^2=0.05; p= 0.823; DF=1). However, there were statistically significant differences in the

genotypic distribution (p=0.023) between both groups, related to a higher frequency of heterozygotes among cancer patients and an increase of proline homozygotes among healthy women (Table 2). There was an increased risk in heterozygous for Arg/Pro compared with proline homozygotes (OR=5.82; IC95%=1.218-30.780; p=0.024). Similar results were obtained in Peruvian patients (29).

Table 2. Codon 72 polymorphism from p53 gene

Sample		N	Genotype (%)			A1
			A1/A1	A1/A2	A2/A2	
Cancer	HPV +	51	7.8	31.4	60.8	0.235
	HPV-	22	0.0	72.7	27.3	0.364
	Total	73	5.5	43.8	50.7	0.274
Healthy		47	17.0	23.4	59.6	0.287

A1=Pro; A2= Arg

In summary, although our study was performed in a small sample, our data show the presence of differences in allelic frequencies in populations and ethnias from different regions. It also demonstrates that the miscigenation can influence and make it difficult to understand the role of these polymorphism as prognostic factors in cervical cancer.

After sequencing all cancer samples, some discordant points were found in relation to the TP53 sequence (U94788). All sequences displayed a cytosine insertion after positions 11818 and 11874 and the presence of a thymine, instead of an adenine, at position 12400 of the gene in both, cancer patients and healthy women samples. Such divergences at intron 2 from *TP53* sequence were also found in esophageal cancer samples and healthy individuals (35) and in different cell lineages (29).

REFERENCES

1. Abramson J.H., WINPEPI (PEPI-for-Windows): Computer programs for epidemiologists. *Epidemiol. Perspect. Innov.* 2004, **1**(1):6.
2. Bauer, H.M., Yi Ting, M.S., Greer, C.E., Chambers, J.C., Tashiro, C. J., Chimera, J., Reingold, A. and Manos, M.M., Genital human papillomavirus infection in female university students as determinated by PCR-based methods. *JAMA* 1991, **265**:472.
3. Bosch, F.X., Lorincz, A., Muñoz, N., Meijer, C.J.L.M. and Shah, K.V., The causal relation between human papillomavirus and cervical cancer. *J. Clin. Pathol.* 2002, **55**: 244-265
4. Calhoun, E.S., McGovern, R.M., Janney, C.A., Cerhan, J.R., Iturria S.J., Smith D.I., Gostout, B.S. and Persing, D.H., Host Genetic Polymorphism Analysis in Cervical Cancer. *Clin. Chem.* 2002, **48**:1218-1224.
5. Dumont, P., Leu, J.I., Della Pietra 3rd, A.C., George, D.L. and Murphy, M., The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat. Genet.* 2003, **33**:357-365.
6. Felix, C.A., Brown, D.L., Mitsudomi, T., Ikagaki, N., Wong, A., Wasserman, R., Womer, R.B. and Biegel, J.A., Polymorphism at codon 36 of the p53 gene. *Oncogene* 1994, **9**:327-328.
7. Felley-Bosco, E., Weston, A., Cawley, H.M., Bennett, W.P. and Harris, C.C., Functional studies of a germ-line polymorphism at codon 47 within the p53 gene. *Am. J. Hum. Genet.* 1993, **53**:752-759.
8. Gaspar, P.A., Hutz, M.A., Salzano, F.M. and Weimer, T.A., TP53 polymorphism and haplotypes in south ameridians and neo-brazilians. *Annals Hum. Biol.* 2001, **28**:184-194.
9. Ge, H., Lam, W.K., Lee, J., Wong, M.P., Fu, K.H., Yew, W.W. and Lung M.L., Detection and evaluation of p53 intron2 polymorphism in lung carcinomas in Hong Kong. *Int. J. Cancer* 1996, **69**:120-124.
10. http://www.technelysion.com.au
11. Huang, X. A contig assembly program based on sensitive detection of fragment overlaps. *Genomics* 1992, **14**:18-25.
12. IARC, *IARC monographs on the evaluation of the carcinogenic risks to humans*. IARC Scientific Publications, Lyon, 1995.
13. Josefsson, A.M., Magnusson, P.K., Ylitalo, N., Quarforth-Tubbin, P., Ponten, J., Adami, H.O. and Gyllensten, U.B., P53 polymorphism and risk of cervical cancer. *Nature* 1998, **396**:531.
14. Kim, J., Lee, C.G., Park, Y.G., Kim, K.S., Kim, I.K., Sohn, Y.W., Min, H.K., Lee, J.M. and Namkoong, S.E., Combined analysis of germline polymorphism of p53 GSTM1, GSTT1, CYP1A1 and CYP2E1: relation to the incidence rate of cervical carcinoma. *Cancer* 2000, **88**:2082-2091.
15. Klug, S.J., Wilmotte, R., Santos, C., Almonte, M., Herrero, R., Guerrero, I., Caceres, E., Peixoto-Guimaraes, D., Lenoir, G., Hainaut, P., Walboomers, J.M.M. and Muñoz, N., TP53 polymorphism, HPV infection and risk of cervical cancer. *Cancer Epidemiol. Biomarkers Prev.* 2001, **10**:1009-1012.
16. Koushik, A., Platt, R.W. and Franco, E.L., p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review. *Cancer Epidemiol. Biomarkers Prev.* 2004, **13**:11-22.
17. Lahiri, D.K. and Nurnberger Junior, J.I., A rapid non-enzimatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 1991, **19**:5444.
18. Lazar, V., Hazard, F., Bertin, F., Janin, N., Bellet, D. and Bressac, B., Simple sequence repeat polymorphism within the p53 gene. *Oncogene* 1993, **8**:1703-1705.
19. Lorenzato, F., Ho, L., Terry, G., Singer, A., Santos, L.C., Batista, R.L., et al.: The use of human papillomavirus typing in detection of cervical neoplasia in Recife (Brazil). *Int. J. Gynecol. Cancer* 2000, **10**(2): 143-150.
20. Makni, H., Franco, E.L., Kaiano, J. Villa, L.L., Labrecque, S., Dudley, R., Storey, A. and Matlashewski, G., p53 polymorphism in codon 72 and risk of human papilomavirus-induced cervical cancer: effect of inter-laboratory variation. *Int. J. Cancer* 2000, **87**:528-533.
21. Malcolm, E.K., Baber, G.B., Boyd, J.C. and Stoler, M.H., Polymorphism at codon 72 of p53 is not associated with cervical cancer risk. *Mod. Pathol.* 2000, **13**:373-378.

22. Manos, M.M., Ting, Y., Wright, D.K., Lewis, A.J., Broker, T.R. and Wolinsky, S.M., The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cell Mol. Diagnos. Hum. Cancer* 1989, **7**:209-214.
23. Mitra, S., Misra, C., Singh, R.K., Panda, C.K. and Roychoudhury, S., Association of specific genotype and haplotype of p53 gene with cervical cancer in India. *J. Clin. Pathol.* 2005, **58**:26-31.
24. *Molecular cloning: a laboratory manual*, Sambrook, J. and Russel, D.W. (eds.), 3rd ed, Cold Spring Harbor Laboratory Press, New York, 2001.
25. Olivier, M., Eeles, R., Hollstein, M., Khan, M.A., Harris, C.C. and Hainaut, P., The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum. Mutat.* 2002, **19**:607-614.
26. Petitjean, A., Mathe, E., Kato, S., Ishioka, C., Tavtigian, S.V., Hainaut, P. and Olivier, M., Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum. Mutat.* 2007, **28**(6):622-629.
27. Sakamuro, D., Sabbatini, P., White, E. and Prendergast, G.C., The polyproline region of p53 is required to activate apoptosis, but not gowth arrest. *Oncogene* 1997, **15**:887-898.
28. Sanguinetti, C.J., Dias Neto, E. and Simpson, A.J.G., Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 1994, **17**:915-916.
29. Shamsher, M. and Montano, X., Analysis of intron 4 of the p53 gene in human cutaneous melanoma. *Gene* 1996, **176**: 259-262.
30. Själander, A., Birgander, R., Saha, N., Beckman, L. and Beckman, G. p53 polymorphisms and haplotypes show distinct differences between major ethnic groups. *Hum. Hered.* 1996, **46**:41-48.
31. Storey, A., Thomas, M., Kalita, A., Harwood, C., Gardiol, D., Mantovani, F., Breuer, J., Leigh, I.M., Matlashewski, G. and Banks, L., Role of a p53 polymorphism in the development of human papilomavírus-associated cancer. *Nature* 1998, **393**:229-234.
32. Thomas, M., Kalita, A., Labrecque, S., Pim, D., Banks, L. and Matlashewski, G., Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol. Cell. Biol.* 1999, **19**:1092-1100.
33. Unger, T., Nau, M.M., Segal, S. and Minna, J.D., p53: a transdominant regulator of transcription whose function is ablated by mutations occurring in human cancer. *EMBO J.* 1992, **11**:1383-1390.
34. van Duin, M., Snijders, P.J.F., Vossen, M.T.M., Klaassen, E., Voorhorst, F., Verheijen, R.H.M., Helmerhorst, T.J., Meijer, C.J.L.M. and Walboomers, J.M.M., Analysis of human papillomavirus type 16E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. *J. Clin. Virol.* 2000, **81**:317-325.
35. Vos, M., Adams, C.H., Victor, T.C. and van Helden, P.D., Polymorphirms and mutations found in the regions flanking exons 5 to 8 of the TP53 gene in a population at high risk for esophageal cancer in South Africa. *Cancer Genet. Cytogenet.* 2004, **140**:23-30.
36. Walboomers, J.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J., Meijer, C.J. and Munoz, N., Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* 1999, **189**:12-19.
37. Wu, X., Zhao, H., Amos, C.I., Shete, S., Makan, N., Hong, W.K., Kadlubar, F.F. and Spitz, M.R., p53 genotypes and haplotypes associated with lung cancer susceptibility and ethnicity. *J. Nat. Cancer Inst.* 2002, **94**:681-690.
38. zur Hausen, H., Papillomaviruses and cancer: from basic studies to clinical application. *Nat. Rev. Cancer* 2002, **2**:342-350.