Association of p53 polymorphisms with breast cancer: a case-control study in Slovak population

M. FRANEKOVÁ[1*], P. ŽÚBOR[2], A. ŠTANCLOVÁ[3], C. A. DUSSAN[2], T. BOHUŠOVÁ[4], S. GALO[2], D. DOBROTA[4], K. KAJO[3], M. PÉČ [1], P. RAČAY[4]

[1]Department of Biology, Comenius University in Bratislava, Jessenius Faculty of Medicine, Malá Hora 4, 03754 Martin, Slovak Republic, e-mail: franekova@jfmed.uniba.sk; [2]Department of Obstetrics and Gynaecology, Comenius University in Bratislava, Jessenius Faculty of Medicine, Kollárova 2, 03659 Martin, Slovak Republic; [3]Department of Pathological Anatomy, Comenius University in Bratislava, Jessenius Faculty of Medicine, Kollárova 2, 03659 Martin, Slovak Republic; [4]Department of Biochemistry, Comenius University in Bratislava, Jessenius Faculty of Medicine, Malá Hora 4, 03754 Martin, Slovak Republic

Received June 8, 2006

Protein p53 is the tumor suppressor involved in cell cycle control and apoptosis. As a transcription factor p53 controls many cell processes and helps in prevention of cancer development. The p53 gene is polymorphic. Polymorphisms can affect the important regions involved in protein tumor suppressor activity. The well-known polymorphisms are the polymorphisms BstUI in exon 4 and MspI in intron 6. Both are supposed to be associated with cancer development. The purpose of this study was to investigate the genotype frequencies and associations of these polymorphisms with breast cancer in Slovak population.

We observed the prevalence of $BstUI^{Pro}$ (27.47 %) and $MspI^{A1}$ (17.58 %) alleles and $BstUI^{Pro/Pro}$ (8.79 %) and $MspI^{A1/A1}$ (5.49 %) genotypes in breast cancer patients in comparison with controls 23.40 %, 14.10%, 5.77 %, 1.92 % respectively. However the differences were not significant. After division of the cases and controls according to the age the prevalence of the risk alleles and genotypes in women at the age 50 years or less was higher as compared to women older than 50 years. In the younger women group, the p53 BstUI polymorphism genotype frequencies were 6.2 % for $BstUI^{Pro/Pro}$, 31.0 % for $BstUI^{Arg/Pro}$ and 62.8 % for $BstUI^{Arg/Arg}$ in controls and 11.11 %, 40.74 % and 48.15 % in cases respectively. The risk of disease for $BstUI^{Pro/Pro}$ genotype was more than two-fold higher in comparison with the $BstUI^{Arg/Arg}$ (OR=2.34, 95% CI=0.53–10.24). In p53 MspI the genotype frequencies were 1.77 % for $MspI^{A1/A1}$, 24.78 % for $MspI^{A1/A2}$ and 73.45 % for $MspI^{A2/A2}$ in controls and 11.11 %, 18.52 % and 70.37 % in cases respectively. The risk of disease for $MspI^{A1/A1}$ genotype was more than six-fold higher in comparison with the $MspI^{A2/A2}$ (OR=6.55, 95% CI=1.02–41.98). When we evaluated the association of both polymorphisms together with the breast cancer risk we observed that the highest risk was connected with the genotype $BstUI^{Pro/Pro}$ / $MspI^{A1/A1}$ (OR=2.99, 95% CI=0.69 – 13.06).

Our results indicate that both BstUI and MspI p53 polymormphisms might play the role in the breast cancer development especially in women younger than 50 years.

Key words: *p53, polymorphism, BstUI, MspI*

Protein p53 plays important role in human body. It is a sequence-specific transcription factor that can mediate many of its downstream effects by the activation or repression of target genes [1]. Protein is not essential for normal growth and development and it is present at almost undetectable levels in most normal cells [2]. But it responds to the different types of stress signals that can cause oncogenic alterations, such as DNA damage, or conditions that lead into tumor cells development, such as abnormal proliferation [3]. Activation of p53 by these stress signals inhibits cell growth by inducing apoptosis [4] or by arresting cell proliferation in either G1 [5] or G2 phase [6, 7] of the cell cycle. p53 also regulates the process of DNA repair [8] or the process of angiogenesis and metastasis [9].

Several mutations of the p53 gene affecting mainly the core DNA binding region of the protein were described [10]. Mutations of p53 gene, which are the most frequent genetic

* *Corresponding author*

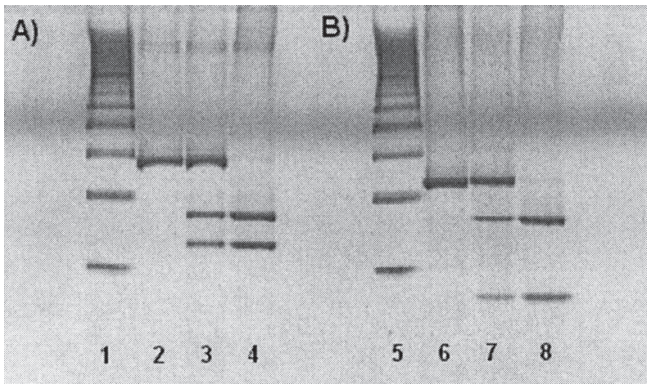

Figure 1. Polyacrylamide gel electrophoresis of p53 MspI PCR products digested with restriction enzymes. A) p53 BstUI polymorphism; lines 1 – 100 bp DNA ladder, 2 – homozygote for Pro allele, 3 – heterozygote, 4 – homozygote for Arg allele; B) p53 MspI polymorphism; lines> 5 – 100 bp DNA ladder, 6 – homozygote for A1 allele, 7 – heterozygote, 8 – homozygote for A2 allele.

alterations detected in human cancers, inactivate the growth regulatory functions and cause a loss of protein tumor suppressor activity. Mutations can also confer tumor-promoting functions, such as the transcriptional activation of genes involved in cell proliferation, cell survival and angiogenesis. Consequently, cells expressing some forms of mutant p53 show enhanced tumorigenic potential with increased resistance to chemotherapy and radiation [11].

Several polymorphisms were detected in wild type p53 both in coding and non-coding regions of the p53 gene [12]. Well known are two types of single nucleotide polymorphism (SNP) BstUI and MspI. The p53 BstUI codon 72 SNP in exon 4 causes amino acid replacement of arginine (CGC) to proline (CCC). Among Caucasians the arginine form is prevalent. It was observed that these two forms are functionally distinct. For example the p53 $BstUI^{Pro}$ is stronger inducer of transcription than p53 $BstUI^{Arg}$ and p53 $BstUI^{Arg}$ appears to induce apoptosis with faster kinetics and suppress transformed cell growth more efficiently than p53 $BstUI^{Pro}$ [13]. p53 $BstUI^{Arg}$ is significantly more susceptible to the degradation induced by human papillomavirus E6 protein than p53 $BstUI^{Pro}$ [14]. p53 BstUI polymorphism influences also individual responsiveness to cancer chemotherapy [15, 16]. It was found that this polymorphism has been associated with several types of cancer, for example lung [17], prostate [18], gastric cancer [19] and others. The p53 MspI SNP in intron 6 represents a polymorphic site within the non-coding region of the p53 gene carrying two alleles, allele A1 and allele A2. A1 allele does not create MspI restriction site. Presence of CCGG sequence in A2 allele creates MspI restriction site [20]. It was indicated that this polymorphism is associated with lung [21] and ovarian cancer [22]. These polymorphisms were also studied in connection with the breast cancer however the results are contradictory [36–48].

In this study we examined the genotypic distribution of p53 codon 72 polymorphism in exon 4 (BstUI) and polymorphism in intron 6 (MspI) in breast carcinoma patients, to investigate the problem whether and how these genetic alterations are associated with an increased breast cancer risk in Slovak women. Although the incidence of breast cancer increases rapidly with age during the reproductive years and then increases at a slower rate after about age 50 years, the average age of menopause [23], it is known that in young women population the breast carcinoma has more aggressive biologic features [24]. It seems that the age is the independent risk factor for survival [25] and for relapse in operable breast cancer patients [26]. Therefore we determined genotypic distribution of both polymorphisms separately among women at the age 50 years or less and among women over 50 years as well.

Material and methods

Sample collection. The studied population included 91 women with histologically proven diagnosis of breast cancer aged 34–82 years (mean 58). Peripheral blood samples were collected at the Department of Gynaecology, University Hospital, Martin. As a control group we used peripheral blood from 156 unselected healthy women population aged 20–71 years (mean 44) recruited at the Department of Gynaecology and at the Department of Haematology and Transfusiology, University Hospital, Martin. The controls had no history of gynaecological and breast disease. Ethics committee of the Jessenius Faculty of Medicine approved the protocol of this study and all samples were obtained with written informed consent. All patients and controls were of Slavic origin (Caucasians) from different regions of Slovakia.

DNA extraction and PCR. Genomic DNA was isolated from 4 ml peripheral blood by salting-out method [27]. Genomic DNA was used for PCR (polymerase chain reaction). The amplification of two fragments of p53 gene was performed in total volume 20 µl. For *BstUI* polymorphism this volume contained 100 ng of genomic DNA, 2 µl of 10 × PCR Mg^{2+}-free buffer, 0,8 µl of $MgCl_2$ solution (50 mM), 0,15 µl of each primer at 40 pM/µl, 0,5 µl of a mixture of dNTPs (each at 10 mM), 0,5 U of Taq polymerase and demineralised water was added to a final volume of 20 µl. Every PCR amplification was carried out with 35 cycles each consisiting of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C. We used sense primer 5'-TTT CAC CCA TCT ACA gTC CC-3' and antisense primer 5'-ACC Tag GCT Cag ggC AAC TgA CCg-3' for amplification of 318-bp PCR product. For *MspI* polymorphism the PCR reaction volume contained the same constituents as mentioned before with addition of 1 µl of DMSO for each reaction. Every PCR amplification was carried out with 35 cycles each consisiting of 1 min at 94 °C, 45 sec at 62 °C and 1 min at 72 °C. We used sense primer 5'-TAT gAg CCg CCT gAg gTC Tgg-3' and antisense primer 5'-TAC Agg CAT gAg CCA CTg

CgC-3' for amplification of 240-bp PCR product. The PCR-products were controlled on 1,5% agarose gel.

Digestion. The PCR products were digested with restriction endonucleases Bst1236I (Fermentas) and MspI (Fermentas) at 37 °C for 2 h. BstUI^{Pro} allele does not contain the BstUI resticion site (318 bp product), BstUI^{Arg} allele was digested into two fragments (182 bp and 136 bp), MspI^{A1} allele does not contain the MspI restriction site (240 bp product), MspI^{A2} allele was digested into two fragments (164 bp and 76 bp). Digested fragments were separated on a vertical 10 % polyacrylamide gel. DNA fragments were stained with ethidium bromide and analysed on UV transluminator using the image analysis system.

Statistical analysis. The Chi-square (χ^2) test was used to determine the significance of differences from the Hardy-Weinberg equilibrium and the independence of genotype frequency between patients and controls. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained from an unconditional logistic regression model. A level of $P < 0.05$ was accepted as statistically significant. All statistical calculations were performed using Microsoft Excel and MedCalc v.5 software for Windows.

Results

All patients and controls were analysed for both polymorphisms. The genotype distribution and alleles frequencies of both polymorphisms are shown in Tables 1 and 2.

The alleles distributions were tested for fit of Hardy-Weinberg equilibrium in controls ($\chi^2 = 0.01$, P = 0.99) for p53 BstUI and ($\chi^2 = 0.02$, P = 0.99) for p53 MspI and in patients ($\chi^2 = 0.13$, P = 0.94) for p53 BstUI and ($\chi^2 = 0.50$, P = 0.78) for p53 MspI. The genotypic distribution values in both groups were in a good agreement with Hardy-Weinberg equilibrium.

In p53 BstUI polymorphism the genotype distributions were 5.77 % for homozygous Pro, 35.26 % for heterozygous and 58.97 % for homozygous Arg in controls and 8.79 %, 37.36 % and 53.85 % in patients respectively. The frequencies of alleles were 23.40 % for Pro allele and 76.60 % for Arg allele in controls and 27.47 % and 72.53 % in patients respectively. The BstUI Pro allele and Pro/Pro genotype frequencies were higher in breast cancer patients group but the differences were not significant (P>0,05). The risk of disease for Pro/Pro genotype was higher in comparison with the genotype Arg/Arg (OR=1.67, 95% CI = 0.61 – 4.60).

In p53 MspI polymorphism the significant differences between cases and controls were also not found (P>0,05). The genotype distributions were 1.92 % for homozygous A1, 24.36 % for heterozygous and 73.72 % for homozygous A2 in controls and 5.49 %, 24.18 % and 70.33 % in patients respectively. The frequencies of alleles were 14.10 % for A1 allele and 85.90 % for A2 allele in controls and 17.58 % and 82.42 % in patients respectively. The risk of disease for A1/A1 genotype was three-fold higher in comparison with the genotype A2/A2 (OR=2.99, 95% CI = 0.69 – 12.94).

Then we conducted a subset analysis according to age. Both cases and controls were divided into two groups. The first group included women at the age 50 years or less, the second group included women over 50 years. The genotype distributions at investigated age groups in both p53 polymorphisms are shown in Tables 1 and 2.

Table 1: The genotype and allele frequencies in p53 BstUI polymorphism

Genotype/Group	**Patients** [n (%)]	**Controls** [n (%)]	P value	χ^2	ORs	95% CI
Frequency distribution						
Allele (n/ %)						
Arg	132 (72.53)	239 (76.60)	0.367	0.815*	1.0	(Ref.)
Pro	50 (27.47)	73 (23.40)			1.24	0.82 – 1.88
Genotype (n/ %)						
Arg / Arg	49 (53.85)	92 (58.97)	0.321	0.985#	1.0	(Ref.)
Arg / Pro	34 (37.36)	55 (35.26)			1.16	0.67 – 2.01
Pro / Pro	8 (8.79)	9 (5.77)			1.67	0.61 – 4.60
Arg/Pro+Pro/Pro	42 (46.15)	64 (48.03)			1.23	0.73 – 2.08
Age						
≤ 50						
Arg/Arg	13 (48.15)	71 (62.8)	0.144	2.136#	1.0	(Ref.)
Arg/Pro	11 (40.74)	35 (31.0)			1.72	0.70–4.22
Pro/Pro	3 (11.11)	7 (6.2)			2.34	0.53–10.24
Arg/Pro + Pro/Pro	14 (51.85)	42 (37.2)			1.82	0.78–4.24
> 50						
Arg/Arg	36 (56.25)	21 (48.8)	0.726	0.122#	1.0	(Ref.)
Arg/Pro	23 (35.94)	20 (46.5)			0.67	0.30–1.50
Pro/Pro	5 (7.81)	2 (4.7)			1.46	0.26–8.19
Arg/Pro + Pro/Pro	28 (43.75)	22 (51.2)			0.74	0.34–1.61

(*P* value, two sided, from χ^2 test for trend)

* (*P* value, two sided, from χ^2 test)

Table 2. The genotype and allele frequencies in p53 MspI polymorphism

Genotype/Group	**Patients** [n (%)]	**Controls** [n (%)]	P value	$\chi 2$	ORs	95% CI
Frequency distribution						
Allele (n/ %)						
A2 150 (82.42)	268 (85.90)	0.366	0.819*	1.0	(Ref.)	
A1 32 (17.58)	44 (14.10)			1.30	0.79 – 2.14	
Genotype (n/ %)						
A2 / A2	64 (70.33)	115 (73.72)	0.317	1.002#	1.0	(Ref.)
A1 / A2	22 (24.18)	38 (24.36)			1.04	0.57 – 1.91
A1 / A1	5 (5.49)	3 (1.92)			2.99	0.69 – 12.94
A1/A1+A1/A2	27 (29.67)	41 (26.28)			1.18	0.67 – 2.10
Age						
≤ 50						
A2/A2	19 (70.37)	83 (73.45)	0.277	1.183#	1.0	(Ref.)
A1/A2	5 (18.52)	28 (24.78)			0.78	0.27–2.28
A1/A1	3 (11.11)	2 (1.77)			6.55	1.02–41.98
A1/A1 + A1/A2	8 (29.63)	30 (26.55)			1.16	0.46–2.94
> 50						
A2/A2	45 (70.31)	32 (74.42)	0.632	0.230#	1.0	(Ref.)
A1/A2	17 (26.56)	10 (23.26)			1.21	0.49–2.98
A1/A1	2 (3.13)	1 (2.32)			1.42	0.12–16.36
A1/A1 + A1/A2	19 (29.69)	11 (25.58)			1.23	0.51–2.93

(*P* value, two sided, from χ^2 test for trend)
* (*P* value, two sided, from χ^2 test)

In p53 BstUI polymorphism we observed the higher risk of disease for $BstUI^{Pro/Pro}$ genotype in comparison with the genotype $BstUI^{Arg/Arg}$ in the group at the age 50 years or less (OR=2.34, 95% CI=0.53–10.24). In the group over 50 years the higher risk of disease for $BstUI^{Pro/Pro}$ genotype was also found but the difference was not as considerable as in the younger women group (OR=1.46, 95% CI=0.26–8.19). These results were not significant for both groups (P>0,05).

In p53 MspI polymorphism the results were similar to that of the BstUI polymorphism. We observed higher frequency of $MspI^{A1/A1}$ genotype in breast cancer patients in both groups. The difference was more prominent in the younger women group. The risk of disease for $MspI^{A1/A1}$ genotype in this group was more than six-fold higher in comparison with the genotype $MspI^{A2/A2}$ (OR=6.55, 95% CI=1.02–41.98). In the group over 50 years the risk of disease for $MspI^{A1/A1}$ genotype was only little higher as compared to $MspI^{A2/A2}$ genotype (OR=1.42, 95% CI=0.12–16.36). However, in both groups the results were not significant (P>0,05). Although the risk of disease for genotype $MspI^{A2/A2}$ among younger women is high, this data are only preliminary because of small number of cases. It is necessary to evaluate larger group of patients and controls to confirm these results.

Simultaneously we evaluated the association of both polymorphisms together with the breast cancer risk. The results are shown in Table 3. The most prevalent genotypes in both patients and controls were $BstUI^{Arg/Arg}$ / $MspI^{A2/A2}$ (53.85 %), $BstUI^{Arg/Pro}$ / $MspI^{A1/A2}$ (20.88 %) and $BstUI^{Arg/Pro}$ / $MspI^{A2/A2}$ (16.48%) in patients and 56.41 %, 18.59%, 16.67% in controls respectively. We observed that the highest risk factor was the genotype p53 $BstUI^{Pro/Pro}$ / $MspI^{A1/A1}$. The risk of disease for $BstUI^{Pro/Pro}$ / $MspI^{A1/A1}$ genotype was almost three-fold higher in comparison with the genotype $BstUI^{Arg/Arg}$ / $MspI^{A2/A2}$ (OR=2.99, 95% CI=0.69–13.06). With regard to small number of subjects we did not evaluate the association of both polymorphisms together in connection with the age to avoid the biased results.

Discussion

Breast cancer is the most common malignancy among females affecting approximately one of ten women. Several molecular alterations have been associated with the development of the disease. The most common are alterations in genes BRCA1 and BRCA2, ATM, p53 [28], H-ras-1 [29], CYP17 [30], CYP19 [31] and others.

p53 is one of the most important tumor suppressor protein. It performs its tumor suppressor function through transcriptional activation or repression of the target genes [32]. Many molecular alterations have been observed in the p53 gene. Mutations in p53 gene are the most frequent molecular alterations detected in human tumors [33]. The IARC TP53 Mutation Database contains the informations about 21 512 somatic and 283 germline mutations of the p53 gene [34]. Human cancers that contain a p53 mutation are more aggressive, more prone to metastasize, and more often fatal [35]. Besides mutations the p53 gene contains some polymorphisms both in coding and non-coding regions [12]. Well known are polymorphisms BstUI and MspI.

In our study we observed the higher frequency of $BstUI^{Pro}$ and $MspI^{A1}$ alleles and $BstUI^{Pro/Pro}$ and $MspI^{A1/A1}$ genotypes in

Table 3. The genotype frequencies in p53 BstUI and MspI polymorphisms

BstUI – MspI Genotype	Patients [n(%)]	Controls [n(%)]	P value	χ^2	ORs	95% CI
Arg/Arg – A2/A2	49 (53.85)	88 (56.41)	0.380	0.770	1.0	(Ref.)
Arg/Arg – A1/A2	0	4 (2.56)				
Arg/Arg – A1/A1	0	0				
Arg/Pro – A2/A2	15 (16.48)	26 (16.67)			1.04	0.50 – 2.14
Arg/Pro – A1/A2	19 (20.88)	29 (18.59)			1.18	0.60 – 2.31
Arg/Pro – A1/A1	0	0				
Pro/Pro – A2/A2	0	1 (0.64)				
Pro/Pro – A1/A2	3 (3.30)	5 (3.21)			1.08	0.25 – 4.70
Pro/Pro – A1/A1	5 (5.49)	3 (1.92)			2.99	0.69 – 13.06

P value, two sided, from χ^2 test for trend

breast cancer patients in comparison with healthy women, however the differences were not significant.

Several authors studied the associations of the BstUI polymorphism with the breast cancer but the results are contradictory. Some authors referred the presence of p53 BstUI^{Pro} allele in genotype as a risk factor for breast cancer development and for the natural history of the disease or patient survival. Noma et al. [36] observed the significant risk of BstUI$^{Pro/Pro}$ genotype for estrogen receptor (ER) positive breast cancer development in Japanese population, whereas the association between BstUI$^{Pro/Pro}$ homozygous genotype and the risk of ER negative breast cancer was not found. Breast cancer patients with BstUI$^{Pro/Pro}$ genotype also seemed to be less sensitive to anthracycline-based chemotherapy [37].

To our knowledge presented results represents for the first time obtained for Middle Europe Caucasian breast cancer patients population. In relation to Slavic origin only one study was published before [38]. In comparison to this Russian study we observed almost the same genotype distributions in the group of breast cancer patients and BstUI^{Pro} allele was considered as a risk allele. The prevalence of BstUI$^{Pro/Pro}$ genotype and BstUI^{Pro} allele in breast cancer patients in comparison to healthy women was observed also in German study [39], significant association with increased risk of disease for BstUI^{Pro} allele carriers was observed in Japanese [40] and Swedish population [41].

Although Tommiska et al. [42] didn't find any association between p53 BstUI polymorphism and breast cancer risk in Finish population, they observed a significantly reduced survival for BstUI$^{Pro/Pro}$ homozygous breast cancer patients. In addition the association between reduction of disease-free and overall survival in BstUI$^{Arg/Pro}$ heterozygous breast cancer patients has been observed as well [43].

On the other hand studies from countries situated in the area of Mediterranean sea (Turkey, Greece and Israel) refer the high prevalence of BstUI$^{Arg/Arg}$ genotype in breast cancer patients. These results observed Papadakis et al. [44] and Kalemi et al. [45] in Greek population, Buyru et al. [46] in Turkish population and Ohayon in Israeli Jewish population [47].

There are not many studies concerning the MspI polymorphism and breast cancer risk. Some authors showed the prevalence of MspI^{A1} allele as the risk factor for breast cancer development as it was in German population [39]. Similarly Weston et al. [48] observed the association of the MspI$^{A1/A1}$ genotype with breast cancer risk in Caucasians. Our results are in the line with these studies. On the other hand Suspitsin et al. [38] didn't find this association in Russian population as well as Sjalander et al. [41] in Swedish population.

Several factors can influence the contradiction of these results. Ethnicity is one possible factor. It was found that the genotype distributions were strongly dependent on ethnicity [12,49]. Small numbers and selection of cases or different methods used in studies could also cause the result differences.

In conclusion our preliminary results showed that both BstUI and MspI polymormphisms could play considerable role in the breast cancer development especially in women younger than 50 years. As the risk alleles we considered BstUI^{Pro} and MspI^{A1} alleles. The highest risk genotypes were genotypes homozygous for these alleles. With regard to fact that there are not many known results from the population of women of Slavic origin, the larger studies are needed to evaluate the role of these polymorphisms in breast carcinogenesis in Slavic population. Especially, in order for assessing the p53 polymorphism status in combination with reproductive and environmental risk factors when counselling individual cancer risk.

Authors are grateful to all patients for their collaboration and technical staff subjects, especially Mrs. Jolana Benčatová and Mrs. Zdenka Cetlová for their technical assistance. This work was supported in part by grants no. aAV/1106/2004, UK/24/2004 and UK/41/2005 by Ministry of Education and by grant no. 2005/14-MFN-06 by the Ministry of Health.

References

[1] RYAN KM, PHILLIPS AC, VOUSDEN KH. Regulation and function of the p53 tumor suppressor protein. Curr. Opin. Cell Biol. 2001; 13: 332–7

[2] DONEHOWER LA, HARVEY M, SLAGLE BL et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356: 215–21
[3] VOUSDEN KH. Activation of the p53 tumor suppressor protein. Biochim. Biophys. Acta 2002; 14: 47–59
[4] SCHULER M, GREEN DR. Mechanisms of p53-dependent apoptosis. Biochem. Soc. Trans. 2001; 29: 684–8
[5] FEI P, EL-DEIRY WS. P53 and radiation responses. Oncogene 2003; 22: 5774–83
[6] TAYLOR WR, DEPRIMO SE, AGARWAL A et al. Mechanisms of G2 arrest in response to overexpression of p53. Mol. Biol. Cell. 1999; 10: 3607–22
[7] FLATT PM, TANG LJ, SCATENA CD et al. p53 regulation of G(2) checkpoint is retinoblastoma protein dependent. Mol. Cell. Biol. 2000; 20: 4210–23
[8] ADIMOOLAM S, FORD JM. p53 and regulation of DNA damage recognition during nucleotide excision repair. DNA Repair (Amst). 2003; 18: 947–54
[9] GASCO M, SHAMI S, CROOK T. The p53 pathway in breast cancer. Breast Cancer Res. 2002; 4: 70–6
[10] BULLOCK AN, FERSHT AR. Rescuing the function of mutant p53. Nat Rev Cancer 2001; 1: 68–76
[11] CADWELL C, ZAMBETTI GP. The effects of wild-type p53 tumor suppressor activity and mutant p53 gain-of-function on cell growth. Gene 2001; 277: 15–30
[12] WU X, ZHAO H, AMOS CI et al. p53 Genotypes and Haplotypes Associated With Lung Cancer Susceptibility and Ethnicity. J. Natl. Cancer Inst. 2002; 94: 681–90
[13] THOMAS M, KALITA A, LABRECQUE S et al. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol. Cell. Biol. 1999; 19: 1092–100
[14] STOREY A, THOMAS M, KALITA A et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998; 393: 229–34
[15] BERGAMASHI D, GASCO M, HILLER L et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003; 3: 387–402
[16] SULLIVAN A, SYED N, GASCO M et al. Polymorphism in wild-type p53 modulates response to chemotherapy in vitro and in vivo. Oncogene 2004; 23: 3328–37
[17] FAN R, WU MT, MILLER D et al. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol. Biomarkers Prev. 2000; 9: 1037–42
[18] WU HC, CHANG CH, CHEN HY et al. p53 gene codon 72 polymorphism but not tumor necrosis factor-alpha gene is associated with prostate cancer. Urol Int. 2004; 73: 41–6
[19] PEREZ-PEREZ GI, BOSQUES-PADILLA FJ, CROSATTI ML et al. Role of p53 codon 72 polymorphism in the risk of development of distal gastric cancer. Scand J Gastroenterol. 2005; 40: 56–60
[20] BIROS E, KALINA I, KOHUT A et al. Allelic and haplotype frequencies of the p53 polymorphisms in brain tumor patients. Physiol Res. 2002; 61: 59–64
[21] BIROS E, KALINA I, KOHUT A et al. Germ line polymorphisms of the tumor suppressor gene p53 and lung cancer. Lung Cancer 2001; 31: 157–62
[22] WANG-GOHRKE S, WEIKEL W, RISCH H et al. Intron variants of the p53 gene are associated with increased risk for ovarian cancer but not in carriers of BRCA1 or BRCA2 germline mutations. Br J Cancer 1999; 81: 179–83
[23] FOTI E, MANCUSO S. Early breast cancer detection. Minerva Ginecol. 2005; 57: 269–92
[24] GONZALEZ-ANGULO AM, BROGLIO K, KAU S-W et al. Women age < or = 35 years with primary breast carcinoma: disease features at presentation. Cancer 2005; 103: 2466–72
[25] JAYASINGHE UW, TAYLOR R, BOYAGES J. Is age at diagnosis an independent prognostic factor for survival following breast cancer? ANZ J Surg 2005; 75: 762–7
[26] HAN W, KIM SW, PARK IA et al. Young age: an independent risk factor for disease-free survival in women with operable breast cancer. BMC Cancer 2004; 4: 82–89
[27] MILLER SA, DYKES DD, POLESKY HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988; 11: 1215
[28] IMYANITOV EN, HANSON KP, Mechanisms of breast cancer. Drug Discov. Today 2004; 1: 235–45
[29] WESTON A, GODBOLD JH. Polymorphisms of H-ras-1 and p53 in breast cancer and lung cancer: a meta-analysis. Environ Health Perspect 1997; 105: 919–26
[30] HONG C-C, THOMPSON HJ, JIANG C et al. Association between the T27C polymorphism in the cytochrome P450 c17alpha (CYP17) gene and risk factors for breast cancer. Breast Cancer Res Treat 2004; 88: 217–30
[31] AHSAN H, WHITTEMORE AS, CHEN Y et al. Variants in estrogen-biosynthesis genes CYP17 and CYP19 and breast cancer risk: a family-based genetic association study. Breast Cancer Res 2005; 7: 71–81
[32] LU X. p53: a heavily dictated dictator of life and death. Current Opinion in Genetics & Development 2005; 15: 27–33
[33] GOMEZ-LAZARO M, FERNANDEZ-GOMEZ FJ, JORDAN J. p53: twenty five years understanding the mechanism of genome protection. J Physiol Biochem 2004; 60: 287–307
[34] OLIVIER M, EELES R, HOLLSTEIN M et al. The IARC TP53 Database: new online mutation analysis and recommendations to users. Hum Mutat. 2002; 19: 607–14 (R10, July 2005 is the latest version of the database)
[35] MORI N, DELSITE R, NATARAJAN K et al. Loss of p53 Function in Colon Cancer Cells Results in Increased Phosphocholine and Total Choline. Molecular Imaging 2004; 4: 319–323
[36] NOMA C, MIYOSHI Y, TAGUCHI T et al. Association of p53 genetic polymorphism (Arg72Pro) with estrogen receptor positive breast cancer risk in Japanese women. Cancer Lett 2004; 210: 197–203
[37] XU Y, YAO L, OUYANG T et al. p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005; 11: 7328–33
[38] SUSPITSIN EN, BUSLOV KG, GRIGORIEV MY et al. Evidence against involvement of p53 polymorphism in breast cancer predisposition. Int J Cancer 2003; 103: 431–3

[39] WANG-GOHRKE S, BECHER H, KREIENBERG R et al. Intron 3 16 bp duplication polymorphism of p53 is associated with an increased risk for breast cancer by the age of 50 years. Pharmacogenetics 2002; 12: 269–272

[40] HUANG XE, HAMAJIMA N, KATSUDA N et al. Association of p53 codon Arg72Pro and p73 G4C14-to-A4T14 at exon 2 genetic polymorphisms with the risk of Japanese breast cancer. Breast Cancer 2003; 10: 307–11

[41] SJALANDER A, BIRGANDER R, HALLMANS G et al. p53 polymorphisms and haplotypes in breast cancer. Carcinogenesis 1996; 17: 1313–1316

[42] TOMMISKA J, EEROLA H, HEINONEN M et al. Breast Cancer Patients with p53 Pro72 Homozygous Genotype Have a Poorer Survival. Clinical Cancer Research 2005; 11: 5098–5103

[43] BONAFÉ M, CECCARELLI C, FARABEGOLI F et al. Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. Clin Cancer Res 2003; 9: 4860–4

[44] PAPADAKIS EN, DOKIANAKIS DN, SPANDIDOS DA. p53 codon 72 polymorphism as a risk factor in the development of breast cancer. Mol Cell Biol Res Commun 2000; 3: 389–92

[45] KALEMI T, LAMBROPOULOS A, GUEORGUIEV M et al. 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; 10: 57–65

[46] BUYRU N, TIGLI H, DALAY N. P53 codon 72 polymorphism in breast cancer. Oncol Rep. 2003; 10: 711–4

[47] OHAYON T, GERSHONI-BARUCH R, PAPA MZ et al. The R72P P53 mutation is associated with familial breast cancer in Jewish women. Br J Cancer. 2005; 92: 1144–8

[48] WESTON A, PAN CF, KSIESKI HB et al. p53 haplotype determination in breast cancer. Cancer Epidemiology Biomarkers & Prevention 1997; 6: 105–112

[49] SIDDIQUE MM, BALRAM C, FISZER-MALISZEWSKA L et al. Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer development. Cancer Epidemiol Biomarkers Prev 2005; 14: 2245–52