Significance of amino acid substitution variants of DNA repair genes in radiosusceptibility of cervical cancer patients; a pilot study

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The present pilot study was designed to elucidate the functional significance of amino acid substitution variants of DNA repair genes. Using the peripheral blood lymphocytes (PBLs) from healthy donors and cervical cancer patients, the contribution of four non-synonymous single nucleotide polymorphisms (SNPs) in three base excision repair genes (BER), *XRCC1* (*Arg194Trp* and *Arg399Gln*), *hOGG1* (*Ser326Cys*), and *APE1* (*Asp148Glu*), to the susceptibility to ionizing radiation were evaluated. The level of initial, oxidative and residual DNA damage produced by 2 Gy was measured by the alkaline single cell gel electrophoresis (the comet assay), and the SNPs were determined by PCR-restriction fragment length polymorphism (RFLP) assay.

No significant differences in the allele frequencies between cancer patients and controls for any of these four SNPs were detected. Although the initial DNA damage levels were approximately similar, significantly higher level of Fpg-sensitive sites were found in patients compared with controls ($p<0.001$) irrespective of genotype distribution. A trend towards increased values of EndoIII-sensitive sites was determined in PBLs from cancer patients compared with healthy women, mainly carriers of the *XRCC1* and *OGG1* variant alleles; however, the mean value of EndoIII-sensitive sites does not reach any significance. A substantial delay in DNA strand-break rejoining was ascertained in patients who carried *APE1 Glu* variant allele in comparison with healthy donors 15 and 60 minutes after irradiation ($p< 0.05$ and $p< 0.01$, respectively). In contrast, slightly higher but statistically significant level of residual DNA damage was estimated in controls (*APE1Asp/Asp*) compared with patients.

An association between single nucleotide polymorphism (SNP) of two DNA repair genes functioning in the same biochemical pathway and susceptibility to radiation was found. In the combined genotype *APE1/XRCC1* and *APE1/hOGG1*, a decreased level of residual DNA damage was detected in carriers of wild type *APE1* genotype. In addition, a possible modulating effect of *hOGG1* gene on the kinetics of strand-break rejoining was estimated. The lowest residual DNA damage level was determined in subjects with the combined *APE1(Asp/Asp)/hOGG1(Ser/Cys+Cys/Cys)* genotypes.

Based on these preliminary data we suppose that a combination of several amino acid substitution variants of DNA repair genes involved in the same repair pathway rather than one low-penetrance SNP in a single gene may contribute to DNA repair outcomes. Larger study with more subjects is needed to verify these findings.

Key words: single nucleotide polymorphism, gene-gene interactions, DNA repair genes, radiosensitivity, cervical cancer patients

Ionizing radiation (IR) produces different types of damage into DNA. Besides single and double strand breaks, there are various base and sugar-phosphate backbone modifications, as well as DNA-DNA and DNA-protein cross-links. Elimination of these lesions is essential for cell survival therefore different repair systems have been developed during the evolution, which are preferentially involved in the removal of one or several types of DNA damage. At least three DNA repair mechanisms are participating in the elimination of DNA lesions produced by IR. Single strand breaks and base modifications are repaired by the base excision repair mechanism (BER) while double strand breaks and cross-links, depending on the cell cycle, are removed by homologous recombination (HR) or non-homologous end joining (NHEJ) mechanisms. Around 130 genes are involved in the repair of various types of DNA damages [1]. Some genes play a key role in particular DNA repair mechanism (core genes) others participate in several repair pathways

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(auxiliary genes). In many of these genes, the single nucleotide polymorphisms (SNPs) have been determined which are responsible for differences in phenotypic expression of the same gene [2]. SNPs were found in protein-coding regions, in intron regions which are in close proximity to exons and in 5´and 3´ non-transcribed (UTR) gene regions. SNPs located in non-coding, regulatory regions of genes may be functional by affecting the binding of transcription factors. Therefore the repair capacity of the proteins could be impacted at different levels of gene expression (transcription, splicing, and translation). Numerous epidemiological studies have consistently demonstrated that the SNP of DNA repair genes may predispose individuals to certain types of cancer and even might underlie the variability of individual responses to cancer treatment [3].

The apurinic/apyrimidinic endonuclease/redox-factor 1 (Ape1/Ref-1) is a bifunctional protein which is involved in the repair of DNA damage as well as in the transcriptional regulation of genes. The repair function of the protein is located on its C-terminal region and plays a central role in the repair of AP sites that arise from the loss of bases through spontaneous processes or during the DNA repair process, via the endonuclease and 3´-phosphodiesterase activities [4]. A ~6 kDa N-terminal domain is of important relevance for the redox activation of different transcription factors such as AP-1 or p53 involved in regulation of gene expression [5]. The *APE1* gene is located on chromosome 14q11.2-q12 and encodes a protein of 318 amino acids. Several sequence variants have been identified in the *APE1* gene from which a G→T change in exon 5, codon 148, leads to an amino acid change from aspartic acid to glutamic acid (*Asp148Glu*). This polymorphism was shown to be associated with increased risk of lung cancer in Japanese smokers [6], however, a non-significant decrease in the risk of non-small cell lung cancer was determined in a case-control study in Germany [7]. On the other hand no overall association has been found between the *APE1* genotype and bladder cancer risk [8].

The 8-oxoguanine DNA glycosylase (hOgg1) is a key repair enzyme in base excision repair (BER) pathway. The *hOGG1* gene is located on chromosome 3p26.2. Two kinds of hOgg1 proteins, αhOgg1 and βhOgg1 (345 and 424 amino acids) which are targeted to the nucleus and the mitochondrion, respectively, are formed due to an alternative splicing after transcription [9]. The nuclear αhOgg1 protein is a DNA glycosylase/AP lyase which incises one of the most mutated lesions among base modifications, 7,8-dihydro-8-oxoguanine (8-oxoG) from damaged DNA. At least 20 sequence variants have been described in the *hOGG1* gene (http://egp.gs.washington.edu) from which a C→G sequence variant leading to an amino acid change from serine to cysteine in exon 7, codon 326 (*Ser326Cys*) has been studied most frequently. Inconsistent data have been obtained from the epidemiological studies. Significantly increased risk for variant *hOGG1* genotypes was observed in all non-small cell lung cancer patients [10], orolaryngeal cancer [11] or prostate cancer [12], however, no contribution of *Ser326Cys* polymorphism to the risk of hepatocellular carcinoma among Japanese [13], breast cancer [14] and lung cancer [15] was found. An inversely association, i.e. a reduce risk for prostate cancer, was estimated for *326Cys/Cys* genotype among men with more aggressive prostate cancer [16].

The X-ray cross complement group 1 (Xrcc1) protein is essential for mammalian viability. This protein has any known enzymatic activity but act as a scaffold protein for both single-strand break repair and base-excision activities. Three interactive domains have been identified in Xrcc1, plus a nuclear localization signal and a phosphorylation site for Ck2 [17]. The Xrcc1 protein physically interact with DNA polymerase β via N-terminal domain, (NTD), polyadenosine diphosphate-ribose polymerases (PARP) 1 and 2 bind to Brct1 domain and Brct2 domain is an interactive binding site for DNA ligase 3 [18]. The *XRCC1* gene lies on the chromosome 19q13.2-13.3 and spans a genomic distance of 32 kb. More than 60 single nucleotide polymorphisms in *XRCC1* are listed (http://egp.gs.washington.edu) from which a C→T variant on exon 6, codon 194 which leads to amino acid change from arginine to tryptophan (*XRCC1 Arg194Trp*) and a G→A variant on exon 10, codon 399 resulting in amino acid change from glutamine to arginine (*Arg399Gln*) are the most studied. Although an association was found between the *Arg399Gln* polymorphism and increased risk for pancreatic cancer [19], gastric cancer [20] and prostate cancer [6], no association was determined for esophageal and bladder cancer [21], breast cancer [22], lung cancer [23] and malignant lymphoma [24]. In contrast, the *Arg194Trp* variant is associated with a reduced risk of lung cancer among smokers [25] and a weak association was found with head and neck cancer [26]. Moreover, significant protective effects of this sequence variant against cervical and endometrial cancer but increased risk for radiation toxicity have been reported [27].

Radiotherapy (RT) is the key treatment modality used in an effort to improve survival rate of cervical cancer patients. The main obstacle to augment the patient outcome is a substantial heterogeneity of patients in response to RT. The inter-individual distinctions may arise from differences in the molecular events taking place during the biochemical cascade triggered by exposure to ionizing radiation [28]. It is thought that part of these differences are probably due to (a) genetically determined intrinsic differences in the cellular response to damage produced by radiation and (b) individual capacity to repair DNA damage in particular.

The objective of this study was to investigate the relationship between polymorphisms in the DNA repair genes *XRCC1*, *APE1* and *OGG1* and the DNA damage level and rate of DNA repair. The level of initial, oxidative and residual DNA lesions in peripheral blood lymphocytes from cervical cancer patients (18) and healthy subjects (30) irradiated in vitro with 2 Gy was measured by the alkaline single cell gel electrophoresis (the comet assay). The aims of this pilot study were first to investigate the impact of particular sequence variant of DNA repair genes on DNA damage removal and second to

evaluate the effect of gene-gene interactions on cellular response to IR.

Materials and Methods

Subjects. Blood samples from 18 consecutive cervical cancer patients were collected at the Department of Radiotherapy of the National Cancer Institute, Bratislava, Slovak Republic. Tumor characteristics and therapy regimen of cervical cancer patients was described elsewhere [29]. As the control group we included 30 women, who were registered at the National Blood Transfusion Station, Ružinov Hospital, Bratislava. Approval by the local ethics committee was provided before recruitment of the patients and only those women (patients and healthy donors) who provided written informed consent were included in this study. Distribution of the genotypes within cancer patient group and healthy donors is shown in Table 1. A structured questionnaire was used to obtain all the relevant information about patient age, health status and dietary habits.

Cell preparation. Blood samples were immediately transported to the laboratory. Peripheral blood lymphocytes (PBL) were isolated by centrifugation in a density gradient of Lymphocyte Separation Medium. The pellet containing PBL was re-suspended twice in Phosphate Buffered Saline (PBS) and centrifugated. The sediment containing PBL after the last centrifugation was re-suspended carefully in a 10% DMSO in fetal bovine serum. The lymphocytes were kept on ice for 30 minutes and after that were frozen at -80°C.

DNA isolation and genotyping. Genomic DNA was isolated from lymphocytes using the phenol/chloroform extraction method. The polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were used for genotyping analyses. Polymorphisms in *XRCC1* codon 194 and codon 399 were analyzed according to Lunn et al. [30], *APE1* polymorphism was determined by method of Hu et al [31], and *hOGG1* polymorphism as described by Le Marchand et al. [32].

Table 1. Distribution of genotypes and allele frequence in patients and healthy controls

	Cases (%)	Controls (%)
XRCC1 codon 194	N=17	N=30
Arg/Arg	14 (82)	24 (80)
Arg/Trp + Trp/Trp	3 (18)	6 (20)
Frequency of variant allele	0.09	0.1
XRCC1 codon 399	N= 18	N=30
Arg/Arg	8 (44)	11 (37)
Arg/Gln + Gln/Gln	10 (56)	19 (63)
Frequency of variant allele	0.3	0.35
hOGG1	N=18	N=25
Ser/Ser	10 (55)	10 (40)
Ser/Cys + Cys/Cys	8 (45)	15 (60)
Frequency of variant allele	0.24	0.30
APE1	N=16	N=22
Asp/Asp	5 (31)	3 (14)
Asp/Glu + Glu/Glu	11 (69)	19 (86)
Frequency of variant allele	0.345	0.45

Single cell gel electrophoresis. Single cell gel electrophoresis (comet assay) was performed as described by Singh et al. [33] with modifications by Gábelová et al. [34]. A base layer of 100 µl of 1.0 % normal melting point (NMP) agarose in phosphate buffer (PBS, Ca^{2+} and Mg^{2+} free) was placed on microscope slides precoated with 1% NMP agarose in water and dried at 50°C. Lymphocytes suspended in 0.75% low-melting point (LMP) agarose were spread on a base layer and irradiated on slides with a dose of 2 Gy. Immediately after irradiation the slides were placed in a lysis mixture (2.5 mM NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 1% Triton X-100, pH 10) for 1h at 4°C to remove cellular proteins (zero min) or put into RPMI 1640 culture medium to allow DNA damage removal. Later on, 15 and 60 minutes after irradiation they were processed as described above. After the lysis, slides were transferred to an electrophoretic box containing an alkaline solution (300 mM NaOH, 1 mM Na2EDTA, pH>13). After 40 min unwinding time, at 4°C, a voltage of 25 V (current 300 mA) was applied for 30 min. The slides were removed, neutralized with Tris-HCl (0.4 mM, pH 7.5) and stained with 20µl ethidium bromide (EtBr, 10 µg/ml). The slides were examined with the Olympus BX51 fluorescence microscope by image analysis using the software Komet 5.5 (Kinetic Imaging, Ltd., Liverpool, UK). The percentage of DNA in the tail (% tail DNA) which is linearly related to the frequency of DNA breaks was used to assess the DNA damage level. A total of 100 nucleoids (triplicate of slides) were scored per each sample in one electrophoretic run. PBLs of one healthy female were used as a reference sample; lymphocytes were irradiated and treated in parallel with the patient and healthy cohort samples in each experiment. Data of the reference sample were used to calculate experimental variability and the cut-point for exclusion of the experimental data from the statistical analysis [35].

The level of residual DNA damage (RD) was calculated according to Aka et al. [36]. The percentage of DNA damage remaining at a given repair time (tx) was defined as follows:

$$RD= [TD_{(tx)} - TD_{(c)}/TD_{(0)} - TD_{(c)}] \times 100$$

Where: RD is residual DNA damage, TD(c) is the % tail DNA prior to irradiation, $TD_{(0)}$ is the % tail DNA immediately after irradiation, $TD_{(tx)}$ is the % tail DNA at a given time x (15 and 60 min) after irradiation.

Oxidative DNA damage detection. The procedure of Collins et al. [37] was used to determine the level of oxidative DNA damage in PBLs after irradiation with 2 Gy. After lysis the slides were washed two times in endonuclease enzyme buffer (40 mM HEPES-KOH, 0.1 KCl, 0.5 mMEDTA, 0.2 mg/ml bovine serum albumin, pH 8.0), drained and incubated with

Table 2. Mean values of DNA damage and oxidative DNA damage in control and patient cohorts according to individual's genotypes

		Initial DNA damage		EndoIII		FPG	
Genotype		Controls	Patients	Controls	Patients	Controls	Patients
XRCC1 194	wt	19.47 ± 3.04	22.85 ± 5.91	4.90 ± 3.47	9.09 ± 5.52**	5.99 ± 4.54	10.21 ± 6.85
	Variants	20.31 ± 2.89	21.49 ± 1.85	7.24 ± 5.14	15.27 ± 4.74	8.39 ± 6.00	12.51 ± 1.44
XRCC1 399	wt	19.52 ± 3.11	22.29 ± 6.36	5.35 ± 3.78	8.59 ± 6.06	6.27 ± 4.79	10.48 ± 7.19
	Variants	19.75 ± 2.98	22.10 ± 5.15	4.78 ± 3.34	11.12 ± 5.55**	6.13 ± 4.38	9.82 ± 5.41
OGG1	wt	20.10 ± 4.21	23.81 ± 6.24	5.70 ± 4.22	9.98 ± 5.25	7.07 ± 5.57	10.63 ± 5.37
	Variants	19.35 ± 2.65	20.16 ± 3.98	4.99 ± 4.16	10.88 ± 7.10*	5.83 ± 4.6	11.37 ± 7.7
APE1	wt	19.25 ± 0.82	23.82 ± 5.76	13.64 ± 4.37	5.41 ± 3.06*	5.03 ± 4.86	10.31 ± 5.59
	Variants	20.19 ± 3.12	22.41 ± 5.56	9.53 ± 5.53	5.4 ± 4.18*	7.05 ± 4.97	11.43 ± 6.75
Mean values		19.74 ± 0.41	22.37 ± 1.2	7.02 ± 3.12	9.47 ± 3.22	6.47 ± 1.01	10.85 ± 0.87***

Student t-test control vs. exposed population for a given genotype
Values represent mean ± SD, * p< 0.05; ** p< 0.01; *** p< 0.001

Table 3. The level of residual damage (RD) in PBLs from cancer patients 15 (RD15) and 60 (RD60) minutes after irradiation

		RD15 (%)		RD60 (%)	
Genotype		Controls	Patients	Controls	Patients
XRCC1 194	wt	44.34 ± 15.2	52.87 ± 21.53	11.58 ± 11.25	16.13 ± 9.97
	Variants	45.43 ± 10.56	43.3 ± 17.9	8.27 ± 2.03	15.93 ± 9.985
XRCC1 399	wt	42.74 ± 18.61	66.68 ± 29.53	11.07 ± 7.32	13.78 ± 10.26
	Variants	45.73 ± 10.87	45.26 ± 17.34	11.41 ± 12.04	17.95 ± 9.29
hOGG1	wt	43.81 ± 8.66	57.9 ± 18.79	23 ± 4.65	18.71 ± 10.68
	Variants	44.01 ± 18.29	50.88 ± 32.62	20.62 ± 10.95	12.83 ± 7.65
APE1	wt	62.3 ± 4.5†	49.34 ± 18.29	25.3 ± 24.8†	13.22 ± 8.84
	Variants	43.35 ± 13.97	61.61 ± 27.3*	10.14 ± 6.1	19.56 ± 8.85**

Student t-test control vs. exposed population for a given genotype (*), between wt vs. variants in control or patients (†). Values represent mean ± SD, * p< 0.05; ** p< 0.01, †p <0.05

repair specific DNA endonucleases, endonuclease III (EndoIII) for 45 min and formamidopyrimidine glycosylase (Fpg) for 30 min at 37° C. Then the slides were processed as described above. The relative level of oxidative damage was expressed as net endo III- and Fpg-sensitive sites by subtracting the value of DNA damage determined in nucleoids incubated with endonuclease buffer alone.

Statistics

Data are given as mean values with ±SD. The inter-patient variation and differences between healthy donors and cancer patients were evaluated by the Student's *t*-test. The threshold of statistical significance was set at p<0.05.

Results and Discussion

Initial, oxidative and residual DNA damage and the genetic polymorphism. No significant difference in the allele frequencies between cervical cancer patients and age-matched healthy women for any of the four SNPs was found (Table 1); however, the limited number of subjects involved in this pilot study might affect the result. Although the association between an inherited defective DNA repair and susceptibility to various forms of cancer has been consistently documented [38, 39], there are studies showing no contribution of SNPs in DNA repair genes to the risk of cancer [8, 14, 15, 13].

Inter-individual variations in the initial, oxidative and residual DNA damage were estimated within both control and patient cohorts. A slightly higher level of initial DNA damage detected in cancer patient group compared to controls was statistically insignificant (Table 2). No substantial variation in the initial DNA damage with respect to genotype distribution was determined among controls and cases. The incubation of whole cell DNA with repair specific DNA endonucleases (Endo-III and Fpg), which cleaves DNA at the sites of oxidized pyrimidines and purines, respectively, has shown differences in the values of oxidative DNA lesions

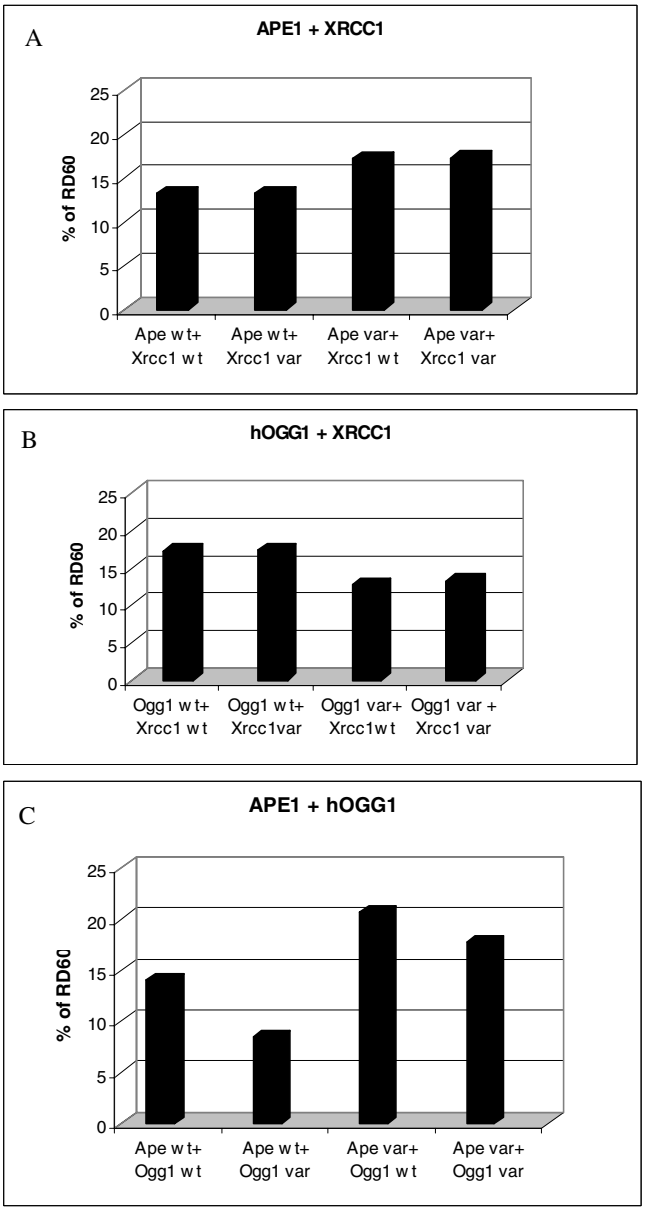

Fig. 1 Association of combined genotypes with the level of residual DNA damage 60 minutes after irradiation (RD60)

between controls and patients in dependence on the genotypes (Table 2). Significantly higher level of Fpg-sensitive sites was found in cervical cancer patients compared with healthy subjects ($p<0.001$) irrespective of the genotype distribution. Moreover, a trend towards substantially higher level of EndoIII-sensitive sites in cancer patients who are carriers of *hOOG1 326Cys* (10.88 ± 7.1 vs. 4.99 ± 4.16; respectively, P=0.03), *XRCC1* 399*Gln* (11.12 ± 5.55 vs. 4.78 ± 3.34, respectively; P=0.002) and *XRCC1 194Trp* (15.27 ± 4.74 vs. 7.24 ± 5.14, respectively) variant alleles was observed. Consistent with our data, Blasiak et al. [40] reported an elevated level of oxidative DNA lesions in cells from cancer patients compared to healthy controls. On the other hand, lower level of Endo-III sensitive sites was detected in patient *APE1* wild type and variant allele carriers (P=0.03 and P=0.034, respectively) compared to control cohort. The level of residual DNA damage (RD), a generally accepted biomarker of cellular repair capacity [35], was utilized to measure an association between PBL repair capacity and distribution of genotypes. The RD values of both cancer patients and healthy subjects was measured 15 (RD15) and 60 (RD60) minutes after irradiation (Table 3). Variations in the RD levels were found between controls and cases as well as within individual groups in dependence on the distribution of the *hOGG1* and *XRCC1* (*codon 194* and *399*) genotypes; however, these differences do not reach any statistical significance. In contrast, an association between RD and *APE1* polymorphism was determined. A substantial delay in DNA strand-break rejoining was ascertained in cancer patients who carried *APE1 Glu* variant allele compared to *APE1* wild type genotype. The RD values determined in cancer patients carrying *APE1* variant allele were significantly higher compared to healthy donors with the same genotype at both time intervals (RD15: 61.61 ± 27.3 vs. 43.35 ± 13.97; respectively, P=0.02; RD60: 19.56 ± 8.85 vs. 10.14 ± 6.1, respectively, P=0.001). On the other hand, significantly slower kinetics of DNA strand break rejoining was detected in healthy subjects with *APE1*wild type compared to carrier of variant genotype 15 min (RD15: 62.3 ± 4.5 vs. 43.35 ± 13.97, respectively; P= 0.03) and 60 min (RD60: 25.3 ± 24.8 vs. 10.14 ± 6.1, respectively; P=0.007) after irradiation.

Despite a great effort to measure the influence of SNPs on the risk of cancer and inter-individual variation in response to cancer therapy [28], the impact of one low-penetrance SNP in a single gene on cancer susceptibility and on cellular radiosensitivity is rather disputable. Several lines of evidence have shown that the combination of several variant alleles may be more effective [41]. Moreover, the combination of multiple sequence variants in the same gene or in various genes functioning in the same biochemical pathway might be more important for the cancer susceptibility or individual sensitivity to radiation [42].

Gene-gene interactions between APE1Asp/Gln, XRCC1Arg/Gln and *hOGG1Ser/Cys.* Consistent with this hypothesis we have focused on the gene-gene interactions between *APE1*, *XRCC1* (*codon 399*) and *hOGG1* genes which are involved in the same BER pathway. The impact of combined genotype on the DNA repair capacity was analyzed only within cancer patient group due to limited number of healthy subjects who were carriers of SNP in individual genes. From the same reason, the statistical analysis was not performed as well.

The association between RD60 and combined genotype of two genes (*APE1/XRCC1*, *hOGG1/XRCC1* and *APE1/hOGG1*) is showed in the Figure 1. It is evident that the *APE1148Glu* variant allele substantially affected the rate of DNA damage removal. A delay of strand-break rejoining (~20%) was detected in subjects who were carrying this vari-

ant allele in combined *APE1/XRCC1(Arg399Gln)* and *APE1/hOGG1* genotypes (Fig. 1A and 1C). These data are in line with the finding of Hu et al. [31] who have determined higher sensitivity to ionizing radiation in subjects carrying the *APE1 148Glu* allele. A small but non-significant decrease of enzymatic activity (94% of wild type) and DNA binding activity (K_d 20.3 vs. 25.8 nM in wild type) was determined for the Ape1-Glu^{148} protein [43]. We can hypothesize that the *APE1* variant allele, despite the insignificant reduced function, may have a lower ability to communicate with other BER proteins giving rise to reduced BER efficacy and thus a potential link to both cancer susceptibility and perhaps radiation sensitivity. Hu et al. [31] assumed that the lower K_d of the Ape1-Glu^{148} protein may imply a higher affinity between APE1 protein and damaged DNA after catalysis and in this way, it can impede normal AP site repair. A potential joint effect of *APE1 148Glu* and *XRCC1 Arg399Gln* variant alleles on increased risk of lung cancer in smokers [6] and cutaneous melanoma [44] has been reported. On the other hand, the *APE1 148Glu* and *XRCC1 399Gln* variant alleles may be protective against the development of acute side effects after radiotherapy among breast cancer patients [45]. An association between *APE1(148Asp/Asp)/XRCC1(399Arg/Gln+Gln/Gln)* combined genotypes and prostate cancer risk in white men but not in black men was found [16].

In contrast to *APE1 148Glu* variant allele, a beneficial effect of the *hOGG1 326Cys* variant allele on the repair phenotype was determined in combined *hOGG1/XRCC1* and *hOGG1/APE1* genotypes (Fig. 1B and 1C). The lowest level of residual DNA damage was detected in subjects with the combined *APE1(Asp/Asp)/hOGG1(Ser/Cys+Cys/Cys)* genotype (Fig. 1C). Although there is no evidence for a direct hOgg1-Ape1 physical interaction, the enzymatic activity of both proteins can be regulated by redox dependent mechanism [46, 5]. The hOgg1 protein possesses eight cysteine residues which can be modulated by oxidative modifications. Two of these cysteines, Cys253 and 255 are present in the active site [47]. In addition these cysteines are surrounded by positively charged amino acids, making them more susceptible to oxidation by the stabilization of the thiolate anion (Cys-S^-) [48]. Oxidation of Ogg1 led to nearly complete inhibition of the DNA glycosylase activity. It is tempting to speculate that the variant hOGG1-Cys^{326} protein howbeit its catalytic constant (k_{cat}/K_m) is approximately 1.6-2-fold lower than the wild type protein [49], is (i) less sensitive to the redox-modifications induced by IR than the wild type protein or (ii) prone to more easily revert the oxidized cysteine forms to yield a non-modified protein more rapidly. Moreover Ape1/Ref-1, the specific redox regulatory bifunctional protein plays role in controlling of protein thiol oxidation and reversion reaction [50]. However other explanation including the impact of the Xrcc1 protein cannot be excluded. Although devoid of known enzymatic activity, Xrcc1 functions as a scaffold protein and through multiple protein-protein interactions extends its coordinating role from the base excision step to the resealing of the repaired DNA strand. It is proposed that Ape1 and hOgg1 both interact with Xrcc1 via the linker central area between the NTD and BRCT1 domains [51]. The Xrcc1-hOgg1 interaction results in a 2- to 3-fold stimulation of the DNA glycosylase activity and stabilization of hOgg1 on the AP site generated by the excision of the modified base until Ape1 is able to bind to the DNA allowing a coordinated transfer of the DNA substrate from the DNA glycosylase product to the AP endonuclease, i.e. Ape1 displaces hOgg1 from the AP site [51]. We can hypothesize that another gene-gene interaction or linkage to another relevant polymorphism can explain the protective role of *hOGG1* variant allele. Several studies have clearly demonstrated that *XRCC1Arg194Trp* polymorphism is associated with reduced risk of cancer [18, 25, 42] though the 194 position is paradoxically within the Ape1 and hOgg1 binding domain [51] that is rich in proline, serine, arginine and lysine residues. The change to tryptophan represents a transition from positively charged arginine to a hydrophobic tryptophan, which could affect binding and DNA repair efficiency. The limited number of subjects does not allow evaluate the genotype-phenotype relationship of these three *APE1/hOGG1/XRCC1* genes. Cellular functional studies will be, however, necessary to help resolve this issue. The genetic variant of *XRCC1Arg399Gln* is localized within the Brct1 C-terminal domain, which interacts with PARP1 and 2 proteins [18, 25, 42], therefore this SNP may have functional significance. In the meta-analysis review by Hu et al. [25], there was no overall association of genotype to cancer risk or cancer protection. In contrast, reduced risk of prostate cancer was determined in Caucasians who were carrying the combined *hOGG1 326Cys* and *XRCC1 codon399 Arg/Arg* or *Arg/Gln* genotype [16]. In agreement with this fact a reduced level (~20%) of residual DNA damage was detected in carriers of the *hOGG1* variant allele in combined *hOGG1/XRCC1399* genotype (Fig. 1B). Moreover, the protective effect of combined *XRCC1 194Trp* and *XRCC1 399Gln* variant alleles has been shown in bladder cancer and colorectal cancer [45].

In addition to genetic polymorphisms other factors including a viral infection may impact the cellular repair capacity. The E6 oncoprotein of the high-risk human papillomavirus (HPV) 16, which is present in at least 50% of all cervical carcinomas worldwide, interacts with the Xrcc1 protein [52]. A potential interacting binding site for E6 oncoprotein is the NTD between amino acides 107 and 170 which partially overlaps with the binding domain for DNA polymerase β [53]. Therefore interaction between the E6 oncoprotein and Xrcc1 could result in a displacement of the polymerase from the complex and consequently in the inhibition of DNA repair [52].

In brief, no significant difference in the allele frequencies between patients and controls for any of the four SNPs and the level of initial DNA damage was found. A trend towards higher level of oxidative DNA lesions was observed in cancer patients who are carriers of variant alleles compared to con-

trols which might suggest an impairment of oxidative damage removal. A possible gene-gene interaction between *APE1* and *hOGG1* genes in association with DNA repair phenotype has been determined. Because of limited number of subjects it was not possible to evaluate the contribution of combined *APE1/XRCC1/hOGG1* genotype on repair capacity. Future larger studies are warranted to further verify the functional significance of these genotypes. In addition, the enlargement of the sample size subject would be helpful in stratifying the individuals according to age, gender and smoking status.

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