Radiosensitivity of cervical cancer cell lines: the impact of polymorphisms in DNA repair genes

S. GURSKÁ, T. FARKAŠOVÁ, A. GÁBELOVÁ*

Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute SAS, Vlárska 7, 833 91 Bratislava, Slovakia e-mail: exongaba@savba.sk

Received October 2, 2006

The aim of this study was to evaluate radiosensitivity of cervical cancer cells *in vitro* and to assess the relationship between genetic polymorphisms in DNA repair genes and the response of cells to ionizing radiation. The alkaline comet assay as a predictive assay of radiosensitivity was used to examine the susceptibility of four human cervical cancer cell lines (CaSki, C-33A, HeLa and SiHa) to radiation damages. The initial DNA damage and the residual DNA damage at 15, 30, 45 and 60 min after irradiation were assessed. Genotypes of DNA repair genes (*XRCC1, hOGG1, PARP, XPD, XRCC3* and *XRCC4*) were analyzed by PCR-RFLP assays. The comet data clearly indicate a variable but dose-dependent increase in the initial DNA damage in all cell lines. The highest slope of dose response curve was observed in C-33A cells and this cell line was assumed to be radiosensitive. All cell lines repaired DNA damage in a similar manner, the level of DNA strand breakage has returned near the background level within 45 min after irradiation. According to the genotype we found that C-33A cells are polymorphic in the majority of analyzed DNA repair genes. This pilot study indicated associations between polymorphisms in DNA repair genes and cell radiosensitivity.

Keywords: cellular radiosensitivity, DNA damage, DNA repair, DNA repair gene polymorphisms

Radiation therapy (RT) is common approach to the treatment of malignant tumors and is often the only one possible approach in the therapy. Nearly 70 % of all cancer patients have to undergo RT during the treatment and despite recent radiotherapy protocols, the cancer deaths still occurred as a direct result of in-field treatment failure. Therefore the problem of predicting radiosensitivity is still of essential importance for cancer patients.

Actually ionizing radiation (IR) is even an ever-present hazard to humans primarily due to its mutagenic, carcinogenic and cell killing ability. Biologically the most significant lesions produced directly by IR in DNA are double-strand breaks (DSB). Indirect effects of IR cause a broad range of different DNA lesions through reactive species generated by radiation energy. In addition to causing DNA damage, irradiation initiates a plethora of signal transduction cascades responsible for maintaining cellular homeostasis and promoting interactions with neighboring cells.

The inter-individual variations in response to RT may be inherited, result of alteration in gene expression induced by epigenetic factors, or determined by the variations in genes involved in the DNA damage recognition, DNA repair and or in regulation of cell cycle. As the DNA repair system plays an important role in protection against mutagenesis and carcinogenesis, the single nucleotide polymorphisms (SNP) occurred within functional domains of essential DNA repair proteins may significantly contribute to patients' inter-individual difference to the radiation treatment. SNP, when located within the coding and/or regulating regions of the gene, can also harm DNA repair capacity due to the diminished protein expression or amino acid substitution and consequently in changes of enzymatic or binding activity. Several studies confirmed the association between clinical radiosensitivity and polymorphisms of some genes, for instance *ATM* [1], *XRCC1* [2, 3], *XRCC3* [3, 4], *XRCC5* [4] and *TGFB1* [5].

Abbreviations: BER, base excision repair; DSB, double strand breaks; HPV, human papilloma virus; IR, ionizing radiation; NER, nucleotide excision repair; PCR, polymerase chain reaction; RD, percentage of residual DNA damage; RFLP, restriction fragment length polymorphism; RT, radiation therapy; SNP, single nucleotide polymorphism; SSB, single strand breaks; TD, percentage of DNA in the tail

* Corresponding author

Even though there are used various techniques for studying radiosensitivity, the molecular mechanisms responsible for sensitivity to IR are still not clearly understandable. Previous studies proved that the SF2 parameter (surviving fraction of tumour cells at 2 Gy) is significantly associated with intrinsic radiosensitivity [6, 7]. Unfortunately, the SF2 assay (colony-forming assay) is time-consuming what is inconsistent with planning of patient's treatment. Therefore, there is increasing interest for the design of a rapid, noninvasive test for measurement of radiation exposure sensitivity. There were developed several assays using different biological endpoints, such as DNA damage [8, 9], chromosomal aberrations [10], DSB [11, 12], DNA repair capacity [13, 14], cell cycle and apoptosis [15], gene expression [16], oxidative stress [17] and others. In particular, the comet assay (single cell gel electrophoresis, SCGE) has been shown to be suitable for the study of radiation-induced DNA damage [8, 18]. Since this method is very sensitive, rapid and only a small number of cells is required, it seems to be very suitable for clinical application.

In the present study, the comet assay was applied for the evaluation of cervical cancer cells radiosensitivity *in vitro*. Initial DNA damage as well as DNA repair kinetics was measured in irradiated human cervical cancer cell lines C-33A, CaSki, HeLa and SiHa. The aim of this study includes the assessment of the relation between SNP in various DNA repair genes (*XRCC1*, *PARP, hOGG1, XRCC3*, *XRCC4* and *XPD*) and the response of cells to IR.

Material and methods

Cells and culture conditions. Cervical cancer cell lines (C-33A, CaSki, HeLa and SiHa) were obtained from Prof. Pastoreková (Institute of Virology, Slovakia). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 4.5 g/l glucose, 10% fetal bovine serum and antibiotics (penicillin 200 U/ml, streptomycin and kanamycin 100 µg/ml). All cell lines were cultured in glass or plastic Petri dishes in a CO_2 incubator at 37 °C. Growth media and other chemicals used for cell cultivation were purchased from GIBCO.

Irradiation. Cells were irradiated on microscope slides with a 6 MV linear accelerator CLINAC 2300C (Varian) with doses 0.5, 1, 1.5, 2, 3 and 4 Gy. During irradiation, cells were kept in cold Hank's solution. For studies of initial DNA damage, cells were lysed immediately after irradiation. For DNA repair studies, cells were incubated for 15, 30, 45 and 60 min in DMEM at 37 °C.

Single cell gel electrophoresis. For the presented study the procedure of Singh et al. [19] with minor modifications by Gábelová et al. [20] was used. Assayed cells were suspended in 0.75% low-melting-point agarose and spread on a base layer (100 µl of 1 % mormal-melting-point agarose in Ca^{2+} and Mg^{2+} free PBS buffer) onto a microscope slide. The agarose was allowed to solidify and then slides were irradiated. Immediately after the irradiation or after prescribed repair 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 1 h at 4°C to remove cellular proteins. Then the slides were transfered to an electrophoresis solution (300 mM NaOH, 1 mM Na_2EDTA, pH 13), and were kept in this solution for a 40 min. unwinding time at 4°C. A current of 25 V (300 mA) was then applied for 30 min. The slides were removed, neutralised with tris-HCl (0.4 mM, pH 7.5), and stained with 20 µl ethidium bromide (EtBr, 10 µg/ml). EtBr-stained nucleoids were evaluated with a Olympus BX51 fluorescence microscope by computerized image analysis (Komet 5.5, Kinetic Imaging, Ltd., Liverpool, UK) determining the percentage of DNA in the tail (% tail DNA) which is linearly related to the frequency of DNA breaks. Percentage of residual DNA damage (RD) was defined according to Aka et al. [21]:

$$RD = \frac{TD(x) - TD(c)}{TD(0) - TD(c)} \times 100$$

where TD(c) is percentage of DNA in the tail unirradiated cells, TD(0) is percentage of DNA in the tail immediately after irradiation, TD(x) is precentage of DNA in the tail at anytime (15, 30, 45 and 60 min) after irradiation.

Genotyping

Genomic DNA was isolated from the cell lines using the phenol/chloroform extraction method. The polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were used for genotyping analyses. The components for PCR reactions were purchased from Promega, PCR products were digested with specific restriction enzymes obtained from Fermentas.

Polymorphisms in *XRCC1* exons 6 (Arg194Trp) and 10 (Arg399Gln) were analyzed according to Lunn et al. [22]. *PARP* genotyping was performed as described by Zhang et al. [23]. *hOGG1* polymorphism was analyzed according to Biroš et al. [24]. Polymorphism in *XPD* gene exon 6 (Arg156Arg) was identified according to Biroš [25], *XPD* exons 10 (Asp312Asn) and 23 (Lys751Gln) polymorphisms were determined according to the protocol described by Hemminki et al. [26]. *XRCC3* genotyping was performed as described by Shen et al. [27]. *XRCC4* polymorphism was analyzed according to Relton et al. [28].

Statistics. Results of the comet assay are representing the mean of nine parallel samples at least (three independent experiments) ± standard deviation (SD). The differences in radiosensitivity of cell lines were tested for statistical significance using Student's t-test and one-way analysis of variance (ANOVA). For comparing dose-response effects linear regression analysis was employed.

Results

Initial DNA damage of cervical cancer cell lines after irradiation (0 – 4 Gy) measured by the comet assay is illustrated in Fig. 1. In all cell lines the evident dose – dependent in-

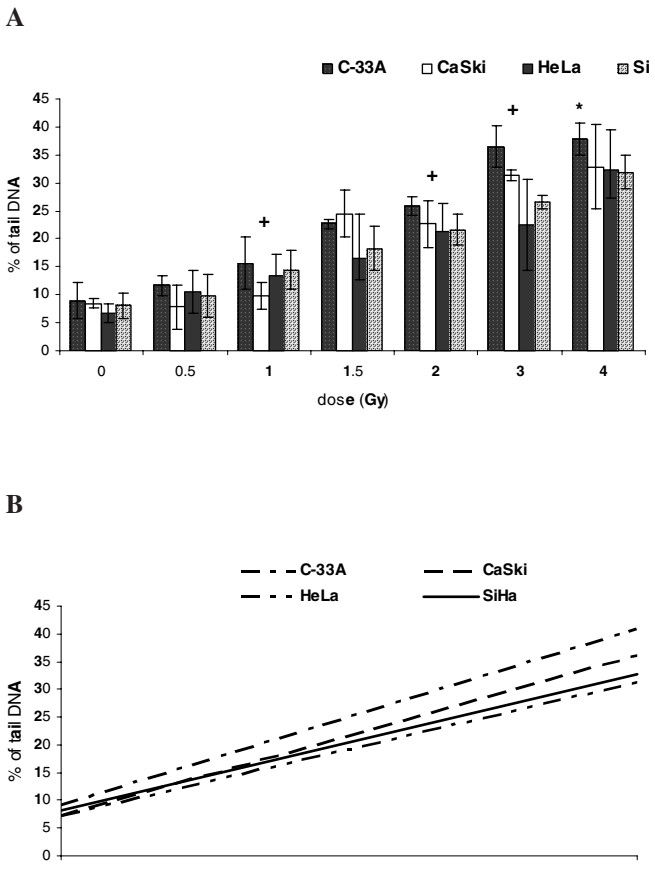

Figure 1. A Initial DNA damage in C-33A, CaSki, HeLa and SiHa cells after irradiation measured by the comet assay. Each data point is the mean of three independent experiments ± SD, statistical significance is as follows: + P < 0.05 for comparison of cell lines by ANOVA analysis; * P < 0.05 for comparison of C-33A cells with rest cell lines by the student t-test B Dose response curves for initial DNA damage induced by irradiation and measured by the comet assay. The lines are fitted by linear regression.

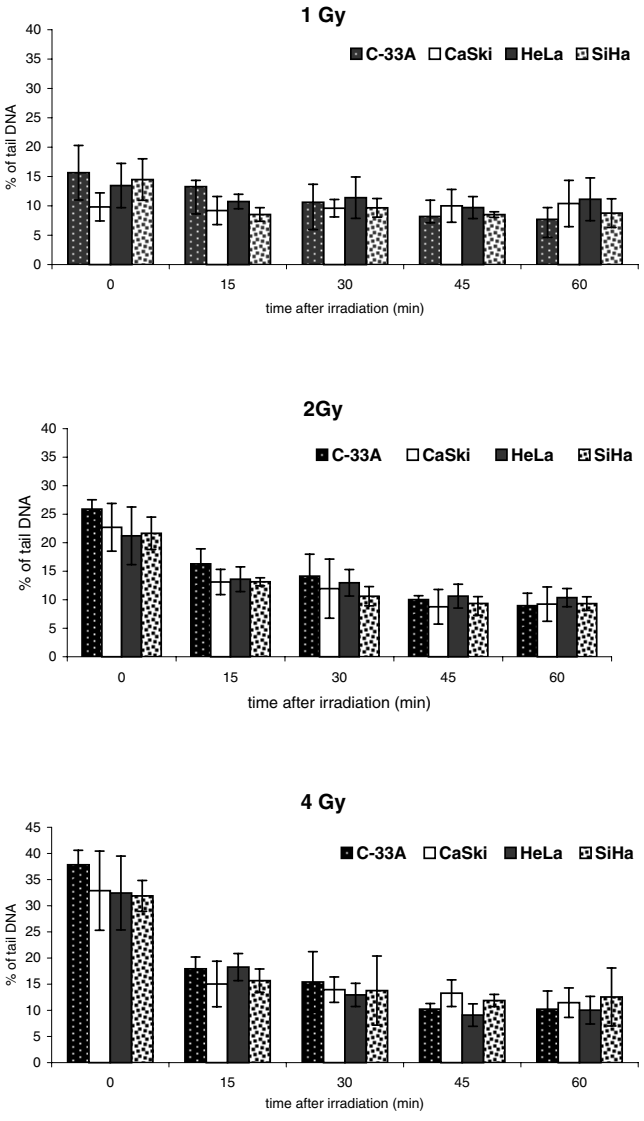

Figure 2. DNA repair kinetics in C-33A, CaSki, HeLa and SiHa cells irradiated with 1, 2 and 4 Gy. The cells were then incubated in fresh medium and scored by the comet assay. Each data point is the mean of three independet experiments ± SD

crease of initial DNA damage was observed (fig. 1A). There were found significant differences between each cell line at 1, 2 and 3 Gy ($P < 0.05$) by ANOVA analysis. At 4 Gy the level of DNA damage was significantly higher in C-33A cells than in the rest (t-test, $P < 0.05$). For each cell line the data were well described by a linear fit (correlation coefficients ≥ 0.93) (fig. 1B). The slopes of the fitted linear regression varied by a factor of 1.3 (range 6.05 – 7.86). The steepest slope of the linear regression calculated for C-33A cells classifies this cell line as the most radiosensitive. The shallower slopes determined for HeLa and SiHa cells indicated that these two cell lines might belong to radioresistant.

The DNA repair kinetics of the cells incubated in fresh medium at 37 °C for 15, 30, 45 and 60 min after the irradiation (1, 2 and 4 Gy) detected by the comet assay is shown in Figure 2. In all used cell lines rapid repair of DNA damage induced by irradiation was observed. All cell lines repaired DNA damage in the same manner; the majority of repair occurs within 15 min and residual DNA damage of all cell lines reached the same near the control level within 45 min and remaind equal at 60 min. The percentage of residual damage (RD) in cells irradiated with 2 and 4 Gy after a given repair time is shown in Table 1. The highest RD was found in HeLa cells during the whole repair time after irradiation with 2 Gy and within 30 min after irradiation with 4 Gy. The lowest level of RD at 45 and 60 min after irradiation (2 and 4 Gy) was observed in radiosensitive C-33A cell line.

Genetic analysis was performed for polymorhic sites in DNA repair genes included in various repair pathways (*XRCC1*, *PARP, hOGG1, XRCC3*, *XRCC4* and *XPD*) (tab. 2)

Table I. % of residual DNA damage (RD) 15, 30, 45 and 60 min post *in vitro* irradiation with 2 and 4 Gy

	2 Gy				4 Gy			
	RD15	RD30	RD45	RD60	RD15	RD30	RD45	RD60
C-33A	43.27 ± 2.36	30.58 ± 3.85	6,32 ± 0.68	0,01 ± 2.18	31.13 ± 2.25	22.41 ± 5.79	4,43 ± 1.07	4,39 ± 3.48
CaSki	32.56 ± 2.21	24.41 ± 5.18	7,97 ± 3.03	5,27 ± 3.01	26.89 ± 4.36	22.42 ± 2.43	19,67 ± 2.55	12,25 ± 2.82
HeLa	47.63 ± 2.17	43.37 ± 2.33	27,28 ± 2.09	25,5 ± 1.59	45.05 ± 2.59	24.41 ± 2.22	9,43 ± 2.15	13,08 ± 2.64
SiHa	37.18 ± 0.71	18.48 ± 1.69	9,02 ± 2.07	8,87 ± 1.19	31.78 ± 2.24	23.82 ± 6.61	15,78 ± 1.17	18,64 ± 5.54

Each data point is the mean of three independent experiments ± SD.

Table II. Distribution of XRCC1, PARP, hOGG1, XPD, XRCC3 and XRCC4 genotypes in the studied cell lines and comparison with slopes of dose response curves and % of residual DNA damage 60 min after irradiation (RD 60).

	C-33A	CaSki	HeLa	SiHa
XRCC1 Arg194Trp	Arg/Arg	Arg/Arg	Arg/Arg	Arg/Arg
XRCC1 Arg399Gln	**Arg/Gln***	Arg/Arg	Arg/Arg	Arg/Arg
PARP Val762Ala	Val/Val	**Ala/Ala**	**Val/Ala**	**Val/Ala**
hOGG1 Ser326Cys	**Cys/Cys**	**Ser/Cys**	Ser/Ser	**Ser/Cys**
XPD Arg156Arg	**Arg/Arg**	Arg/Arg	Arg/Arg	Arg/Arg
XPD Asp312Asn	**Asp/Asn**	Asp/Asp	Asp/Asp	Asp/Asp
XPD Lys751Gln	**Gln/Gln**	Lys/Lys	Lys/Lys	Lys/Lys
XRCC3 Thr241Met	**Thr/Met**	**Thr/Met**	**Thr/Met**	**Thr/Met**
XRCC4 Ile401Thr	Ile/Ile	Ile/Ile	Ile/Ile	Ile/Ile
slope of dose response curve	7.861	7.036	6.051	5.965
RD 60 (2 Gy)	0.011 ± 2.185**	5.271 ± 3.013	25.502 ± 1.595	8.874 ± 1.196
RD 60 (4 Gy)	4.393 ± 3.479	12.254 ± 2.819	13.082 ± 2.638	18.644 ± 5.543

* results printed in bold indicate the presence of variant allele in the gene
** each data point is the mean of three independent experiments ± SD.

in all studied cell lines. No variants were found in *XRCC1*-194 and *XRCC4* gene, while the variant allele in gene *XRCC3* was detected in all cell lines. In comparison with CaSki, HeLa and SiHa cells, C–33A cell line does not contain variant allele in *PARP* gene. Apart from *PARP* and *XRCC3* genes, HeLa cells does not contain other variants in studied DNA repair genes, SiHa and CaSki cells were polymorphic in *hOGG1* gene. But in most radiosensitive C33–A cells the variants in *hOGG1*, *XPD* at codons 156, 312 and 751, *XRCC3* and *XRCC1* gene at codon 399 were identified. To investigate whether the found polymorphisms are associated with differences in the level of initial and residual DNA damage of irradiated cells, DNA repair genes genotype and the level of DNA damage were compared (tab. 2).

Discussion

The aim of this study was to assess the relation between cancer cell radiosensitivity and genetic polymorphisms in various DNA repair genes (*XRCC1*, *PARP, hOGG1, XRCC3*, *XRCC4* and *XPD*). The comet assay was used for evaluation of *in vitro* cell radiosensitivity of four cervical cancer cell lines C-33A, CaSki, HeLa and SiHa. This sensitive method is able to detect single-strand breaks (SSB) and alkali labile sites at clinically relevant doses of radiation. It is known that among the DNA damage induced by IR mainly DSB are produced. El-Awady et al. [29] found that tumor cell lines with a high number of induced DSB are much more sensitive to IR than cells with a low number of induced DSB. However induction of DSB on measurable level requires higher radiation doses than those used clinically. In comparison to DSB, the amount of SSB is much higher after irradiation [30] and induction of SSB can be considered as an indicator of DSB induction. For this reason, the cells were irradiated with lower doses (0.5 – 4 Gy) and the comet assay was applied as a suitable method for evaluation the cell radiosensitivity.

According to our results, the highest level of initial DNA damage after irradiation was observed in C-33A cell line (Fig. 1). Therefore it is supposed that these cells are the most radiosensitive. The response of remaining cell lines to radiation was nearly similar. Hypersensitivity of C-33A cells to IR was already previously detected by neutral comet assay [31] and H2AX foci [14] and confirmed by SF2 assay.

As differences in DNA repair capacity could contribute to inter-individual differences in cells in response to IR [32], in the next step the kinetics of DNA rejoining was studied. All cells were allowed to repair induced DNA damage for 15, 30, 45 and 60 min. (Fig. 2) and residual DNA damage was calcu-

lated (Tab. 1). In general, most radiosensitive cells showed fastest repair after 4 Gy, while in HeLa cells considered as radioresistant higher values of residual damage were detected. Similarly to our results, no significant correlation between the cellular radiosensitity and repair capacity was observed by El-Awady et al. [33] in fibroblasts. It is necessary to note that the comet assay measures rejoining but not fidelity of DNA repair.

Results obtained by DNA repair study could be affected by human papilloma virus (HPV). Epidemiologic studies have established that infection by HPV is a risk factor for cervical cancer [34]. Interestingly, the radiosensitive C-33A cell line is the only HPV negative from studied cells. Remaining cell lines contain an integrated HPV 16 and 18. In infected cells the E6 and E7 proteins are expressed. These proteins bind to and inactivate P53 and PRB and consequently cause disruption of the P53-mediated cellular response to DNA damage [35, 36]. Inactivation and/or degradation of P53 by proteins E6 and E7 could affect not only its role as transcription factor and regulator of cell cycle, but also interactions with proteins in DNA repair pathways, mainly nucleotide excision repair (NER) [37] and homologous recombination [38]. For that reason, HPV infection could be responsible for slower DNA repair observed in HeLa, CaSki and SiHa cells when comparing to radiosensitive C-33A cells.

It is supposed that in the response to RT could participate genetic variations in genes and the important step is to reveal the candidate genes and their polymorphisms responsible for radiosensitivity/radioresistance. In our study, the polymorphisms in the DNA repair genes involved in base excision repair (BER), NER and repair of DSB were analyzed.

DSB are the most important lesions induced by IR therefore genes (*XRCC3* and *XRCC4*) which products are involved in repair of such DNA damage were genotyped. There were no differences in polymorphisms in genes playing role in the repair of DSB between cell lines even though there are studies which confirmed association between polymorphism in *XRCC3* and sensitivity to IR [4, 21].

As IR induces broad range of DNA oxidative damage, polymorphisms in *PARP*, *hOGG1* and two polymorphic sites in *XRCC1* gene as candidate genes for BER were analyzed. In SiHa, HeLa and CaSki, the polymorphism was detected in *PARP* only, while in C-33A cells the variants were found in *XRCC1* (Arg399Gln) and *hOGG1*. Polymorphisms in *XRCC1* gene are extensively studied in cancer epidemiology but results are often conflicting [39]. Hu et al. [40] and De Ruyck et al. [3] found that variants of XRCC1 may contribute to IR hypersensitivity. Similarly, variants in hOGG1 are associated with decreased DNA repair of 8-oxoguanine [41, 42] but other works did not prove this presumptions [43, 44]. It was found that cysteine at the position 326 in hOGG1 causes lower ability of hOGG1 to suppress mutations than serine at this position [45]. The interactions between products of these genes are important for stimulation of their activities in BER and SSBs repair [46]. The polymorphism resulted in exchange Arg to Gln in codon 399 in XRCC1 is probably in PARP and hOGG1 binding region [2] and consequently could affect activity of these enzymes.

Except the role of XPD protein in NER, this helicase plays role in regulation of cell cycle and apoptosis and has structural function in transcription [47, 48]. For that reason, the variant alleles in *XPD* could affect DNA repair capacity as well as other crucial pathways in living organisms. We found that only C-33A cells are polymorphic in all studied polymorphic sites of *XPD*. Influence of polymorphisms in *XPD* gene on DNA damage induced by IR was previously found by Rzeszowska-Wolny et al. [49]. But on the other hand, some articles pointed out protective effects of polymorphisms in *XPD* [50, 51].

The contribution of the polymorphisms in DNA repair genes to the response of cells to IR is summarized in tab. 2. The highest slope of dose response curve was calculated in C-33A cells and therefore this line was assumed to be radiosensitive. After genotyping we found that C-33A cells are polymorphic in the majority of analyzed DNA repair genes but interestingly, residual damage measured 60 min after irradiation with 2 and 4 Gy was the lowest. It is supposed that there is the association between polymorphisms in some DNA repair genes and initial DNA damage but no correlation was detected between polymorphisms and DNA repair. Our results indicate that monitoring of polymorphism in a single gene is not sufficient for prediction of the individual susceptibility to ionizing radiation. As shown by Aka et al. [21] and Naccarati et al. [51] an investigation of combination of genotypes of DNA repair genes involved in several repair pathways is more beneficial.

In conclusion, this pilot study found the association between several genetic polymorphisms in DNA repair genes and cell radiosensitivity and may contribute to design the larger studies of patient‘s tumor radiosensitivity and their inter-individual differences.

This work was supported by project 2003 SP 510280800/0280801 from the National Programme Use the Cancer Genomics to Improve the Human Population Health. The authors wish to thank Mrs. A. Vokáliková for excellent technical assistance and Mrs. A. Stehlíková for her help with cell irradiation.

References

[1] ANGELE S, ROMESTAING P, MOULLAN N, VUILLAUME M, CHAPOT B ET al. AMT haplotypes and cellular response to DNA damage: Association with breast cancer risk and clinical radiosensitivity. Cancer Res 2003; 63: 8717–8725.

[2] MOULLAN N, COX DG, ANGELE S, ROMESTAING P, GERARD JP, HALL J. Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. Cancer Epidemiol Biomarkers Prev 2003; 12: 1168–1174.

[3] DE RUYCK K, VAN EIJKEREN M, CLAES K, MORTHIER R, DE PAEPE A et al. Radiation-induced damage to normal tissues after radiotherapy in patients treated for gynecologic

tumors: Association with single nucleotide polymorphisms in XRCC1, XRCC3 and OGG1 genes and in vitro chromosomal radiosensitivity in lymphocytes. Int J Radiat Oncol Biol Phys 2005; 62: 1140–49.

[4] PRICE EA, BOURNE SL, RADBOURNE R, LAWTON PA, LAMERDIN J et al. Rare microsatellite polymorphisms in the DNA repair genes XRCC1, XRCC3 and XRCC5 associated with cancer in patients of varying radiosensitivity. Somat Cell Mol Genet 1997; 23: 237–247.

[5] ANDREASSEN CN, ALSNER J, OVERGAARD M, OVERGAARD J. Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. Radiother Oncol 2003; 69: 127–135.

[6] WEST CM, DAVIDSON SE, ROBERTS SA, HUNTER RD. Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. Br J Cancer 1993; 68: 819–823.

[7] JOHANSEN J, BENTZEN SM, OVERGAARD J, OVERGAARD M. Relationship between the in vitro radiosensitivity of skin fibroblasts and the expression of subcutaneous fibrosis,telangiectasia, and skin erythema after radiotherapy. Radiother Oncol 1996; 40: 101–109.

[8] BACOVA G, HUNAKOVA L, CHORVATH M, BOLJESIKOVA E, CHORVATH B et al. Radiation-induced DNA damage and repair evaluated with comet assay in human ovarian carcinoma cell lines with different radiosensitivities. Neoplasma 2000; 47: 367–374.

[9] MARCON F, ANDREOLI C, ROSSI S, VERDINA A, GALATI R et al. Assessment of individual sensitivity to ionizing radiation and DNA repair efficiency in a healthy population. Mutat Res 2003; 541: 1–8.

[10] VRAL A, THIERENS H, BAEYENS A, DE RIDDER L. Chromosomal aberrations and in vitro radiosensitivity: intra-individual versus inter-individual variability. Toxicol Lett 2004; 149: 345–352.

[11] BRAMMER I, HERSKIND C, HAASE O, RODEMANN HP, DIKOMEY E. Induction and repair of radiation-induced DNA double-strand breaks in human fibroblasts are not affected by terminal differentiation. DNA Repair 2004; 3: 113–120.

[12] MAHRHOFER H, BURGER S, OPPITZ U, FLENTJE M, DJUZENOVA CS. Radiation induced DNA damage and damage repair in human tumor and fibroblast cell lines assessed by histone H2AX phosphorylation. Int J Radiat Oncol Biol Phys 2006; 64: 573–580.

[13] POPANDA O, EBBELER R, TWARDELLA D, HELMBOLD I, GOTZES F et al. Radiation-induced DNA damage and repair in lymphocytes from breast cancer patients and their correlation with acute skin reactions to radiotherapy. Int J Radiat Oncol Biol Phys 2003; 55: 1216–1225.

[14] BANATH JP, MACPHAIL SH, OLIVE PL. Radiation sensitivity, H2AX phosphorylation, and Kinetics of repair of DNA strand breaks in irradiated cervical cancer cell lines. Cancer Res 2004; 64: 7144–7149.

[15] GASINSKA A, ADAMCZYK A, KOJS Z, SZUMIEL I. Are stromal fobroblasts from cervical tumors suitable to predict normal tissue radiation reaction? Neoplasma 2004; 51: 228–238.

[16] AMUNDSON SA, GRACE MB, MCLELAND CB, EPPERLY MW, YEAGER A et al. Human in vivo radiation-induced biomarkers: gene expression changes in radiotherapy patients. Cancer Res 2004; 64: 6368–6371.

[17] SHANKAR B, KUMAR SS, SAINIS KB. Generation of reactive oxygen species and radiationresponse in lymphocytes and tumor cells. Radiat Res 2003; 160: 478–487.

[18] MONEEF MAL, SHERWOOD BT, BOWMAN KJ, KOCKELBERGH RC, SYMONDS RP et al. Measurements using the alkaline comet assay predict bladder cancer cell radiosensitivity. Br J Cancer 2003; 89: 2271–2276.

[19] SINGH NP, MCCOY MT, TICE RR, SCHNEIDER EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184–191.

[20] GABELOVA A, SLAMENOVA D, RUZEKOVA L, FARKASOVA T, HORVATHOVA E. Measurement of DNA strand breakage and DNA repair induced with hydrogen peroxide using single cell gel electrophoresis, alkaline DNA unwinding and alkaline elution of DNA. Neoplasma 1997; 44: 380–388.

[21] AKA P, MATEUCA R, BUCHET JP, THIERENS H, KIRSCH-VOLDERS M. Are genetic polymorphisms in OGG1, XRCC1 and XRCC3 genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionizing radiations? Mutat Res 2004; 556: 169–181.

[22] LUNN RM, LANGLOIS RG, HSIEH LL, THOMSON CL, BELL DA. XRCC1 polymorphisms: Effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 1999; 59: 2557–2561.

[23] ZHANG X, MIAO X, LIANG G, HAO B, WANG Y et al. Polymorphisms in DNA base excision repair genes ADPRT and XRCC1 and risk of lung cancer. Cancer Res 2005; 65: 722–726.

[24] BIROS E, BESKID O, BINKOVA B, RÖSSNER P JR, SRAM RJ. The hOGG1 Ser326Cys polymorphism: significance for chromosomal aberrations. In: Cebulska-Wasilewska A, Au WW, Sram RJ. Human monitoring for genetic effects. NATO Sci Series I, IOS Press 2003; 351: 174–180.

[25] BIROS E. Polymorphisms in XRCC1, XPD, hOGG1, p53 and MS genes. Standard Operation Procedure Institute of Experimental Medicine 2003 Prague 15.

[26] HEMMINKI K, XU G, ANGELINI S, SNELLMAN E, JANSEN CT et al. XPD exon 10 and 23 polymorphisms and DNA repair in human skin in situ. Carcinogenesis 2001; 22: 1185–1188.

[27] SHEN H, WANG X, HU Z, ZHANG Z, XU Y et al. Polymorphisms of DNA repair gene XRCC3 Thr241Met and risk of gastric cancer in a Chinese population. Cancer Lett 2004; 206: 51–58.

[28] RELTON CL, DANIEL CP, HAMMAL DM, PARKER L, TAWN EJ et al. DNA repair gene polymorphisms, pre-natal factors and the frequency of somatic mutations in the glycophorin-A gene among healthy newborns. Mutat Res 2004; 545: 49–57.

[29] EL-AWADY RA, DIKOMEY E, DAHM-DAPHI J. Radiosensitivity of human tomour cells is correlated with the

induction but not with the repair of DNA double-strand breaks. Br J Cancer 2003; 89: 593–601.

[30] WARD JF. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 1988; 35: 95–125.

[31] MARPLES B, LONGHURST D, EASTHAM AM, WEST CML. The ratio of initial/residual DNA damage predicts intrinsic radiosensitivity in seven cervix carcinoma cell lines. Br J Cancer 1998; 77: 1108–1114.

[32] DIKOMEY E, BORGMANN K, BRAMMER I, KASTEN-PISULA U. Molecular mechanisms of individual radiosensitivity studied in normal diploid human fibroblasts. Toxicology 2003; 193: 125–135.

[33] EL-AWADY RA, MAHMOUD M, SALEH EM, EL-BAKY HA, LOTAYEF M et al. No correlation between radiosensitivity or double-strand break repair capacity of normal fibroblasts and acute normal tissue reaction after radiotherapy of breast cancer patients. Int J Radiat Biol 2005; 81: 501–508.

[34] CHEN JR, LEE YJ, CHEN T, WANG KL, DANG CW et al. MHC class I chain-related gene A (MICA) polymorphism and the different histological types of cervical cancer. Neoplasma 2005; 52: 369–373.

[35] WESIERSKA-GADEK J, SCHLOFFER D, KOTALA V, HORKY M. Escape of p53 protein from E6-mediated degradation in HeLa cells after cisplatin therapy. Int J Cancer 2002; 101: 128–136.

[36] BRAUN K, EHEMANN V, WALDECK W, PIPKORN R, CORBAN-WILHELM H et al. HPV18 E6 and E7 genes affect cell cycle, pRb and p53 of cervical tumor cells and represent prominent candidates for intervention by use peptide nucleic acids (PNAs). Cancer Lett 2004; 209: 37–49.

[37] ROBLES AI, HARRIS CC. P53-mediated apoptosis and genomic instability diseases. Acta Oncol 2001; 40: 696–701.

[38] LINKE SP, SENGUPTA S, KHABIE N, JEFFRIES BA, BUCHHOP S et al. P53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination. Cancer Res 2003; 63: 2596–2605.

[39] LADIGES WC. Mouse models of XRCC1 DNA repair polymorphisms and cancer. Oncogene 2006; 25: 1612–1619.

[40] HU JJ, SMITH TR, MILLER MS, MOHRENWEISER HW, GOLDEN A et al. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001; 22: 917–922.

[41] KOHNO T, SHINMURA K, TOSAKA M, TANI M, KIM SR et al. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 1998; 16: 3219–3225.

[42] YAMANE A, KOHNO T, ITO K, SUNAGA N, AOKI K et al. Differential ability of polymorphic OGG1 proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell in vitro. Carcinogenesis 2004; 25: 1689–1694.

[43] PARK YJ, CHOI EY, CHOI JY, PARK JG, YOU HJ et al. Genetic changes of hOGG1 and the activity of oh8Gua glycosylase in colon cancer. Eur J Cancer 2001; 37: 340–346.

[44] LI D, FIROZI PF, ZHANG W, SHEN, DIGIOVANNI J et al. DNA adducts, genetic polymorphisms, and K-ras mutation in human pancreatic cancer. Mutat Res 2002; 513: 37–48.

[45] LUNA L, ROLSETH V, HILDERSTRAND GA, OTTERLEI M, DANTZER D et al. Dynamic relocalization of hOGG1 during the cell cycle is disrupted in cells harbouring the hOGG1-Cys326 polymorphic variant. Nucleic Acid Res 2005; 33: 1813–1824.

[46] MARSIN S, VIDAL AE, SOSSOU M, MENISSIER-DE MURCIA J, LE PAGE F et al. Role of XRCC1 in the coordination and stimulation of oxidative DNA damage repair initiated by the DNA glycosylase hOGG1. J Biol Chem 2003; 278: 44068–44074.

[47] LEHMANN AR. DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Biochimie 2003; 85: 1101–1111.

[48] BENHAMOU S, SARASIN A. ERCC2/XPD gene polymorphisms and lung cancer: a HuGE review. Am J Epidemiol 2005; 161: 1–14.

[49] RZESZOWSKA-WOLNY J, POLANSKA J, PIETROWSKA M, PALYVODA O, JAWORSKA J et al. Influence of polymorphisms in DNA repair genes XPD, XRCC1 and MGMT on DNA damage induced by gamma radiation and its repair in lymphocytes in vitro. Radiat Res 2005; 164: 132–140.

[50] SPITZ MR, WU X, WANG Y, WANG LE, SHETE S et al. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res 2001; 61: 1354–1357.

[51] NACCARATI A, SOUCEK P, STETINA R, HAUFROID V, KUMAR R et al. Genetic polymorphisms and possible gene-gene interactions in metabolic and DNA repair genes: Effects on DNA damage. Mutat Res 2006; 593: 22–31.