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QUARTERLY

Repair of γ-ray-induced base damage in L5178Y sublines is damage type-dependent and unrelated to radiation sensitivity✪

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The L5178Y (LY) murine lymphoma sublines LY-R and LY-S are differentially sensitive to ionizing radiation. The high radiation sensitivity of LY-S cells is related to impaired rejoining of DNA double strand breaks. We found previously that the γ-ray-induced base damage is higher in the more radiosensitive LY-S subline. Here, we examine the role of the repair of ionizing radiation induced base damage in relation to the radiosensitivity difference of these sublines.

We used the GS/MS technique to estimate the repair rates of six types of base damage in γ-irradiated LY cells. All modified DNA bases identified in the course of this study were typical for irradiated chromatin. The total amount of initial base damage was higher in the radiation sensitive LY-S subline than in the radiation resistant LY-R subline. The repair rates of 5-OHMeUra, 5-OHCyt, 8-OHAde were similar in both cell lines, the repair rates of FapyAde and 8-OHGua were higher in the radiosensitive LY-S cell line, whereas the repair of 5-OHUra was faster in its radio resistant counterpart, the LY-R.

Altogether, the repair rates of the γ-ray-induced DNA base damage in LY sublines are related neither to the initial amounts of the damaged bases nor to the differential lethal or mutagenic effects of ionizing radiation in these sublines.

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Abbreviations: 5-OHHyd, 5-hydroxyhydantoin; 5-OHCyt, 5-hydroxycytosine; 5-OHMeUra, 5-hydroxymethyluracil; 5,6-diOHUra, 5,6-dihydroxyuracil; FapyAde, 4,6-diamino-5-formamidopyrimidine; 8OHAde, 8-hydroxyadenine; 8-OHGua, 8-hydroxyguanine; BSTFA, bis(trimethylsilyl)-trifluoroacetamide; DSB, double strand break; GC/MS, gas chromatography/mass spectrometry.

Ionizing radiation-induced DNA lesions are locally clustered [1]. As reviewed by Wallace [2], when clustered base damage is processed by base excision repair, a DNA double strand break (DSB) can result. Logically, the delayed rejoining of DSB that is observed in some cell lines can be caused by the low efficiency of the DSB repair system and/or by DSB generation during the post-irradiation period, as indicated by Wallace [2].

The aim of this study was to investigate the repair of base damage induced by γ-rays in two related cell sublines differing in the sensitivity to oxidative stress. The respective L5178Y sublines, LY-S and LY-R, display a unique feature of inverse cross-sensitivity to X rays and hydrogen peroxide [3–7]. The high sensitivity of LY-S cells to X rays (D_0 = 0.5 Gy) is explained by the impairment of DSB rejoining [8] and high initial DNA damage [9–11]. In the case of hydrogen peroxide treatment the reasons for the enhanced sensitivity of LY-R cells are more complex. These are: a less efficient antioxidant defence system [5], and a higher content of iron ions (available for entering the Fenton reaction [12] and generating the damaging hydroxyl radicals). Hence, a significantly higher amount of initial DNA lesions than that in LY-S cells [6, 13].

The induction of base damage in γ-irradiated or hydrogen peroxide-treated LY sublines has been described previously; the extent of the initial damage was found to be related to the subline's sensitivity to the damaging agent [13]. Interestingly, a similar relation to sensitivity was described by Mori & Dizdaroglu [14] for the parent L5178Y line and its radiosensitive mutant M10.

We undertook base damage determination by gas chromatography–mass spectrometry, which allowed us to discern and quantitate various base damage types. This, however, is only possible after irradiation with a supralethal dose (400 Gy). The enzymatic repair activity that is detectable after such a massive dose gives a good reason to assume that it also is functional after irradiation with lower doses. With the different end-points examined previously (survival, DNA strand break induction and repair, mutation frequency) and in this report, it is unavoidable to use a broad range of radiation doses in order to obtain an optimal damage range for each method. Such a discrepancy in the dose range applied previously and in this study seems to be acceptable, as we compare relative responses in the two cell sublines rather than absolute relations between damage estimated at the molecular, subcellular and cellular levels.

Although it is not possible to directly discern between the primary and secondary DSBs, examination of repair of the ionizing radiation-induced base damage in LY sublines may give some indication as to the role of base damage in the delayed repair of DSBs in LY-S cells.

MATERIALS AND METHODS

Chemicals. Triton X-100 was purchased from Sigma Chemical Company. Internal standards were a gift from Dr. M. Dizdaroglu from the National Institute of Standards and Technology (Gaithersburg, MD, U.S.A.). Acetonitrile and bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosiliane were obtained from Pierce Chemical Co. Formic acid was from Mallincrodt.

Cell cultures. Murine leukaemic lymphoblasts LY-R and LY-S were maintained in suspension cultures in Fischer's medium supplemented with 8% bovine serum, as described by Szumiel [15]. Asynchronous populations in exponential phase of growth were used in all experiments.

Irradiation. Cells were collected by centrifugation and resuspended in cold Fisher's medium containing 8% bovine foetal serum (4×10^6 cells/ml). ^{60}Co γ rays were applied in an icebath, at a dose rate of 39.2 Gy/min (MINEOLA, INCT), as previously described

[13]. After irradiation, cell suspension aliquots were placed at 37°C for repair periods ranging from 15 to 360 min and than frozen in liquid nitrogen and stored at –80°C until chromatin isolation. To avoid artifactual oxidation of chromatin from dead cells, the cell membrane integrity was monitored by the nigrosine test. Throughout the whole repair period more than 90% of irradiated cells had cell membrane not permeable to nigrosine.

Isolation of chromatin and base damage determination. This was carried out as described previously [13]. In brief, chromatin was isolated according to the modified procedure of Mee & Adelstein [16]. Chromatin samples containing 100 μg of DNA (as determined by spectrophotometry) were supplemented with internal standards, lyophilized and hydrolysed with 0.5 ml of 60% formic acid in evacuated and sealed tubes for 30 min at 140°C. The hydrolysates were lyophilized and then trimethylsilylated in polytetrafluoroethylene-capped hypovials (Pierce Chemical Co.) with 100 μl of a mixture of BSTFA and acetonitrile (4:1, v/v) by heating for 30 min at 130°C under nitrogen. After hydrolysis and derivatization, the samples were analyzed by gas chromatography/isotope-dilution mass spectrometry with selected ion-monitoring according to the method described by Dizdaroglu [17].

A Hewlett Packard Model 5890 Series II Model gas chromatograph interfaced to a Hewlett Packard Model 5972 mass selective detector was used. The injection port and GC/MS interface were both maintained at 250°C and the ion source at about 200°C. Separations were carried out using a fused-silica capillary column (Ultra 2, 12.5 m × 0.2 mm, Hewlett Packard) coated with cross-linked 5% phenylmethylsilicone gum phase (film thickness 0.33 μm). An aliquot of each derivatized sample (4 μl) was injected without any further treatment into the injection port of the gas chromatograph by means of an autosampler.

Data processing and statistical analysis. The DNA repair curves described by the equation $y = a\ e^{-bt} + c$ were fitted (by the least square method) to the experimental values. In this statistical model a is the reparable damage induced by radiation, c is the irreparable damage (total damage is $a + c$) and b (=$1/\tau$) is the time constant for the repair of that damage, τ is the time required to reduce the reparable damage to 37% of its initial amount. The significance of the difference in mean values was estimated by the Student's t-test for independent samples. All statistical evaluation and curve fitting were performed with the use of Statistica v. 5.1 software (StatSoft Inc. Tulsa, U.S.A.).

RESULTS

Figure 1 presents the initial amounts of six types of base damage and their repair in LY cells that were irradiated with 400 Gy of γ rays. The following altered bases were determined: 5-hydroxyuracil (5-OHUra), 5-hydroxymethyluracil (5-OHMeUra), 5-hydroxycytosine (5-OHCyt), 8-hydroxyadenine (8-OHAde), 4,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyAde), and 8-hydroxyguanine (8-OHGua).

Generally, the total amount of initial base damage was higher in the radiation sensitive LY-S subline than in the radiation resistant LY-R subline. Although the products identified in the course of this study were typical for radiation treated chromatin, the amount of base damage reported is higher than that reported in the literature (reviewed in [18]). However, it is in excellent agreement with the previously reported results [13]. The repair rates differed between sublines and were not related to the initial amount of the given altered base. The data were fitted to the equation $y = a\ e^{-bt} + c$. The parameters for all repair curves are presented in Table 1. Although the repair rates of the given altered bases differ between sublines, the repair rates of the total base damage (the sum of all altered bases) were similar in both cell lines (Fig. 2A, Table 1). However, if the relative amounts of the

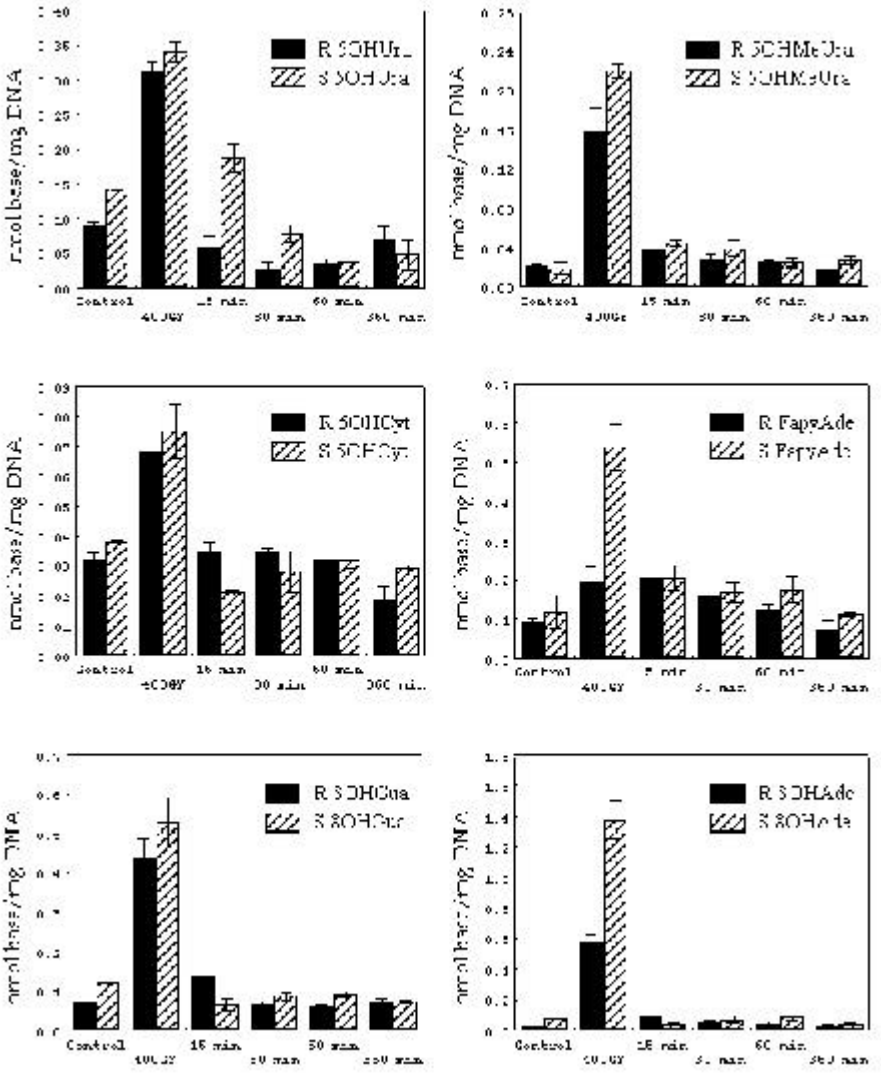

Fig ure 1. Ini tial DNA base dam age and its re pair in γ-irradiated (400 Gy) LY-R and LY-S cells.

Mean re sults from 3 ex per i ments ± SEM.

to tal base dam age are plot ted, slower re pair in LY-R cells is noticeable (Fig. 2B).

The most marked difference between LY-R and LY-S cells was in the initial amount of FapyAde and in the rate of its removal: the amount was con sid er ably higher in LY-S cells than in LY-R cells, whereas LY-R cells re-moved it much more slowly than LY-S cells. As shown in Fig. 1, the re pair in LY-S cells was almost completed after 15 min; at that time, no dam age was re paired in LY-R cells. A two-fold higher content of 8-OHAde was found upon irradiation in LY-S cells than in LY-R cells, how ever, the rates of re pair did not sig-nificantly differ. In the case of 8-OHGua, the re pair rate and the ini tial amount of the dam-aged base were higher in LY-S cells than in LY-R cells. As can be seen in Fig. 1, the dif fer-ence in damage re moval con cerns mainly the 15 min point. In con trast, 5-OHUra was gen er-ated in equal amounts in both sublines, but the repair rate in this case was markedly lower in LY-S than in LY-R cells. To make this com pli cated pat tern eas ier to fol low, we pres-ent the dif fer ences be tween the LY sublines in a simplified way in Table 2.

The con trol lev els were strik ingly high in the case of 5-OHUra (LY-S cells) and FapyAde (LY-R cells; cf. Fig. 1). Com paring the con trol lev els and the re pair rates in these cases, one

can see that where the repair was slow – the control content was high, a perfectly logical outcome. However, there was no statistically sensitive LY-S subline, its repair rates in the LY sublines seem to be unrelated to the differential lethal effect of irradiation. The repair

Table 1. Initial amounts (*a*), time constants of repair (τ) and residual amounts (*c*) of various types of base damage in LY-R and LY-S cells irradiated with 400 Gy of γ-rays

	LY-R			
	a	*b*	*c*	τ
5-OHUra	0.28 ± 0.02	0.16 ± 0.143[1]	0.03 ± 0.013	6.2
5-OHMeUra	0.13 ± 0.01[1]	0.14 ± 0.033	0.02 ± 0.004	7.1
5-OHCyt	0.04 ± 0.01	0.09 ± 0.099	0.03 ± 0.007	11.1
FapyAde	0.13 ± 0.03[1]	0.01 ± 0.006[1]	0.07 ± 0.020	100.0
8-OHAde	0.54 ± 0.01[1]	0.16 ± 0.020	0.04 ± 0.006	6.2
8-OHGua	0.37 ± 0.01[1]	0.11 ± 0.012[1]	0.06 ± 0.007[1]	9.1
Total	1.44 ± 0.02[1]	0.11 ± 0.007	0.30 ± 0.014[1]	3.3
	LY-S			
	a	*b*	*c*	τ
5-OHUra	0.30 ± 0.02	0.05 ± 0.01[1]	0.03 ± 0.015	20.0
5-OHMeUra	0.19 ± 0.01[1]	0.16 ± 0.04	0.03 ± 0.004	6.2
5-OHCyt	0.04 ± 0.001	0.22 ± 0.04	0.03 ± 0.001	4.5
FapyAde	0.39 ± 0.04[1]	0.12 ± 0.05[1]	0.14 ± 0.021	8.3
8-OHAde	1.31 ± 0.02[1]	0.29 ± 0.13	0.06 ± 0.013	3.4
8-OHGua	0.44 ± 0.01[1]	0.25 ± 0.05[1]	0.08 ± 0.004[1]	4.0
Total	2.67 ± 0.09[1]	0.19 ± 0.05	0.40 ± 0.040[1]	2.5

Equation $y = a\,e^{-bt} + c$ was fitted to the experimental values; *a* is the measure of the reparable damage induced by radiation; *c* is their reparable damage (total damage is $a + c$) and *b* ($=1/\tau$) is the time constant for the repair of that damage; τ is the time required to reduce the reparable damage to 37% of its initial amount. Results represent estimated value ± S.E. [1]Significant difference, LY-R *versus* LY-S.

significant correlation between the control base damage levels and the values of *b*, when the data for all types of base damage were considered.

DISCUSSION

The yield of DNA base damage induced by low-LET ionizing radiation has been estimated to be 2.7 times the yield of single strand break, that is, 2700 damaged bases per cell per Gy. This type of damage seems unimportant for the lethal effect of irradiation in mammalian cells (reviewed in [19]). Although the base damage is higher in the more radio-

rates of various types of base damage in the radiation sensitive LY-S cell line are either equal to those in the radioresistant counterpart, LY-R, or higher (Tables 1 and 2), with one exception (5-OHUra). In spite of irradiation with a very high dose (400 Gy), about 80% of damage usually is removed during the first 15 min, as can be seen in Fig. 1.

The most striking difference between LY-R and LY-S cells found in this study was in the initial amount of FapyAde. This difference may be due to the enhanced induction of the damage or to the enhanced rate of its removal. The former is rather unlikely in the case of L5178Y cells, since the amount of FapyAde is considerably higher in LY-S cells,

but its removal is slower in LY-R cells. Whether these two closely related cell lines can differ so much in the induction of initial DNA damage needs to be further clarified. The potential factors that can preferentially modify the induction of DNA base damage are intracellular redox environment and transition metal ion content [20–23].

Although the damaged bases seem to be very efficiently removed, their location in the vicinity of other lesions gives rise to multiply damaged sites, and thus adds to the lethal effect of irradiation [19]. The delay in repair of such sites may be the ultimate death cause: as suggested by Aldridge and Radford [24], the time period available for DNA repair prior to potential activation of apoptosis is a critical determinant of radiosensitivity in some cell lines. Thus, base damage may indirectly contribute to the overall lethal effect of radiation. Estimation of this contribution would be rather difficult without applying a much more sensitive analytical method. However, judging from the data on base damage repair in the LY sublines (Tables 1 and 2), the rate of repair is not related to the radiation sensitivity. This result does not support the assumption that DSB generation due to clustered base damage excision contributes to the delayed rejoining of DSB in LY-S cells; the delayed DSB rejoining obviously is caused by a defect in the functioning of the DSB repair system. Even the removal rate of the potentially lethal formamidopyrimidine that effectively blocks DNA polymerases [2], is 12 times lower in the radioresistant LY-R subline than in the radiosensitive LY-S subline.

Table 2. DNA base damage (initial amount and repair rate) in LY cells γ-irradiated with 400 Gy

Damaged base	Initial amount	Repair rate	Residual damage
	LY-R *versus* LY-S		
5-OHUra	Equal	Higher	Equal
5-OHMeUra	Lower	Equal	Equal
5-OHCyt	Equal	Equal	Equal
FapyAde	Lower	Lower	Equal
8-OHAde	Lower	Equal	Equal
8-OHGua	Lower	Lower	Lower
Total	Lower	Equal	Lower

The role of base damage in mutagenesis is a matter of debate [2, 19, 25]. There is strong evidence that multiply damaged sites are the causative lesions in mutagenesis (reviewed in [19]). On the other hand, oxidized bases are abundant in cellular DNA and are implicated in various pathological processes [26, 27] and

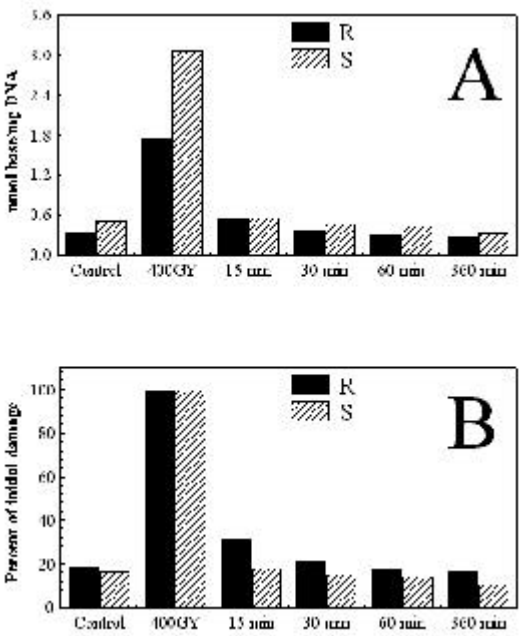

Figure 2. Total initial DNA base damage (sum of all altered bases) and its repair in γ-irradiated (400 Gy) LY-R and LY-S cells expressed as the amount of altered bases (A) or the percentage of the initial damage (taken as 100%) (B).

ageing [28]. In bacteria, base-excision repair enzymes are anti-mutagenic, as indicated by enhanced mutation frequencies in strains defective in the activity of glycosylases involved in the repair of oxidised bases [25]. Whether there is an analogy between bacteria and mammalian cells, remains to be seen when suitable mutant mammalian cell lines are obtained.

Since the locally multiply damaged sites also comprise base damage, the relative amounts of base damage and strand breaks and their repair rates, especially under conditions of low dose rate irradiation, may affect the yield of lethal or mutagenic lesions. In this respect, the LY sublines fit this general pattern. The higher level of radiation induced DNA base damage in LY-S cells is compensated by higher rates of repair of the potentially lethal formamidopyrimidine, and of the highly mutagenic 8-OHGua (the latter seen when the percentage of damage at the 15 min time point is compared in the LY sublines, Fig. 2B). However, since only about 30% of X-ray induced mutations are point mutations [2] the higher rates of repair of FapyAde and 8-OHGua do not sufficiently explain the hypomutability of LY-S cells exposed to ionizing radiation [3, 7]; hence, other cellular processes may be responsible for the low mutability of these cells. The remaining 70% of X-ray induced mutations are deletions and chromosomal rearrangements; if it happens that the target locus is in a close vicinity to that of essential genes – their loss causes cell kill, thus excluding mutations in the target locus from the analysis and resulting in an apparent hypomutability (as proposed by Evans to explain the hypomutability of LY-S cells [29, 30]).

In summary, the repair rates of the γ-ray-induced DNA base damage in the LY sublines are related neither to the initial amounts of the damaged bases nor to the differential lethal or mutagenic effects of ionizing radiation in these sublines. Although there is no doubt that the impairment of DSB rejoining is the main cause of LY-S susceptibility to ionizing radiation [8], our result does not support the assumption that DSB generation due to excision of clustered base damage contributes to the delayed rejoining of DSB in these cells.

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REFERENCES

1. Ward, J.F. (1999) Nature of lesions formed by ionizing radiation; in *DNA Damage and Repair, vol 2: DNA Repair in Higher Eucaryotes* (Nickoloff, J.A. & Hoekstra, M.F., eds.) pp. 65–84, Humana Press, Totowa, New Jersey.

2. Wallace, S.S. (1998) Enzymatic processing of radiation-induced free radical damage in DNA. *Radiat. Res.* **150**, S60–S79.

3. Beer, J.Z., Budzicka, E., Niepokojczycka, E., Rosiek, O., Szumiel, I. & Walicka, M. (1983) Loss of tumorigenicity with simultaneous changes in radiosensitivity and photosensitivity during *in vitro* growth of L5178Y murine lymphoma cells. *Cancer Res.* **43**, 4736–4742.

4. Bouzyk, E., Buraczewska, I., Rosiek, O., Sochanowicz, B. & Szumiel, I. (1991) Hydrogen peroxide induced reproductive and interphase death in two strains of L5178Y murine lymphoma differing in radiation sensitivity. *Radiat. Environ. Biophys.* **30**, 105–116.

5. Bouzyk, E., Iwanenko, T., Jarocewicz, N., Kruszewski, M., Sochanowicz, B. & Szumiel, I. (1997) Antioxidant defense system in differentially hydrogen peroxide sensitive L5178Y sublines. *Free Radical Biol. Med.* **22**, 697–704.

6. Kruszewski, M., Green, M.H., Lowe, J.E. & Szumiel, I. (1994) DNA strand breakage, cytotoxicity and mutagenicity of hydrogen peroxide treatment at 4°C and 37°C in L5178Y sublines. *Mutat. Res.* **308**, 233–241.

7. Kruszewski, M., Green, M.H., Lowe, J.E. & Szumiel, I. (1995) Comparison of effects of iron and calcium chelators on the response of L5178Y sublines to X-rays and H_2O_2. *Mutat. Res.* **326**, 155–163.

8. Wlodek, D. & Hittelman, W.N. (1987) The repair of double-strand DNA breaks correlates with radiosensitivity of L5178Y-S and L5178Y-R cells. *Radiat. Res.* **112**, 146–155.

9. Wlodek, D. & Hittelman, W.N. (1988) The relationship of DNA and chromosome damage to survival of synchronized X-irradiated L5178Y cells. II. Repair. *Radiat. Res.* **115**, 566–575.

10. Wlodek, D. & Hittelman, W.N. (1988) The relationship of DNA and chromosome damage to survival of synchronized X-irradiated L5178Y cells. I. Initial damage. *Radiat. Res.* **115**, 550–565.

11. Kapiszewska, M., Szumiel, I. & Lange, C.S. (1992) Damage at two levels of DNA folding measured by fluorescent halo technique in X-irradiated L5178Y-R and L5178Y-S cells. I. Initial lesions. *Radiat. Environ. Biophys.* **31**, 311–322.

12. Szumiel, I., Kapiszewska, M., Kruszewski, M., Iwanenko, T. & Lange, C.S. (1995) Content of iron and copper in the nuclei and induction of pH 9-labile lesions in L5178Y sublines inversely cross-sensitive to H_2O_2 and X-rays. *Radiat. Environ. Biophys.* **34**, 113–119.

13. Zastawny, T.H., Kruszewski, M. & Olinski, R. (1998) Ionizing radiation and hydrogen peroxide induced oxidative DNA base damage in two L5178Y cell lines. *Free Radical Biol. Med.* **24**, 1250–1255.

14. Mori, T. & Dizdaroglu, M. (1994) Ionizing radiation causes greater DNA base damage in radiation-sensitive mutant M10 cells than in parent mouse lymphoma L5178Y cells. *Radiat. Res.* **140**, 85–90.

15. Szumiel, I. (1979) Response of two strains of L5178Y cells to *cis*-dichlorobis (cyclopentylamine)platinum(II). I. Cross-sensitivity to *cis*-PAD and UV light. *Chem.-Biol. Interact.* **24**, 51–72.

16. Mee, L.K. & Adelstein, S.J. (1981) Predominance of core histones in formation of DNA–protein crosslinks in gamma-irradiated chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2194–2198.

17. Dizdaroglu, M. (1994) Chemical determination of oxidative DNA damage by gas chromatography-mass spectrometry. *Methods Enzymol.* **234**, 3–16.

18. Halliwell, B. & Dizdaroglu, M. (1992) The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. *Free Radical Res. Commun.* **16**, 75–87.

19. Ward, J.F. (1995) Radiation mutagenesis: The initial DNA lesions responsible. *Radiat. Res.* **142**, 362–368.

20. Dizdaroglu, M., Rao, G., Halliwell, B. & Gajewski, E. (1991) Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions. *Arch. Biochem. Biophys.* **285**, 317–324.

21. Gajewski, E., Rao, G., Nackerdien, Z. & Dizdaroglu, M. (1990) Modification of DNA bases in mammalian chromatin by radiation-generated free radicals. *Biochemistry* **29**, 7876–7882.

22. Martins, E.A., Chubatsu, L.S. & Meneghini, R. (1991) Role of antioxidants in protecting cellular DNA from damage by oxidative stress. *Mutat. Res.* **250**, 95–101.

23. Meneghini, R. (1997) Iron homeostasis, oxidative stress, and DNA damage. *Free Radical Biol. Med.* **23**, 783–792.

24. Aldridge, D.R. & Radford, I.R. (1998) Explaining differences in sensitivity to killing by

ionizing radiation between human lymphoid cell lines. *Cancer Res.* **58**, 2817–2824.

25. Laval, J., Jurado, J., Saparbaev, M. & Sidorkina, O. (1998) Antimutagenic role of base-excision repair enzymes upon free radical-induced DNA damage. *Mutat. Res.* **402**, 93–102.

26. Beckman, K.B. & Ames, B.N. (1997) Oxidative decay of DNA. *J. Biol. Chem.* **272**, 19633–19636.

27. Loft, S. & Poulsen, H.E. (1996) Cancer risk and oxidative DNA damage in man. *J. Mol. Med.* **74**, 297–312.

28. Bohr, V.A. & Anson, R.M. (1995) DNA damage, mutation and fine structure DNA repair in aging. *Mutat. Res.* **338**, 25–34.

29. Evans, H.H., Ricanati, M., Di Salvo, C., Horng, M.F. & Mencl, J. (1991) Induction of multilocus lesions by UVC-radiation in mouse L5178Y lymphoblasts. *Mutation Res.* **251**, 79–90.

30. Yi, P.N., Evans, H.H., Beer, J.Z. & Rha, C.K. (1994) Relationships between mitotic delay and the dose rate of X radiation. *Radiat. Res.* **140**, 387–392.