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Sirtuin inhibition increases the rate of non-homologous end-joining of DNA double strand breaks

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Sirtuins (type III histone deacetylases) are an important member of a group of enzymes that modify chromatin conformation. We investigated the role of sirtuin inhibitor, GPI 19015, in double strand break (DSB) repair in CHO-K1 wt and xrs-6 mutant cells. The latter is defective in DNA-dependent protein kinase (DNA-PK)-mediated non-homologous end-joining (D-NHEJ). DSB were estimated by the neutral comet assay and histone γH2AX foci formation. We observed a weaker effect of GPI 19015 treatment on the repair kinetics in CHO wt cells than in xrs6. In the latter cells the increase in DNA repair rate was most pronounced in G1 phase and practically absent in S and G2 cell cycle phases. The decrease in the number of histone γH2AX foci was faster in xrs6 than in CHO-K1 cells. The altered repair rate did not affect survival of X-irradiated cells. Since in G1 xrs6 cells DNA-PK-dependent non-homologous end-joining, D-NHEJ, does not operate, these results indicate that inhibition of sirtuins modulates DNA-PK-independent (back-up) non-homologous end-joining, B-NHEJ, to a greater extent than the other DSB repair system, D-NHEJ.

Keywords: NHEJ, DSB, DNA-PK, histone H2AX, GPI, comet assay, X-irradiation

INTRODUCTION

Histone deacetylases (HDACs) are an important member of a group of enzymes that modify chromatin conformation. In mammalian cells, homologues of the yeast gene *SIR2* code type III sirtuins that are dependent on NAD^+ and inhibited by nicotinamide. It is assumed that in mammalian cells with damaged DNA, HDACs, including certain sirtuins, may modify chromatin structure and thus alter the accessibility of the damaged sites for repair enzymes (Kruszewski & Szumiel, 2005). One possibility to alter chromatin condensation is acetylation of histone "tails". This is a way to decondense the chromatin fibre and to open a further possibility to remodel the structure of the particular chromatin fibre fragment, e.g. by ATP-dependent chromatin remodelling factors (known as SWI/SNF remodelers). In turn, deacetylation opens the way to histone methylation and formation of condensed, transcriptionally inactive chromatin.

Both deacetylation and acetylation of histones seem important for DNA double strand break (DSB) repair in yeast and mammalian cells (reviewed in Kruszewski & Szumiel, 2005). The importance has recently been greatly emphasised by the discovery

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Abbreviations: ATM, ataxia telangiectasia mutated; B-NHEJ, DNA-PK-independent (backup) non-homologous end-joining; DNA-PK, DNA-dependent protein kinase; D-NHEJ, DNA-PK-dependent non-homologous end-joining; DSB, double strand break; GPI, 2-(2,4-dinitro-phenylsulfanyl)-thiophene; γH2AX, gamma phosphorylated histone H2AX; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDAC III, type III histone deacetylase (sirtuin); HRR, homologous recombination repair; PARP-1, poly(ADP-ribose) polymerase-1; *SIR2,* silent information regulator yeast gene; Tip60, Tat interactive protein; XRCC1, X-ray repair complementing defective repair in Chinese hamster cells 1.

that histone acetyltransferase (HAT) Tip60 modifies the key kinase of the DNA damage signalling system – ataxia telangiectasia mutated (ATM). This acetylation step is essential for ATM activation and for developing by the mammalian cell of an efficient checkpoint and repair response to ionising radiation (Sun *et al*., 2005). The respective ATM-specific HDAC has not been identified so far.

HDAC inhibition increases radiosensitivity of tumor cells *in vitro* and *in vivo.* At least three cellular mechanisms lead to this effect:
(1) the inhibitors attenuate the expression of apoptosis antagonists (e.g., Goh *et al*., 2001);
(2) expression of DSB repair-related genes is reduced, among others, of those coding for the catalytic subunit of the DNA-dependent protein kinase DNA-PKcs, as well as Ku70, Ku80, and Rad50, thus affecting the repair after exposure to radiation (Goh *et al*., 2001; Munshi *et al*., 2005);
(3) slower DSB repair takes place in HDAC inhibitor-treated cells (suggested by prolonged expression of histone γH2AX foci in X-irradiated human tumor cells *in vitro* (Camphausen *et al*., 2004a; 2004b; 2005; Munshi *et al*., 2005)).

In the latter studies, however, DNA repair was examined only by the host cell reactivation assay and only HDAC class I and II inhibitors were applied in combination with ionising radiation. So far, there are no data directly confirming the effect of specific sirtuin inhibition on DSB repair processes in mammalian cells. Since it has long been known that various DNA repair processes proceed differently in various genome regions (e.g. Chakalova & Russev, 1998; Sak & Stuschke, 1998) and the acetylation status of histones affects the conformation of chromatin, and thus modifies the accessibility of the damaged sites to the DNA repair machinery (reviewed in Kruszewski & Szumiel, 2005), it seemed plausible to assume that DSB repair may be modified by sirtuin inhibitors. Therefore, we undertook an investigation of the role of sirtuins in the repair of X-ray-induced DSB using two Chinese hamster cell lines: a wild type (CHO-K1) and a radiation sensitive DSB repair-defective mutant line, xrs-6. The latter is defective in DNA-dependent protein kinase (DNA-PK)-mediated non-homologous end-joining (D-NHEJ) due to the deficiency in Ku80 protein. Hence, comparison of the effect of sirtuin inhibition in these two cell lines could provide information not only on the DSB repair rate under conditions of deacetylase inhibition but also on the identity of the repair system involved. Whereas D-NHEJ functions normally in CHO-K1 cells, the xrs6 cells use an alternative not yet sufficiently characterised non-homologous end-joining system for DSB repair in non-replicated DNA. Here, we present results of experiments with a specific sirtuin inhibitor, GPI 19015.

MATERIALS AND METHODS

Materials. GPI 19015 (2-(2,4-dinitro-phenylsulfanyl)-thiophene (relative molecular weight 282.30) was synthesized at MGI Pharma Inc., USA (Zhang & Xu, 2005). It has an IC_{50} of 4 μM against recombinant human SIRT2 in a fluorimetric assay (SIRT2 Fluorescent Activity Assay/Drug Discovery Kit, Bimol® International, LP, USA). For SIRT1, its IC_{50} is about 100 μM. GPI 19015 does not inhibit HDAC I or HDAC II with <4% inhibition at 100 μM in an assay for HDAC I and II (HDAC Fluorescent Activity Assay/Drug Discovery Kit, Bimol® International, LP, USA).

Cell lines. Experiments were carried out using a pair of Chinese hamster ovary (CHO) lines: a wild type one (CHO-K1), and a radiation sensitive, DSB repair defective mutant line, xrs6 (obtained from Dr. Penny A. Jeggo). The cells were grown in minimal essential medium (MEM, Sigma) supplemented with 10% fetal calf serum (GIBCO) and antibiotics in humidified air incubator at 37°C, 5% CO_2. Asynchronous populations in the exponential growth phase were used in all experiments.

Treatment. Exponentially growing cells were incubated at 37°C for 1 h with the sirtuin inhibitor GPI 19015 at 20 μM and X-irradiated with a 10 Gy (comet assay) or a 1 Gy (histone γH2AX foci estimation). In experiments with the use of comet assay or repair foci analysis there was no medium change after irradiation. X-Irradiation (ANDREX, Holger Andreassen, Denmark, 200 kVp, 5 mA) was at a dose rate of 1.2 Gy/min.

Clonogenicity assay. Survival of CHO-K1 and xrs6 cells subjected to combined treatment (GPI 19215+X) was determined by cloning. After irradiation with an approximately equitoxic dose (3 Gy, CHO-K1 cells; 0.3 Gy, xrs6 cells) the medium was replaced by a fresh portion. Clones were scored after 5 days. Data were normalized to the respective controls (irradiated cells to unirradiated, untreated; irradiated and GPI-treated cells to GPI-treated). The cloning efficiency of both cell lines was about 50%.

Neutral comet assay. To determine the initial DNA damage, cells were irradiated on ice. For time course experiments cells were irradiated at room temperature and incubated at 37°C for 30, 60 and 120 min. DSB were estimated by the neutral comet assay in a recently modified version (Wojewodzka *et al*., 2002). Briefly, the cell suspension (4×10^5 cells/ml) was mixed with on equal volume of low melting point agarose Type VII at a final concentration of 0.75%. The suspensions were cast on microscope slides pre-coated with 0.5% regular agarose Type IA and allowed to set under cover slips on an ice-cooled metal plate. After solidification the slides were left at 4°C in dark for 1–2 h in the lysing buffer. The lys-

ing buffer consisted of 2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, 1% *N*-lauroylsarcosine, pH 9.5. Immediately before use Triton X-100 and dimethylsulphoxide (DMSO) were added to the buffer to 0.5% and 10%, respectively, and mixed for 20 min. After lysis the slides were washed 3 times with the electrophoresis buffer (300 mM sodium acetate, 100 mM Tris/HCl, pH 8.5) and left in a fresh portion of the buffer for 1 h, then placed in a horizontal gel electrophoresis unit filled with fresh electrophoretic buffer. Electrophoresis conditions were 0.7 V/cm, 12 mA, for 1 h at 8°C. Slides were stained with 1 μM 4′,6-diamidine-2-phenylindole dihydrochloride (DAPI, 50 μl per slide). Image analysis was performed by the Comet version 3 software package (Kinetic Imaging, Liverpool, UK).

To evaluate DNA damage repair in CHO-K1 and xrs6 cells in different phases of the cell cycle, the results obtained for single cells in each experiment were grouped according to the distribution in the cell cycle. The data for each cell line were obtained by flow cytometry, carried out as described in Buraczewska *et al.* (2000). Using as reference the cell cycle distribution, each cell population was divided into subpopulations corresponding to cell cycle phases on the basis of DNA content assessed from the total comet fluorescence. Mean values of tail moment were then calculated for each cell-cycle phase from four to six independent experiments.

Immunofluorescence. The procedure was taken from (Cowell *et al*., 2005). Briefly, cells were seeded on coverslips and grown to about 60% confluence (estimated under inversed microscope). GPI 19015 treatment or sham treatment for 1 h was followed by X-irradiation with 1 Gy and by a 4 h incubation in the presence or absence of the inhibitor. The cells on coverslips were subsequently washed with PBS (phosphate-buffered saline), fixed in methanol at –20°C for 5 min, washed 3 times for 10 min with PBS, blocked overnight at 4°C with a blocking solution (120 mM KCl, 20 mM NaCl, 10 mM Tris/HCl, pH 8.0, 1 mM EDTA, 0.1% (w/v) Triton X-100 containing 10% (w/v) dried milk powder and 2% (w/v) bovine serum albumine). Incubation with mouse monoclonal anti-γH2AX antibody diluted 1:200 (Upstate, UK) was carried out in the same solution for 1–2 h at room temperature. After three washes with buffer (120 mM KCl, 20 mM NaCl, 10 mM Tris/HCl, pH 8.0, 1 mM EDTA, 0.1% (w/v) Triton X-100) a fluorescent 2nd antibody (Alexafluor 546 anti-mouse IgG (H+L); Molecular Probes) was pipetted onto the coverslips and incubated for 1 h at room temperature in the dark. After three washes, the coverslips were mounted on slides using mounting medium (90% glycerin in PBS, 200 mM DABCO (Sigma)) with 1 μM DAPI. Images were captured using a Leitz fluorescent microscope equipped with a CCD camera and Metasystem software. Subsequent image examination was carried out in Paint. One-hundred cells were scored per experimental point.

Statistical analysis. Data analysis was based on the mean population response or on the distribution of damage among cells. Statistical evaluation (two-tailed Student's *t*-test) and plots were prepared with Statistica 5.1 software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

We compared the rate of DSB repair in GPI+X-treated cells and X-irradiated ones. Figure 1 shows the percentage of DNA in comet tail *versus* time after X-irradiation, fitted to a standard equation describing DSB rejoining kinetics (Kruszewski *et al*., 1998). The equation parameters and statistical evaluation

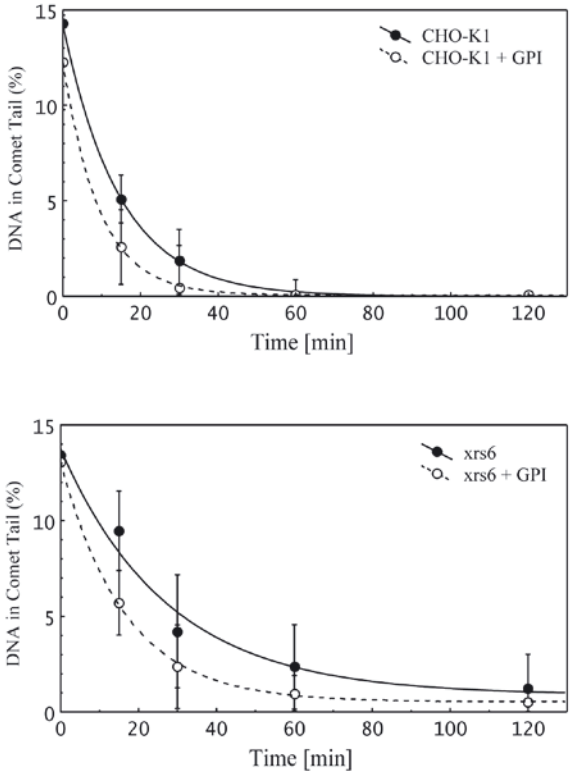

Figure 1. Repair of DNA in CHO-K1 and xrs6 cells treated with GPI 19015.
DSB repair estimated by the neutral comet assay in CHO-K1 and xrs6 cells incubated with 20 μM sirtuin inhibitor GPI 19015 at 37°C for 1 h and X-irradiated with 10 Gy without medium change. The value for control (unirradiated) cells was subtracted from that for irradiated cells. Four or five independent experiments were carried out for each treatment and percentages of DNA in the comet tail in 50 comets per experimental point were measured. Data were computer-fitted (least square method) to the equation $y=a\bullet e^{-bt}+c$, where a=initial repairable damage, b=reciprocal of the time constant, t=time and c=residual damage. See Table 1 for equation parameters.

Table 1. Parameters of repair curve of CHO cells treated with GPI 19015

Cells and treatment	a ±S.D.	b ±S.D.	c ±S.D.	Correlation coefficient
CHO-K1 + 10 Gy	**14.27 ± 0.33**	0.07 ± 0.33	0.0001 ± 0.21	0.99
CHO-K1 + GPI + 10 Gy	**12.19 ± 0.24**	0.11 ± 0.01	0.06 ± 0.13	0.99
xrs6 + 10 Gy	12.85 ± 3.19	**0.04 ± 0.02**	0.89 ± 2.44	0.98
xrs6 + GPI + 10 Gy	12.54 ± 0.51	**0.06 ± 0.01**	0.54 ± 0.33	0.99

Parameters of equation $y=a\bullet e^{-bt}+c$, describing DNA repair estimated by the neutral comet assay in CHO-K1 and xrs6 cells incubated with 20 μM sirtuin inhibitor GPI 19015 at 37°C for 1 h and X-irradiated with 10 Gy without medium change (data from Fig. 1). Significantly different parameters between GPI-treated and untreated cells $P<0.05$ (two-tailed *t*-test) are printed in bold.

of differences are given in Table 1. Generally, a statistically significant effect of GPI 19015 treatment in CHO-K1 cell line was on the initial repairable damage (parameter a), whereas in the DSB repair-defective mutant cell line xrs6, there was an alteration in the time constant of DSB repair (parameter b). The residual damage (parameter c) differences were not statistically significant.

A better resolution of the differences between CHO-K1 and xrs6 cells was possible when we evaluated DSB rejoining in different phases of the cell cycle (Fig. 2). The results obtained for single cells in each experiment were grouped according to their DNA content and hence, to the distribution in the cell cycle. The differences in parameters of repair curves are statistically evaluated in the same way as in the case of whole cell populations (Table 2). It can be seen that in CHO-K1 cells a difference in the initial damage due to inhibitor treatment is seen in G1 cells only. Additionally, an alteration in the time constant of DSB repair (parameter b) could be revealed in G1 phase cells, which was masked in the whole cell population analysis. Also in G1 phase, where the NHEJ deficiency in xrs6 cells shows most clearly, the GPI+X treated xrs6 cells rejoin DSB significantly more quickly than the GPI-untreated, X-irradiated cells. In S and G2 phases, where HRR can act with the replicated DNA as a template for correct repair, the difference between the GPI-treated and untreated X-irradiated xrs6 cells becomes statistically insignificant. Interestingly, there is a decrease in the initial damage in S phase xrs6 cells after GPI treatment. The negative sign in some data for parameter c in Table 2 is due to the fact that control values were subtracted from treated sample values; they illustrate the fact that repair lowers the damage level to that not discernible from the control within the sensitivity limits of the method.

We also checked the effect of a continuous (up to 4 h) presence of the inhibitor on the dynamics of γ-H2AX focus disappearance in X-irradiated cells, since it is assumed (e.g., Antonelli *et al*., 2005) that a longer persistence of γH2AX foci is consistent with a slower repair. Figure 3 shows that at the 4 h interval, the number of foci induced by X-irradiation in untreated and inhibitor-treated CHO-K1 cells is equal. In xrs6 cells, the number of foci is lower after inhibitor treatment, in agreement with the comet assay data (Fig. 1). It should be noted that DSB rejoining measured with the comet assay proceeds much faster than the decrease in foci frequency (Banath *et al*., 2004); hence, there is a discrepancy between the time intervals for measuring DSB repair by the comet assay and for foci scoring.

Table 2. Parameters of repair curve in different phases of the CHO cells cycle

Cells and treatment	Phase	a ±S.D.	b ±S.D.	c ±S.D.	Correlation coefficient
CHO-K1 + 10 Gy	G1	**15.46 ± 1.22**	**0.05 ± 0.01**	–1.52 ± 0.83	0.99
	S	17.82 ± 2.24	0.05 ± 0.01	–2.49 ± 1.48	0.99
	G2	14.87 ± 3.20	0.09 ± 0.06	–0.23 ± 1.88	0.99
CHO-K1 + GPI + 10 Gy	G1	**12.96 ± 0.94**	**0.08 ± 0.02**	–1.18 ± 0.57	0.99
	S	15.41 ± 2.18	0.10 ± 0.04	–1.69 ± 1.27	0.99
	G2	13.62 ± 1.47	0.09 ± 0.03	–0.50 ± 0.87	0.99
xrs6 + 10 Gy	G1	12.70 ± 3.75	**0.03 ± 0.02**	1.09 ± 3.00	0.98
	S	**15.61 ± 0.19**	0.05 ± 0.01	**–0.50 ± 0.13**	1.00
	G2	12.12 ± 2.85	0.05 ± 0.03	–0.07 ± 1.87	0.98
xrs6 + GPI + 10 Gy	G1	12.53 ± 0.86	**0.06 ± 0.01**	0.69 ± 0.57	0.99
	S	**13.21 ± 1.24**	0.07 ± 0.01	**0.48 ± 0.76**	0.99
	G2	11.31 ± 1.09	0.07 ± 0.02	–0.96 ± 0.70	0.99

Parameters of equation $y=a\bullet e^{-bt}+c$, describing DNA repair estimated by the neutral comet assay in CHO-K1 and xrs6 cells in relation to cell cycle distribution (pooled data from Fig. 1 divided according to DNA content). Significantly different parameters between GPI-treated and untreated cells $P<0.05$ (two-tailed *t*-test) are printed in bold.

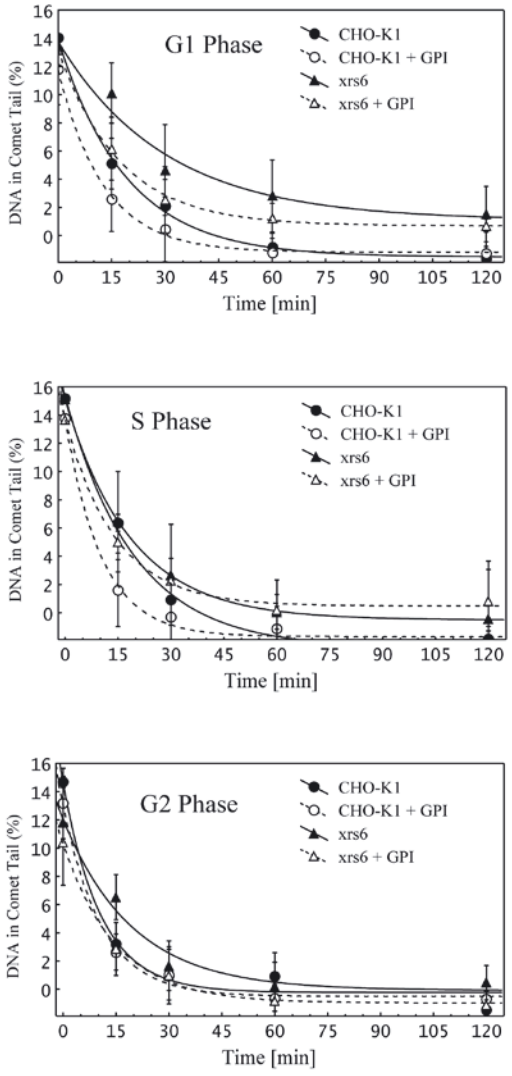

Figure 2. Repair of DNA estimated by the neutral comet assay in CHO-K1 and xrs6 cells in relation to cell cycle distribution.
To evaluate DNA damage repair in CHO-K1 and xrs6 cells in different phases of the cell cycle, the results obtained for single cells in each experiment were grouped according to the distribution in the cell cycle, as described by Kruszewski *et al.* (1998). Using as reference the cell cycle distribution data obtained for each cell line by flow cytometry (not shown), cell population was divided into subpopulations corresponding to cell cycle phases on the basis of DNA content assessed from the total comet fluorescence. Mean values of the percentage of DNA in the comet tail were then calculated for each cell cycle phase. Data were computer-fitted (least square method) to the equation as in Fig. 1. See Table 2 for equation parameters.

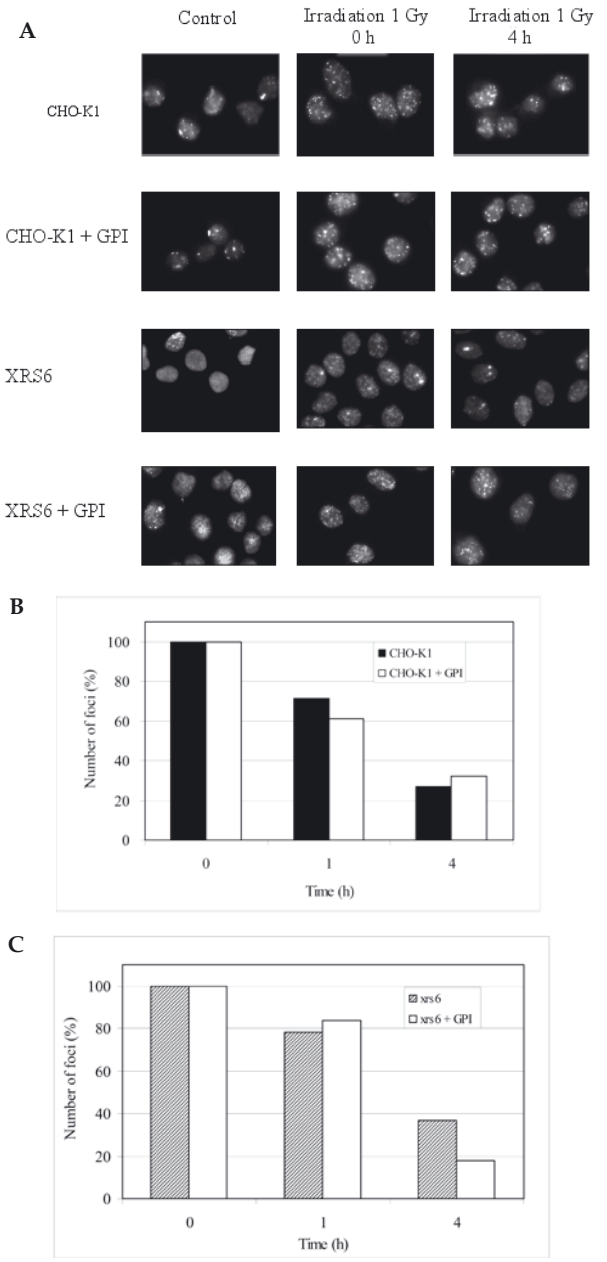

Figure 3. (A) γH2AX histone foci in control cells and cells irradiated with 1 Gy of X-rays. (B) Time-dependent decrease of the number of γH2AX histone foci in untreated and inhibitor-treated CHO-K1 cells. (C) Time-dependent decrease of the number of γH2AX histone foci in untreated and inhibitor-treated xrs6 cells.
On panels B and C the numbers of foci are expressed as the percentage of the mean initial foci number in irradiated cells. The number of foci in controls was subtracted. Foci in 100 cells were scored for each time interval.

In order to see the effect of the altered DSB rejoining rate on cell survival, cloning experiments were carried out. The choice of dose for each cell line was such as to obtain a comparable survival level (50–60%). As shown in Fig. 4, a 1 h treatment with the sirtuin inhibitor prior to X-irradiation did not change the cell survival. This result must be treated as preliminary, since a single radiation dose was applied; within a wider range of doses, differences in the survival could be revealed.

DISCUSSION

A comparison of the parameters of DNA repair curves for whole cell populations indicates that

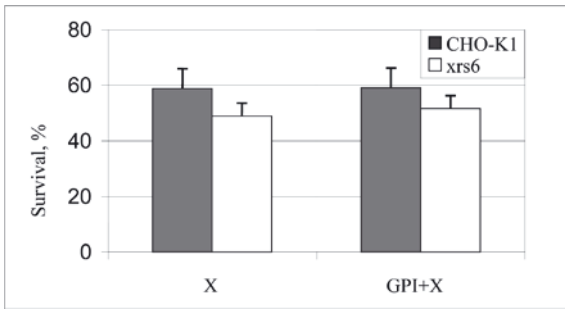

Figure 4. Survival of the CHO and xrs6 cells treated with GPI 19015.
No effect on clonogenic ability of CHO and xrs6 cells treated with 20 μM sirtuin inhibitor GPI 19015 at 37°C for 1 h, X-irradiated with 3 Gy (CHO-K1 cells) or 0.3 Gy (xrs6 cells) and cloned in fresh culture medium. Clones were scored after 5 days. Data were normalized to the respective controls (irradiated cells to unirradiated, untreated; irradiated and GPI-treated cells to GPI-treated). Cloning efficiency of both cell lines was about 50%.

after X-irradiation, the DSB repair-competent CHO-K1 cells are less affected by GPI 19015 treatment than xrs6 cells (Fig. 1, Table 1). In CHO-K1 cells, however, an effect can clearly be discerned in G1 phase (Fig. 2, Table 2). In contrast, in xrs6 cells, accelerated DSB repair is seen both in the whole cell population and in G1 phase cells. This result is supported by the decreased γH2AX foci frequency in X-irradiated xrs6 cells after GPI 19015 treatment. Persistent foci mark unrepaired or mis-repaired DSB. For this reason, the foci scoring is applied at late (4–24 h) intervals; then, the frequency of foci can be treated as a measure of repair ability and radiosensitivity (Taneja *et al*., 2004).

A possible reason of the difference between wild type CHO and xrs6 cells may lie in the impaired D-NHEJ due to mutated Ku80. DSB repair in xrs6 cells has to rely on homologous recombination repair (HRR) or DNA-PK independent (backup) B-NHEJ. The latter system is active in cells with impaired D-NHEJ (Wang *et al*., 2003; 2005; Perrault *et al*., 2004) in G1 phase, is suppressed in wild type cells by DNA-PK, and involves ligase III instead of ligase IV (Audebert *et al*., 2004).

Apparently, the important HDAC involved in DSB repair can be a sirtuin. There are several possible ways of exerting an effect on DNA repair by modifying the acetylation status of histones: (a) specific histone modifications may be necessary for components of the DNA repair systems to recognize the damaged DNA site; (b) hyperacetylation may facilitate access of repair enzymes to the damaged sites or affect DNA repair indirectly through controlling the expression of repair factors; and (c) whenever repair replication is involved in the particular repair pathway, there is a need for re-assembly of the newly synthesised DNA "patch" into chromatin. The H3 and H4 histone molecules imported from the cytoplasm must be acetylated prior to being deposited onto DNA and immediately thereafter they are deacetylated (reviewed by Tyler, 2002).

The possibilities (a) and (b) seem relevant to our experimental system, as shown above. Recently, experimental support for (b) and (c) came mainly from work on yeast (Qin & Parthun, 2002; Ai & Parthun, 2004; Linger & Tyler, 2005; Morrison & Shen, 2005). The first study with the use of vertebrate cells (chicken DT40) mutated in *SIRT1* or *SIRT2* or both (Matsushita *et al*., 2005) confirmed a relation of *SIRT1* to radiation sensitivity due to increase in apoptotic death. *SIRT1*$^{-/-}$ cells appeared to be unimpaired in DSB repair measured by plasmid re-ligation, but were more apoptosis-prone following X-irradiation than wild-type cells. Furthermore, both *SIRT1*- and *SIRT2*-deficient cells as well as double mutants were more sensitive to apoptosis-inducing agents, cisplatin and staurosporine, in a p53-independent manner. It should be noted, however, that the plasmid re-ligation assay does not reflect the conformational aspects that are important when the repair substrate is chromatin, in contrast with "naked" DNA.

The results reported here are consistent with the assumption (b) and may indicate that inhibition of sirtuins affects the access of repair enzymes to the damaged site and thus facilitates the NHEJ pathway that operates mainly in G1 phase. Presumably, increased acetylation is favourable for NHEJ to a greater extent than for other cell cycle-specific DSB repair systems, as can be judged from a comparison of the repair curves for CHO-K1 and xrs6 cells (Fig. 2). Interestingly, support comes from a recent paper (Yaneva *et al*., 2005) indicating that NHEJ, but not HRR, enhances survival of cells exposed to a histone deacetylase inhibitor.

The experimental schedule applied for cloning was designed to enable differentiation between the immediate effects on DSB repair and delayed the effects on the expression of DSB repair-related genes as well as on the late post-irradiation processes such as pro-apoptotic signalling which is known to depend on sirtuins (reviewed in Kruszewski & Szumiel, 2005; and Seo *et al*., 2005). We found that short treatment with GPI 19015 before and during X-irradiation did not significantly alter the survival when a dose that reduced survival to about 60% was used. It remains to be checked whether GPI 19015 treatment would cause a change in the survival when used for longer times or when paired with higher X-ray doses. So far, we have no direct or indirect information about the fidelity of the accelerated end-joining nor on the possible effect of GPI 19015 on apoptosis.

In conclusion: the DSB repair system, NHEJ, that operates in G1 phase, is the DSB repair system

affected by sirtuin inhibition; the functioning of the alternative (backup) route of DSB repair (B-NHEJ) apparently is facilitated to a greater extent than the other DSB repair system, D-NHEJ.

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