International Journal of Radiation Biology, 2013; Early Online: 1–10
© 2013 Informa UK, Ltd.
ISSN 0955-3002 print / ISSN 1362-3095 online
DOI: 10.3109/09553002.2013.800248

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Impact of dose-rate on the low-dose hyper-radiosensitivity and induced radioresistance (HRS/IRR) response*

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Abstract

Purpose: **To ask whether dose-rate influences low-dose hyper-radiosensitivity and induced radioresistance (HRS/IRR) response in rat colon carcinoma progressive (PRO) and regressive (REG) cells.**

Methods: **Clonogenic survival was applied to tumorigenic PRO and non-tumorigenic REG cells irradiated with ^{60}Co γ-rays at 0.0025–500 mGy.min^{-1}. Both clonogenic survival and non-homologous end-joining (NHEJ) pathway involved in DNA double-strand breaks (DSB) repair assays were applied to PRO cells irradiated at 25 mGy.min^{-1} with 75 kV X-rays only.**

Results: **Irrespective of dose-rates, marked HRS/IRR responses were observed in PRO but not in REG cells. For PRO cells, the doses at which HRS and IRR responses are maximal were dependent on dose-rate; conversely exposure times during which HRS and IRR responses are maximal (t_{HRSmax} and t_{IRRmax}) were independent of dose-rate. The t_{HRSmax} and t_{IRRmax} values were 23 ± 5 s and 66 ± 7 s (mean ± standard error of the mean [SEM], $n = 7$), in agreement with literature data. Repair data show that t_{HRSmax} may correspond to exposure time during which NHEJ is deficient while t_{IRRmax} may correspond to exposure time during which NHEJ is complete.**

Conclusion: **HRS response may be maximal if exposure times are shorter than t_{HRSmax} irrespective of dose, dose-rate and cellular model. Potential application of HRS response in radiotherapy is discussed.**

Keywords: Hyper-radiosensitivity (HRS) response, induced radioresistance (IRR) response, dose-rate, DSB repair, tumor cells, radiotherapy

Introduction

It is now well documented that cells irradiated at single low-dose fraction can show marked hyper-radiosensitivity (HRS) and induced radioresistance (IRR) response (Table I). The HRS/IRR response is a representative example of a non-linear dose-dependent event. The HRS/IRR response was originally observed in vivo in mice using acute skin tissue damage as an endpoint (Joiner et al. 1986). Thereafter, the HRS response, mostly observed by using in vitro survival assay in single tumor cells, was shown to result in a significant reduction of about 25% cell survival between 0.1 and 0.8 Gy. The dose at which the maximal HRS response is observed (D_{HRSmax}) depends on the cell line (Table I). The HRS response generally occurs in tumor or transformed cells (Marples and Collis 2008). At doses higher than D_{HRSmax}, cell survival increases progressively and this phenomenon was called IRR response. Despite a number of studies, the mechanisms of the HRS and IRR responses, whether taken separately or together, remain unclear. It has been suggested that the HRS response may depend upon changes in chromatin conformation (Joiner et al. 2001), failure of the Ataxia Telangiectasia Mutated protein (ATM)-dependent G_2/M checkpoint (Marples et al. 2004), or defects in DNA double-strand breaks (DSB) (Vaganay-Juery et al. 2000, Short et al. 2005). It was notably suggested that the HRS response may reflect apoptotic death of tumor cells that failed to arrest in cell cycle whereas the IRR response may reflect early cell cycle G_2-phase arrest allowing time for repair and increased cell survival (Marples and Collis 2008). In 2008, we pointed out that the HRS response may be caused by impairments in the non-homologous end-joining (NHEJ) repair pathway that targets G_1 cells and in lack of control of the RAD51-dependent recombination repair pathway that targets S-G_2/M cells; the consequences of such impairments are failure to arrest in the cell cycle, propagation of damage through the cell cycle, mitotic death, but not p53-dependent apoptosis (Thomas et al. 2008).

The HRS/IRR response is more marked in cells displaying genomic instability: In fact, this response was mostly

*The authors dedicate this work to Bernard Fertil for his contribution to radiobiology.

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(Received 20 November 2012; revised 10 April 2013; accepted 22 April 2013)

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Table I. Major radiobiological studies on HRS/IRR response. Most studies used tumor cell lines. Few studies used transformed cell lines (V79, CHO-K1, MR4, GM0639, EBS7YZ5) or a normal fibroblast cell line (BJ). The doses at which the maximal HRS and IRR response are observed ($D_{HRSmax,}$ D_{IRRmax}) and the time at which the maximal HRS and IRR response are observed ($t_{HRSmax,}$ t_{IRRmax}) were obtained from survival data reported in the quoted references.

Irradiation	Dose rate (mGy.min^{-1})	D_{HRSmax} (mGy)	t_{HRSmax} (s)	D_{IRRmax} (mGy)	t_{IRRmax} (s)	Cell line	Reference
240 KV X-rays	180	200	67	600	200	Human HT29	Lambin et al. (1993)
240 KV X-rays	118	120	40	500	167	Human Be11	Lambin et al. (1996)
240 KV X-rays	180	250	83	500	167	Human RT112	Lambin et al. (1996)
^{60}Co γ-rays	580	200*	21	600	62	Human variant 1 (T1p26)	Thomas et al. (1997)
^{60}Co γ-rays	580	100*	10	200	21	Human clone 4 (T1C3)	Thomas et al. (1997)
^{60}Co γ-rays	1000	440	26	750	45	Rodent V79	Tsoulou et al. (2001)
9.5Mev α-rays	1000	340	20	500	30	Rodent V79	Tsoulou et al. (2001)
300 KV X-rays	500	110	13	500	60	Rodent subline CHO-K1	Barkowiak et al. (2001)
^{60}Co γ-rays	2000	100	3	500	15	Human BMG1	Chandna et al. (2002)
^{60}Co γ-rays	2000	200	6	500	15	Human U87	Chandna et al. (2002)
^{60}Co γ-rays	2000	300	9	500	15	Human PECA4451	Chandna et al. (2002)
^{60}Co γ-rays	2000	300	9	1000	30	Human PECA4197	Chandna et al. (2002)
10 MV X-rays	2430	800	20	950	23	Human G5	Beauchesne et al. (2003)
10 MV X-rays	2430	700	17	800	20	Human G111	Beauchesne et al. (2003)
10 MV X-rays	2430	700	17	950	23	Human G142	Beauchesne et al. (2003)
10 MV X-rays	2430	800	20	950	23	Human G152	Beauchesne et al. (2003)
^{137}Cs γ-rays	220	100	27	200	54	Human A549	Enns et al. (2004)
^{137}Cs γ-rays	220	250	68	750	205	Human T98G	Enns et al. (2004)
320 KV X-rays	750	180	14	300	24	Rodent MR4	Wykes et al. (2006)
320 KV X-rays	750	105	8	400	32	Human M059K	Wykes et al. (2006)
320 KV X-rays	750	140	11	400	32	Human EBS7YZ5	Wykes et al. (2006)
^{60}Co γ-rays	660	280	25	1000	91	Human T47D	Edin et al. (2007)
320 KV X-rays	750	310	25	750	60	Human T98G	Krueger et al. (2007a)
^{60}Co γ-rays	1800	100	3.3	300	10	Human BJ	Nuta & Darroudi (2008)
^{60}Co γ-rays	500	190	23	500	60	Rodent PRO	Thomas et al. (2008)
200 KV X-rays	500	100	12	300	36	Human GM0639	Xue et al. (2009)
290 Mev ^{6}C	500	170	20	400	48	Human GM0639	Xue et al. (2009)
62 Mev protons	15000	2000	8	4000	16	Human HTB140	Petrovic et al. (2010)
250 KV X-rays	855	250	18	500	35	Human A549	Wera et al. (2012)

*These numbers correspond to a reanalysis of our raw data for exposure times less than 10 min.

observed in tumor and in some transformed normal cell lines (Table I). Furthermore, we have previously reported that human and rodent tumorigenic cells with high metastatic potential preferentially show the HRS response (Thomas et al. 1997, 2008). We have therefore suggested that the HRS response may find applications in radiotherapy, notably for unvascularized and isolated micrometastasis (Thomas et al. 2001, 2007). On the other hand, the occurrence of the HRS/IRR response in primary normal cells is still controversial and may depend on the differentiation and/or proliferation status. As an example, six among nine primary explants of uroepithelium showed HRS/IRR response with a 14 days post-irradiation proliferative assay as endpoint (Mothersill et al. 1995). The HRS/IRR response assessed by micronuclei assay was also observed in about 10% of primary keratinocytes and fibroblasts from cervix carcinoma patients (Slonina et al. 2007).

Interestingly, the literature shows that the HRS/IRR response of tumor cells irradiated with low-energy transfer (LET) radiation was investigated at dose-rates ranging from 0.18–2.43 Gy.min^{-1} (Table I). These data raise the question of a dose-rate-dependence of the HRS/IRR response. In order to answer this question, we investigated clonogenic cell survival at seven dose-rates (from 0.0025–500 mGy.min^{-1}) in two rat colon carcinoma sublines progressive (PRO) and regressive (REG) cells that were shown to be HRS positive and negative, respectively (Thomas et al. 2008).

Materials and methods

Cells and irradiation

Rat colon carcinoma PRO and REG cells were kindly provided by Dr F. Martin (Dijon, France). PRO and REG sublines were isolated from the parental tumor cell line DHD-K12, established from dimethylhydrazine-induced colon carcinoma in syngeneic BDIX rats (Martin et al. 1983). PRO and REG sublines were isolated according to their sensitivity to trypsin-mediated detachment from plastic surface (PRO subline is more trypsin-resistant than REG subline). When grafted subcutaneously in BDIX rats, REG cells produced regressive tumors disappearing within 3–4 weeks while PRO cells produced progressive tumors in 60% of animals with metastases to lungs, kidney or lymph nodes (Martin et al. 1983). PRO and REG sublines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with 2 mM glutamine, 10% decomplemented fetal bovine serum, 1% [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (HEPES) and antibiotics (1% penicillin, streptomycin) (Gibco-Invitrogen-France, Cergy-Pontoise, France). Cells were mycoplasma-free and

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maintained at 37°C at 5% CO_2 for no more than five passages after defrost. For all the assays described below, confluent PRO and REG cultures were softly detached with 0.025% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) (Gibco-Invitrogen-France, Cergy-Pontoise, France) to obtain single cell suspensions. Since the HRS/IRR response is suppressed under condition of increased cell-cell contact (Chandna et al. 2002), the number of aggregates (no more than 5 cells) was kept as low as possible. Irradiations were performed at European Synchrotron Radiation Facility (ESRF, Grenoble, France) with X-rays produced by a clinical irradiator (75 kV, 14 mA) at a dose-rate of 25 mGy/min and at Institut de Recherche Biomédicale des Armées (IRBA, Grenoble, France) with ^{60}Co γ-rays at dose-rates of 230, 60, 44, 25, 0.3 or 0.0025 mGy.min^{-1}. This range of dose-rate corresponds to space radiation (0.0025 mGy.min^{-1}), nuclear medicine (0.3 mGy.min^{-1}), radiodiagnosis (25, 44, 60 mGy.min^{-1}) and radiotherapy (230 mGy.min^{-1}). These four groups of dose-rate are evenly distributed on log scale. The 25 mGy.min^{-1} dose-rate was chosen to evaluate cell survival at two different clinically relevant radiation type (Cobalt 60 γ-rays and 75 kV X-rays). Dose and their homogeneity in the irradiation field were routinely verified with Physikalisch Technische Werkstatten (PTW) ionization chambers (0.3 cm^3 type TM23332 for dose-rates higher than 25 mGy.min^{-1} and 30 cm^3 type TM23361 for dose-rates lower than 25 mGy.min^{-1} at IRBA and semiflex chamber type TW31010–03907 for dose-rate of 25 mGy.min^{-1} at ESRF). The relative dose error was 10%. The error committed on exposure times (given digitally) was negligible. For all the dose-rates applied in this study, the exposure times were always shorter than 10 min (Table II).

Clonogenic survival assay

Clonogenic survival was assessed as previously described (Thomas et al. 2008). Briefly, 250 cells were seeded in six-well plates and irradiated 24 h after plating at various dose-rates. Colonies were fixed and stained with standard crystal violet solution (Sigma-Aldrich-France, l'Isle d'Abeau, France) after 10 days incubation without change of medium. Only colonies showing more than 50 cells were considered. Plating efficiencies of unirradiated REG and PRO cells at IRBA were 39 ± 6% (mean ± standard error of the mean [SEM], $n = 8$ independent experiments) and 25 ± 2% (mean ± SEM, $n = 17$ independent experiments), respectively. Plating efficiencies of unirradiated REG and PRO cells at ESRF were 29 ± 3% (mean ± SEM, $n = 3$ independent experiments) and 14 ± 1% (mean ± SEM, $n = 2$ independent experiments), respectively. The impact of cell proliferation before irradiation on HRS response was previously investigated; we showed that the HRS response was similar in PRO cells whether irradiated 2 or 24 h after plating; cell multiplicity (i.e., the number of cells per colony-forming unit) 24 h after plating was found to be close to one (Thomas et al. 2008).

Table II. Dose-rates, exposure times and doses investigated in this study with ^{60}Co γ-rays (data shown in Figure 1A–F).

Dose-rate (mGy/min)	Exposure times (s)	Doses (mGy)
230	2.6; 6.5; 13; 26; 65; 78; 130	10; 25; 50; 100; 250; 300; 500
60	5; 10; 25; 50; 100; 200; 300; 500	5; 10; 25; 50; 100; 200; 300; 500
44	7; 14; 27; 70; 140; 270; 341	5; 10; 20; 50; 100; 200; 250
25	6; 12; 24; 60; 120; 180; 240	2.5; 5; 10; 25; 50; 75; 100
0.3	5; 10; 20; 40; 100; 200	0.025; 0.05; 0.1; 0.2; 0.5, 1
0.0025	2.4; 6; 12; 24; 48; 120; 240	0.0001; 0.00025; 0.0005; 0.001; 0.002; 0.005; 0.01

Survival curves analysis

Using the JMP software (version 2.0.5. SAS institute, Cary, NC, USA), the surviving fractions (SF) were fitted to two models: The one population linear-quadratic (LQ) model and the induced repair (IR) model (Thomas et al. 2008) defined by, respectively:

$$SF(D) = e^{-(\alpha.D + \beta.D^2)} \quad (1)$$

$$SF(D) = e^{-\alpha_r\left[1+\left(\frac{\alpha_s}{\alpha_r}-1\right)\cdot e^{-\frac{D}{d_c}}\right]\cdot D - \beta_r\cdot D^2} \quad (2)$$

The IR model is a modified version of the LQ model in which the α term is dependent on dose (D): at very low doses, α is large, and it decreases with increasing dose in an exponential manner at a rate determined by a constant d_c. The parameter α_s represents the initial slope of the survival curve at very low doses; α_r represents the initial slope of the survival curve extrapolated from the conventional high-dose response described by the LQ model; d_c represents the dose that induced the change from HRS to IRR response and β_r represents the distal slope of the survival curve. The occurrence of the HRS/IRR response is mathematically deduced from α_s and α_r values that do not coincide and d_c values significantly greater than zero (Table II). Since some data reported in Table I were not always fitted to the IR model, we deliberately chose to rename the d_c parameter D_{HRSmax} since it corresponds to the maximal extent of the HRS response. Similarly, we defined the D_{IRRmax} parameter that corresponds to the maximal extent of the IRR response (Table I).

Immunofluorescence assay

The assay which is described elsewhere (Thomas et al. 2008), was applied with minor modifications to measure the number of γ-pH2AX foci per cell 15 min, 1 h , 4 h and 24 h after irradiation. Briefly, 10^4 cells were seeded on slides in six-well plates and incubated for 24 h in complete medium at 37°C. After irradiation at 75 kV X-rays at 10 mGy (24 s) and 100 mGy (240 s), plates were incubated at 37°C for 10 min and 24 h. Cells were then fixed in paraformaldehyde solution for 15 min at room temperature and permeabilized for 90 s at 4°C in lysis solution (20 mM HEPES) [pH 7.4], 50 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, 0.5% Triton X-100) (Sigma-Aldrich-France, l'Isle d'Abeau, France). Primary antibody incubations were performed for 40 min at 37°C. Anti-γ-*pH2AX*^{ser139} antibody (#05636; Upstate Biotechnology-Euromedex, Mundolsheim, France) was used at 1:800. Incubation with anti-mouse fluorescein

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(green) secondary antibody was performed at 1:100 at 37°C for 20 min. Slides were mounted in 4′,6-diamidino-2-phenylindole (DAPI)-stained Vectashield (Abcys, Paris, France) and the number of γ-pH2AX foci per cell in 126-209 cells (15 min experiments) or 142-198 cells (24 h experiments) were examined with Olympus BX51 fluorescence microscope. DAPI staining permitted to indirectly evaluate yield of G_1 cells (nuclei with homogeneous DAPI staining), S cells (nuclei showing numerous γ-pH2AX foci), G_2 cells (nuclei with heterogeneous DAPI staining) and metaphase (visible chromosomes). DAPI staining permitted also to quantify the percentage of cells with micronuclei by examining 100 cells at least. In order to avoid any bias by using imaging analysis software, the number of foci per cell was determined after eye-scoring in about 50 cells in G0/G1 per slide.

Results

HRS/IRR response of PRO cells irradiated with ^{60}Co γ-rays

In 2008, we demonstrated the existence of a HRS/IRR response in PRO cells but not in REG cells irradiated at 500 mGy.min^{-1}. The D_{HRSmax} value that reflects the transition between the HRS and IRR response (i.e., the lowest survival data) was 190 (± 8) mGy (Thomas et al. 2008). This dose corresponds to an exposure time of 23 (± 1) s at 500 mGy.min^{-1} (Table I). In order to examine whether dose-rate influences the HRS/IRR response, we investigated clonogenic survival of PRO cells irradiated at six different dose-rates between 0.0025 and 230 mGy.min^{-1}. For all the dose-rates applied in this study, a HRS/IRR response was systematically observed in PRO cells (Figure 1A–F). Since the distal part of the survival curves obtained at 0.3 and

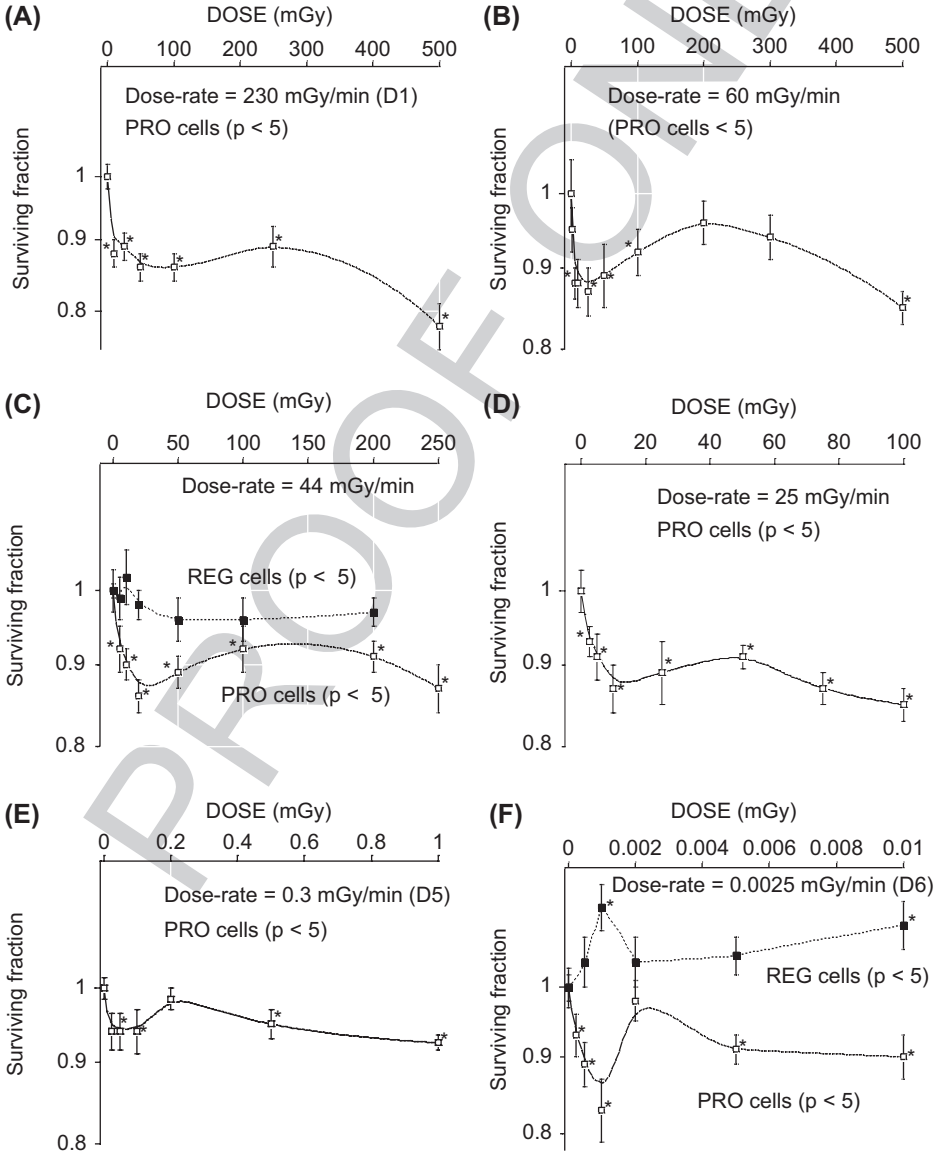

Figure 1. Impact of dose-rate on the HRS/IRR response. Survival curves of PRO cells (A–F) and REG cells (C and F) irradiated with cobalt 60 γ-rays at low-doses. Experiments were performed with cells cultured for no more than five passages ($p < 5$). Each data represents the mean ± SEM of three (A), three (B), two (PRO cells) and two (REG cells) (C), two (D), four (E) and three (F) experiments for cells irradiated 24 h after plating. Six data points per dose are included in each experiment. Figures were fitted to a smooth function. $^{*}p < 0.05$ for comparison between irradiated cells and unirradiated cells, using the *t*-test.

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Table III. Values of parameters obtained from the survival data fit to the IR model; α_s represents the initial slope of the curve at very low doses; α_r represents the low-dose slope of the survival curve extrapolated from high-doses; d_c represents the dose that induced the change from HRS to IRR response and β_r represents the distal slope of the survival curve. Numbers in parentheses are the standard errors given by the JMP software; nc = no convergence.

	α_s	α_r	α_s/α_r	d_c (mGy)	β_r
Figure 1A	9.96 (2.1)	0.43 (0.23)	18	42 (8)	0.124 (0.5)
Figure 1B	21.8 (3.5)	0.21 (0.13)	106	21 (2.6)	0.21 (0.31)
Figure 1C (PRO cells)	17.2 (1.2)	0.5 (0.17)	34	23 (1.7)	0.11 (0.74)
Figure 1C (REG cells)	nc	nc	nc	nc	nc
Figure 1D	31.5 (1.6)	1.5 (0.3)	21	11 (0.7)	1.22 (3.45)
Figure 1E	4.05 (0.69)	0.1 (0.03)	41	0.045 (0.006)	−0.024 (0.033)
Figure 1F (PRO cells)	0.59 (0.2)	0.017 (0.016)	35	0.00071 (0.0002)	−0.0006 (0.0018)
Figure 1F (REG cells)	nc	nc	nc	nc	nc
Figure 3A (75 kV X-rays)	55.8 (5.7)	1.36 (1)	41	11 (1.2)	4.9 (10.4)
Figure 3A (^{60}Co γ-rays)	31.5 (1.6)	1.5 (0.3)	21	11 (0.7)	1.22 (3.45)

0.0025 mGy.min^{-1} showed negative β_r parameter with the IR model (Table III), all data were fitted to a smooth function (Figure 1A–F). Irrespective of the dose-rates, the HRS/IRR response was observed systematically, but not at the same dose range. For example, the lowest survival was 86 ± 1% irrespective of dose-rate, but D_{HRSmax} ranged between 190 mGy at 500 mGy.min^{-1} and 0.00071 mGy at 0.0025 mGy.min^{-1} (Table IV). The D_{HRSmax} values appeared to be a linear function of dose-rate with $D_{HRSmax} = 0.4428 \times$ dose-rate ($R^2 = 0.89$) (Figure 2A). The slope of this linear function corresponds to the exposure time required for the maximal HRS response. For convenience, we called it t_{HRSmax}. Its average value was 0.4428 ± 0.05 min or 26.57 ± 3 s, and independent of dose-rate (Figure 2B). Similarly, if D_{IRRmax} and t_{IRRmax} are defined as the dose and the exposure time required for the maximal IRR response, respectively, our data showed that D_{IRRmax} is linearly dependent on dose-rate with $D_{IRRmax} = 0.997 \times$ dose-rate ($R^2 = 0.99$) (Figure 2C). The slope t_{IRRmax} was found to be 0.997 ± 0.07 min or 59.8 ± 4.2 s, and independent of dose-rate (Figure 2D). Thus, it appears that the maximal HRS and IRR responses in PRO cells correspond to exposure times that are independent of dose-rates.

Table IV. Values of the HRS/IRR response parameters obtained with PRO cells irradiated with ^{60}Co γ-rays. D_{HRSmax} and D_{IRRmax} are the doses at which the maximal HRS and IRR response are observed, respectively; t_{HRSmax} and t_{IRRmax} are the time at which the maximal HRS and IRR response are observed, respectively.

Dose-rate (mGy.min^{-1})	D_{HRSmax} (mGy)	t_{HRSmax} (s)	D_{IRRmax} (mGy)	t_{IRRmax} (s)
500	190 ± 8.1[a]	23 ± 1[a]	500[a]	60[a]
	250[b]	30[b]	500[b]	60[b]
230	42 ± 8[a]	11 ± 2[a]	175[a]	46[a]
	37 ± 12[b]	10 ± 3[b]	217 ± 17[b]	65 ± 4[b]
60	21 ± 2[a]	21 ± 2[a]	125[a]	125[a]
	28 ± 12[b]	28 ± 12[b]	83 ± 17[b]	83 ± 17[b]
44	23 ± 2[a]	31 ± 3[a]	100[a]	136[a]
	27[b]	37[b]	55[b]	75[b]
25	11 ± 1[a]	26 ± 2[b]	50[a]	120[a]
	17[b]	41[b]	38[b]	91[b]
0.3	0.045 ± 0.006[a]	9 ± 1[a]	0.2[a]	40[a]
	0.031 ± 0.006[b]	6 ± 1[b]	0.19 ± 0.1[b]	38 ± 20[b]
0.0025	0.00071 ± 0.0002[a]	17 ± 5[a]	0.004[a]	96[a]
	0.0005 ± 0.00025[b]	12 ± 6[b]	0.002 ± 0.0009[b]	48 ± 22[b]

[a]Parameters obtained from survival data fit to the IR model. [b]Experimental parameters obtained from raw survival data shown in Figure 1 except those at 500 mGy.min^{-1} (taken from Thomas et al. 2008).

In agreement with our previous data obtained at 500 mGy.min^{-1}, it is noteworthy that REG cells did not show marked HRS/IRR response at 44 mGy.min^{-1} and 0.0025 mGy.min^{-1} (Figure 1C and 1F, respectively). Conversely, REG cells displayed significant radio-stimulation at 0.0025 mGy.min^{-1} (Figure 1F). Such very low dose-rate is known to stimulate the division potential in normal cells (e.g., Croute et al. 1986, Planel et al. 1987). However, these hormetic-like responses and their possible cellular mechanisms – that were recently reviewed (Szumiel 2012) – are beyond the scope of this paper (Supplementary Material to be found online at http://informahealthcare.com/abs/doi/10.3109/09553002.2013.800248).

Comparison with the literature

We reviewed the HRS/IRR responses obtained in the literature from 1993–2012 (Table I). As a first step, only low-LET radiation (X- and γ-rays) data obtained at single dose-rate with short exposure times less than 10 min were considered. With regard to the HRS/IRR response parameters, no significant difference was observed between human and rodent cells. By pooling rodent and human data shown in Table I, the HRS/IRR responses were obtained at an average dose-rate of 1000 mGy.min^{-1}. At such dose-rate, the D_{HRSmax} and D_{IRRmax} values obtained in the literature are in agreement with our data (Figure 1A and C). The t_{HRSmax} value obtained in the literature [23 ± 4 s (mean ± SEM, $n = 25$)] was not significantly different from the experimental t_{HRSmax} value obtained in this study [23.4 ± 5.3 s (mean ± SEM, $n = 7$)] (Table IV). Similarly, the t_{IRRmax} value obtained in the literature [59 ± 12 s (mean ± SEM, $n = 25$)] was not significantly different from the experimental t_{IRRmax} value obtained in this study [66 ± 7.1 s (mean ± SEM, $n = 7$)] (Table IV). By pooling literature and our data, over a very large range of dose-rates (0.0025–2430 mGy.min^{-1}) t_{HRSmax} and t_{IRRmax} were found to be 23 ± 3 s and 60 ± 9 s [mean ± SEM ($n = 32$)], respectively.

HRS/IRR response of PRO cells irradiated with 75 kV X-rays

Since radiodiagnosis exams like computed tomography (CT) scans involve low-energy X-rays rather than high-energy γ-rays, we examined whether the HRS/IRR response of PRO cells also exists with 75 kV X-rays. With regard to dose-rate, we chose to work at 25 mGy.min^{-1} since this dose-rate

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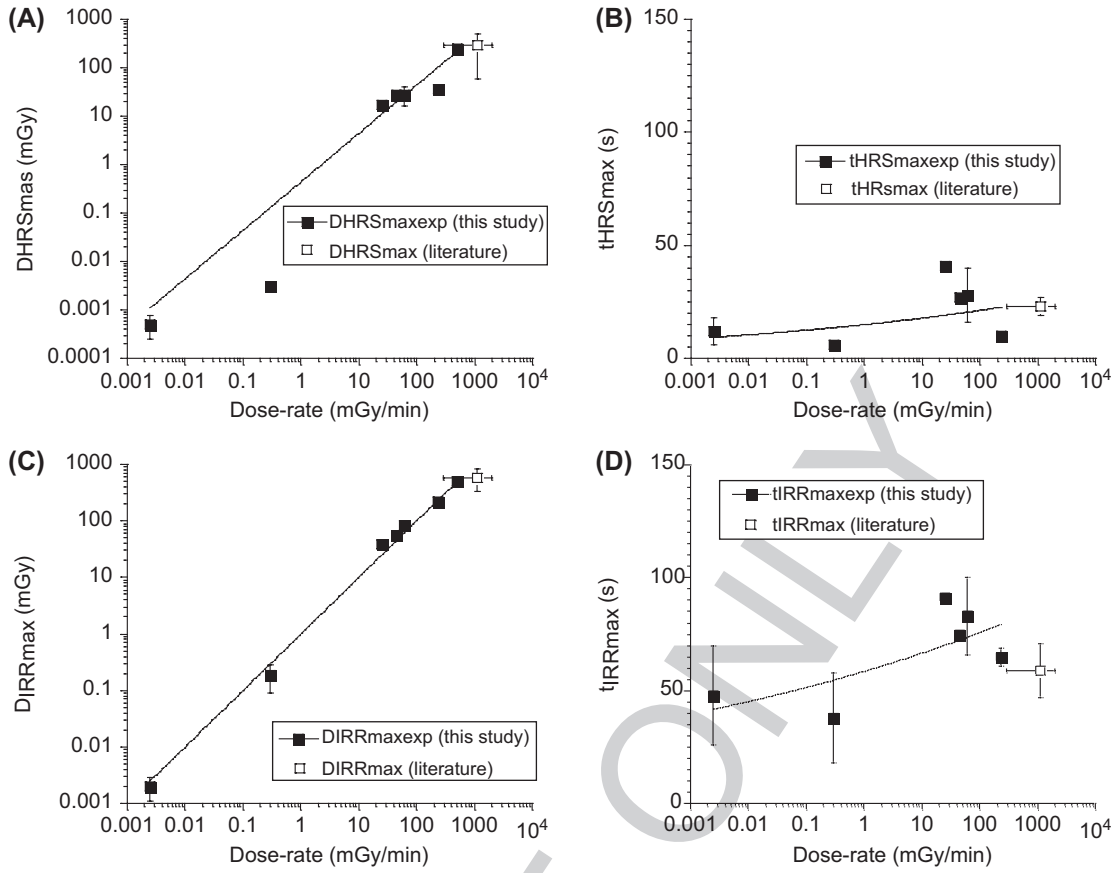

Figure 2. Relationships between the HRS/IRR response experimental parameters (shown in Table IV) and the dose-rates in PRO cells. (A) Significant linear correlation between D_{HRSmax} and dose-rate was found ($y = 0.4428.x$, $R^2 = 0.89$, $p < 0.05$). (B) t_{HRSmax} was not significantly correlated to dose-rate. (C) Significant linear correlation between D_{IRRmax} and dose-rate was found ($y = 0.997.x$, $R^2 = 0.99$, $p < 0.05$). (D) t_{IRRmax} was not significantly correlated to dose-rate. Error bars indicate the SEM for $n = 2$–4 independent experiments taken from Figures 1A–F (■) and for 25 independent experiments obtained with low-LET radiation taken from Table I (□).

generally applied in CT scan exams. Figure 3A shows that in the 5–100 mGy range, the HRS/IRR response occurs in PRO cells. Although the extent of the HRS response in PRO cells appeared to be larger with 75 kV X-rays than with ^{60}Co γ-rays, the survival data were not found significantly different (Figure 3A). Accordingly, the HRS and the IRR response parameters fitted with the IR model were found similar with 75 kV X-rays and ^{60}Co γ-rays (Table III). Finally we confirmed that REG cells irradiated with 75 kV X-rays did not display significant HRS/IRR response (data not shown).

DSB repair features of HRS/IRR response

Thereafter, by using 75 kV X-rays delivered at 25 mGy.min^{-1}, we examined the radiation-induced DSB reflected by γ-H2AX foci in two representative conditions: After 10 mGy, corresponding to the maximal HRS response (D_{HRSmax}) and an exposure time lower than t_{HRSmax}; after 100 mGy, corresponding to dose higher than the maximal IRR response (doses higher than D_{IRRmax}) and exposure time longer than t_{IRRmax}. Figure 3B showed that for both doses, the kinetics of appearance/disappearance of γ-H2AX foci elicited the same biphasic shape: (i) An increase of the number of γ-H2AX foci corresponding to the recognition of radiation-induced DSB managed by NHEJ; (ii) a decrease of the number of γ-H2AX foci corresponding to the repair of recognized DSB. However, while the maximal number of γ-H2AX foci ranged from 7–11 nuclear foci for both doses, the incubation times at which it was reached differed significantly, i.e., 4 h and 1 h post-irradiation after a dose of 10 mGy and 100 mGy, respectively. Furthermore, while the DSB repair is completed after 100 mGy, the DSB induced by 10 mGy appeared to be more severe with 5.5 ± 0.7 residual γ-H2AX foci 24 h after irradiation (Figure 3B). These data suggest that t_{HRSmax} may be associated with deficient NHEJ repair and maximal HRS response while t_{IRRmax} may be associated with full NHEJ repair and maximal IRR response.

Discussion

Impact of dose-rate on the HRS/IRR response

By investigating one of the largest ranges of dose-rates applied in HRS/IRR studies, our data show that the maximal HRS and IRR responses obtained with low-LET radiation correspond to exposure times of about 20 s and 60 s, respectively. To our knowledge, the impact of dose-rate and exposure time on the HRS/IRR response have not been investigated *per se*, notably with short exposure times less than 10 min. Exposure time is basically dependent on dose and dose-rate since these three parameters are linked mathematically. The dose-rates applied in the published studies ranging from 0.18–2.43 Gy.min^{-1} (Table I), have rarely been explained: Their choice generally results from

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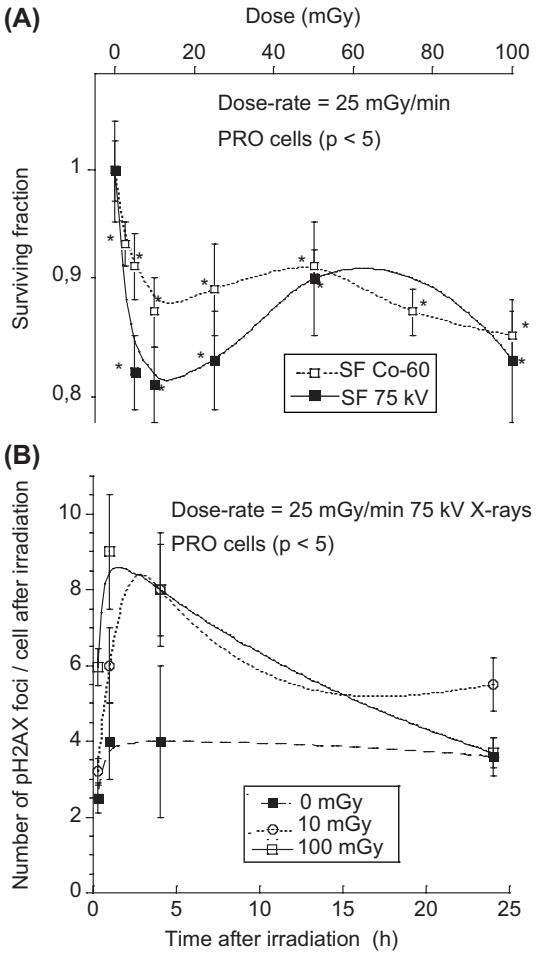

Figure 3. HRS/IRR response of PRO cells irradiated with 75 kV X-rays. (A) Comparison between ^{60}Co γ-rays survival data (dashed line) and 75 kV X-rays survival data (continuous line). Experiments were performed with cells cultured for no more than 5 passages. Each data represents the mean ± SEM of two independent experiments for cells irradiated 24 h after plating. Six data points per dose are included in each experiment. $^{*}p < 0.05$ for comparison between unirradiated cells and irradiated cells using the *t*-test. (B) Kinetic of DSB repair at 10 mGy (HRS response) or 100 mGy (IRR response). Each data represent the mean ± SEM of 3–5 independent experiments for cells irradiated 24 h after plating. All data in Figure 3 were fitted to a smooth function.

a practical compromise between the availabilities of the irradiator in the laboratory, a short exposure time to avoid artifacts and the possibility to expose cells during a minimal time. For example, some authors used several dose-rates for completing a single survival curve (e.g., Marples and Joiner 1993, Martin et al. 2009). We deliberately chose not to include the studies using several dose-rates in our review shown in Table I. Similarly, HRS/IRR responses obtained with long exposure times (generally longer than 1 h) were not considered (e.g., Enns et al. 2004).

Some HRS/IRR responses were also observed with other radiation than X- or γ-rays. This is notably the case of neutrons (Dionnet et al. 2000), α-rays (Tsoulou et al. 2001), protons (Petrovic et al. 2010), heavy ions (Xue et al. 2009) and β-rays (Wéra et al. 2012). Interestingly, D_{HRSmax}, D_{IRRmax}, t_{HRSmax}, t_{IRRmax} are also in agreement with the values range of our review (Table I), which consolidates our conclusions showing that the maximal HRS and IRR responses would correspond, (by pooling literature and our data), to average exposure times of 31 ± 8 s (SEM, $n = 35$) and 58 ± 9 s (SEM, $n = 35$), respectively, irrespective of the radiation type (low and high-LET radiation). Thus our data suggest that the HRS response is not limited to low-doses since t_{HRSmax} can theoretically be reached with high-doses. Accordingly, tumor cells irradiated at 2 Gy with protons at 15 Gy.min^{-1} (exposure time = 8 s) showed HRS response (Petrovic et al. 2010). However, since most HRS/IRR responses were obtained with low-LET radiation corresponding to cell survival of 75 ± 18 % (mean ± SD, $n = 28$) with doses ranging from 100–800 mGy (Table I), we stressed that the validity of the HRS/IRR response may not be relevant for higher doses and lower cell survival.

Biological significance of t_{HRSmax}

The findings that t_{HRSmax} and t_{IRRmax} are constant and common to human and rodent cells, tumor and transformed normal cells suggest that exposure times corresponding to the maximal HRS and IRR responses may not entirely depend on cellular parameters like cellular model or cell death pathways. Furthermore, a drastic decrease of cell survival was shown to be correlated with DSB repair impairments with a number of cellular models and conditions (e.g., Joubert et al. 2008). In mammalian cells, DSB are mainly recognized and repaired by the NHEJ pathway. Particularly, alterations in NHEJ induce hyper-radiosensitivity at high-doses. This is the case of ATM-, ligase (LIG) 4-, DNA-protein kinase (PK)-mutated cell lines that exhibit a survival fraction at 2 Gy (SF2) of about 1% (Joubert et al. 2008). Interestingly, the α parameter of the LQ model and the surviving fractions corresponding to these hyper-radiosensitive cell lines are very similar to those observed in the initial part of the survival curve in PRO cells and in other HRS-positive cell lines sorted in Table I. We suggest therefore that the [0–t_{HRSmax}] exposure time interval may correspond to an incapacity of NHEJ to recognize and repair efficiently the induced DSB, as it is the case for the ATM-, LIG4-, DNA-PK-mutated cells. It was shown that the ATM kinase produces a cascade of phosphorylations of proteins involved in the radiation response (Foray et al. 2003). The NHEJ repair pathway requires several steps such as: (i) DSB recognition, (ii) interaction between ATM and γ-H2AX, and (iii) complete H2AX phosphorylation. In our hands, at least 10 min post-irradiation are required to observe the maximal number of γ-H2AX foci. Besides, some authors applied 30 min post-irradiation to assess the number of recognized DSB (e.g., Joubert et al., 2008). Hence, DSB recognition and repair steps likely require much more than 20 s. Since residual DSB is observed 24 h after irradiation at 10 mGy delivered either at 25 mGy.min^{-1} (exposure time of 24 s) (this study) or at 70 mGy.min^{-1} (exposure time 8.5 s) (Grudzenski et al. 2010), we suggest that t_{HRSmax} may be consistent with the time corresponding to deficient NHEJ repair.

Biological significance of t_{IRRmax}

With regard to the second part of the survival curve ranging from t_{HRSmax} to t_{IRRmax}, an increase of cell survival is observed: induced-radioresistance (IRR) is the major interpretation of this part of the survival curve (Krueger et al. 2007b).

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The t_{IRRmax} exposure time would therefore correspond to the time necessary for a fully active NHEJ pathway. Our data in Figure 3B show that NHEJ repair is complete 24 h after irradiation at 100 mGy delivered at 25 mGy.min^{-1} which corresponds to an exposure time larger than t_{IRRmax}. Accordingly, t_{IRRmax} may be compatible with kinetic of change in chromatin structure and nucleo-shuttling of pATM forms (Bakkenist and Kastan 2003), the earliest time to detect γ-H2AX foci after irradiation (Rothkamm and Löbrich 2003) and the time required for induced repair after low-dose X-rays [e.g., 68 s or 80 mGy delivered at 70 mGy.min^{-1} (Grudzenski et al. 2010)]. Altogether, our data are compatible with three exposure time phases and N-shaped dose-response curve regarding DSB and cell survival (Figure 4):

- $t < t_{HRSmax}$: Incomplete DSB recognition by NHEJ and decrease of cell survival (HRS response);
- $t_{HRSmax} < t < t_{IRRmax}$: Progressive activation of NHEJ and increase of cell survival (IRR response);
- $t > t_{IRRmax}$: All DSB are recognized but they are so numerous that they cannot be all repaired; decrease of cell survival (beyond the HRS/IRR response).

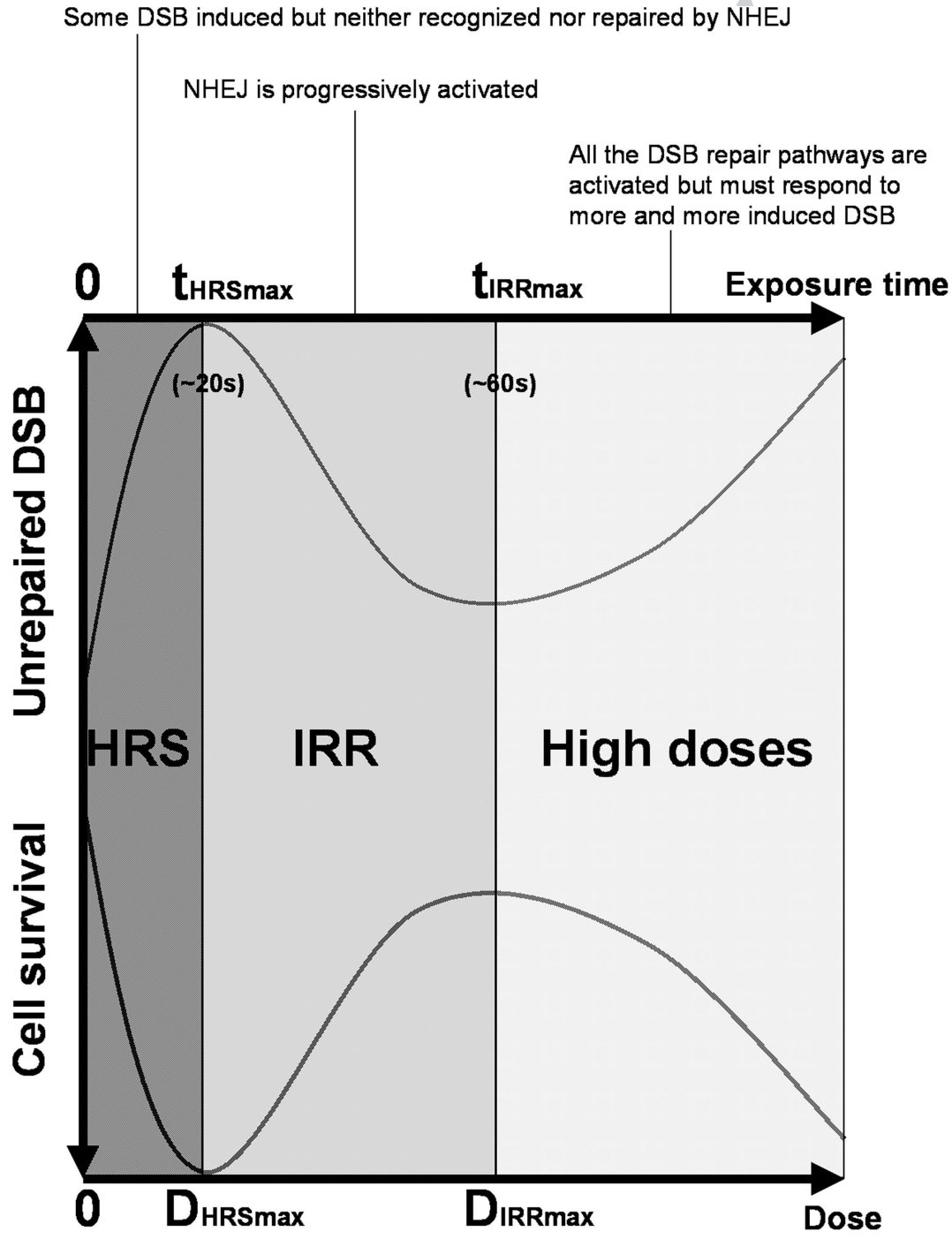

Figure 4. Model for the HRS/IRR response. From 0 to D_{HRSmax}, radiations induce physical DSB that are not all recognized biologically and therefore unrepaired. Consequently, cell survival decreases. From D_{HRSmax} to D_{IRRmax}, DSB are all recognized biologically and progressively repaired and cell survival increases. For doses higher than D_{IRRmax}, DSB are all recognized but their amount is so large that some DSB are not repaired and cell survival decreases.

Potential impact of the HRS/IRR response in radiotherapy

Our findings suggest that significant decrease of cell survival could be reached independently of dose-rate provided that exposure times are shorter than 30 s. This may be notably the case of the cyberknife™ radiotherapy technique that delivers non-uniform patterns of intermittent radiation using a compact miniaturized 6 MV nominal linear accelerator with high doses-rates of 4, 6 or 8 Gy.min^{-1}. The dose per fraction is delivered using 80–150 non-coplanar sequential mini-beams with $< 0.1\%$ leakage at 1 m from the beam path. For example, for a dose per fraction of about 7 Gy to the brain, the peripheral dose is less than 5 mGy at 80 cm from the target (Di Betta et al. 2010). Interestingly, cyberknife delivers a single fraction of the total dose in 1–36 s with an interval between two beams of 5 s (Murphy et al. 2007). Furthermore, Lin and Wu reported that not all 2 Gy fractions are equivalent: Human and rodent cells irradiated with ^{60}Co γ-rays at 1.3–1.5 Gy.min^{-1} in 10 fractions of 0.2 Gy (corresponding to about 8 s per fraction with an interval of 16 s between fractions) showed higher radiosensitivity than a single fraction of 2 Gy (corresponding to an exposure time of 86 s at 1.4 Gy.min^{-1}) (Lin and Wu 2005). Thus data suggest that intermittent irradiation delivered in multiple fractions or continuous irradiation delivered in a single fraction with exposure time per fraction shorter than 20 s may show maximal HRS response independently of dose-rate. However, further investigations are required to examine whether the time between fractions impacts significantly on the HRS response.

Finally and consistently with our previous reports (Thomas et al. 1997, 2001, 2007, 2008), we suggest that the HRS response may be relevant to target unvascularized micrometastases with peripheral doses received at a distance from the clinical target volume irradiated with intermittent radiation. In the context of oligometastatic disease, local ablative stereotactic irradiation can be used to eradicate gross tumor while the potential microscopic disease is managed using systemic treatments (chemotherapy) or left untreated (Thariat et al. 2012). We suggest that the HRS response driven by short exposure times such as used with stereotactic radiotherapy may find also application to manage micrometastatic disease at distance from the irradiated gross tumor. More experimental and clinical investigations with additional highly metastatic human cell lines will be needed to verify this medical hypothesis.

Acknowledgements

We are grateful to the reviewers for their helpful comments and to Dr Christian Mazars and his team [Unité Mixte de Recherche (UMR) Université Paul Sabatier (UPS)-Centre National de la Recherche Scientifique (CNRS) 5546, Toulouse] for helpful discussions. We thank also Madame Beaufrère for her assistance in editing English.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This work is supported by the Alliance de la Vie et de la Santé (AVIESAN), the Agence Nationale de la Recherche (ANR), the Institut National du Cancer (INCa), the Centre National d'Etudes Spatiales (CNES) and the Association pour la Recherche sur l'ataxie Telangiectasie (APRAT).

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Supplementary material available online

Survival fraction data for PRO cells irradiated at 230 mGy/min (D1), 60 mGy/min (D2), 44 mGy/min (D3), 25 mGy/min (D4), 0.3 mGy/min (D5) and 0.0025 mGy/min (D6); survival data for REG cells irradiated at 44 mGy/min (D3) and 0.0025 Gy/min (D6).

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Supplementary material for Thomas C, et al. Impact of dose-rate on the low-dose hyper-radiosensitivity and induced radioresistance (HRS/IRR) response. *International Journal of Radiation Biology*, 2013; doi: 10.3109/09553002.2013.800248.

PRO cells D1.

Control	10 mGy	25 mGy	50 mGy	100 mGy	250 mGy	500 mGy
1.18	1.02	0.89	0.98	0.93	0.8	0.8
0.98	0.93	0.93	0.89	1.02	1.1	0.69
1.09	0.84	0.93	0.98	0.8	0.8	0.98
0.98	0.93	0.89	0.84	0.98	1.05	0.8
0.93	0.84	0.84	0.89	0.84	0.8	0.8
0.89	0.89	0.98	0.89	0.89	0.85	0.69
0.98	0.8	1	0.9	0.6	0.98	0.75
0.93	0.6	0.9	0.8	0.8	0.91	0.7
0.98	0.85	0.95	0.75	0.9	0.84	0.55
1.13	0.9	0.7	0.8	1	0.84	0.75
1.09	0.85	0.65	0.75	0.9	0.77	0.75
0.93	0.75	0.95	0.6	0.95	0.91	0.5
1	0.95	0.84	0.84	0.84		0.88
1	1.09	1.05	0.91	0.77		0.95
1.2	0.81	0.88	0.91	0.81		0.7
0.8	0.84	0.84	0.91	0.84		0.77
1.1	0.98	0.98	0.95	0.84		0.98
0.9	0.91	0.91	0.91	0.84		0.98
1.12						
1.12						
1.09						
0.91						
0.91						
0.84						

PRO cells D2.

Control	1 mGy	5 mGy	10 mGy	25 mGy	50 mGy	100 mGy	200 mGy	300 mGy	500 mGy
0.91	0.94	0.75	0.84	0.81	0.75	0.78	0.94	0.75	0.84
1.03	1.06	0.88	0.81	0.69	0.63	0.97	0.84	0.91	0.94
1	0.88	0.75	0.78	0.75	0.78	1.03	0.94	0.78	0.94
0.88	1	0.78	0.72	0.81	0.75	0.97	0.84	0.84	0.81
0.94	1.12	0.72	0.81	0.81	0.66	0.75	0.88	0.94	0.75
1.25	0.94	0.69	0.66	0.84	0.72	0.81	0.94	0.88	0.69
0.94	0.83	0.82	1	1.06	1	1.18	1	0.94	0.82
1.18	1	1	1.06	1.24	0.82	0.82	0.94	1.12	0.88
0.94	0.83	1	0.71	1.06	0.94	1.06	0.82	1.18	0.76
0.94	0.83	0.94	0.94	0.82	1	1	0.82	0.88	0.71
1.06	0.92	0.88	1.12	0.94	0.82	0.76	1.12	0.82	0.82
0.94	1.04	1	0.94	0.94	1.06	1	0.82	1	0.76
1		0.92	1	0.83	1.04	0.83	1.04	1	1
1.04		0.92	1	0.75	1	0.92	1.21	1	0.83
0.92		1	0.83	0.75	1	0.75	1.04	1	0.92
0.92		0.92	0.75	1	1.12	1	1	1.04	1
1.13		1	1	0.75	0.92	0.92	1	0.83	0.75
1		0.92	0.92	0.75	1.04	1	1	1	1

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PRO cells D3.

Control	5 mGy	10 mGy	20 mGy	50 mGy	100 mGy	200 mGy	250 mGy
1.04	0.95	0.96	0.86	0.72	0.88	0.9	0.72
1.2	1	0.88	0.9	0.8	0.76	0.88	0.96
1.2	1.05	0.8	1	0.8	0.76	0.9	0.88
1.04	0.86	0.8	0.9	0.8	0.88	0.95	0.8
0.88	0.9	0.8	0.86	0.76	0.76	0.95	0.88
1.28	1	0.8	0.9	0.8	0.8	1	0.8
0.88	1.05	0.95	0.72	0.9	1.1	0.98	0.92
1.04	0.85	0.95	0.92	0.81	1	0.92	0.85
0.8	0.85	0.86	0.72	0.86	1	0.79	0.98
0.88	0.72	0.86	0.85	1	1	0.85	0.92
0.96	0.85	0.9	0.82	0.95	0.9	0.85	0.72
0.96	0.92	0.86	0.92	0.95	1.05	0.92	0.98
0.95	1	1.05	0.83	0.85	0.92	1.08	1.25
1.05	1.17	0.98	0.92	0.85	0.79	1	0.96
0.9	0.92	0.98	0.92	0.85	0.85	1	0.83
1.05	0.83	0.85	1.08	0.92	0.79	1	0.92
1.1	1.08	0.72	0.92	0.85	0.79	0.83	0.92
0.95	0.83	0.85	1.17	0.85	0.89	1.08	1
0.92	0.65	0.88	1.22	1.17	0.92	1.04	0.83
1.1	0.96	0.92	1.09	1.25	0.96	0.87	0.74
0.98	1.22	0.88	0.91	1.08	1	0.91	1
0.92	0.87	1	1.04	0.92	0.83	1.13	0.96
1	0.96	1	0.96	1	0.92	0.74	0.91
1.17	0.91	1	1	1	0.92	1.3	0.91
0.92	1	0.91	0.95	0.87	1.09	1.24	1.07
1.04	1.02	1.13	0.95	1	1.22	1.24	0.95
1	0.95	1	1.19	0.96	0.83	1.07	0.83
0.92	1.07	1.22	0.95	1.09	0.83	0.88	0.95
1.04	1.19	1	1.95	1	1	1.07	0.95
1	0.9	1	1.19	0.96	1	1.07	1.19
1		0.95		1	1		
0.92		0.95		1.07	1.19		
1		1.07		1.07	0.95		
0.83		1		0.95	0.95		
1.17		0.95		1.19	0.9		
1.04		1.07		1			
1.09							
1.13							
1.04							
0.83							
1.09							
0.96							
1.13							
0.83							
1							
0.96							
0.82							
0.87							
1.09							
0.87							
1							
0.96							
1.07							
0.95							
0.95							
0.98							
1.24							
1							
1.07							
0.9							
1.09							
1							
0.95							
0.98							
1							
0.95							
0.95							
0.98							
0.95							
1.07							

PRO cells D4.

Control	2.5 mGy	5 mGy	10 mGy	25 mGy	50 mGy	75 mGy	100 mGy
1.1	0.96	0.74	0.96	0.74	0.96	0.9	0.74
0.96	0.92	0.74	0.81	0.81	0.89	0.9	0.81
1.1	0.96	0.89	0.74	0.74	0.96	0.76	0.81
0.89	0.81	0.81	0.74	0.96	0.81	0.88	0.89
1.04	1.1	1.04	0.81	0.74	0.89	0.86	0.81
0.93	0.92	0.89	0.78	0.78	0.81	0.9	0.85
1.05	0.95	1.05	0.9	1	0.95		0.95
1	0.86	1.05	0.95	0.86	0.9		0.86
1.1	0.9	1	0.95	1	0.93		0.9
1.1	0.95	0.95	1	0.95	0.95		0.88
0.86	0.9	0.9	1	1.05	0.93		0.86
0.9	0.9	0.93	0.81	1.05	0.9		

PRO cells D5.

Control	0.025 mGy	0.05 mGy	0.1 mGy	0.2 mGy	0.5 mGy	1 mGy
1.17	0.91	0.78	1.09	1.04	1.04	0.91
1.04	0.87	1.04	0.83	1.04	0.96	0.91
1.09	0.83	1.04	0.91	0.91	0.78	0.74
1.13	0.87	1.04	1	0.96	0.87	0.83
1.04	0.91	0.87	0.87	1.13	0.87	1.09
0.83	0.87	1	0.96	0.96	0.78	1
1.09	1.17	0.91	0.78	0.96	1	0.65
0.96	0.96	0.83	0.96	0.83	0.91	0.91
1.13	0.91	1.04	1	0.87	0.91	0.87
0.83	0.78	0.87	0.65	0.87	0.96	0.96
1	0.65	1.09	0.74	1.04	0.61	0.91
0.96	0.96	0.83	0.74	0.87	0.78	0.87
0.83	1.14	1.05	1.12	1.02	0.98	1.07
0.87	1.02	1.02	1	1	0.98	1
1.09	0.93	1.07	1.19	1.07	0.95	1.1
0.87	0.98	1	0.95	1	1.14	1.02
1	0.9	1.02	1.07	0.95	1	1
0.96	1.12	1.07	1	0.95	1	1.05
1.07	0.94	1	0.7	1.03	0.9	0.7
0.95	1.03	0.7	1	1.03	0.9	0.7
0.95	1.13	0.6	0.95	1	0.95	0.7
0.98	0.86	0.6	0.7	1.03	0.85	0.8
1.24	0.84	0.75	0.8	1.06	0.8	0.75
1	0.96	0.85	0.95	1.03	0.9	0.85
1.07		1.13	1.23		1.08	1.23
0.9		1.09	0.97		1.13	0.92
1.09		0.97	0.87		1.18	0.97
1		0.97	1.03		0.92	1.13
0.95		0.92	1.13		1.23	1.08
0.98		0.94	1.18		1.03	1.03
1						
0.95						
0.95						
0.98						
0.95						
1.07						
1						
1						
1.2						
0.8						
1.1						
0.9						
1.02						
0.97						
0.92						
1.13						
0.97						
0.97						

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PRO cells D6.

Control	0.25 uGy	0.5 uGy	1 uGy	2 uGy	5 uGy	10 uGy
1	1	0.85	0.45	0.95	0.9	0.9
1	0.95	0.75	0.7	1.12	0.8	0.8
1.2	1.07	0.65	0.75	1.07	0.95	0.6
0.8	1	0.85	0.7	1	0.7	0.7
1.1	0.83	0.65	0.65	0.98	0.95	0.7
0.9	0.95	0.85	0.5	1.14	0.8	0.9
1.07	1	1.07	1	1.05	0.9	1.05
0.95	0.8	1.05	1	0.95	1	1.1
0.95	1	0.95	0.98	0.8	0.95	1.1
0.98	0.9	1.05	0.95	0.9	0.93	0.98
1.24	0.85	0.98	0.86	0.9	0.95	1.02
1	0.8	0.9	0.88	0.9	0.95	1.05
1.07		0.85	1		1.05	0.9
0.9		0.75	1.05		0.95	1
1.09		0.9	0.95		0.95	0.95
1		1	0.8		1	0.85
0.95		1	0.85		0.9	0.9
0.98		0.9	0.9		0.8	0.8
1						
0.95						
0.95						
0.98						
0.95						
1.07						
0.9						
1.1						
1.2						
1						
0.9						
0.9						

REG cells D6.

Control	0.5 uGy	1 uGy	2 uGy	5 uGy	10 uGy
1.09	0.85	1.21	1.23	1.03	0.85
0.97	1.21	1.15	0.85	1.21	1.03
1.03	0.73	1.27	1.23	0.97	1.21
0.97	0.97	1.39	0.85	1.03	1.27
1.09	0.85	1.33	0.92	1.27	1.51
0.85	0.96	1.09	1.15	0.97	0.97
0.73	1.15	1.08	1.08	1.08	1.08
0.92	1.15	1.23	0.85	1.23	1.08
0.85	1.23	0.92	1.08	0.92	1.08
0.92	1.08	0.88	1.15	0.81	1.15
1	1.15	1.15	1.08	0.92	1.27
1.15	0.85	1.15		1	1.04
0.92	1	1.08		0.85	1.15
1	1	1.23		1.15	1.08
1.23	1.15	1.23		1.23	0.95
1.15	1.15	0.85		1	1.14
1	1	1		1.14	1.33
1	1.31			1.33	0.95
0.86				0.95	0.95
0.76				0.95	0.95
0.81				1.14	
0.81				0.95	
0.86					
1.05					
1.33					
1.24					
0.95					
1.14					
1.14					
1.14					

REG cells D3.

Control	5 mGy	10 mGy	20 mGy	50 mGy	100 mGy	200 mGy
1	1.13	1	1.07	0.93	0.93	1
0.9	1	1	1.07	1.1	0.9	1
1	0.93	1	1	1.07	1	1
1.07	1	1	1	1	0.9	1.07
1	1.07	1	1.07	1	0.93	1
1.07	0.93	1.13	0.87	0.87	0.8	1.13
1.19	0.97	1.3	0.97	0.97	0.97	0.86
0.97	0.86	0.86	0.97	0.81	0.97	0.92
1.08	1.08	1.08	0.86	0.97	1.03	0.81
0.92	0.86	1.08	0.92	1.03	0.76	0.97
0.86	1.14	0.97	1.03	0.92	1.19	0.92
0.97	0.86	0.76	0.97	0.86	1.08	0.97

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