Available online at www.sciencedirect.com

SCIENCE DIRECT®

Life Sciences 75 (2004) 623–632

Life Sciences

www.elsevier.com/locate/lifescie

Changes in G1-phase populations in human glioblastoma and neuroblastoma cell lines influence p(66)/Be neutron-induced micronucleus yield

John M. Akudugu[a,*], Anke Binder[a], Antonio Serafin[a], Jacobus Slabbert[b], Alf Giese[c], Lothar Böhm[a]

[a] *Department of Radiation Oncology, Faculty of Health Sciences and Tygerberg Hospital, University of Stellenbosch, Tygerberg, South Africa*

[b] *Radiation Physics Division, iThemba LABS, Faure, South Africa*

[c] *Laboratory for Brain Tumour Biology, Department of Neurosurgery, University Hospital Eppendorf, Hamburg, Germany*

Received 16 September 2003; accepted 13 January 2004

Abstract

Some photon resistant tumours are sensitive to neutrons but no predictive methods exist which could identify such tumours. In a recent study addressing this clinically important issue, we demonstrated that relative biologic effectiveness (RBE) values for p(66)/Be neutrons estimated from micronucleus (MN) data correlate positively with RBE values obtained from conventional clonogenic survival data. However, not all photon-resistant cell lines showed high RBE values when the MN endpoint was used. Now, we examine how the functional status of the p53 tumour suppressor gene and radiation-induced changes in cell cycle phase populations may contribute to this discrepancy. No significant association was established between p53 status and MN yield for both photon and neutron irradiation. The data demonstrated that neutron-, but not photon-, induced MN yield is dependent on the intrinsic ability of cells to activate a G1-phase arrest. In cell lines of comparable photon sensitivity, those showing more extensive depletion of the G1 population express significantly more micronuclei per unit dose of neutrons. These results suggest that differences in cell cycle kinetics, and not the p53 status, may constitute an important factor in damage induction by high linear energy transfer (LET) irradiation and need to be considered when radiation toxicity in clinical radiobiology or radiation protection is assessed using damage endpoints.

Keywords: G1 population; Micronucleus yield; LET

* Corresponding author. Ontario Cancer Institute, Division of Experimental Therapeutics, Room 10-112, 610 University Ave., Toronto, Ontario M5G 2M9, Canada. Tel.: +1-416-946 2000x4920, fax: +1-416-946-2984.

E-mail address: jakudugu@uhnres.utoronto.ca (J.M. Akudugu).

0024-3205/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2004.01.013

Introduction

Tumour therapy with fast neutrons has not yielded much clinical success (Joiner and Field, 1988; Schmitt and Wambersie, 1990; West et al., 1993). It is generally accepted that fast neutron treatment is likely to benefit select pathologies represented by only 10% of patients (Schmitt and Wambersie, 1990; West et al., 1993; Britten et al., 2001). The reasons for this are not yet clear, and assays for identifying neutron sensitive tumours, clearly would be of great interest.

Difficulties in harnessing the full clinical potential of neutron irradiation may be due to the choice of the neutron beam energy. It has previously been shown that low energy first generation neutrons (mean energy of 6 MeV) discriminate between photon resistant and photon sensitive cells and that a significant increase of neutron RBE with photon resistance can be demonstrated (Slabbert et al., 2000). Using the same neutron beam, we have shown in a more recent study that RBE values obtained from MN data correlates significantly with RBE values derived from cell survival data, and that the MN assay shows potential as a useful tool for identifying photon-resistant tumours that may show neutron sensitivity (Akudugu et al., 2003). However, not all photon-resistant cell lines yielded expected RBE values from MN data. Studies aimed at elucidating this discrepancy are desirable.

Tumour response to irradiation is characterised by several factors including cell cycle checkpoint activation (Paulovich et al., 1997) and DNA damage repair (Weichselbaum, 1986; Fox and McNally, 1988; Peak et al., 1991; Hu and Hill, 1996). The role of the p53 tumour suppressor gene in modulating cellular responses to ionising radiation is well documented (Kastan et al., 1991; Lane, 1992; Hartwell and Kastan, 1994; Amundson et al., 1998; Bunz et al., 1998; Ryan and Vousden, 1998). Although the LET-dependence of p53 activity is unclear, it is known that fission neutrons and α-particles induce a p53-dependent build-up of $p21^{WAF1}$ mRNA and protein (Balcer-Kubiczek et al., 1999; Takahashi et al., 2000). Apoptosis induced in human glioblastoma cells by carbon beams is p53-independent (Tsuchida et al., 1998). A study on human breast cancer cell variants has suggested that the p53 status influences cytotoxicity induced by photons but not by fission neutrons (Balcer-Kubiczek et al., 1995). Micronuclei are a reflection of residual DNA damage and high-LET irradiation has been shown to induce higher MN yield per unit dose than low-LET irradiation (Tates et al., 1989; Darroudi et al., 1992; Huber et al., 1994; Heimers, 1999). Although numerous attempts have been made to reconstruct intrinsic radiosensitivity, based on clonogenic survival, from radiation-induced damage (Streffer et al., 1982; Bush and McMillan, 1993; Villa et al., 1994; Abend et al., 1995, 2000; Akudugu et al., 2000; Akudugu and Böhm, 2001), the impact of the functional status of p53 and radiation-induced changes in cell cycle phase population on MN formation and on the relationship between MN yield and intrinsic radiosensitivity is yet to be elucidated. In this study, we examine how p53 status and radiation-induced cell cycle alterations affect MN yield in eight human tumour cell lines as such information will be a valuable input to the understanding of the mechanisms underlying an apparent association between DNA damage induced by different radiation modalities and subsequent cell kill.

Materials and methods

Cell lines and culture maintenance

The glioblastoma cell lines G-28, G-60, G-112 (p53 mutant) and G-44, G-62, G120 (p53 wild-type) were cultured as previously described (Akudugu et al., 2001). Briefly, cells were grown at 37°C in

monolayers in Eagle's modified minimum essential medium supplemented with 10% foetal bovine serum, 10 μg/ml streptomycin, 10 μg/ml penicillin and 2.2 μg/ml sodium pyruvate. The neuroblastoma cell lines KELLY and SK-N-SH were grown in the same medium, but in the absence of sodium pyruvate.

Immunochemical detection of $p21^{WAF1}$ in neuroblastoma cell lines

To assess the functional status of the p53 tumour suppressor gene in the KELLY and SK-N-SH cell lines, the induction of $p21^{WAF1}$ gene after 7 Gy of ^{60}Co γ-irradiation was detected using a protocol modified from that described elsewhere (Deptala et al. 1999). At 0, 2.5, 5 and 7.5 hours after irradiation, cells were fixed in 1% methanol-free formaldehyde in PBS at 4 °C for 15 min, washed with PBS and permeabilised with 80% ethanol at − 20 °C. After re-swelling in PBS at room temperature (22 °C) cells were lysed in 0.25% Triton X100 on ice for 5 min. Cells were then washed with PBS containing 1% bovine serum albumin (BSA) and incubated overnight at 4 °C with 100 μl 1% BSA/PBS containing 1 μg anti-$p21^{WAF1}$ antibody (PharMingen, clone 2G12, cat. No. 15441A) or 3 μl mouse anti-IgG1 (Dako, X0931) in a 1:30 titre as a negative control. The cells were then washed in 1% BSA/PBS containing 3 μg mouse anti-IgG1 secondary antibody (Sigma, F-2012) for 1 hour in the dark at room temperature. $p21^{WAF1}$ induction was assessed by flow cytometry measuring green fluorescein isothiocyanate (FL1-H) versus red PI (FL3-H) on a fluorescence-activated cell sorter.

Determination of cell cycle phase population changes

Changes in radiation-induced cell cycle phase population were assessed by flow cytometry (Ormerod, 1994). Asynchronous cell populations in 25-cm^2 cell culture flasks in exponential growth were irradiated at 7 Gy with ^{60}Co γ-rays as described elsewhere (Binder et al., 2000; Theron and Böhm, 2000) or at 4 Gy with p(66)/Be neutrons. The relative biologic effectiveness of the neutron beam in this panel of cell lines based on clonogenic survival ranges between 1.02 and 2.78 (Akudugu et al., 2003a). Cells were trypsinised, centrifuged and fixed in 70% ethanol at 0, 6, 12, 14, 16, 18, 20, 22 and 24 hours after irradiation and stored at − 20 °C. For analysis, pre-fixed cells were centrifuged, washed with PBS and resuspended in a volume of PBS containing 0.1% glucose, 50 μg/ml PI and 100 μg/ml RNase. Samples of about 2×10^6 per ml were incubated in the dark at 37 °C for 30 minutes and analysed within four hours in a Becton Dickinson FACScan flow cytometer. The score of the DNA content in each cell cycle phase was based on a sample of 10,000 cells per aliquot and was displayed as a frequency distribution histogram. The percentages of cells in the different cell cycle phases were determined using marker statistics (LYSIS II software; Becton Dickinson). Cell debris, nuclei, doublets and triplets were excluded by gating. No significant differences were observed in the proportions of cells progressing through the various cell cycle phases after photon and neutron irradiation over the time course investigated. Therefore, the ratios: G1(t)/G1(0), S(t)/S(0) and G2M(t)/G2M(0) following photon irradiation were used to reflect intrinsic cell cycle checkpoint integrity in subsequent analyses. G1(t), S(t) and G2M(t) denote the minimum G1- and corresponding S- and G2/M-populations. G1(0), S(0) and G2M(0) are the respective populations in unirradiated cultures at the time of flow cytometric analysis. *t* represents the time at which the minimum G1 population occurs. A ratio of less than 1.0 was considered as a population depletion, while a value of greater than 1.0 implied an accumulation of cells in the corresponding phase.

Table 1
Summary of flow cytometric, radiosensitivity and micronucleus data for eight human tumour cell lines

Cell line	p53 status	$G1_t/G1_0$	S_t/S_0	$G2M_t/G2M_0$	MID_γ (Gy)*	γ-MN yield (Gy^{-1})*	*n*-MN yield (Gy^{-1})*
G-112 (t = 14h)	*mt*	0.37	2.70	1.96	2.79 ± 0.17	0.24 ± 0.01	1.27 ± 0.03
G-60 (t = 14h)	*mt*	0.36	1.18	2.25	2.25 ± 0.09	0.23 ± 0.00	0.97 ± 0.07
G-28 (t = 12h)	*mt*	0.73	0.67	1.82	3.21 ± 0.11	0.35 ± 0.01	0.82 ± 0.03
KELLY (t = 20h)	*mt*	0.46	1.41	2.00	1.25 ± 0.11	0.20 ± 0.01	0.48 ± 0.03
		0.48 ± 0.15	1.49 ± 0.75	2.01 ± 0.16			
G-120 (t = 14h)	*wt*	0.64	0.24	2.40	2.59 ± 0.13	0.39 ± 0.03	0.56 ± 0.06
G-44 (t = 12h)	*wt*	0.40	0.42	2.00	2.74 ± 0.07	0.35 ± 0.02	1.45 ± 0.10
G-62 (t = 14h)	*wt*	0.59	0.33	2.19	2.94 ± 0.07	0.21 ± 0.01	0.67 ± 0.04
SK-N-SH (t = 14h)	*wt*	0.89	0.66	1.71	1.52 ± 0.03	0.04 ± 0.00	0.19 ± 0.01
		0.63 ± 0.17	0.41 ± 0.16	2.08 ± 0.25			

G1(t)/G(0), S(t)/S(0) and G2M(t)/G2M(0) denote the ratio of the minimum G1-population, and corresponding S- and G2/M-populations after exposure to 7 Gy of ^{60}Co γ-rays to the control populations, respectively. *t* is the time at which the minimum G1-population occurs. The mean ± SD of each ratio for each group is also presented. *mt* and *wt* denote mutated and wild-type p53, respectively. MID_γ is the photon mean inactivation dose. γ-MN yield and *n*-MN yield represent the slope of the micronucleus-dose response after ^{60}Co γ- and p(66)/Be neutron irradiation, respectively.

*Data adapted from previous study (Akudugu et al., 2003).

Radiosensitivity and micronucleus yield

The cell survival and micronucleus formation data used in the present discussion were adapted from a preceding study (Akudugu et al., 2003) and are summarised in Table 1. Cellular radiosensitivity was based on mean inactivation dose and cytokinesis-block micronucleus yield was defined as the slope of the micronucleus frequency (MNF)-dose response curves.

Statistical analysis

For non-parametric analysis, the Student's *t*-test was used and for parametric associations, linear regression analysis was used.

Results

The glioblastoma cell lines were of known p53 status and were classified as p53 wild-type (G-44, G-62 and G-120) and as p53 mutant (G-28, G-60 and G-112) (Akudugu et al., 2001). Induction of $p21^{WAF1}$ gene after ^{60}Co γ-irradiation was used to assess p53 function in the neuroblastoma cell lines. Fig. 1 shows that the SK-N-SH cell line expresses functional p53 as indicated by the induction of the p21 target gene. On the other hand, no significant p21 induction was evident in the KELLY cell line. Therefore, SK-N-SH and KELLY were deemed to be p53 wild-type and p53 mutant, respectively.

A *t*-test analysis of flow cytometric data in Table 1 demonstrates that the p53 mutants tended to show greater, but not significant, G1-phase depletion ($P = 0.30$) and S-phase accumulation ($P = 0.05$) than the p53 wild-type cell lines. On average, a 2-fold p53-independent accumulation was observed in the G2/M-phase.

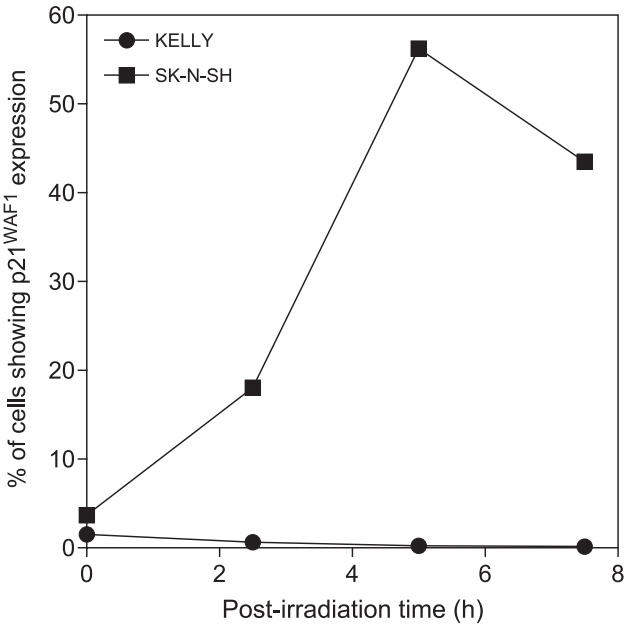

Fig. 1. A plot of the nuclear accumulation of $p21^{WAF1}$ in 2 human neuroblastoma cell lines as a function of time after 7 Gy of ^{60}Co γ-irradiation.

A plot of MN yield as a function of G1(t)/G1(0) shows a significant correlation for neutrons but not for photons (Fig. 2). The linear regression coefficients for neutron and photon irradiation were 0.74 (P = 0.035) and 0.32 (P = 0.439), respectively. No useful link was established between MN yield and changes in G2/M- and S-populations. It is also apparent from Fig. 2 that p53 wild-type cell lines tended to show lower MN yields than their mutant counterparts after neutron irradiation. However, while the p53 mutant KELLY cell line shows a relatively low MN yield, the p53 wild-type G-44 cell line exhibits a high MN yield. These data seem to suggest that p53-independent radiation-induced changes in G1-population may influence the inter-cell line variability in MN yield.

To test this hypothesis, the cell lines were grouped according to the criterion that cell lines with G1(t)/G1(0) > 0.50 show low to moderate depletion (group I: G-28, G-62, G-120 and SK-N-SH) while those with G1(t)/G1(0)<0.50 exhibit high G1 depletion (group II: G-44, G-60, G-112 and KELLY). The mean ± SD G1(t)/G1(0)-ratios for groups I and II were significantly different and were found to be 0.71 ± 0.11 and 0.40 ± 0.04, respectively (r = 0.88, P = 0.004). Cell lines in group II showed an accumulation (S(t)/S(0) = 1.43 ± 0.82) in S-phase and those in group I exhibited a depletion (S(t)/S(0) = 0.48 ± 0.19), but the difference did not reach statistical significance (r = 0.62, P = 0.10). Both groups showed a 2-fold elevation in the G2/M population (r = 0.05, P = 0.90).

MN yield is plotted against intrinsic radiosensitivity based on photon mean inactivation dose in Fig. 3. Interestingly, while photon irradiation did not distinguish between cell lines (Fig. 3a), neutron irradiation puts the cell lines in the same groups based on G1 depletion (Table 1, Fig. 3b), indicating that changes in cell cycle phase population may be important in damage assessment after high LET irradiation.

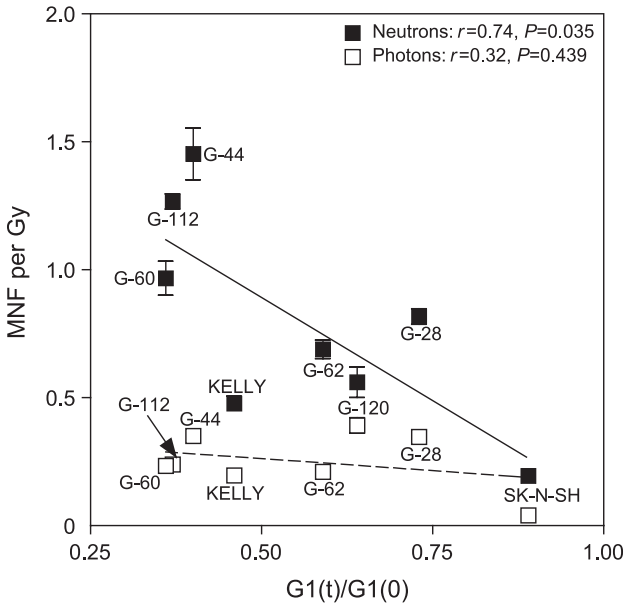

Fig. 2. Relationship between photon (open symbols and dotted line) or neutron (solid symbols and solid line) induced MN yield and the maximum depletion in the G1-phase population following a 7 Gy photon irradiation.

Regression analysis yielded a positive correlation between neutron-induced MN yield and intrinsic radiosensitivity with coefficients of 0.99 (P = 0.002) and 0.97 (P = 0.028) for groups I and II, respectively. Cell lines in group II expressed significantly more micronuclei per unit dose than those of comparable photon sensitivity in group I.

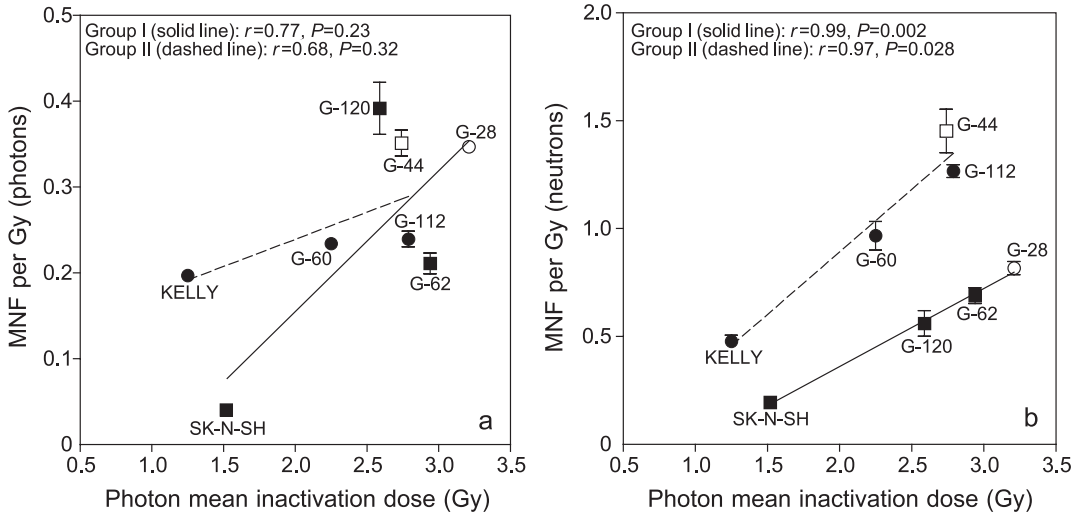

Fig. 3. A plot of MN yield (slope of MN frequency-dose response) after: (a) photon and (b) neutron irradiation against cellular intrinsic radiosensitivity based on photon mean inactivation dose.

Discussion

The ability of the MN assay to sufficiently distinguish radiosensitive cell lines from radioresistant cell lines is a longstanding controversy (Streffer et al., 1982; Shibamoto et al., 1991; Bush and McMillan, 1993; Villa et al., 1994; Akudugu et al., 2000), and the observation that some radioresistant cell lines show high MN yield remains an unresolved issue. It has been suggested that differences in radiation-induced apoptosis constitute an important component of the mechanisms underlying this anomaly (Abend et al., 1995; Guo et al., 1998, 1999). Recently, it has been demonstrated by our group and other investigators that cell death via pathways other than micronucleus formation, apoptosis and abnormal morphology is a critical determinant in the relationship between MN yield and intrinsic radiosensitivity (Abend et al., 2000; Akudugu and Böhm, 2001; Akudugu et al., 2002).

Interestingly, studies attempting to establish the MN assay as a predictive method for radiosensitivity have been predominantly performed with low-LET irradiation. There is evidence that high-LET irradiation induces higher MN yield per unit dose than low-LET irradiation (Tates et al., 1989; Darroudi et al., 1992; Huber et al., 1994; Heimers, 1999). Similarly, the data in Table 1 demonstrate that neutron irradiation leads to a higher MN yield of 0.19-1.45 MN per cell per Gy compared with photon irradiation which results in 0.04–0.39 MN per cell per Gy. In a preceding study, it was established that a significant correlation exists between RBE calculated from clonogenic and MN data (Akudugu et al., 2003a). However, not all radioresistant cell lines showed high RBE-values derived from MN yield, indicating that other factors may be important in the processes leading to MN formation.

The dependence of cellular response to irradiation on cell cycle checkpoint activation is noteworthy (Paulovich et al., 1997). Despite the numerous attempts to relate clonogenic cell survival to radiation-induced damage (Streffer et al., 1982; Shibamoto et al., 1991; Bush and McMillan, 1993; Villa et al., 1994; Abend et al., 1995, 2000; Akudugu et al., 2000, 2002; Akudugu and Böhm, 2001), the influence of changes in cell cycle phase populations on MN formation is yet to be fully elucidated. While p53 is known to control G1-phase arrest via trans-activation of p21 (Lane, 1992; Hartwell and Kastan, 1994; Amundson et al., 1998), it is not required for induction of G2-phase arrest (Kastan et al., 1991). p53 wild-type cells are expected to exhibit both G1- and G2-phase blocks following ionising radiation, whereas cells that are deficient in p53 function (i.e. p53 mutant or null) would show only a G2-phase block. Our data in Table 1 support this notion except in the case of the p53 wild-type G-44 and the p53 mutant G-28 cell lines. The reasons for the unexpectedly high G1 depletion in G-44 as illustrated in Fig. 2 are unknown. The relatively low G1 depletion in G-28 may be attributable to the suggestion that not all p53 mutants lose cell cycle control (Ryan and Vousden, 1998).

Interestingly, classification of this panel of cell lines based on the level of depletion of the G1-population, demonstrates that cell lines that showed greater G1-phase depletion (i.e. $G1(t)/G1(0) < 0.5$) after irradiation also exhibited higher neutron-induced MN yield than those of comparable intrinsic radiosensitivity with lesser G1 depletion (i.e. $G1(t)/G1(0) > 0.5$) (Table 1, Fig. 3b). This seems to suggest that the ability of a cell line to invoke a p53-independent G1-phase block after radiation exposure is an important role player in MN formation. Clearly, the uniform and significant ($\sim$ 2-fold) increase in the G2/M population indicates that the differences observed in MN yield between the two groups cannot be due to disparities in G2/M phase arrests. Similarly, the absence of a significant difference between the mean S-phase populations of the groups suggests that S-phase arrests may be a minor contributor to the observed differences in MN yield.

An arrest in G1-phase is expected to permit the repair of the damaged DNA template before replication in S-phase, and would result in low levels of residual damage. This phenomenon is demonstrated in Fig. 2 where the cell lines with the least G1 depletion showed less MN yield after neutron irradiation, and is consistent with the suggestion that in cell lines lacking a G1-block, there is a progression of DNA damage through S-phase and this incurs a high level of genomic instability (Paulovich et al., 1997). In another study using the glioblastoma cell lines, we demonstrated that while total (20 hours) DNA double-strand-break (DSB) rejoining reflects clonogenic cell survival, fast (2 hours) rejoining influences MN yield (Akudugu et al., 2004). Mammalian cells repair DNA damage mainly by both homologous recombination (HR) and non-homologous end joining (NHEJ) (Sargent et al., 1997; Liang et al., 1998). If repair immediately after irradiation is dominated by the faster error prone NHEJ, high proportions of G1 cells may misrepair DSB damage since no sister-chromatids exist in this phase. It is therefore likely that group II cells progress into S phase with high levels of misrepaired damage that interfere with replication and lead to increased MN yield. On the other hand, it can be speculated that as large proportions of group I cells reside in G1 for extended periods, most misrepaired damage may be corrected by other mechanisms including HR and thus lead to low yields of micronuclei. It is also possible that these repair phenomena are enhanced in a LET-dependent manner. Although the rate of DNA repair has been shown to be similar after high and low LET irradiation, neutrons induce more than double DSBs compared to photons and therefore produce high levels of residual unjoined strand-breaks (Ritter et al., 1977; Roots et al., 1990; Goodhead, 1994).

Notably, MN yield after low LET irradiation cannot distinguish the cell lines according to the extent of G1 depletion (Fig. 2). The absence of significant differences in the extent of photon- and neutron-induced cell cycle arrests cannot corroborate the finding that more human skin fibroblasts are blocked in G1 after photon irradiation than after exposure to α-particles (Gadbois et al., 1996). It is likely that a LET threshold exists above which this phenomenon is observed, and that neutrons which have a much lower LET than α- particles exhibit photon-like characteristics in the induction of cell cycle checkpoints.

In conclusion, these data demonstrate that while the functional status of p53 may not be an important factor in radiation-induced MN formation, G1 checkpoint activation efficiency plays a central role in the yield of high-LET induced micronuclei. Therefore, changes in cell cycle progression need to be considered when damage assays are assessed for predictive purposes.

Acknowledgements

This work was supported by fellowships from iTHEMBA LABS and the Medical Research Council (MRC) of South Africa to JMA, and grants from the National Research Foundation of South Africa (NRF), the Becker Trust and the Cancer Association of South Africa (CANSA) to LB.

References

Abend, M., Rhein, A., Gilbertz, K.-P., Blakely, W.F., van Beuningen, D., 1995. Correlation of micronucleus and apoptosis assays with reproductive cell death. International Journal of Radiation Biology 67, 315–326.

Abend, M., Kehe, K., Kehe, K., Riedel, M., van Beuningen, D., 2000. Correlation of micronucleus and apoptosis assays with reproductive cell death can be improved by considering other modes of death. International Journal of Radiation Biology 76, 249–259.

Akudugu, J.M., Abend, M., Böhm, L., 2002. A unifying model for reconstructing radiosensitivity from micronucleus formation, apoptosis and abnormal morphology. Radiation and Environmental Biophysics 41, 267–274.

Akudugu, J.M., Böhm, L., 2001. Micronuclei and apoptosis in glioma and neuroblastoma cell lines and the role of other lesions in the reconstruction of cellular radiosensitivity. Radiation and Environmental Biophysics 40, 295–300.

Akudugu, J.M., Gerde, G., Böhm, L., 2001. Cytotoxicity of azadirachtin A in human glioblastoma cell lines. Life Sciences 68, 1153–1160.

Akudugu, J.M., Slabbert, J.P., Roos, W.P., Böhm, L., 2003. Micronucleus response of human glioblastoma and neuroblastoma cells toward low-LET photon and high-LET p(66)/Be neutron irradiation. American Journal of Clinical Oncology (CCT) 26, e1–e6.

Akudugu, J.M., Slabbert, J.P., Serafin, A., Böhm, L., 2000. Frequency of radiation-induced micronuclei in neuronal cells does not correlate with clonogenic survival. Radiation Research 153, 62–67.

Akudugu, J.M., Theron, T., Serafin, A.M., Böhm, L., 2004. Influence of DNA double-strand break rejoining on clonogenic survival and micronucleus yield in human cell lines. International Journal of Radiation Biology 80, 93–104.

Amundson, S.A., Myers, T.G., Fornace, A.J., 1998. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 17, 3287–3299.

Balcer-Kubiczek, E.K., Yin, J., Lin, K., Harrison, G.H., Abraham, J.M., Meltzer, S.J., 1995. p53 mutational status and survival of human breast cancer MCF-7 cell variants after exposure to X-rays and fission neutrons. Radiation Research 142, 256–262.

Balcer-Kubiczek, E.K., Zhang, X.F., Harrison, G.H., Zhou, X.J., Vigneulle, R.M., Ove, R., McCready, W.A., Xu, J.F., 1999. Delayed expression of hpS2 and prolonged expression of CIP1/WAF1/SDI1 in human tumour cells irradiated with X-rays, fission neutrons or 1 GeV/nucleon Fe ions. International Journal of Radiation Biology 75, 529–541.

Binder, A.B., Serafin, A.M., Böhm, L.J.F., 2000. Abrogation of G2/M-phase block enhances the cytotoxicity of daunorubicin, melphalan and cisplatin in TP53 mutant human tumour cells. Radiation Research 154, 640–649.

Britten, R.A., Peters, L.J., Murray, D., 2001. Biological factors influencing the RBE of neutrons: implications for their past, present, and future use in radiotherapy. Radiation Research 156, 125–135.

Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J.P., Sedivy, J.M., Kinzler, K.W., Vogelstein, B., 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 282, 1497–1501.

Bush, C., McMillan, T.J., 1993. Micronucleus formation in human tumour cells: lack of correlation with radiosensitivity. British Journal of Cancer 67, 102–106.

Darroudi, F., Farouqi, Z., Benova, D., Natarajan, A.T., 1992. The mouse splenocyte assay, an in vivo/in vitro system for biological monitoring: studies with X-rays, fission neutrons and bleomycin. Mutation Research 272, 237–248.

Deptala, A., Li, X., Bender, E., Cheng, W., Traganos, F., Darzynkiewicz, Z., 1999. Differences in induction of p53, $p21^{WAF1}$ and apoptosis in relation to cell cycle phase of MCF-7 cells treated with camptothecin. International Journal of Oncology 15, 861–871.

Fox, J.C., McNally, J.J., 1988. Cell survival and DNA double-strand break repair following X-ray or neutron irradiation of V79 cells. International Journal of Radiation Oncology Biology Physics 54, 1021–1030.

Gadbois, D.M., Crissman, H.A., Nastasi, A., Habbersett, R., Wang, S., Chen, D., Lehnert, B.E., 1996. Alterations in the progression of cells through the cell cycle after exposure to alpha particles or gamma rays. Radiation Research 146, 414–426.

Goodhead, D.T., 1994. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. International Journal of Radiation Biology 65, 7–17.

Guo, G.Z., Sasai, K., Oya, N., Takagi, T., Shibuya, K., Hiraoka, M., 1998. Simultaneous evaluation of radiation-induced apoptosis and micronuclei in five cell lines. International Journal of Radiation Biology 73, 297–302.

Guo, G.Z., Sasai, K., Oya, N., Shibata, T., Shibuya, K., Hiraoka, M., 1999. A significant correlation between clonogenic radiosensitivity and the simultaneous assessment of micronucleus and apoptotic cell frequency. International Journal of Radiation Biology 75, 857–864.

Hartwell, L.H., Kastan, M.B., 1994. Cell cycle control and cancer. Science 266, 1821–1828.

Heimers, A., 1999. Cytogenetic analysis in human lymphocytes after exposure to simulated cosmic radiation which reflects the in-flight radiation environment. International Journal of Radiation Biology 75, 691–698.

Hu, Q., Hill, R.P., 1996. Radiosensitivity, apoptosis and repair of DNA double-strand breaks in radiation-sensitive Chinese hamster ovary cell mutants treated at different dose rates. Radiation Research 146, 636–645.

Huber, R., Schraube, H., Nahrstedt, U., Braselmann, H., Bauchinger, M., 1994. Dose-response relationships of micronuclei in human lymphocytes induced by fission neutrons and by low LET radiation. Mutation Research 306, 135–141.

Joiner, M.C., Field, S.B., 1988. The response of mouse skin to irradiation with neutrons from the 62 MeV cyclotron at Clatterbridge, UK. Radiotherapy and Oncology 12, 153–166.

Kastan, M.B., Onyekwere, O., Sidransky, D., Vogelstein, B., Craig, R.W., 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Research 51, 6304–6311.

Lane, D.P., 1992. p53, guardian of the genome. Nature 328, 15–16.

Liang, F., Han, M., Romanienko, P.J., Jasin, M., 1998. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. Proceedings of the National Academy of Sciences USA 95, 5172–5177.

Ormerod, M.G., 1994. Analysis of DNA – general methods. In: Rickwood, D., Himes, B.D. (Eds.), Flow Cytometry: a Practical Approach, 2nd ed. Oxford University Press, Oxford, pp. 120–135.

Paulovich, A.G., Toczyski, D.P., Hartwell, L.H., 1997. When checkpoints fail. Cell 88, 315–321.

Peak, M.J., Wang, L., Hill, C.K., Peak, J.G., 1991. Comparison of repair of DNA double-strand breaks caused by neutron or gamma radiation in cultured human cells. International Journal of Radiation Biology 60, 891–898.

Ritter, M.A., Cleaver, J.E., Tobias, C.A., 1977. High-LET radiations induce a large proportion of non-rejoining DNA breaks. Nature 266, 653–655.

Roots, R., Holley, W., Chaterjee, A., Irizarry, M., Kraft, G., 1990. The formation of strand breaks in DNA after high-LET irradiation: a comparison of data from in vitro and cellular systems. Interantional Journal of Radiation Biology 58, 55–69.

Ryan, K.M., Vousden, K.H., 1998. Characterization of structural p53 mutants which show selective defects in apoptosis but not cell cycle arrest. Molecular and Cellular Biology 18, 3692–3698.

Sargent, R.G., Brenneman, M.A., Wilson, J.H., 1997. Repair of site-specific double-strand breaks in a mammalian chromosome by homologous and illegitimate recombination. Molecular and Cell Biology 17, 267–277.

Schmitt, G., Wambersie, A., 1990. Review of the clinical results of fast neutron therapy. Radiotherapy and Oncology 17, 47–56.

Shibamoto, Y., Streffer, C., Fuhrmann, C., Budach, V., 1991. Tumour radiosensitivity prediction by the cytokinesis-block micronucleus assay. Radiation Research 128, 293–300.

Slabbert, J.P., Theron, T., Zölzer, F., Streffer, C., Böhm, L., 2000. A comparison of the potential therapeutic gain of p(66)/Be neutrons and d(14)/Be neutrons. International Journal of Radiation Oncology Biology Physics 47, 1059–1065.

Streffer, C., van Beuningen, D., Molls, M., 1982. Possibilities of the micronucleus test as an assay in radiotherapy. In: Kärcher, J.H., Kogelnik, H.D., Reinartz, G. (Eds.), Progress in Radio-Oncology II. Raven Press, New York, pp. 243–251.

Takahashi, A., Ohnishi, K., Tsuji, K., Matsumoto, H., Aoki, H., Wang, X., Tamamoto, T., Yukawa, O., Furusawa, Y., Ejima, Y., Tachibana, A., Ohnishi, T., 2000. WAF1 accumulation by carbon-ion beam and alpha-particle irradiation in human glioblastoma cultured cells. Interantional Journal of Radiation Biology 76, 335–341.

Tates, A.D., de Vogel, N., Rotteveel, A.H., Leupe, F., Davids, J.A., 1989. The response of spermatogonia and spermatocytes of the Northern vole Microtus oeconomus to the induction of sex-chromosome nondisjunction, diploidy and chromosome breakage by X-rays and fast fission neutrons. Mutation Research 210, 173–189.

Theron, T., Böhm, L., 2000. Influence of the G2 cell cycle block abrogator pentoxifylline on the expression and subcellular location of cyclin B1 and $p34^{cdc2}$ in HeLa cervical carcinoma cells. Cell Proliferation 33, 39–50.

Tsuchida, Y., Tsuboi, K., Ohyama, H., Ohno, T., Nose, T., Ando, K., 1998. Cell death induced by high-linear-transfer energy carbon beams in human glioblastoma cell lines. Brain Tumour Pathology 15, 71–76.

Villa, R., Zaffaroni, N., Gornati, D., Costa, A., Silvestrini, R., 1994. Lack of correlation between micronucleus formation and radiosensitivity in established and primary cultures of human tumours. British Journal of Cancer 70, 1112–1117.

Weichselbaum, R.R., 1986. Radioresistant and repair proficient cells may determine radiocurability in human tumours. International Journal of Radiation Oncology Biology Physics 12, 637–639.

West, C.M., Davidson, S.E., Roberts, S.A., Hunter, R.D., 1993. Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. British Journal of Cancer 68, 819–823.