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The relationship between radiation-induced G_1 arrest and chromosome aberrations in Li-Fraumeni fibroblasts with or without germline *TP53* mutations

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Summary We previously showed that cultured fibroblasts from patients with the cancer-prone Li-Fraumeni (LF) syndrome, having heterozygous germline *TP53* mutations, sustain less ionizing radiation-induced permanent G_1 arrest than normal fibroblasts. In contrast, fibroblast strains from LF patients without *TP53* mutations showed normal G_1 arrest. We have now investigated the relationship between the extent of G_1 arrest and the level of structural chromosome damage (mainly dicentrics, rings and acentric fragments) in cells at their first mitosis after G_1 irradiation, in 9 LF strains with *TP53* mutations, 6 without *TP53* mutations and 7 normal strains. Average levels of damage in the mutant strains were 50% higher than in normals, whereas in non-mutant LF strains they were 100% higher. DNA double strand breaks (dsb) are known to act as a signal for p53-dependent G_1 arrest and to be the lesions from which chromosome aberrations arise. These results suggest that a minimal level of dsb is required before the signal for arrest is activated and that p53-defective cells have a higher signal threshold than p53-proficient cells. Dsb that do not cause G_1 blockage can progress to mitosis and appear as simple deletions or interact to form exchange aberrations. The elevated levels in the non-mutant strains may arise from defects in the extent or accuracy of dsb repair. In LF cells with or without *TP53* mutations, the reduced capacity to eliminate or repair chromosomal damage of the type induced by ionising radiation, may contribute to cancer predisposition in this syndrome. © 2001 Cancer Research Campaign http://www.bjcancer.com

Keywords: Li-Fraumeni syndrome; p53; Chk2; ionizing radiation; G_1 arrest; chromosome aberrations; fibroblasts

When normal human fibroblasts are exposed in vitro to ionizing radiation, a proportion of the cells in the G_1 phase of the cell cycle suffer a dose-dependent reduction in their ability to progress further through the cell cycle (Little and Nagasawa, 1985). We have shown (Williams et al, 1997; Boyle et al, 1999) that the extent of this permanent G_1 arrest is less pronounced in fibroblasts of patients with the Li-Fraumeni (LF) syndrome carrying inherited, heterozygous mutations of the *TP53* gene which confer a high risk of cancer (reviewed by Varley et al, 1997). Our results are consistent with the hypothesis that p53 is responsible for monitoring the integrity of the genome (Lane, 1992) and eliminating genetically damaged cells that might otherwise be predisposed to malignant conversion.

In some cell types, apoptosis appears to be the functional equivalent of G_1 arrest and is reduced or eliminated in p53-defective cells after exposure to DNA-damaging agents (references in Williams et al, 1996, 1997; Camplejohn et al, 1995). For example, a cell line of human lymphocyte origin carrying a *TP53* mutation showed less radiation-induced apoptosis than an isogenic cell line without the mutation (Schwartz et al, 1995) although neither line sustained G_1 arrest (Little et al, 1995). The mutant cell line exhibited more structural chromosome damage at the first mitosis after G_1 irradiation than the wild type cells and Schwartz and colleagues (Schwartz et al, 1995; Schwartz and Jordan, 1997) suggested that the cells with the most chromosome damage were selectively eliminated by pre-mitotic apoptosis in the p53-proficient line.

The hypothesis of Schwartz et al, if extended to cells with reduced permanent G_1 arrest, would predict that fibroblasts of LF patients with germline *TP53* mutations would exhibit more chromosome damage at their first mitosis after G_1 irradiation than normal fibroblasts. Only about half of the families with the LF syndrome are found to carry inherited *TP53* mutations (Varley et al, 1997) and we have shown that fibroblasts from non-mutation cases have no defect in G_1 arrest after irradiation (Boyle et al, 1999) and might not, therefore, be expected to show enhanced chromosomal radiosensitivity of G_1 cells. In the present study we have investigated the G_1 chromosomal radiosensitivity of fibroblasts from 7 normal donors, 9 LF patients with *TP53* mutations and 6 without mutations.

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MATERIALS AND METHODS

Full details of the cell strains used are given in Boyle et al (1998, 1999, 2000). The maximum number of in vitro population doublings (PD) was previously established for each strain and all were irradiated before they had reached 80% of their maximum PD, because LF strains tend to become chromosomally unstable during the last 20% of their proliferative lifetime (Boyle et al 1998, 2000).

Designations of the strains, which were all established in this laboratory, are as follows.

Group 1 (normal controls); 83MA, 84MA, 105MA, 120MA, 162MA, 177MA, 187MA.

Group 2 (mutation carriers); FH1, 109MA, 110MA, 124MA, 138MA, 178MA, 185MA, 191MA, 193MA. These 9 strains were established from 7 LF families each with different *TP53* mutations (Boyle et al, 1999). LF families have been subdivided into classical (LFS) and LF-like (LFL) depending upon the number of individuals with cancer, the nature of the cancers, their time of onset and other criteria (Varley et al, 1997). Group 2 comprised 5 LFS and 2 LFL families.

Group 3 (non-mutation carriers); 80MA, 107MA, 121MA, 126MA, 128MA, 147MA. The 6 strains were from different LF families (3 LFS and 3 LFL) and all were from patients with experience of cancers typical of those in LF families (e.g. sarcomas and breast cancer), except strain 121MA which was from an unaffected brother of a patient with a typical LF cancer. Strain 121MA was included because at late passage (>90% maximum PD) it showed the spontaneous chromosomal instability seen in ageing LF strains from cases with typical LF-type cancers (Boyle et al, 2000).

To produce a G_1 population, cells were grown to confluence in Minimal Essential Medium with Earle's salts (MEM) and 15% fetal calf serum (FCS) without antibiotics and maintained at confluence with twice weekly medium changes for 10–14 days. 2 days before irradiation the medium was changed to MEM with 0.1% FCS. On the day of irradiation, cells were harvested by trypsinisation, counted, and exposed in suspension (approximately 1×10^6 per ml) to 0 or 3 Gy ^{137}Cs gamma irradiation at a dose rate of 3.3 Gy per min. Cells were then seeded at 1×10^6 per T150 flask (Corning) in complete medium. At 16 h after seeding, before cells had entered their first mitosis (Williams et al, 1997), colcemid (150 ng ml^{-1}) was added to arrest cells at their first metaphase and incubation was continued for a further 50 h at which time metaphase preparations were made by normal procedures. Cells were stained with Giemsa.

For each cell strain 50–100 metaphases were scored for structural aberrations from coded slides of irradiated and non-irradiated cells with 45 or 46 chromosomes.

Measurement of permanent G_1 arrest was made previously (Williams et al, 1997; Boyle et al, 1999). Briefly, G_1 cells were irradiated or mock-irradiated, subcultured in medium containing tritiated thymidine to label cells entering the S phase, harvested at intervals between 60–120 hours post-irradiation and prepared for autoradiography. The extent of permanent arrest was determined by comparing the proportions of labelled cells in irradiated and non-irradiated samples.

The Mann–Whitney U test was used to test the significance of differences in endpoints between groups of strains.

RESULTS

Mean aberration yields and the range of values for irradiated and non-irradiated cells of each of the 3 groups are given in Table 1, together with values for the degree of permanent G_1 arrest.

The spontaneous aberration yield in the normal stains was approximately 5% and was slightly, but not significantly, higher in the other 2 groups. Further details of spontaneous structural and numerical aberrations in these strains as they progress to senescence are given in Boyle et al (1998, 2000).

The majority of induced aberrations (subtracting the spontaneous yields) were of the chromosome type, comprising dicentrics, centric rings and excess acentric fragments (see footnote 4 of Table 1), typical of cells irradiated in G_1. Approximately 15% of the induced aberrations were of the chromatid type (mainly breaks and large gaps), possibly indicating the presence of a small proportion of cells in S and G_2 at the time of irradition and/or arising from DNA base damage in G_1 cells (Schwartz and Russell, 1999).

As we reported earlier (Williams et al, 1997; Boyle et al, 1999), the average degree of G_1 arrest was significantly less ($P < 0.01$) in mutation-carrying strains (group 2, 44.6 ± 16.3%) than in normals (group 1, 75.0 ± 13.0%). In contrast, the mean frequency of total aberrations induced in group 2 strains (74.4 ± 12.7 per 100 cells) was approximately 50% *higher* ($P = 0.02$) than in normals (50.0 ± 16.2). This difference was seen for all types of aberrations.

The average extent of G_1 arrest in non-*TP53* mutation-carrying strains (group 3, 73.0 ± 7.4%) was very similar ($P = 0.67$) to that of normals (75.0 ± 13.0%), as previously reported (Boyle et al, 1999). However, the mean yield of total aberrations (98.7 ± 42.6 per 100 cells) in group 3 was almost 100% higher ($P < 0.01$) than in normals (50.0 ± 16.2), for all aberration types (Table 1).

Table 1 Chromosomal aberration yields and the extent of G1 arrest in the 3 groups of cell strains

Group	Dose (Gy)	Chromosome-type aberrations[1]				Chromatid aberrations	Total aberrations	G1 arrest (%)[2]
		Dicentrics	Rings	Acentrics[4]	Total			
1. Normals	0	0 ± 0	0 ± 0	1.3 ± 1.3	1.3 ± 1.3	3.2 ± 2.1	4.6 ± 2.2	–
(*n* = 7)				(0 – 3)	(0 – 3)	(0 – 6)	(2 – 8)	
	3	17.1 ± 15.5	5.4 ± 5.4	19.6 ± 8.1	42.1 ± 19.5	11.1 ± 5.8	53.1 ± 20.4	75.0 ± 13.0
		(4 – 44)	(0 – 16)	(12 – 34)	(20 – 68)	(5 – 18)	(30 – 85)	(50.2 – 90.9)
2. Mutation carriers	0	0.67 ± 1.4	0 ± 0	1.0 ± 2.6	1.7 ± 2.7	3.6 ± 2.9	5.2 ± 3.8	
(*n* = 9)[5]		(0 – 4)		(0 – 8)	(0 – 8)	(0 – 10)	(2 – 14)	
	3	29.4 ± 15.0	7.9 ± 2.5	29.0 ± 15.9	66.3 ± 12.4	15.0 ± 9.7	81.0 ± 14.7	44.6 ± 16.3[3]
		(14 – 59)	(4 – 12)	(4 – 54)	(54 – 88)	(6 – 26)	(64 – 114)	(14.9 – 63.5)
3. Non-mutation carriers	0	0 ± 0	0 ± 0	1.3 ± 1.6	1.3 ± 1.6	6.3 ± 4.1	7.7 ± 4.1	–
				(0 – 4)	(0 – 4)	(2 – 14)	(6 – 16)	
	3	39.7 ± 20.9	10.7 ± 6.2	37.0 ± 10.3	87.3 ± 27.7	19.7 ± 17.8	106.3 ± 46.5	73.0 ± 7.4
(*n* = 6)		(16 – 72)	(4 – 20)	(22 – 50)	(58 – 140)	(6 – 58)	(66 – 198)	(58.7 – 79.4)

[1] Aberration yields are expressed per 100 cells ± SD. Ranges are given in brackets. 50–100 cells scored from each sample except for irradiated cells from normal strain 187MA where only 30 cells could be analysed. [2] Data from Boyle et al (1999). [3] G1 arrest data for 185MA are not included because they were obtained after a dose of 4.0 Gy (Varley et al, 1998), but the 3.0 Gy data for 178MA are from the same family. [4] One acentric fragment was allocated to each dicentric and ring. The remaining (excess) acentric fragments are given in this column. [5] Details of mutations given in Boyle et al (1999) except for strain 185MA, which was from family 2635 (see Table 1 in Boyle et al, 1999).

Because of the wide range of aberration yields in group 3, giving a comparatively large standard deviation, the mean yield was not significantly greater than that of group 2 ($P = 0.15$). Within group 3, strain 80MA showed a considerably higher frequency of both spontaneous (16/100 cells) and induced (182/100 cells) aberrations than the 5 remaining strains, all of which had a spontaneous yield of 6/100 cells and a range of induced yields from 60–96/100 cells. The mean frequency in the 5 strains was 82.0 ± 13.4 per 100 cells which was still significantly higher ($P = 0.01$) than the normals. Strain 80MA also had the lowest level of G_1 arrest (58.7%) amongst group 3, the remaining 5 of which had a range from 72.8 to 79.4% (mean 73.0 ± 7.4%). Strain 80MA has now been found to carry a germline mutation in the *hCHK2* gene (Bell et al, 1999) which is likely to be involved in G_1 checkpoint control because of its influence on p53 transcription (see Discussion).

DISCUSSION

Our results support the hypothesis of Schwartz and colleagues (Schwartz et al, 1995; Schwartz and Jordon, 1997) that p53-proficient cells have a mechanism for selectively eliminating cells with high levels of chromosome damage induced in the G_1 phase of the cell cycle. However, whereas in the cells of lymphoid origin used by Schwartz et al this selection process occurs by apoptosis, in human fibroblasts the mechanism appears to involve permanent G_1 arrest. During the course of our studies, Schwartz and Russell (1999) have reported finding increased levels of chromosome damage at the first mitosis after gamma-irradiation of a human adenocarcinoma cell line in which permanent G_1 arrest was eliminated by transfection with the human papillomavirus type 16 E6 gene which inactivates p53 expression.

There is abundant evidence that radiation-induced DNA double strand breaks (dsb) can act as a signal for p53-dependent permanent G_1 arrest (references in Schwartz and Russell, 1999). Our observations and those of Schwartz and Russell on non-lymphoid cells provide additional support for this concept, since radiation-induced chromosome aberrations arise mainly from dsb (reviewed by Pfeiffer et al, 2000). These results suggest that a certain minimal level of dsb is required before the signal for arrest is activated and that p53-defective cells have a higher signal threshold than p53-proficient cells. Dsb that do not cause G_1 blockage can progress to mitosis and appear as simple deletions (excess fragments) or interact to for, exchange aberrations (e.g. dicentrics and rings). Schwartz and Russell (1999) argue that the increase in chromatid aberrations, which they also observed in irradiated HPV16-E6 cells compared with non-transfected cells, is a consequence of deficient base excision repair that has been reported in p53-defective human fibroblasts (Ford et al, 1998).

There have been several other studies (Parshad et al, 1993, Lee et al, 1994, Williams, et al, 1997), including our own (Wang et al, 1996, Mitchell and Scott, 1997, Varley et al, 1998) in which p53-defective cells have been found to have elevated levels of radiation-induced structural chromosome damage compared with p53-proficient cells. However, in these investigations, cells were either irradiated as asynchronous populations or in the G_2 phase of the cell cycle. In the former, the specific role of G_1 arrest in determining these increased yields could not be assessed. The latter results demonstrate that in p53-defective cells it is not only those that are in the G_1 phase at the time of irradiation that are at increased risk of enhanced chromosome damage, but the mechanism of G_2 radiosensitization is at present unknown.

There is compelling evidence that the degree or accuracy of DNA double strand break repair can determine the levels of chromosome damage in cells exposed to ionizing radiation (Pfeiffer et al, 2000). We suggest that defects in these mechanisms may explain the elevated levels of chromosome damage in cells strains without *TP53* mutations and with normal G_1 arrest. However, for strain 80MA, with a mutation in the *hCHK2* gene, impaired G_1 arrest is a more likely explanation since it had the lowest G_1 arrest value of the group 3 strains, overlapping the group 2 range. The Chk2 protein is activated in response to ionizing radiation (Tominaga et al, 1999) and is required to stabilize p53 transcription (Chehab et al, 2000) so will have an influence on G_1 arrest. The fact that the induced aberration frequency in 80MA is almost twice as high as that of the most sensitive *TP53* mutation-carrying strain suggests that Chk2 has a function in addition to the stabilization of p53 and that this additional function influences the aberration production after irradiation. However, this interpretation of the abnormal behaviour of strain 80MA must remain tentative until the other non-*TP53* mutant strains have been tested for germline *hCHK2* mutations. So far, only strain 107MA has been tested and found to be without a mutation of this gene.

In Li-Fraumeni cells with or without *TP53* mutations, a reduced capacity to eliminate or repair chromosomal damage of the type induced by ionizing radiation, from endogenous or exogenous sources, may contribute to cancer predisposition in this syndrome.

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