Absence of a Radiation-induced First-Cycle G₁-S Arrest in p53⁺ Human Tumor Cells Synchronized by Mitotic Selection¹

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ABSTRACT

It is well known that normal human diploid fibroblasts undergo a significant, p53-dependent arrest in the G₁ phase of the cell cycle after exposure to ionizing radiation. The presence and magnitude of a G₁ arrest in human tumor cell lines, however, has been controversial, particularly in cells derived from solid tumors and irradiated during exponential growth. To examine this question more precisely, we synchronized cells by mitotic selection and irradiated them in very early G₁ prior to any of the described G₁ checkpoints. Progression of cells from G₁ into the S phase was monitored by autoradiographic measurement of cumulative labeling indices and by flow cytometric analysis. Three different human tumor cell lines confirmed as expressing normal p53 function were examined, i.e., lines derived from an adenocarcinoma of the colon (RKO), a breast cancer (MCF-7), and a squamous cell carcinoma (SCC61). Following irradiation with 4–8 Gy, there was a transient delay in progression from G₁ into S phase, lasting approximately 2 h, and in two of the three cell lines (RKO and MCF-7), a small fraction of cells (5–8%) never entered the first S phase. Although there was no evidence for a prolonged G₁ arrest, the expected G₁ delay was observed in all three cell lines. When irradiated RKO cells were resynchronized at the next mitosis, approximately 30% of the cells did not enter the second S phase. This latter finding is consistent with earlier reports on the kinetics of radiation-induced reproductive failure in mammalian cells. These results indicate that cells derived from human solid tumors that express normal p53 may respond to irradiation quite differently than do normal cells in terms of G₁ checkpoint control.

INTRODUCTION

Information on radiation-induced cell cycle delays at the G₁-S and G₂-M checkpoints in mammalian cells has been accumulating for over 30 years (1). In some studies with transformed rodent cells and HeLa cells exposed to moderate doses of ionizing radiation, a transient reduction that lasted for 1–4 h occurred in the rate at which cells progressed from G₁ into S phase (2–4), particularly when synchronized cells were irradiated in G₁ (3, 4), whereas in other studies, no such reduction was observed (1, 5). On the other hand, a prolonged G₁ arrest occurred in slowly growing human diploid fibroblasts following exposure to 3–10 Gy, an arrest that, in some cases, lasted for several days (6).

In later studies with human diploid fibroblasts, the semisynchronous movement of cells from G₀-G₁ into S phase was examined in cells irradiated and then released from the confluent, density-inhibited phase of growth (7–10). Two distinct phenomena emerged. (a) A dose-dependent delay in the movement of cells from G₁ into S phase occurred in irradiated as opposed to nonirradiated cultures; the delay was observed on the order of 4 h with a radiation dose of 4 Gy. (b) A significant fraction of the cells appeared to be irreversibly arrested in G₀-G₁. In some cell strains, as many as 80% of the cells were subject to this G₀-G₁ arrest; these strains included moderately radiosensitive human skin fibroblasts from patients with retinoblastoma (7, 8) and AT³ heterozygotes (9, 10), as well as others (11), all of which express wild-type p53. Cells derived from AT homozygotes showed neither the G₁-S delay nor the G₀-G₁ arrest (7–10). Similarly, no radiation-induced G₁ delay or G₀-G₁ arrest was observed for human diploid fibroblasts transfected with the human papilloma virus E6 gene (11–13) or in fibroblasts that had lost p53 function derived from a patient with the Li-Fraumeni syndrome (14). When cells from normal individuals were released from density-inhibited growth and then irradiated near the G₁-S border, no radiation-induced G₁ delay or G₀-G₁ arrest occurred (14), presumably because the cells had progressed beyond the critical checkpoints in G₁. On the basis of these findings, it has been proposed that the expression of functional p53 plays an important role in G₁ checkpoint control and the induction of an irreversible arrest in irradiated human diploid fibroblasts (11–13, 15).

The occurrence and nature of the G₁ arrest in human tumor cells, however, is much less clear. It is well known that tumor cells undergo multiple genetic changes involving loss of normal growth controls during the development of a fully malignant, invasive tumor (16). However, several investigators have reported the occurrence of a prolonged, radiation-induced first-cycle G₁ arrest that is p53 dependent in asynchronously growing cell lines derived from human lymphomas (17–21) and solid tumors (22–24). We, on the other hand, have been unable to demonstrate the presence of such a prolonged first cycle G₁ arrest in p53⁺ human tumor cell lines irradiated during exponential growth and examined by flow cytometry (25, 26) or following irradiation of density-inhibited G₁ phase cells subsequently released and examined by continuous labeling autoradiographic techniques (27).

To gain definitive information on the effects of radiation on the progression of cells from G₁ into S phase during the first postirradiation cell cycle, we studied highly synchronized tumor cell populations obtained by mitotic selection and irradiated in very early G₁ prior to any of the described G₁ checkpoints (28). Cell lines derived from three different types of human solid tumors were used, all of which were confirmed to express wild-type p53. Although there was a transient delay in progression from G₁ into S phase, lasting approximately 2 h, there was no evidence for a prolonged radiation-induced G₁ arrest in any of these tumor cell lines.

MATERIALS AND METHODS

Cell Culture and Synchrony. Three human tumor cell lines with normal p53 function (MCF7, RKO, and SCC61) were used in these studies. They were cultured in Eagle’s MEM supplemented with 10% heat-inactivated FBS (56° C), 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cell lines derived from three different types of human solid tumors were used, all of which were confirmed to express wild-type p53. Although there was a transient delay in progression from G₁ into S phase, lasting approximately 2 h, there was no evidence for a prolonged radiation-induced G₁ arrest in any of these tumor cell lines.

Received 10/20/97; accepted 3/3/98.

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1 This work was supported by NIH Grants CA-47542 and CA73725 and Center Grant ES-00002; Massachusetts Department of Public Health Grant 3408799 DO35; and a British Cancer Research Award (to C. M.).

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Synchronous cells were obtained by shaking loose the mitotic cells from the actively growing asynchronous cultures (29, 30). The shaking procedure (2–4 shakes/s on a reciprocating platform shaker for a total of 10–20 s, depending upon the cell line) was carried out at 37°C in a walk-in incubator. The synchronous cells were immediately cooled to 0–4°C by pouring the medium containing the mitotic cells into a glass Erlenmeyer flask placed in an ice bath. Ten ml of conditioned medium were then added to the T-150 culture flasks in preparation for the next shake. Conditioned medium was obtained from near confluent cultures 24 h after incubation in fresh medium. The shaking procedure was repeated every 10 min until sufficient cells were collected (3–5 × 10^6 cells for autoradiographic and 10^7 cells for flow cytometric analysis). At each shake, the cell suspension was added to the Erlenmeyer flask in the ice bath. An aliquot of the cell suspension was removed after each shake and monitored for the cell count and percentage mitotic cells. If the mitotic index was less than 80%, the cell suspension from that shake was discarded. The pooled suspension of mitotic cells was concentrated immediately after the last shake by centrifugation at 1000 rpm (300 × g) for 10 min. The mitotic indices were 80–99.5%, as shown in Table 1. A representative photomicrograph of such a synchronized population is shown in Fig. 1. We verified that most of these mitotic cells completed cytokinesis within 1 h at 37°C, as previously reported (30). To control for any effects of the procedure whereby mitotic cells were accumulated and pooled, the cells in some experiments were studied immediately after a single mitotic shake. The results of these latter experiments did not differ from those with the pooled suspension derived from multiple shakes.

**Cell Cycle Analysis.** The mitotic cells were seeded onto 35-mm plastic Petri dishes in complete medium containing 1 µCi/ml [H]thymidine (specific activity, 20 Ci/mmol) at 37°C to score the labeling index as a measure of the progression of G1 cells into S. The cells were irradiated 1–4 h after seeding when cytokinesis was complete with a 60Co γ ray source (United States Nuclear model GR-9) at a dose rate of 0.12 Gy/sec. At regular intervals after seeding, one dish was removed, and the cells were fixed directly on the dish (7). Kodak nuclear emulsion NTB2 (Eastman Kodak, Rochester, NY) was applied to the fixed dishes for autoradiography by standard techniques, as described previously (7). After development, the cells were stained through the emulsion with 0.1% crystal violet; labeled cells were clearly recognized, and 200 total cells were scored on each dish to determine the labeling index.

**Flow Cytometric Analysis.** For analysis of cell cycle distributions of control and irradiated samples by PI staining, cells were removed by trypsinization at various times after irradiation, washed twice with PBS, and then fixed in 75% ice-cold ethanol by centrifugation, cells were treated with RNase (1 µg/ml) for 30 min and then stained with PI (10 µg/ml) for 15 min. DNA fluorescence of PI-stained cells was measured with an EPICS Elite ESP flow cytometer/cell sorter (Coulter Electronics, Hialeah, FL). An argon ion laser operating at a 488-nm wavelength and 15 mW was used for excitation. DNA fluorescence was monitored through a 610-nm band pass filter. The percentages of G1, S, and G2-M cells were determined from the DNA histograms using the Multiple AV program of the Phoenix Flow System (San Diego, CA).

For two-parameter analysis of cell cycle progression, cells were continuously cultured with 10–15 µM BrdUrd in complete medium. At various times after labeling, cells were removed from flasks by trypsin and fixed in 75% ice-cold ethanol. The DNA needed to be denatured to allow the anti-BrdUrd antibody access. Therefore, cell pellets were resuspended and incubated in 3 ml of 75% ethanol for 24 h before DNA analysis. After the removal of ethanol by centrifugation, cells were permeabilized by 1 ml of PBS with 0.5% FBS and 0.5% Triton X-100, spun down, and then resuspended in 1 ml of PBS with 2% FBS for 20 min to block nonspecific binding. The cells were pelleted and stained with 10 µl anti-BrdUrd/FITC (Boehringer Mannheim) for 45 min.

Then, cells were treated with 1 ml of RNase (1 mg/ml) prior to being counterstained with PI (10 µg/ml) for flow cytometric analysis (31). Fluorescence distributions of at least 10,000 cells/sample were collected by a EPICS Elite ESP flow cytometer. The distribution of green fluorescence from FITC expressed on a logarithmic scale was collected as a measure of BrdUrd content, and the distribution of red fluorescence from PI on a linear scale was collected as a measure of DNA content. Filters used were 550-nm dichroic, 525-nm band pass for green fluorescence and 610-nm band pass for red fluorescence.

Two-parameter distributions of BrdUrd content and DNA content were generated using the Elite program (Coulter Electronics) on a PC workstation. The actual percentage of BrdUrd-positive cells, as defined by the box shown in Fig. 5, was determined by subtracting the percentage of cells from the negative control. The distribution of G1, S, and G2-M cells in each sample was analyzed by the Multicycle AV program (31).

**Western Blot Analysis.** Exponentially growing cells were irradiated with 6.0 Gy. Protein extracts were prepared 5.5 h after irradiation treatment (32). Cells were washed twice with PBS, scraped into 0.5 ml of lysis buffer [50 mM Tris (pH 8.0), 5 mM EDTA, 150 mM NaCl, 0.5% NP40, and 1 mM phenylmethylsulfonyl fluoride] and incubated on ice for 15 min with occasional mild vortexing. Cells were then sonicated for 10 pulses at setting 5, 50% output, with a Branson 450 sonifier. Lysates were spun at 15,000 × g for 15 min to remove cellular debris. One hundred µg of protein extract from the resulting supernatants were resolved by SDS-PAGE and transferred to Immobilon-P membranes (Millipore) for detection with the p53 monoclonal antibody Ab-6 (Oncogene Science) or the p21 polyclonal antibody 15431E (PharMingen; Ref. 32).

**PCR/Single-Strand Conformation Polymorphism Analysis.** Five exons (exons 5–9) of the p53 gene were amplified by PCR using oligonucleotide primers as follows: exon 5, 5′-TTCATGCCTGTGCCGCCC-3′ (forward) and 5′-CTCATGTGCTGTA CGTCCTGT-3′ (reverse); exon 6, 5′-ACDOACA- GGG CGTGGTTCCCCA-3′ (forward) and 5′-CTCCAGACCATG CCTG-3′ (reverse); exon 7, 5′-GCCCTCATCTTG GGCCGTGG-3′ (forward) and 5′-CATGTCGACGCTGGTGG CAGT-3′ (reverse); exon 8, 5′-CTCGC CTCTGTCTGCTTCTT3′-3′ (forward) and 5′-TCTTCTTCACGCGTCTT-TGT3′-3′ (reverse); and exon 9, 5′-GCATTGATGCTCAGATTCA-3′ (forward)

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**Table 1 Characteristics of human tumor cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>SSCPa,b</th>
<th>Functionc</th>
<th>MPDT (h)</th>
<th>Radiosensitivity, D0 (Gy)</th>
<th>Mitotic index after selection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RKO</td>
<td>Colon adenocarcinoma</td>
<td>WT</td>
<td>+</td>
<td>15</td>
<td>1.05</td>
<td>85–93</td>
</tr>
<tr>
<td>MCF7</td>
<td>Breast adenocarcinoma</td>
<td>WT</td>
<td>+</td>
<td>20</td>
<td>0.95</td>
<td>80–85</td>
</tr>
<tr>
<td>SCC61</td>
<td>Squamous cell carcinoma</td>
<td>WT</td>
<td>+</td>
<td>20</td>
<td>1.07</td>
<td>95–99.5</td>
</tr>
</tbody>
</table>

a Five exons (exons 5–9) of the p53 gene were amplified by PCR using oligonucleotide primers, as described previously (20).
b SSCP, single-strand conformation polymorphism; MPDT, mean population doubling time; WT, wild type.
c Induction with 6 Gy of γ irradiation (Fig. 2).
cells showed little or no enhancement in p53 protein levels after individual experiment ± SE.

RESULTS

A 6% polyacrylamide gel with 10% glycerol. The gel was run for 16 h at 6 W (94°C, 1 min at 58°C, and 1 min at 72°C), the PCR product was analyzed on (Perkin-Elmer/Cetus, Norwalk, CT). After 30 cycles of reaction (1 min at dCTP, dGTP, and dTTP; 10 pmol of each primer; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; and 1 unit of AmpliTaq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, CT). After 30 cycles of reaction (1 min at 94°C, 1 min at 58°C, and 1 min at 72°C), the PCR product was analyzed on a 6% polyacrylamide gel with 10% glycerol. The gel was run for 16 h at 6 W and was exposed for 15 h for autoradiographic detection of bands. (33).

The three p53+ tumor cell lines studied were derived from a squamous cell carcinoma (SCC61), an adenocarcinoma of the colon (RKO), and an adenocarcinoma of the breast (MCF-7). These lines are all relatively radioresistant as compared with those from many human tumors (34), with the $D_0$ (inverse of the slope of the survival curve) ranging from 0.95 to 1.07 Gy (Table 1). Their wild-type p53 status was confirmed by single-strand conformation polymorphism analysis of exons 5–9, the DNA-binding region (Table 1). Each of these cell lines was irradiated, and p53 and p21 were levels examined by Western blot analysis. As can be seen in Fig. 2, p53 and p21 protein levels were significantly increased in RKO and MCF-7 cells 5.5 h after irradiation, the time of maximal induction. Although SCC61 cells showed little or no enhancement in p53 protein levels after irradiation, there was a significant enhancement in p21 expression postirradiation. To examine the effect of irradiation on the progression of cells from G₁ into S phase (G₁ phase checkpoint), synchronized cell populations obtained by mitotic selection were incubated with [³H]thymidine and irradiated 1, 2, or 4 h later when cytokinesis was complete, and their movement into S phase monitored by measuring the cumulative labeling index by autoradiography. As can be seen in Table 2, where the maximum cumulative labeling indices are shown, 5 and 8% of RKO and MCF7 cells, respectively, did not enter the first S phase after irradiation with 4 Gy 1 h after mitosis. Although there was a small difference in the fraction of MCF-7 cells arrested in G₁ after irradiation at 4 h as compared with 1 h after mitosis, the maximum cumulative labeling indices appeared otherwise to be independent of the time of irradiation up to 4 h after mitosis.

The effect of increasing radiation doses on the entry of cells into S was examined in RKO cells. As can be seen in Table 3, there was no significant increase in the fraction of cells arrested in the first G₁ phase with increasing doses of radiation; the slight increase seen following 8 Gy is not statistically significant. When synchronized RKO cells and with and without irradiation reached the second mitosis, the mitotic cells were harvested by a second mitotic selection, and the movement of cells through the second postirradiation cell cycle was monitored. Of the cells initially irradiated with 6 Gy, 28% never entered the S phase during the second cell cycle after irradiation (Table 3). The kinetics of progression of cells from G₁ into S during the first cycle were monitored by measuring the cumulative labeling index at multiple time points up to 80 h following mitotic selection and irradiation 1 h later with 6 Gy. Results for the RKO and SCC61 cell lines are shown in Fig. 3. As can be seen, there was a transient delay of approximately 2 h in the movement of cells into the S phase in both cell lines. In addition, as shown in Table 2, a small fraction of RKO cells never entered S phase, as evidenced by the maximum continuous labeling index. The progression of cells through the entire life cycle was examined up to 27 h postirradiation by flow cytometric techniques. As can be seen in Fig. 4, synchronous RKO and SCC61 cells exhibited a single peak of G₁ DNA content at 2 or 3 h after mitosis (Fig. 4, A, E, I, and M). These G₁ cell populations progressed into the S phase over the next 6–9 h (Fig. 4, B, F, J, and N), the irradiated cells showing evidence of a slight transient delay in progression from G₁ into S phase, consistent with the results in Fig. 3. Nonirradiated cells had begun entering the second cell cycle G₁ phase by 18 h after mitosis (Fig. 4, C and K, and Fig. 5A) and subsequently progressed into the second-cycle S phase (Fig. 4, D and L, and Fig. 5B). Cells irradiated with 6 Gy not only showed a slight transient delay in their movement from G₁ into S phase but also displayed a characteristic G₂-phase arrest 18 h after mitosis (Fig. 4, G and O, and Fig. 5C). These G₂-arrested cells moved slowly into the second cell cycle G₁ phase by 24–27 h after original mitotic selection (Fig. 4, H and P, and Fig. 5D). Two-parameter analysis with continuous labeling with BrdUrd (Fig. 5) indicated that a small fraction of cells did not incorporate BrdUrd in the irradiated cell populations, as compared with controls; however, the percentage of noncycling cells was less than 10% in each cell line.

Table 3 Effects of different radiation doses on maximum cumulative labeling index after release of RKO cells from mitotic synchronization

<table>
<thead>
<tr>
<th>Dose (cGy)</th>
<th>First cell cycle</th>
<th>Second cell cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95.0 ± 4.6 (100)</td>
<td>98.3 ± 1.0 (100)</td>
</tr>
<tr>
<td>4</td>
<td>95.1 ± 0.9 (96 ± 0.7)</td>
<td>70.2 ± 1.2 (72 ± 1.2)</td>
</tr>
<tr>
<td>6</td>
<td>94.0 ± 1.9 (95 ± 1.7)</td>
<td>88.5 ± 5.3 (89 ± 5.4)</td>
</tr>
</tbody>
</table>

* Values given are mean percentage ± SE of results from three or more independent experiments. Figures in parentheses are values normalized to 100% for 0 dose controls.

Table 2 Maximum cumulative labeling index in control cells and after irradiation (4 Gy) at various times after release from mitotic synchronization

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RKO</td>
<td>92.4 ± 2.4 (100)</td>
<td>87.3 ± 3.6 (95 ± 2.0)</td>
<td>87.5 ± 3.6 (95 ± 2.0)</td>
<td></td>
</tr>
<tr>
<td>SCC-61</td>
<td>95.8 ± 0.8 (100)</td>
<td>92.4 ± 0.2 (98 ± 1.0)</td>
<td>95.4 ± 1.2 (100 ± 1.0)</td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>95.9 ± 1.16 (100)</td>
<td>87.5 ± 1.7 (92 ± 1.5)</td>
<td>90.1 ± 1.55 (94 ± 1.2)</td>
<td>93.1 ± 0.86 (97 ± 1.3)</td>
</tr>
</tbody>
</table>

* Values given are mean percentage ± SE of results from three or more independent experiments. Figures in parentheses are values normalized to 100% for controls for each individual experiment ± SE.
of the tumor cell lines, in agreement with the autoradiographic results in Tables 2 and 3 and Fig. 3.

DISCUSSION

In early investigations, it was shown that the primary mode of death after irradiation of most proliferating mammalian cells was reproductive failure; that is, loss of the capacity of cells to continue dividing and form macroscopic colonies in vitro (35). Interestingly, irradiated cells undergoing reproductive failure could progress through one or more rounds of cell division before mitosis ceased (35–37). The number of division cycles nonsurviving cells could complete was dose dependent (36, 37). Many cells did not die during mitosis, however, but eventually became arrested in G1 (38), where they could persist as viable cells for periods of many days (36).

In these early experiments, a reduction in the rate at which cells progressed from G1 into S phase was reduced in some cell lines (2), whereas in others, no effect on entry into S phase was demonstrated (39, 40). However, all of these studies were carried out with either a human tumor cell line (HeLa) or various transformed rodent cell lines. When the progression of irradiated cells from G1 into S phase was examined in human diploid fibroblasts, a prominent G1 arrest was observed; following doses as low as 1–4 Gy, up to 60% of the population became irreversibly arrested in the first postirradiation G1 phase (6–8). Recently, this irreversible G1 arrest has been shown to be p53 dependent (11–13). Moreover, Linke et al. (15) showed that a significant fraction of the irradiated cells that escaped arrest in the first cell cycle become irreversibly arrested in the second G1 phase; this effect continued for at least three rounds of cell division. The progressive loss of damaged cells from the population over multiple generations accounts for the enhanced radiosensitivity of human diploid fibroblasts expressing wild-type p53. The loss of cells in subsequent cycles occurred but to a much lesser extent in p53- cells (15).

The response to irradiation of cell lines derived from human solid tumors, however, appears to differ significantly. First, unlike the case with normal cells, clear evidence is lacking for a systematic effect of p53 expression on radiosensitivity. Although McIlwraith et al. (41) reported that six tumor lines showing normal p53 function were significantly more radiosensitive than were cell lines with p53 mutations, the tumor types in the two groups differed completely. When Brachman et al. (42) examined the radiosensitivity of 24 tumor cell lines derived from squamous cell carcinomas of the head and neck, they found no difference in the range of radiosensitivities for the nine lines expressing wild-type p53, compared with those with mutant p53. Furthermore, abrogation of p53 function in several human tumor cell lines expressing wild-type p53 by transfection with the human papilloma virus E6 gene led to no significant change in their radiosensitivity (19, 43, 44).

The function of the G1 checkpoint in irradiated cell lines derived from human solid tumors, as opposed to lymphomas or cells of hematopoietic origins, has also been controversial. Kuerbitz et al. (22) initially reported that irradiation of several tumor cell lines during exponential growth induced a prolonged, transient first-cycle G1 arrest lasting 24 h or longer. On the other hand, we have been unable to find evidence for such an arrest either in exponentially growing tumor cells

![Fig. 3. Cumulative labeling indices after release of RKO and SCC61 cells, synchronized by mitotic selection and irradiated 1 h later. □, controls; ●, 6-Gy γ rays.](image)

![Fig. 4. Flow cytometric profiles of PI-stained single cell populations. RKO (left) and SCC61 (right) cells were synchronized by mitotic selection, released, and irradiated 1 h later, then examined at the times shown (A–P).](image)
studied under similar conditions (25) or following irradiation of density-inhibited cells, subsequently released by subculture to low density and monitored for progression to S phase by autoradiography (27). In cells studied during exponential growth, it may be argued that most cells in the population will be beyond the G1 checkpoint, thus masking the effect in the small population in early G1. Furthermore, it is conceivable that the colcemid and [3H]thymidine added to the cultures to arrest them in the first mitosis (and thus prevent contamination of the flow cytometric profiles by second-cycle G1 cells) could influence the results. In the second type of experiment, the incubation of the cells in low serum, as is required for tumor cells to attain density inhibition of growth, could result in cells being distributed throughout G1 rather than in early G1, thus bypassing a critical G1 checkpoint. Thus, we carried out the present experiments in which the entire cell population was synchronized in very early G1, by mitotic selection. Clearly, two of the cell lines examined showed normal p53 function (Fig. 2). The lack of apparent induction of p53 by radiation in the SCC61 line may indicate a p53-independent induction of p21 (45). MDM-2 expression was also low in this cell line.

As is evident in Table 1, the mitotic selection technique we have used provides nearly pure mitotic cell populations and thus permits the study of a highly synchronized cell population in very early G1, without the use of any chemical inhibitors or growth manipulations. This high degree of synchrony is visually represented in Fig. 1. When cells were irradiated immediately after cytokinesis was complete, the cells moved synchronously into S phase 7-10 h later. As can be seen in Fig. 3, a transient delay of progression from G1 into S phase of the cells irradiated with 4 Gy completed only one round of cell division, becoming reproductively inactive in the second cycle. It is now generally accepted that tumor cells have undergone a multiplicity of genetic changes that permit them to escape normal growth controls. It is not unexpected, therefore, that the function of the G2 checkpoint did not appear to be reduced or abrogated in these human tumor cells.

ACKNOWLEDGMENTS

We thank K. Moss and R. Harley for technical assistance.

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