Chromosomal Radiosensitivity of Human Tumor Cells during the G2 Cell Cycle Period

Ram Parshad, Raymond Gantt, Katherine K. Sanford,1 and Gary M. Jones

Department of Pathology, Howard University College of Medicine, Washington, DC 20059 [R. P.], and Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205 [R. G., K. K. S., G. M. J.]

ABSTRACT

Thirteen cell lines derived from human tumors of diverse tissue origin and histopathology were compared with 12 lines of normal skin fibroblasts with respect to chromatin damage induced by 25, 50, or 100 R of X-irradiation during the G2 period of the cell cycle. Only cells in metaphase were examined, and these had been irradiated 1.5 hr before fixation. When irradiated under identical conditions, the tumor cells showed significantly more chromatid breaks and gaps than did the normal cells at all doses tested. The data suggest that the increased G2 chromosomal radiosensitivity of the tumor cells is associated with deficient DNA repair during the G2-prophase period of the cell cycle.

INTRODUCTION

Improved banding techniques for the study of human chromosomes have revealed an association between chromosomal aberrations and cancer (1, 27). The chromosomal aberrations are manifestations of a genetic instability associated with the malignant potential of cells. This concept is supported by the observation that cells from individuals genetically predisposed to a high risk of cancer exhibit chromosome instability or hypersensitivity of the genome to environmental mutagenic agents (7). Furthermore, both mouse and human cells that have undergone malignant transformation in culture, when compared with their normal controls, show increased chromatin damage after exposure to visible light or X-irradiation during the G2 period of the cell cycle (17, 19, 21). Addition of the DNA repair inhibitors ara-C or caffeine directly after irradiation of the cells in G2 significantly increases the incidence of chromatin damage in the normal cells of both mouse and human origin but has little or no influence on the malignant cells (17, 19, 21). These observations suggest that the normal cells have ara-C and caffeine-sensitive DNA repair mechanisms operative during G2-prophase that are absent or deficient in their malignant derivatives. A process for repair of DNA damage late in G2 that is sensitive to inhibitors of DNA synthesis or repair, such as caffeine, hydroxyurea, or aphidicolin, and that leads to chromatid aberrations has been suggested from studies with human lymphocytes (10, 14) and Chinesehamster fibroblasts (16, 24).

To determine whether enhanced G2 chromosomal radiosensitivity is a general phenomenon associated with human neoplasia, we compared the cytogenetic responses of cell lines derived from 13 human tumors with 12 cell lines of normal tissue origin. All tumor cell lines, irrespective of the tissue of origin or histopathology of the tumor, when compared with the normal cells, showed a significantly higher incidence of chromatid aberrations following X-irradiation during G2.

Although the increased incidence of chromatid aberrations in the tumor cells could result from deficient DNA repair during G2-prophase, such an increase could also result from greater susceptibility to radiation-induced DNA damage or to a differential radiation-induced delay in the progression of cells through the G2 prophase period; this delay would influence the time available for DNA repair before metaphase. Therefore, we have evaluated, in asynchronous cell populations, the relative susceptibility of tumor and normal cells to DNA breakage by X-irradiation and have compared the cells with respect to the X-irradiation-induced G2 block and rate of flow of cells into metaphase.

MATERIALS AND METHODS

Cell Culture, X-irradiation, and Cytogenetic Procedures. Lines of normal skin fibroblasts were obtained from the American Type Culture Collection, Rockville, MD (CRL), The Institute for Medical Research, Camden, NJ (GM), and from Dr. T. Kakunaga (KD) (B) and R. Trimmer (RJH 4) of this laboratory (NCI). Tumor cell lines were obtained from the Naval Biosciences Laboratory, Oakland, CA and had been established with support from NCI under auspices of the Office of Naval Research and Regents of the University of California. Three additional lines were provided by Dr. S. Aaronson of this laboratory (NCI).

Stock lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Flow Laboratories, Inc., Rockville, MD) in T-25 plastic flasks. Cultures were split, usually 1:2, at weekly intervals by dispersion with trypsin. Medium was renewed 3 times weekly, and cultures were gassed with a humidified mixture of 10% CO2 in air. Stock cultures and medium in our laboratory were never exposed to light of wavelength <500 nm, since all manipulations were carried out under gold or red light. Cell lines examined for Mycoplasma by both direct and indirect tests (Flow Laboratories) were negative except for line HT29.

Prior to irradiation, the cells were grown for at least 1 week in Pyrex T-60 flasks. Leighton tubes, each containing a 9- x 50-mm coverslip (No. 1 thickness; Bellico Glass Co., Vineland, NJ) were inoculated with 4 to 10 x 106 cells in 2 ml of culture medium. After 48 hr of incubation, cultures were irradiated by means of 2 Philips RT250 opposing therapeutic 250-kV potential X-ray tubes operated at 235 kV, 15 mA, with 0.25-mm copper and 0.55-mm aluminum filters (half-value layer, 0.9 mm of copper) and at a dose rate of 126 R/min at a 54-cm target distance. In one experiment, 5 x 10-6 M ara-C (Sigma Chemical Co.) was added to the cultures 1.5 hr before X-irradiation. After irradiation, culture fluid was renewed with or without ara-C within approximately 10 to 30 min, and 0.1 µg of Colcemid/ml (Grand Island Biological Co., Grand Island, NY) was added for 1 hr.

For chromosome analysis, the experimental and control cells were processed in situ on cover slips by techniques described (6). Chromosome analyses were made on randomized, coded preparations; 4 cultures were used for each variable, and 100 to 200 metaphase cells were examined per variable, with the following exceptions: KD, 97 for 50 R; GM 3878, 92, 64, and 90 for 0, 50 and 100 R, respectively; A382, 96

Received March 5, 1984; accepted August 24, 1984.
for 0 and 100 R; ranges were as follows: A388, 75 to 87; A186, 36 to 55; A427, 49 to 65; A101D, 17 to 42; HTB 79, 25 to 31. Abnormalities scored as breaks showed distinct dislocation and misalignment of the chromatid fragment, whereas gaps were achromatic lesions, showing no dislocation in spite of apparent chromatid discontinuity. Statistical analyses were based on both the number of chromatid breaks or gaps per cell and the percentage of cells showing these abnormalities. p values were obtained using t tests based on the proportion of cells with one or more gaps (breaks) after arsine transformation (25). Agents to induce synchrony were not used, since they are usually nonphysiological and/or perturb the cell cycle. Although the cell populations were asynchronous, only metaphase cells were scored for chromatid damage. Metaphase cells fixed at 1.5 hr after irradiation would presumably be in G₂ at the time of X-irradiation. The interval of approximately 30 min after irradiation before Colcemid addition would allow cells in metaphase or prophase at the time of irradiation to complete mitosis and be in interphase by the time of fixation of cells.

**Technique for Determination of Irradiation-induced DNA Strand Breaks.** Cellular DNA size indicative of strand breakage was determined by the alkaline elution method of Kohn (11) with minor variations (5). In this technique, the elution rate is dependent on the DNA strand length; the larger the molecular weight, the slower the elution rate. 1.5 × 10⁶ each of the normal and tumor cells, prelabeled with [2-¹⁴C]thymidine (0.4 μCi/ml) and [methyl-³²P]thymidine (1.0 μCi/ml), respectively, were inoculated into T-25 flasks in 5 ml of culture medium and cocultivated. After 24 hr, the cells were mechanically removed and pooled in 10 ml of culture medium at room temperature, X-irradiated (500 R) in a polyethylene tube, and immersed immediately in ice water. After 15 to 20 min, the cells were collected on a polycrylonitrile membrane filter (pore size, 2.0 μm, Bio-Rad, Richmond, CA) and lysed with 5 ml of 20 mM sodium EDTA (pH 10.2) containing 0.3% Sarkosyl (w/v; Ciba-Geigy, Greensboro, NC) and 2 u NaCl, followed by a burst of air forced through the membrane with a syringe to fragment the DNA (5). The DNA was then eluted at a flow rate of 0.33 ml/min with 20 mM EDTA-tetrapropylammonium hydroxide (free acid form of EDTA titrated to pH 12.2 with tetrapropylammonium hydroxide). Fractions were collected every 15 min for 3.5 to 4 hr. Glacial acetic acid (0.1 ml) was added to each fraction, and the ¹⁴C and ³²P were counted as a gel in 10 ml of Ready-Solv MP (Beckman Instruments, Palo Alto, CA) at 61 and 34% efficiency, respectively, in a Beckman LS250 scintillation counter. The radioactivity remaining on the filter was solubilized with dilute HCl (1 N, 70°C for 30 min) and counted after the elution.

**Chart 1.** Incidence of chromatid breaks and gaps following X-irradiation during G₂ phase in normal human skin fibroblasts (left) and tumor cells (right). No chromatid interchanges were observed in the normal cell lines; only 4 of the 13 tumor cell lines had 1 or 2 interchanges per 100 to 200 cells, and these seemed to be unrelated to X-irradiation.
RESULTS

X-Ray-induced Chromatid Damage in Normal and Tumor Cells. X-irradiation of mammalian cells in G2 phase just prior to mitosis induces chromatid breaks and gaps, apparent at the first posttreatment metaphase. The incidence of such aberrations following exposure of cells to low-level X-irradiation (25, 50, and 100 R) is summarized in Chart 1. In spite of the different tissues of origin and histopathologies, cells from all 13 tumors showed a significantly higher incidence of radiation-induced chromatid breaks and gaps than did the 12 lines of normal cells derived from individuals ranging in age from 1 to 66 years. At each dose, $p < 10^{-6}$ for breaks or gaps in the tumor cell lines as a group, and, for each tumor line individually, $P < 0.002$ for breaks and $<0.05$ for gaps, with the exception of gaps in lines A1095 and A388. For breaks and gaps combined, $p < 10^{-4}$ at each dose for individual tumor lines. The increased incidence of radiation-induced damage in tumor cells apparently does not result from a longer period of growth in culture, since line HTB79, examined at the 16th passage, an in vitro passage level comparable to that of the normal cells, also showed the significantly higher incidence of radiation-induced chromatid damage (Chart 1).

Three types of cytogenetic response to X-irradiation were observed among the tumor cell lines. In 6 (A1095, A388, A632, A186, A549, and HT1080) the incidence of radiation-induced chromatid gaps was lower than that of breaks; 15 to 40% of the damage consisted of gaps. In 5 cell lines (HT29, A253, A172, A382, and A101D) the incidence of radiation-induced chromatid gaps was markedly higher than that of breaks; 70 to 80% of the damage consisted of gaps. In cell lines HTB 79 and A427, induced gaps and breaks occurred with equal frequency (50%).

In cells from normal donors and from a few tumors, the low level of chromatid damage showed no consistent or clear-cut dose response, particularly with respect to radiation-induced chromatid gaps, as is also evident in previous reports (18, 26).

To test whether chromatid gaps result from DNA damage and whether their low level in normal cells results from efficient repair of this damage, the DNA repair inhibitor ara-C was added to cultures of a normal cell line, RJH-4, prior to and following X-irradiation. As seen in Chart 2, addition of the DNA repair inhibitor ara-C had little effect on incidence of radiation-induced chromatid breaks but greatly increased the incidence of radiation-induced chromatid gaps. Under these conditions, the cells showed a clear-cut dose response to X-irradiation.

To evaluate the influence of time after irradiation on the yield of chromatid aberrations, cells of a normal (RJH 4) and tumor line (A172) were exposed to 100 R and fixed for chromosome analysis at 0.5, 1, 2, 3, and 4 hr after irradiation. No metaphase cells were observed at 2 to 4 hr, indicating a distinct radiation-induced G2 mitotic block in both normal and tumor cells between 1 and 2 hr postirradiation. Metaphase cells of the tumor line, accumulated by Colcemid treatment for 0.5 hr after irradiation, showed a higher incidence of both chromatid breaks and gaps than that seen in the normal cells. A lower incidence of both chromatid breaks and gaps was observed in normal cells accumulated by Colcemid during the subsequent 0.5 hr. In contrast,
an increased incidence of both chromatid breaks and gaps was seen in the tumor cells during the corresponding period (Chart 3).

X-Ray-induced DNA Strand Breaks in Normal and Tumor Cells. The susceptibility of normal cells (RJH 4) as compared with tumor cells (A172) was evaluated by determination of the relative molecular weights of DNA in the 2 cell populations immediately after irradiation. The elution rates of the DNA were essentially the same for both cell lines (Chart 4). These results indicate that X-irradiation caused an equivalent number of DNA strand breaks and that the 2 populations were similar in their susceptibility to irradiation as measured by DNA single-strand breaks.

Influence of X-Irradiation on Progression of G2 Cells into Metaphase. As noted earlier, radiation might differentially delay the progression of cells into metaphase and thus alter the time available for repair. In both normal (RJH 4) and tumor cells (A172), X-irradiation produced, within 0.5 to 1 hr, a similar decline in the number of cells entering metaphase, and both cell lines showed an equivalent G2 block by 1.5 hr postirradiation (Chart 5). It may be noted that only a small fraction of cells enter metaphase at 1.5 hr postirradiation. The data in Chart 1, however, represent the chromatid damage in metaphase cells accumulated by Colcemid during the preceding 1.0 hr, i.e., from 0.5 to 1.5 hr after irradiation.

DISCUSSION

A major finding of this study is that cells derived from 13 tumors of diverse tissue origin and histopathology all have an enhanced G2 chromosomal radiosensitivity as compared with normal cells. A similar enhanced G2 chromosomal radiosensitivity has also been reported in mouse and human cells transformed in culture (17, 19, 21) and in skin fibroblasts from individuals genetically predisposed to a high risk of cancer (3, 12, 18, 20, 26). It is thus possible that G2 chromosomal radiosensitivity may be a precondition or an initiating change in the carcinogenic process, though a causal relationship has not yet been established.

The increased incidence of radiation-induced chromatid damage in the tumor cells as compared with the normal cells could result from greater initial DNA damage by irradiation, from a decreased radiation-induced G2 block, allowing less time for DNA repair (15, 28), or from an impaired capacity to repair the DNA damage. Initial DNA damage by irradiation was determined in asynchronous cell populations by measuring the extent of DNA single-strand breakage immediately after X-irradiation. Such single-strand breaks provide a measure of cell susceptibility or the capacity of cells to cope with the free radicals generated within the cell by ionizing radiation. Our results from alkaline elution of DNA immediately after irradiation show that the tumor and normal cells are equally sensitive to DNA single-strand breakage by X-irradiation.

With respect to a possible differential effect of irradiation on traverse of the cells through G2-prophase, both normal (RJH 4) and tumor (A172) cell populations showed a similar G2 mitotic block between 1 and 2 hr postirradiation. Furthermore, the percentage of irradiated cells, relative to nonirradiated, entering
cells per culture in X-irradiated to nonirradiated controls. Cells were arrested at
irradiated control cultures and represents the ratio of mean number of metaphase
metaphase by Colcemid for 0.5 hr. Bars, standard error of the ratio of the means.
Each determination is based on 3 to 4 irradiated and 2 to 3 nonirra-

- It appears, therefore, that the increased incidence of radiation-
-induced chromatid damage in the tumor cells as compared with that in normal cells does not result from a difference in initial susceptibility or from a differential radiation-induced $G_2$ block. The enhanced incidence in the tumor cells could result from deficient repair of the X-ray-induced damage during $G_2$-prophase. Considerable experimental evidence indicates that chromatid aberrations result from unrepaired DNA damage; DNA repair capacity and extent of chromatid damage observed following radiation or treatment with chemical mutagens tend to be inversely related (2, 4, 9, 17, 20, 22, 26). For example, cells from individuals deficient in DNA repair show more chromatid damage following treatment with DNA-damaging agents than do cells from normal individuals (3, 12, 20, 23). Furthermore, in repair-compotent normal cells, known inhibitors of DNA repair, such as ara-C or caffeine, increase the induced chromatid damage (17–19, 21, 22; Chart 2). According to the monomerone theory of chromatid structure, each chromatid contains a single continuous DNA double strand. Therefore, chromatid breaks seen in the first metaphase following $G_2$ irradiation represent unrepaired DNA double-strand breaks. Radiation-induced chromatid gaps apparently result from unrepaired DNA single-strand breaks (2, 13, 18). The DNA breaks could arise directly or indirectly as a result of repair processes (2, 4, 9, 21, 22). The results with the inhibitor of DNA repair, ara-C, indicate that radiation-induced chromatid gaps result from DNA damage that is dose-related and efficiently repaired in normal cells in the absence of ara-C (Chart 2). ara-C

is thought to inhibit polymerase activity in nucleotide excision repair. In accordance with this interpretation, the chromatid gaps seen within 1.5 hr after irradiation result from incomplete excision repair of damage produced by X-irradiation during $G_2$. In view of these findings, the tumor cells appear to be deficient in DNA repair during $G_2$-prophase. This possibility is further supported by data comparing the incidence of chromatid damage in a normal cell line (RJH 4) and a tumor cell line (A172) at 0.5 and 1 hr postirradiation (Chart 3). In the normal cells, the incidence of chromatid damage decreased during the period between 0.5 and 1 hr following irradiation, presumably due to DNA repair. In contrast, both chromatid breaks and gaps in the tumor cells increased during the same postirradiation period, presumably through incomplete nucleotide excision repair with initial incision of DNA without completion of the repair process. This defect would result in the accumulation of DNA single-strand breaks which could subsequently be converted to DNA double-strand breaks by single-strand nuclease (2) and be manifest as chromatid breaks.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Robert E. Tarone, Biometry Branch, NCI, for statistical analysis of data.

REFERENCES

4. Evans, J. H. Molecular mechanisms in the induction of chromosome aberra-

10. Kihlman, B. A., Hansson, K., Paltti, F., Andersson, H. C., and Hartley-Asp, B. Potentiation of induced chromatid-type aberrations by hydroxyurea and caf-

12. Kihlman, B. A., Hansson, K., Paltti, F., Andersson, H. C., and Hartley-Asp, B. Potentiation of induced chromatid-type aberrations by hydroxyurea and caf-


Chromosomal Radiosensitivity of Human Tumor Cells during the G₂ Cell Cycle Period

Ram Parshad, Raymond Gantt, Katherine K. Sanford, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/12_Part_1/5577

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.