Oncogenic Transformation of C3H/10T½ Clone 8 Mouse Embryo Cells by Halogenated Pyrimidine Nucleosides

Peter A. Jones, William F. Benedict, Mary S. Baker, Sukdeb Mondal, Ulf Rapp, and Charles Heidelberger

SUMMARY

Oncogenic transformation has been induced in vitro in the C3H/10T½ clone 8 line of mouse cells by exposure to 5-fluoro-2'-deoxyuridine (FUdR) or 5-fluorouracil. This transformation is both dose and time dependent and can be markedly decreased by simultaneous exposure of the cells to thymidine. The transformation induced by 5-fluorouracil is probably due to its intracellular conversion to FUdR or its monophosphate. Transformation by FUdR was found to be cell cycle dependent with maximum sensitivity to transformation occurring in early S phase. Cell lines that produced sarcomas in antithymocyte-treated syngeneic mice were isolated from FUdR-transformed cultures. Trifluoro-2'-deoxyuridine, 5-bromo-2'-deoxyuridine, and 5-iodo-2'-deoxyuridine induced no transformed foci in the C3H/10T½ clone 8 cell line. Thus, not all mutagens produce oncogenic transformation nor does the lack of mutagenicity, as classically measured, completely exclude the possibility that a given agent is oncogenic. Also, there was no evidence of the "switch on" of oncornaviral information in the FUdR-transformed cell lines.

INTRODUCTION

It has been reported by one of us (20) that hamster fetal cells are transformed by treatment with FUdR, an antineoplastic agent that was developed in one of our laboratories (12, 13). That transformation, however, was difficult to quantitate in mass cultures. Hence, it seemed desirable to engage in a joint collaboration for a quantitative study of the abilities of FUdR, FU, and related nucleosides to produce malignant transformation of the C3H/10T½ CL8 cell line, which was also developed in one of our laboratories (28, 29). This cell line was chosen because, like our earlier mouse prostate cells (8), it is highly sensitive to postconfluent inhibition of division, has a very low incidence of spontaneous transformation (29), and can be transformed by numerous types of compounds including polycyclic hydrocarbons (28), X-rays (31), cigarette smoke condensates (4), and some cancer-chemotherapeutic drugs (3, 19).

In this report we show that the C3H/10T½ cells can be transformed by both FUdR and FU and that the transformation is dependent on the dose and time of exposure. Furthermore, since FUdR is a cell cycle-specific agent (13), it might be anticipated that the transformation event would also be cell cycle dependent. Indeed, we report that the maximum sensitivity to FUdR transformation is in early S phase, unlike the late G2 sensitivity for MNNG transformation (6).

The transformation induced by FUdR is especially interesting because the drug is not incorporated into DNA (7, 12, 13) and is not mutagenic to mammalian cells (18). We wished, therefore, to determine whether pyrimidine nucleoside analogs that are incorporated into DNA (2, 9, 10) and are mutagenic (9, 18) also produce transformation of these cells. These compounds included BUdR, IUdR, and F3TdR, a clinically effective antiviral drug (32) developed in one of our laboratories (12–14). These latter compounds were found to be inactive as transforming agents in the C3H/10T½ cells.

MATERIALS AND METHODS

Chemicals. FUdR and BUdR were obtained from Sigma Chemical Co., St. Louis, Mo.; IUdR was from Calbiochem, Los Angeles, Calif.; FU was from Hoffmann-La Roche, Inc., Nutley, N. J.; and F3TdR was from Burroughs Wellcome and Co., Research Triangle Park, N. C. The drugs were dissolved in calcium- and magnesium-free PBS at 100 times the final concentration tested, sterilized by filtration, and then added to the medium. [methyl-3H]Thymidine (~20 mCi/mmol), [6-3H]FU, and [6-3H]FUdR were purchased from New England Nuclear, Boston, Mass.

Cells and Culture Conditions. The cell line used was the C3H/10T½ CL8 line between the 12th and 18th passage. The cells were handled as originally described (29) and were grown in Eagle's basal medium containing 10% heat-inactivated fetal calf serum and penicillin and streptomycin. Sera and medium were obtained from Grand Island Biological Co., Grand Island, N. Y. Stock cultures were propagated in 75-cm² plastic flasks (Falcon Plastics, Oxnard, Calif.), and transformation assays were carried out in 60-mm plastic
dishes obtained from Falcon or Lux Scientific Corp. (Thousand Oaks, Calif.). Cultures were grown in humidified incubators at 37°C in an atmosphere of 5% CO₂ in air.

**Standard Transformation Assay.** The experimental conditions for this assay have been previously described (28). Briefly, 1000 cells obtained from stock cultures in log phase were seeded into 60-mm dishes. Twenty-four hr after seeding, 12 dishes were treated at each dose for an additional 24 hr. The medium was then removed, the dishes were washed once with 5 ml of PBS, and fresh medium was added. Medium was then changed twice weekly for 4 to 6 weeks, after which the cultures were fixed with methanol and stained with Giemsa. The stained dishes were then examined for dense colonies of crisscrossed cells (type III foci) (28). Cytotoxicity was determined by the reduction in plating efficiency in similarly treated dishes seeded with 200 cells. Three dishes/dose were used and the colonies were stained and counted after 9 to 11 days. Cytotoxicity was calculated by comparing the plating efficiency of the treated cultures with that of untreated controls. The transformation frequency is defined as the percentage of surviving cells giving rise to type III foci.

**Cell Cycle Studies.** Cells were synchronized either by release from postconfluence inhibition of division or by release from isoleucine deprivation as previously described (6). Using the former method, several 75-sq cm flasks were grown to confluence and allowed to stand for 48 hr without medium change. The monolayers were then trypsinized and 10⁴ cells seeded per 60-mm dish. At various times after plating, the cells were treated with 10⁻⁴ M FUdR (12 dishes/group) for 2 hr, after which the medium was removed, the cells were washed with 5 ml PBS, and fresh medium was added. In order to treat the cultures throughout a 36-hr period, replicate 75-cm flasks were trypsinized 12 hr apart, and studies were performed simultaneously on the 2 groups. Dose and time responses to FUdR were also determined in cells synchronized by this method. Cells synchronized as outlined above received various doses of FUdR for either 2- or 4-hr exposure, 24 hr after the cells had been seeded from confluent monolayers.

Cells synchronized by release from isoleucine deprivation were derived from confluent monolayers and plated in medium containing 10% freshly dialyzed serum and no isoleucine (6). Forty-eight hr after seeding, the medium was changed to whole medium, and the cultures (12 dishes/group) were treated for 2 hr with 10⁻⁴ M FUdR at various times throughout the cell cycle. The medium was changed after the drug exposure, the cultures were washed with 5-mI PBS, and fresh medium was added. Medium was subsequently changed twice weekly for 4 to 6 weeks to score for transformation.

These 2 methods used to synchronize the C3H/10T¹/₂ cells have previously been shown to yield a high degree of synchrony (6).

**Measurement of DNA Synthesis and Cytotoxicity.** DNA synthesis was determined as previously described (6). Cytotoxicity was estimated by an indirect method in which the cells were trypsinized immediately after exposure to the chemical and then plated as follows.

The medium was removed from dishes that had just undergone treatment, and the cells were washed rapidly with trypsin. The culture was then trypsinized with 1 ml of enzyme and, after the cells had detached, 1 ml of medium was added. Aliquots (50 μl) of the 2-ml cell suspension were then pipetted into fresh 60-mm dishes containing 5 ml of medium (3/treatment) and the cells allowed to grow as colonies for 9 to 11 days. One ml of the remaining cell suspension was counted on a Coulter counter to estimate the number of cells seeded. From a knowledge of the plating efficiency it was possible to estimate cytotoxicity.

**Tumorigenicity Studies.** Several type III foci that arose in FUdR-treated cultures were isolated by the ring isolation technique and subsequently recloned. These cells were then injected s.c. into litters of 4- to 6-day-old C3H mice obtained from the Drug Development Branch, National Cancer Institute, Bethesda, Md. The mice were immunosuppressed with rabbit anti-mouse thymocyte serum obtained from Microbiological Associates, Bethesda, Md., as previously described (4). Each animal received 2.5 × 10⁶ cells and was examined weekly for the appearance of tumors.

**Metabolism of [6-³H]FU and [6-³H]FUdR.** C3H/10T¹/₂ cells were plated at 10⁴ cells/roller bottle. Twenty-four hr later the bottles were treated with the labeled compounds (10⁻⁴ M; 0.001 μCi/ml) for 24 hr, and the cells were harvested by scraping to yield 2.08 × 10⁴ cells that had been treated with FU and FUdR, respectively. The cells were lysed with distilled water and counted. The extract was then passed through a DEAE-cellulose column (1 × 5 cm). FU and FUdR passed through with the effluent, and the monophosphates were eluted with 2 M pyridine acetic acid. FU (R₄ 0.2) was separated by thin-layer chromatography on silica with chloroform:methanol (1:1).

**Determination on Oncornaviral Antigens and Infectious Virus.** The attempt to detect the gs-1 structural protein antigen was carried out by the indirect immunofluorescence test (25). The search for the production of low levels of infectious MuLV virions was done by cocultivation of 3 FUdR-transformed C3H/10T¹/₂ clones with the ecotropic virus indicator cell line III 6A (11), or a mink xenotropic virus indicator cell line (15) for 9 passages using similar techniques as described in these papers (11, 15). Culture fluids from these cells were also concentrated 500-fold and tested for reverse transcriptase activity (1).

**RESULTS**

**Transformation of Asynchronous Cells.** The transformation of asynchronous C3H/10T¹/₂ cells, which were treated for 24 hr with varying doses of FUdR 24 hr after seeding, is shown in Table 1. In these experiments we have only scored type III foci as transformed, although type II foci were also seen. Type III foci are characterized by spindle cells which grow with a piled crisscrossed morphology and, in general, have a higher tumorigenicity than type II foci (28). Transformation was induced by FUdR over a wide concentration range (10⁻⁷ to 10⁻⁴ M) and was dose dependent. The high transformation frequency observed with 10⁻⁴ M FUdR was of the same order of magnitude as that induced by polycyclic aromatic hydrocarbons in the C3H/10T¹/₂ cells (28).
Table 1 also shows that both the cytotoxicity and transformation produced by $10^{-4}$ and $10^{-3}$ M FUdR were markedly decreased by simultaneous treatment with a 10-fold excess of thymidine. Thymidine itself was somewhat cytotoxic to the cells but did not induce transformation.

The transformation of asynchronous cells by varying doses of FU is shown in Table 2. Transformation was not observed at $10^{-7}$ or $10^{-6}$ M FU, although at these concentrations FUdR induced transformation; the transformation frequencies produced by both $10^{-6}$ and $10^{-4}$ M FU were significantly less than those seen with the same levels of FUdR (Table 1). As with FUdR, simultaneous treatment of the cells with a 10-fold excess of thymidine abolished transformation by FU, although the decrease in cytotoxicity was less dramatic than that seen with FUdR.

It was of interest to determine whether there was appreciable interconversion of FU and FUdR in these C3H/10T1/2 cells. Accordingly, logarithmically growing cells were treated with $10^{-6}$ M [6-3H]FU or with [6-3H]FUdR (0.001 µCi/ml). One day later the cells were harvested and processed (see "Materials and Methods"). As shown in Table 3, FUdR was converted to FU to a small extent and was extensively converted into the biologically active monophosphates. FU was also converted to FUdR and to monophosphates. This experiment suggests that the transformation produced by FU, which required higher concentrations than that produced by FUdR, probably resulted from conversion of FU into FUdR. Similar conversions have been repeatedly demonstrated in other cells and in vivo (12, 13).

Although transformation of cultured cells by polycyclic hydrocarbons has been clearly demonstrated to be a direct transformation and not a selection of preexisting transformed cells (23, 28), the slight possibility existed that the transformation produced by FUdR resulted from a selective process. However, as shown in Table 4, FUdR was not significantly more cytotoxic to the nontransformed C3H/10T1/2 cells than to 2 clones transformed by FUdR.

**Cell Cycle Studies.** Chart 1 shows the incorporation of [3H]thymidine by C3H/10T1/2 cells at various times after $10^4$ cells/60-mm dish were plated from cultures in a state of postconfluence inhibition of growth. No thymidine was incorporated during the first 16 hr after plating. Subsequently,

### Table 1
**Transformation of C3H/10T1/2 CL8 cells by FUdR**

<table>
<thead>
<tr>
<th>Nucleosides added (M)</th>
<th>No. of experiments</th>
<th>Av. plating efficiency (%)</th>
<th>Total type III foci/total dishes</th>
<th>Transformation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (none)</td>
<td>5</td>
<td>24</td>
<td>0/51</td>
<td>0</td>
</tr>
<tr>
<td>FUdR ($10^{-4}$)</td>
<td>3</td>
<td>1.25</td>
<td>13/30</td>
<td>3.5</td>
</tr>
<tr>
<td>FUdR ($10^{-3}$) + TdR ($10^{-3}$)</td>
<td>1</td>
<td>10.7</td>
<td>1/7</td>
<td>0.13</td>
</tr>
<tr>
<td>FUdR ($10^{-3}$)</td>
<td>5</td>
<td>7.6</td>
<td>18/43</td>
<td>0.55</td>
</tr>
<tr>
<td>FUdR ($10^{-4}$) + TdR ($10^{-4}$)</td>
<td>1</td>
<td>16.5</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>FUdR ($10^{-4}$)</td>
<td>2</td>
<td>15.1</td>
<td>5/19</td>
<td>0.17</td>
</tr>
<tr>
<td>FUdR ($10^{-3}$)</td>
<td>1</td>
<td>13.5</td>
<td>3/11</td>
<td>0.20</td>
</tr>
<tr>
<td>TdR ($10^{-3}$)</td>
<td>1</td>
<td>10</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>TdR ($10^{-4}$)</td>
<td>1</td>
<td>19.5</td>
<td>0/9</td>
<td>0</td>
</tr>
</tbody>
</table>

* TdR, thymidine.

### Table 2
**Transformation of C3H/10T1/2 CL8 cells by FU**

<table>
<thead>
<tr>
<th>Nucleosides added (M)</th>
<th>No. of experiments</th>
<th>Av. plating efficiency (%)</th>
<th>Total type III foci/total dishes</th>
<th>Transformation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU ($10^{-4}$)</td>
<td>3</td>
<td>1.25</td>
<td>5/22</td>
<td>1.82</td>
</tr>
<tr>
<td>FU ($10^{-3}$) + TdR ($10^{-3}$)</td>
<td>1</td>
<td>3</td>
<td>0/9</td>
<td>0</td>
</tr>
<tr>
<td>FU ($10^{-3}$)</td>
<td>4</td>
<td>15</td>
<td>3/28</td>
<td>0.07</td>
</tr>
<tr>
<td>FU ($10^{-4}$) + TdR ($10^{-4}$)</td>
<td>1</td>
<td>19</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>FU ($10^{-4}$)</td>
<td>1</td>
<td>17.5</td>
<td>0/12</td>
<td>0</td>
</tr>
<tr>
<td>FU ($10^{-3}$)</td>
<td>1</td>
<td>19.5</td>
<td>0/11</td>
<td>0</td>
</tr>
</tbody>
</table>

* TdR, thymidine.

Percentage of transformed type III foci based on the number of cells surviving.
Table 3
Metabolism of [6-3H]FU and [6-3H]FUDR by C3H/10T1/2 cells

Logarithmically growing cells were exposed to [6-3H]FU or [6-3H]FUDR (0.001 μCi/ml) for 24 hr. The cells were then harvested and 
FU, FUDR, and monophosphates were determined in cell lysates as described in the text.

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Amount recovered (pmoles)</th>
<th>FU (pmoles)</th>
<th>FUDR (pmoles)</th>
<th>Monophosphates (pmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUDR</td>
<td>16.4</td>
<td>1.5</td>
<td>8.7</td>
<td>6.1</td>
</tr>
<tr>
<td>FU</td>
<td>13.0</td>
<td>2.4</td>
<td>6.1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Table 4
Cytotoxicity of FUDR for C3H/10T1/2 cells and 2 FUDR-transformed clones

Cells derived from log-phase cultures of C3H/10T1/2 cells or 2 derivatives transformed by FUDR (13H and 20J) were seeded into
60-mm dishes (200/dish) and treated 24 hr later with the indicated
doses of FUDR. Exposure time was 24 hr, after which the cultures
were washed and kept for 11 days before being stained and the
number of surviving colonies counted.

<table>
<thead>
<tr>
<th>Concentration of FUDR (M)</th>
<th>C3H/10T1/2 (PE° = 24%)</th>
<th>13H* (PE = 19%)</th>
<th>20J (PE = 14%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-4</td>
<td>63</td>
<td>79</td>
<td>44</td>
</tr>
<tr>
<td>10^-3</td>
<td>32</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>10^-2</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

a PE, plating efficiency.
b FUDR-transformed clones that were tumorigenic.

as the cells entered S phase, the incorporation of thymidine
increased and reached a maximum 30 hr after seeding. When
cells were exposed to 10^-4 M FUDR for 2 hr at various
times during this period, the cytotoxicity and trans-
formation results shown in Chart 1 were observed. It can be
seen that there was little cytotoxicity, as defined by inhibi-
tion of colony formation, until the cells entered S phase.
The cell-killing effect of FUDR then increased to a maximum
of 40% cell kill in mid S phase and subsequently declined.
No transformation was seen during the first 12 hr after plat-
ing (G, phase) but was evident as the cells entered S phase.
Maximum transformation was found in early S phase and
reached a minimum in mid S phase when cytotoxicity was
greatest. Thus, there is no direct correlation between cell
killing and cell transformation by FUDR. The results shown
in Chart 1 were reproduced in a separate experiment, al-
though the transformation frequency obtained was lower.
Chart 2 shows that a similar result was obtained when
cells were synchronized by isoleucine deprivation. Cytotox-
icity produced by FUDR was maximal at the peak of DNA
synthesis. Transformation, however, was again highest in
very early S phase.
The experiments shown in Chart 3 were conducted to
determine whether the transformation induced in synchro-
nized cells was dependent on the amount of FUDR used and
the exposure time. Cells derived from confluent monolayers
were treated 24 hr after seeding with the indicated concen-
trations of FUDR for 2 or 4 hr. Under these conditions, no
transformation was seen at 10^-6 M FUDR, but it was clearly
dose dependent as the dose was increased to 10^-4 M. In-
creasing the exposure time to 4 hr also increased the trans-
formation rate. It was again evident that there is no direct
correlation between cell killing and cell transformation,
since the transformation rate increased more rapidly than the cytotoxicity. Furthermore, no difference in cytotoxicity was seen between the 2 exposure times, although the 4-hr exposure was more efficient in inducing transformation.

**Search for Expression of Oncornaviruses and Attempts to Transform Cells with IUdR, BUDR, and F3Tdr.** We have previously demonstrated in one of our laboratories (27) that no infectious oncornaviruses or viral cores are “switched on” when C3H/10T1/2 cells undergo malignant transformation by polycyclic hydrocarbons. We have similarly examined 3 type III clones produced by FuDR for MuLV expression. By indirect immunofluorescence analysis, none of 2 × 10^6 cells were positive for the MuLV P30 structural protein (gs-1 antigen). Similarly, the search for the production of low or transient levels of infectious MuLV by cocultivation with ectropic indicator cells (III 6A cells) or with mink xenotropic indicator cells was entirely negative. In addition, no reverse transcriptase activity was detected in 500-fold diluted cultures from 10^6 ectropic and xenotropic cocultivations.

In previous work, however, we did observe a transient switch on of infectious MuLV following treatment by IUdR under conditions where no oncogenic transformation was detected (27). This prompted us to determine more carefully whether the base analogs, IUdR, BUDR, and F3Tdr, which are incorporated into DNA and are mutagenic in various systems (2, 9, 10, 18), produced oncogenic transformation of C3H/10T1/2 cells. As shown in Table 5, no such transformation could be found.

**Tumorigenicity.** The tumorigenicities of 5 cell lines derived from type III foci are shown in Table 6. The parental C3H/10T1/2 line was not tumorigenic, as has been found in several laboratories (4, 28, 31). Three of the FuDR-transformed lines gave rise to tumors in syngeneic mice. Approximately 60% of the type III foci were therefore tumorigenic, a value that is slightly lower than that found for hydrocarbon-induced transformation (28).

**DISCUSSION**

Our results demonstrate that the C3H/10T1/2 line of mouse cells can reproducibly undergo malignant transformation by FuDR and, at higher concentrations, by FU. This effect is dependent on both the concentration and time of exposure. Our results also suggest that FuDR- and FU-induced transformation is related to thymidine metabolism, since simultaneous exposure of treated cultures to thymidine caused a marked decrease in the transformation frequency. There is little indication from the literature that FU and FuDR are carcinogenic in vivo. Poirier et al. (26) found that FuDR, as well as IUdR and BUDR, did not significantly increase the incidence of lung adenomas in strain A mice, or when administered repeatedly to newborn Swiss mice (L. A. Poirier, J. A. Miller, and E. C. Miller, private communication). On the other hand, Lombart and Marcos (21) reported without data that FU is transplacentally carcinogenic in the rat. There is no explanation for the apparent discrepancy between the in vivo and in vitro results.

It appears that the critical cellular events involved in the FuDR in vitro transformation process occur during the early S-phase of the cell cycle. In cultures synchronized either by release from postconfluence inhibition of growth or by isoleucine deprivation, FuDR produced no transformation in the G2 phase and a minimum transformation rate in the middle of the DNA-synthetic phase when cytotoxicity was greatest. FuDR is S-phase specific in its cancer-chemotherapeutic activity (13). This S-phase specificity contrasts with that observed for MNNG, where the sensitive phase for transformation of C3H/10T1/2 cells takes place 4 hr prior to the onset of DNA synthesis (6). It is similar, but not identical, to the cell cycle dependency of transformation induced by cytosine arabinoside (3).
In all in vitro systems designed to detect malignant transformation, the question of selection of preexisting malignant cells versus their induction must be carefully considered. We believe that the evidence that we have obtained strongly suggests that FUdR directly induces a malignant change in the C3H/10T1/2 cells for the following reasons: (a) these cells have a remarkably low rate of spontaneous transformation (29); (b) there was no significant difference between the cytotoxicity produced by FUdR in the nontransformed clone and in 2 FUdR transformed clones, thus excluding the possibility that the transformed cells have a selective advantage; and (c) no correlation was found between cytotoxicity and transformation, i.e., significant transformation was observed under conditions of little cytotoxicity.

In C3H/10T1/2 cells, FU was less active than FUdR in producing transformation. This finding, as well as the data on the metabolism of [6-3H]FU and [6-3H]FUdR, suggests that FU produced transformation as a consequence of its conversion into FUdR or its monophosphate.

The mechanism by which FUdR induces malignant transformation of C3H/10T1/2 cells is of interest. It has been demonstrated that transformation of these cells by polycyclic hydrocarbons and MNNG does not involve the switch on of C-type RNA tumor viruses or their gs antigens, or reverse transcriptase. Thus, the genetic information for virus production that is present in these cells (27) is not overtly expressed as a function of, or because of chemical transformation. Transformation by FUdR would therefore appear to be direct. Moreover, with the exception of bacteriophage PBS2, which contains uracil instead of thymine in its DNA (22), FUdR is not incorporated into DNA (12, 13). Such incorporation into DNA might have provided a mechanism of transforming and mutagenic activity for FUdR. However, BUdR, IUdR, and F3TdR, which are incorporated into DNA and are mutagenic, do not transform C3H/10T1/2 cells. Thus, not all mutagens produce oncogenic transformation, nor does the lack of mutagenicity as measured by classical methods (18) completely eliminate the possibility that a given agent is potentially oncogenic. The lack of transforming activity by IUdR is also of particular interest, since it does switch on endogenous oncornaviruses in these cells (27).

FUdR inhibits DNA synthesis as a result of inhibition of thymidylicate synthetase by its 5'-monophosphate (12, 13). However, it seems unlikely that inhibition of DNA synthesis per se could produce oncogenic transformation. It seems more likely that the transformation might result from the chromosomal aberrations that FUdR is known to produce (17, 30). This would be in line with recent evidence that shows that chromosomal balance may be important in malignant expression (5, 16). Nomura et al. (24) have also found that FUdR induces flat revertants of murine sarcoma virus-transformed 3T3 cells that, in turn, have different chromosome numbers than the starting cell population.

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REFERENCES


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