Asynchronous DNA Synthesis and Asynchronous Mitosis in Multinuclear Ovarian Cancer Cells

Patrick F. Sheehy, Theresa Wakonig-Vaartaja, Rodger Winn, and Bayard D. Clarkson

SUMMARY

Using autoradiographic techniques, the synchrony of DNA synthesis and mitosis in multinuclear cells from two patients with ovarian carcinomatous ascites was studied. The patients had previously been treated with surgery, radioactive phosphorus (32P), radiotherapy, and Leukeran. The incidence of binuclear, trinuclear, quadrinuclear, and multinuclear (five or more nuclei per cell) in both tumors was estimated. Asynchronous DNA synthesis and asynchronous mitosis were frequent in the binuclear and trinuclear pools in the first patient and in all the cell pools in the second patient. Cytogenetic studies demonstrated aneuploidy in the tumor cells removed from both patients.

The results demonstrate that the nuclei in multinuclear cells from treated ovarian cancer patients are capable of individual DNA synthesis and mitosis and are probably not dependent on cytoplasmic controlling factors as previously reported.

INTRODUCTION

The incidence of multinuclear cells varies markedly in different human tumors. Usually the incidence is low in leukemias and lymphomas while it is often high in solid tumors. Multinuclear cells are formed principally in 2 ways: (a) by nuclear division without cytokinesis (8, 9, 15); and (b) by the fusion of 2 or more mononuclear cells (3, 14). Amitotic division could play a part in the formation of multinuclear cells (3, 14) as could endoreplication with endomitosis but conclusive evidence for these events has not yet been presented.

When like cells are fused in culture by the addition of Sendai viruses, synchronous DNA synthesis and synchronous mitosis occur (4, 10). However, when heterokaryons, produced by the fusion of unlike cells, were labeled with TdR-3H, asynchronous DNA synthesis was not always imposed on the nuclei (4, 6). Burns (1) found a high incidence of asynchronous DNA synthesis in the Ehrlich ascites tumor but no asynchrony in mitosis, and Sandberg et al. (12) noted asynchronous DNA synthesis but synchronous entry into metaphase in some binucleate cells in a human cell line derived from the blood of a patient with acute myeloblastic leukemia. Gallardo et al. (3) found a few cells demonstrating asynchronous mitosis in giant cell osteoclastomas in tissue culture. In order to investigate the synchrony of DNA synthesis and mitosis in multinuclear cells in human tumors, we studied 2 patients with ovarian ascitic tumors.

MATERIALS AND METHODS

A small polyethylene catheter was inserted into the peritoneal cavity. The catheter was anchored in place with small sutures. Through this catheter 100 μCi of TdR-3H (methyl-3H; specific activity, 6.0 to 6.5 Ci/mM) (Schwarz/Mann, Orangeburg, N. Y.) were injected. Samples of cells were withdrawn at 1 and 2 hr after the pulse labeling and every 24 hr for the next 4 days in the 1st patient and at 2 hr after pulse labeling in the 2nd patient. The cells were processed for autoradiography as previously described (2). The slides were dipped in NTB-2 Kodak emulsion and exposed for 14 days at -4°. They were developed in D-19 developer and stained with Giemsa.

Labeling Index. Several thousand cells were counted and divided into mononuclear, binuclear, trinuclear, quadrinuclear, and multinuclear cells. Nuclei with 4 or more grains were counted as labeled. Of each particular cell pool, the labeling index was calculated from counting the number of labeled cells per 1000 cells in both the mononucleated and binucleated cells and from 500 cells in the trinuclear, quadrinuclear, and multinuclear cell pool. In the binuclear, trinuclear, quadrinuclear, and multinuclear cells, the number of labeled nuclei in each cell was counted. To obtain sufficient number of cells in each category, multiple slides were counted for each sample. The incidence of the different cell pools and the labeling index of each category did not differ significantly from slide to slide.

Mitotic Index. Over 5000 mononuclear cells were counted to determine the mitotic index of this cell pool. In the other pools, mitotic figures were scored where they were seen and counted.

Chromosomal Preparations. Samples of freshly obtained ascitic cells were incubated at 37° for 2 hr with Colcemid...
clear cells; moreover, mitotic figures in multinucleated cells
were found only rarely in the direct smears and none was
observed with more than 1 nucleus in mitosis.

Selection of Patients. The patients were selected for study
because they had numerous multinuclear tumor cells. Both
patients had advanced disease and had been treated with
radiotherapy and chemotherapy. The patients and their
families were fully informed both verbally and in writing
that the proposed studies were experimental and no therapeu-
tic gain could be expected; signed consent forms were
obtained.

RESULTS

Case Report Subject I. M. L. was a 49-year-old woman
who had a bilateral salpingo-oophorectomy and partial
omentectomy for papillary adenocarcinoma of the ovary on
July 24, 1970 (13). Fifteen mCi 32P were instilled into the
peritoneal cavity at operation, and she received abdominal
radiotherapy of 2975 rads postoperatively. Because of the
development of ascites in November, Leukeran (Burroughs
Wellcome, Research Triangle Park, N. C.), 6 mg/day, was
given for 3 days, but this was then stopped because of nausea
and vomiting. Ten days after Leukeran was discontinued,
800 ml of peritoneal fluid were removed for symptomatic
relief and the study described above was done. She was sub-
sequently treated with i.p. thio-tepa (Lederle Laboratories,
Pearl River, N. Y.) and intraperitoneal 5-fluorouracil (Roche
Laboratories, Nutley, N. J.) after developing bilateral
pleural effusions in February, but benefit was short lived
and she died on April 28, 1971.

Cytology. Cells were identified as being poorly differenti-
cated cystadenocarcinoma by Dr. Myron Melamed (Depart-
ment of Pathology). There were 2000 cells/cu mm, 5% of
which were mesothelial cells or leukocytes and the remain-
der were tumor cells. Of the tumor cells, 93.2% were
mononuclear, 6% were binuclear, 0.4% were trinuclear,
0.1% were quadrinuclear, and 0.1% had 5 or more nuclei.

Chromosomal Study. The main stem line had 44 chromo-
somes and made up 54% of the 50 cells counted; as
mentioned previously most of these appeared to be mononu-
clear cells. Ten% had 39 to 41 chromosomes; 14% had 42 to
43 chromosomes; 4% had 45 chromosomes; 10% had 52 to
55 chromosomes; 8% had 78 to 80 chromosomes. Three to 5
marker chromosomes were present in many of the cells.
Chromosomes were missing from some groups while others
had additions. A variety of abnormalities, and diplo-
chromosomes were present, some of which could be attrib-
uted to treatment.

Tdr-H Studies. The results of the labeling indices are
given in Table 1. Fig. 1A shows asynchronous labeling in a
binuclear cell and Fig. 1B shows asynchronous labeling in a
trinuclear cell. None of the quadrinuclear or multinuclear
cells was labeled. Table 2 shows the labeling indices of the
binuclear cells of later samples. After 84.0 hr, 8% of the
labeled binuclear cells still had but 1 nucleus labeled.

The incidence of binuclear (labeled and unlabeled) cells
remained constant around 6% throughout the study, but the
labeling index of the binuclear cells doubled after 48 hr. The
most likely explanation for the latter finding is that there
was nuclear division of labeled mononuclear cells without
cytokinesis (or fusion of the daughter cells immediately
after telophase); division of labeled binucleates to produce 2
daughter binucleates cannot be excluded, but this seems less
likely since few binucleates were seen in mitosis and none
with both nuclei in division. Similarly, random fusion of
labeled mononuclears seems unlikely in view of their
infrequency; only 14% were labeled and one would not
expect them to come together selectively. The most likely
reason there was no change in the incidence of the differ-
tent pool is because the division rate among binuclear cells
was lower than among mononuclear cells; thus the rate of
increase of the mononuclear cells kept pace with the
formation of new binucleates by division of mononuclear
cells without cytokinesis or cell fusion. An alternative
possibility is that some binuclear or other multinuclear cells
gave rise to mononuclear cells due to their asynchronous
mitosis, but this again seems less likely.

Asynchronous Mitosis. The mitotic index of the mononu-
clear cells was 0.4%. Of the binuclear cells counted, 9 cells
were seen in which 1 nucleus was in mitosis and the other
nucleus was nonlabeled and in interphase (Fig. 1C). Of the
trinuclear cells counted, 5 cells were seen in which 1 nucleus
was in mitosis and the other 2 nuclei were in interphase and
nonlabeled (Fig. 1D); 1 cell was seen in which 1 nucleus was

I Adenocarcinoma of ovary (Subject I): incidence of asynchronous
DNA synthesis in multinuclear cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cells counted</th>
<th>Labeling index (%)</th>
<th>No. of nuclei labeled (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear</td>
<td>1000</td>
<td>14</td>
<td>0.4%</td>
</tr>
<tr>
<td>Binuclear</td>
<td>1000</td>
<td>6</td>
<td>20.0%</td>
</tr>
<tr>
<td>Trinuclear</td>
<td>500</td>
<td>9</td>
<td>11.5%</td>
</tr>
<tr>
<td>Quadrinuclear</td>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multinuclear</td>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2

Incidence of asynchronous DNA synthesis (Subject I)

<table>
<thead>
<tr>
<th>Time after pulse label (hr)</th>
<th>Cells counted</th>
<th>Labeling index of binuclear cells (%)</th>
<th>% labeled binuclear cells which had 1 nucleus labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>1000</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>48</td>
<td>1000</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>72</td>
<td>1000</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>84</td>
<td>1000</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>
in mitosis (prophase) and 1 of the remaining 2 interphase nuclei was labeled (Fig. 1B). Of the quadrinuclear cells, 3 cells were seen with 1 nucleus going through mitosis and the other nuclei were in interphase and nonlabeled. No cells were seen where there were 2 or more metaphase figures in one cell.

Case Report Subject 2. M. D. had bilateral salpingo-oophorectomy and a hysterectomy on January 11, 1970, for mucinous cystadenocarcinoma of the ovary. A dose of 11.45 mCi of $^{32}$P was instilled into the peritoneal cavity at operation, and postoperatively she was given abdominal irradiation (4000 rads). She was asymptomatic until August 1971 when she developed ascites and was given Leukeran, 6 mg/day, for 2 weeks until she accidentally fractured her left clavicle when it was discontinued. She was hospitalized on August 20, 1971, 1 week after stopping the Leukeran, and the study described above was done. At the conclusion of the study, the fluid was drained off and 10 mg of thio-tepa were injected. However, the fluid reaccumulated, and she developed intestinal obstruction and died on December 13, 1971.

Cytology. The tumor cells were identified as adenocarcinoma of the ovary by Dr. Melamed. The cell count was 220 cells/cu mm and the tumor cells made up 16.5% of the total population, 79% were small lymphocytes, and 4.5% were mesothelial cells. Of the tumor cells, 35.6% were mononuclear cells, 36% were binuclear cells, 11.3% were trinuclear cells, 8.9% were quadrinuclear cells, and 8.2% were multinuclear cells.

Chromosomal Study. Twenty-five cells were examined. No stem line was detected. Four percent of the cells had 34 to 43 chromosomes; 24% had 45 to 48 chromosomes; 32% had 50 to 70 chromosomes; 28% had 70 to 90 chromosomes; 12% had 150 to 220 chromosomes. Four cells were karyotyped. One was normal; 2 had 48 chromosomes with missing and extra chromosomes, markers, fragments, and dicentrics; the remaining cell had 45 chromosomes with missing and extra chromosomes as well as 1 marker and dicentric chromosome. The finding, however, of extra chromosomes in the secondary infective agent may play a part in their etiology (7). They may also be formed either from mutants derived from the neoplastic stem cell or because the secondary infective agent may have a delaying effect on the labeled nuclei entering mitosis since the mean length of G2 was estimated to be 5 hr in this patient (13); or (c) that a labeled mononuclear cell had fused with an unlabeled mononuclear cell to form a multinuclear cell. Unfortunately, we cannot distinguish which of these possible mechanisms was most prevalent from the available data.

The works of Moorehead and Hsu (8), Oftebro (9), and Rao and Johnson (10, 11) show that in the multinuclear HeLa cells in vitro DNA synthesis is synchronized and all nuclei go through mitosis together. The nuclear chromatin condenses into chromosomes simultaneously and at mitosis 1 metaphase is formed. Afterward, depending on the number of poles present and whether cytokinesis takes place, either another multinuclear cell is formed or many mononuclear cells are produced or a combination of both. The factors which initiate DNA synthesis and mitosis are apparently located in the cytoplasm of this particular cell line and they regulate and synchronize homologous nuclei. Similar rigid cytoplasm controls do not appear to be operative in the ovarian cancer cells in the present patients.

The most likely interpretation of our findings is that when a nucleus divides without cytokinesis, the daughter nuclei may have Gi (or G0) periods of unequal length, an interpretation not dissimilar to that proposed by Temin (16) to explain the different temporal responses of stationary cells to stimulation with serum.

The factors which lead to the formation of multinuclear cells in human solid tumors are not understood. Viruses as the secondary infective agent may play a part in their etiology (7). They may also be formed either from mutants derived from the neoplastic stem line or because the neoplastic stem cells are growing in a nonpermissive type environment. Since the genes that control nuclear division and cytokinesis are different and are not interdependent, at least in yeast (5), it is possible that multiple factors are responsible. Regardless of the mechanism by which multinucleated cells are formed, the present observations demonstrate clearly that individual nuclei in some multinuclear ovarian cancer cells are capable of asynchronous DNA synthesis and mitosis and are not dependent on cytoplasmic

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cells counted</th>
<th>Labeling index (%)</th>
<th>No. of nuclei labeled (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear</td>
<td>1000</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Binuclear</td>
<td>1000</td>
<td>22</td>
<td>78  22</td>
</tr>
<tr>
<td>Trinuclear</td>
<td>500</td>
<td>22</td>
<td>71  20  9</td>
</tr>
<tr>
<td>Quadrinuclear</td>
<td>500</td>
<td>29</td>
<td>83  13  4  0</td>
</tr>
<tr>
<td>Multinuclear</td>
<td>500</td>
<td>42</td>
<td>58  35  6  1</td>
</tr>
</tbody>
</table>

DISCUSSION

The results of this study show that asynchronous DNA synthesis and asynchronous mitosis occur in ovarian multinuclear cells. Since both these patients had been heavily irradiated and had received chemotherapy, it is not possible to say whether this asynchrony is the result of treatment or is a normal occurrence in this type of tumor cell. The fact that after 84 hr, in the 1st patient, 8% of the labeled binuclear cells still had 1 nucleus labeled, suggests at least 3 possibilities: (a) that the labeled nuclei in the binuclear cells were arrested in S or in G2; (b) that the unlabeled nuclei may have a delaying effect on the labeled nuclei entering mitosis since the mean length of G2 was estimated to be 5 hr in this patient (13); or (c) that a labeled mononuclear cell had fused with an unlabeled mononuclear cell to form a multinuclear cell. Unfortunately, we cannot distinguish which of these possible mechanisms was most prevalent from the available data.

Asynchronous Mitosis. The mitotic index of the mononuclear cells was 0.4%. Of the binuclear cells counted, 11 cells were seen when 1 nucleus was in metaphase and the other was in interphase and nonlabeled (Fig. 2, C and D). Of the trinuclear cells counted, 3 cells had 1 nucleus in metaphase and the other 2 were in interphase and nonlabeled.
factors as has been demonstrated for various other types of
cells (1, 10, 11).

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Fig. 1. Subject 1. A, asynchronous DNA synthesis; B, asynchronous DNA synthesis and asynchronous mitosis; C, asynchronous mitosis; D, asynchronous mitosis in a trinucleate cell.
Fig. 2. Subj. 2. A. asynchronous DNA synthesis. B. asynchronous DNA synthesis in a multinucleated cell. C and D. asynchronous mitosis in binucleate cells.
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