DNA Synthesis in Tumor-bearing Rats\textsuperscript{1}

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SUMMARY

Thymidine (TdR) incorporation into DNA increased in the livers and spleens of rats bearing Yoshida sarcoma (solid type) or AH130 (solid type). TdR kinase and DNA polymerase activities increased in the serum, liver, and spleen of these rats; while thymidine monophosphate kinase activity increased appreciably only in the liver and spleen. On diethylaminoethyl cellulose column chromatography, 2 peaks of TdR kinase activity were separated from the serum and tumor tissues of rats bearing Yoshida sarcoma (solid type) while only 1 peak was obtained from the liver. TdR kinase activity in the serum decreased abruptly 7 hr after removal of the Yoshida sarcoma, while that in the liver decreased more slowly.

INTRODUCTION

A number of in vivo studies on the host-tumor relationship have been reported (1, 2, 7, 8, 11, 16, 20, 24–26). Several investigators (11, 16, 20, 24) showed an increase in the incorporation of labeled precursors into the DNA of normal tissues of tumor-bearing animals.

Parsens and Warren (20) found an increase in the sizes of some organs, particularly the spleen of tumor-bearing animals. Enlargement of the liver in tumor-bearing animals has also been reported (2, 26). Cerecedo et al. (7, 8) observed an increase in the nucleic acid content of the liver and lung in mice with transplanted tumors. Recently, Morgan and Cameron (16) reported increase in DNA content and DNA synthesis in the livers and spleens of tumor-bearing mice, the latter being 2-fold or more the value in control animals.

Rounds (21) and Rubin (22) found that culture media of Rous sarcoma cells or human cancer cells contained growth-stimulating substances. Nair and DeOme (17, 18) reported that a soluble fraction, prepared from solid Rous sarcoma cells or human cancer cells, contained growth-stimulating substances that increase the growth of density-inhibited mouse embryo cells in monolayer culture. Therefore, tumor tissues may release some growth-stimulating substances that increase the growth of various host tissues by enhancing DNA synthesis.

In this work the possibility that tumor-bearing animals contain growth-stimulating substances was investigated.

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MATERIALS AND METHODS

Male Donryu rats, weighing 100 to 150 g, were given laboratory chow and water \textit{ad libitum}. Yoshida sarcoma and AH130 cells were transplanted into rats i.p. and the cells were used 5 to 6 days after transplantation. Solid-type tumors of Yoshida sarcoma and AH130 were prepared by transplanting $5 \times 10^7$ ascites cells into the subepidermal tissue of the back. Rats bearing a Yoshida or AH130 tumor were killed, and their livers were thoroughly perfused with 0.9% NaCl solution before removal. Solid tumors were used 1 or 2 weeks after transplantation. Partial hepatectomy was performed by the procedure of Higgins and Anderson (10), and the remaining liver was removed 48 hr later.

\textbf{Incorporation of TdR\textsuperscript{2} into DNA.} Tissue slices (200 mg) were incubated in a test tube in 1.8 ml of Krebs-Ringer phosphate buffer, pH 7.4, with 0.5 $\mu$Ci of [2-$^{14}$C]TdR at 37\degree C for 60 min. The reaction was stopped by adding cold 5% trichloroacetic acid. The precipitate was homogenized and centrifuged at 600 $\times$ g for 10 min. This procedure was repeated 3 times in all. Acid-insoluble DNA was extracted from the precipitate by the method of Schmidt and Thannhauser (23), and its radioactivity was measured in a liquid scintillation counter.

\textbf{Enzyme Assay.} Rat tissues were homogenized in 20 mm Tris-HCl (pH 8.0) with a Teflon homogenizer. Enzyme solutions were obtained by centrifugation of 10% homogenates for 30 min at 8000 $\times$ g.

TdR kinase activity was measured by determining the conversion of labeled TdR to labeled dTMP by the DEAE-cellulose disc method, as described previously (9). The reaction system contained the following constituents: enzyme solution, 200 $\mu$l; 1 mm [2-$^{14}$C]TdR, (30,000 cpm), 25 $\mu$l; 100 $\mu$M ATP, 25 $\mu$l; 120 $\mu$M $\alpha$-glycerophosphate, 25 $\mu$l; and 0.2 $\mu$M Tris-HCl buffer, pH 8.0, 200 $\mu$l in a total volume of 0.5 ml. The mixture was incubated in a test tube at 37\degree C for 30 min. Assay of TMP kinase was based on the principle described by Lehman \textit{et al.} (12). The reaction mixture (final volume, 0.25 ml) consisted of 10 $\mu$moles of Tris-HCl (pH 7.5), 2 $\mu$moles of ATP, 4 $\mu$moles of MgCl$_2$, 50 $\mu$moles of [8$^{14}$C]TMP, and enzyme solution. After incubation for 30 min at 37\degree C, the reaction was stopped by heating the mixture in boiling water for 2 min. The products of the reaction were separated by the method of Weissman \textit{et al.} (27). Assay of DNA polymerase was based on the principle described by Mantsavinos (15). The reaction mixture contained 0.05 $\mu$mol of $[14$C]TMP.

\textsuperscript{2} The abbreviation used is: TdR, thymidine.
RESULTS

There are a number of published studies dealing with the incorporation of TdR into DNA of regenerating liver (3, 4, 14). In partially hepatectomized rats, TdR incorporation into DNA increased considerably in the liver (Table 1). No increase was detectable in the spleens of these animals. On the other hand, the activities of TdR kinase, TMP kinase, and DNA polymerase, which are involved in DNA synthesis, increased remarkably in both the liver and spleen. High activities of DNA-synthesizing enzymes were also observed in solid-type Yoshida sarcoma and AH130 tumors 7 and 14 days, respectively, after transplantation (Table 1). In these tumors TdR incorporation into DNA and the activities of TdR kinase, TMP kinase, and DNA polymerase all increased. The activities of enzymes synthesizing DNA were also elevated in the livers and spleens of the tumor-bearing rats (Table 1).

Chart 1 shows that the activities of enzymes synthesizing DNA began to increase in the livers of Yoshida sarcoma-bearing rats 4 days after tumor transplantation and increased abruptly on the 6th day. TdR kinase activity also increased in the livers of mice bearing Sarcoma 180, Ehrlich, or L1210 tumors (unpublished results of the authors).

TdR kinase and DNA polymerase activities rose in the sera of Yoshida sarcoma-bearing rats. The increases were detectable 4 days after tumor transplantation and were rapid on the 6th day, as in the liver of tumor-bearing rats (Chart 2). However, unlike in the liver, TMP kinase activity in the serum showed little increase in tumor-bearing rats. Therefore, the activities of TdR incorporation into DNA were not detected in the sera of either normal or tumor-bearing rats.

As reported previously (19), TdR kinase of tumor tissues, such as Yoshida sarcoma, AH130, and Morris hepatoma, was separated into 2 fractions by DEAE-cellulose column chromatography, while only a single fraction was obtained from normal rat tissues, such as marrow cells, spleen, and embryonic and regenerating liver. On the basis of this finding, studies were made to determine whether TdR kinase in the serum of Yoshida sarcoma-bearing rats originated from tumor tissue. Serum of Yoshida sarcoma-bearing rats was subjected to DEAE-cellulose column

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**Table 1**

Activities of DNA-synthesizing enzymes in various tissues

<table>
<thead>
<tr>
<th>Rats</th>
<th>Tissue</th>
<th>[14C]TdR into DNA (cpm/mg DNA)</th>
<th>Activity (nmole/mg protein)</th>
<th>DNA polymerase (pmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[nCi]</td>
<td>TdR kinase</td>
<td>TMP kinase</td>
</tr>
<tr>
<td>Normal</td>
<td>Liver</td>
<td>410 ± 56</td>
<td>0.056 ± 0.005</td>
<td>0.072 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>403 ± 107</td>
<td>0.568 ± 0.124</td>
<td>0.450 ± 0.035</td>
</tr>
<tr>
<td>Partially hepatectomized</td>
<td>Liver</td>
<td>1614 ± 165</td>
<td>2.82 ± 0.32</td>
<td>8.51 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>419 ± 48</td>
<td>1.35 ± 0.45</td>
<td>1.10 ± 0.09</td>
</tr>
<tr>
<td>Bearing Yoshida sarcoma</td>
<td>Tumor</td>
<td>30170 ± 1560</td>
<td>18.9 ± 2.8</td>
<td>15.5 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>9389 ± 640</td>
<td>23.3 ± 2.6</td>
<td>6.64 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>2360 ± 180</td>
<td>17.0 ± 2.6</td>
<td>3.30 ± 0.07</td>
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<tr>
<td>Bearing AH130</td>
<td>Tumor</td>
<td>26680 ± 1420</td>
<td>0.375 ± 0.008</td>
<td>5.10 ± 0.28</td>
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<td></td>
<td>Liver</td>
<td>1670 ± 250</td>
<td>0.171 ± 0.055</td>
<td>1.22 ± 0.09</td>
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<tr>
<td></td>
<td>Spleen</td>
<td>1620 ± 285</td>
<td>0.940 ± 0.140</td>
<td>1.31 ± 0.09</td>
</tr>
</tbody>
</table>
DNA Synthesis in Tumor-bearing Rats

The activities of various enzymes concerned with DNA synthesis increased in the liver and spleen of tumor-bearing rats (Table 1). TdR kinase and DNA polymerase activities rose in the serum of rats bearing Yoshida sarcomas, as well as in the liver. While the TMP kinase activity increased greatly in the liver, it increased but little in the serum. The TdR kinase activity in the serum of Yoshida sarcoma-bearing rats was separated into 2 peaks by DEAE-cellulose column chromatography, but that of the liver gave only 1 peak. The fact that the chromatographic pattern of the serum TdR kinase was similar to that of the Yoshida sarcoma.

DISCUSSION

The activities of various enzymes concerned with DNA synthesis increased in the liver and spleen of tumor-bearing rats. TdR kinase activity was extremely low in the sera of both partially hepatectomized (12,24, and 48 hr after partial hepatectomy) and normal rats but was high in the serum of Yoshida sarcoma-bearing rats. This suggests that TdR kinase in the liver of Yoshida sarcoma-bearing rats is not derived directly from tumor tissues but is produced by de novo synthesis, which was presumably accelerated by some substances released from tumor tissues.
sarcoma enzyme suggests that the serum enzyme was derived from the Yoshida sarcoma.

There are 2 possible explanations for the increases of TdR kinase, TMP kinase, and DNA polymerase activities in the liver of Yoshida sarcoma-bearing rats. One is that the increases are due to increased de novo syntheses of these enzymes in the liver. The other is that the increases of these enzymes are derived from the enzymes in the serum. The latter possibility seems unlikely because the chromatographic patterns of TdR kinase from the liver and serum on a DEAE-cellulose column were quite different. Moreover TPK kinase activity was elevated in the liver of Yoshida sarcoma-bearing rats but not in the serum. Therefore, it seemed that some substances are released from tumor tissue that increase the de novo syntheses of these enzymes in the liver of tumor-bearing rats. This possibility is supported by the finding that the elevated liver TdR kinase activity decreased on removal of the tumor tissues. Recently, Nair and DeOme (18) reported that a soluble fraction prepared from a solid spontaneous primary mouse mammary tumor stimulated the growth of density-inhibited mouse embryo cells in monolayer culture.

As shown in Chart 5, the half-time of disappearance of liver TdR kinase after removal of Yoshida sarcoma was about 7 hr. This is longer than the half-life of the enzyme reported previously by Bresnick and Burleson (5) and Bresnick et al. (6). They found that the turnover of TdR kinase in Novikoff ascites cells was 3.5 hr, while the turnovers of TdR kinase in regenerating and control livers were 3.7 and 2.6 hr, respectively.

Experiments are now in progress on the mechanism of the increased activity of DNA-synthesizing enzymes in the tissues and sera of tumor-bearing rats.

REFERENCES

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