Enhanced drug-metabolizing capacity within liver adjacent to human and rat liver tumors
(cytochrome P-450/Morris hepatomas)

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ABSTRACT Cytochrome P-450 content (nmol/g of liver) differed within regions of rat liver according to proximity to intrahepatically implanted Morris hepatoma 5123D. Liver adjacent to tumor had higher microsomal cytochrome P-450 content, decreased DNA content (mg/g of liver), and unaltered cytochrome c reductase activity compared to histologically indistinguishable liver far-removed from the tumor. Liver either adjacent to or far-removed from tumor contained markedly more cytochrome P-450 and higher cytochrome c reductase activity but less DNA than transplanted Morris hepatomas 7795 and 5123D that were grown intrahepatically. Compared to intramuscular implants of these same tumors, intrahepatically implanted Morris hepatomas 7795 and 5123D had increased cytochrome P-450 content. Tumor-containing liver from two human subjects revealed regional changes in cytochrome P-450-mediated monooxygenases similar to those observed in rats. These results suggest that histomorphologically nontumorous mammalian liver directly adjacent to intrahepatic tumors exhibits previously unsuspected biochemical alterations.

Previous studies demonstrated that, compared to normal rat liver, hepatomas had lower microsomal cytochrome P-450 monooxygenase activity (1–7). In addition, cytochrome P-450 content declined within host livers after extrahepatic hepatomas became extremely large and necrotic (3, 4). The present study examines cytochrome P-450-mediated monooxygenases in intrahepatically implanted hepatomas rather than extrahepatic hepatomas. Our results extend previous observations in extrahepatic tissues by describing an unanticipated alteration in the hepatic microsomal cytochrome P-450-mediated monooxygenase system within liver adjacent to intrahepatically implanted Morris hepatomas 5123D and 7795. These alterations also occurred in human liver adjacent to intrahepatic tumors. In addition to presenting a novel biological phenomenon, these observations also raise practical problems related to regional differences within a tumor-containing organ with respect to capacity to biotransform drugs, other foreign compounds, and certain endogenous substrates.

MATERIALS AND METHODS

Animals. All animals were male Buffalo rats weighing 200–250 g at the onset of the studies. The rats, supplied by Microbiological Associates (Walkersville, MD), were maintained as described (8).

Pretreatments. In order to test for a potential factor, present in tumor or adjacent tissues, capable of altering cytochrome P-450 content, we injected the 100,000 × g supernatant of such tissues into normal rats as follows. Groups of four rats were pretreated daily for 1, 2, or 3 days with intraperitoneal injections of 0.5, 1.0, or 2.0 ml of 100,000 × g supernatant of tumor or adjacent tissue. Rats were killed 24 hr after the last injection.

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Tumor Implants. Seventeen milligrams of Morris hepatoma 5123D or 7795 (both originally induced in Buffalo rats) was surgically implanted intrahepatically within the left lateral lobe of each rat by means of a 13-gauge trochar. Small portions of Gelfoam (Upjohn) were packed into the needle track to prevent tumor extrusion. Tumors developed intrahepatically until they weighed approximately 1.3 g (30 days for 5123D and 54 days for 7795), at which time the rats were killed. Seventeen milligrams of normal liver was implanted in livers of control animals. However, approximately 1 week later no evidence of implanted liver was apparent in this group of rats, thereby making further analyses impossible. When removed for analyses, intramuscular tumors used in these experiments were also approximately 1.3 g.

Source of Human Tissue. Portions of human liver were obtained by surgical resection of a primary hepatoma. Liver containing a metastatic (mammary) carcinoma was obtained approximately 4 hr after death from respiratory arrest. Neither human subject had recently received medication known to induce hepatic cytochrome P-450-mediated monooxygenases, and neither subject showed evidence of liver cirrhosis. Microsomal fractions were isolated as described (8).

Histology. For light microscopic analyses, total body perfusions were performed under light ether anesthesia by using 10% buffered formalin injected through the left ventricle and exiting via the right atrium. Sections cut from paraffin-embedded blocks were stained with hematoxylin and eosin. Tissues were prepared for electron microscopy according to the method of Wisse et al. (9).

Preparation of Microsomes. After the rats were decapitated, livers were removed immediately and immersed in ice-cold 0.02 M Tris-HCl, pH 7.4/1.15% KCl. Each tumor was dissected free, and all surrounding liver within 0.5 cm of tumor was taken as adjacent tissue. Portions of the remaining unaffected lobes were considered far-removed normal liver. Preliminary experiments using normal male Buffalo rats revealed no detectable inter- or intralobular differences in hepatic cytochrome P-450, DNA, or cytochrome c reductase activity. In every experiment, pooled tissues from at least six animals were required. Hepatic microsomal fractions were isolated as described (8).

Analytical Methods. Cytochrome P-450 content was determined with an Aminco DW2 UV-Vis recording spectrophotometer by the method of Omura and Sato (10). DNA was extracted from whole homogenate as described by Munro and Fleck (11) and determined by the diphenylamine reaction (12) with calf thymus DNA for a standard. Protein was estimated by the Lowry technique (13). Cytochrome c reductase activity was determined by the method of Williams and Kamin (14) using an extinction coefficient of 21,000 M⁻¹ cm⁻¹. Microsomal aniline hydroxylase and ethylmorphine N-demethylase activities were determined as described (15). Dry weight determinations were made after tissues were held in an oven at 40°C for 5 days.
RESULTS

Histological analyses by light and electron microscopy revealed no apparent differences between tumor-adjacent liver and liver far-removed from the intrahepatic hepatoma in rats. Morris hepatomas 7795 and 5123D contained larger, more irregularly shaped hepatocytes with a greater nucleus-to-cytoplasm ratio than did tumor-adjacent or far-removed liver. Dry weight determinations on tissue slices from the three distinct regions indicated the average water content of tumor-adjacent and far-removed liver to be 66 and 66.5%, respectively, whereas that of hepatomas 7795 and 5123D was 79.1 and 80.5%, respectively (Table 1).

Compared to far-removed liver, tumor-adjacent liver had a higher microsomal cytochrome P-450 content and a lower DNA content (Table 1; Fig. 1). No significant differences in cytochrome c reductase activity occurred between these two regions. In contrast, intrahepatic hepatomas 7795 and 5123D had increased DNA levels but markedly decreased cytochrome P-450 content and cytochrome c reductase activity (Table 1; Fig. 1). In comparison to Morris hepatomas 7795 and 5123D implanted intramuscularly, intrahepatic implants of these same tumors contained significantly higher levels of cytochrome P-450 (Table 2). No significant differences in DNA content, cytochrome c reductase activity, or dry weight occurred between intramuscularly and intrahepatically grown hepatomas.

Experiments designed to test for a soluble factor(s) present in tumor or adjacent tissue that might be capable of altering cytochrome P-450 content revealed that pretreatment of nor-
nmal rats with 100,000 X g supernatant of tumor or adjacent tissue had no effect on cytochrome P-450 or DNA contents. In two human samples, microsomal cytochrome P-450 content, aniline hydroxylase activity, and ethylmorphine N-demethylase activity were higher in tumor-adjacent liver than in far-removed liver (Table 3). With respect to these values, both human tumors contained less than did either adjacent or far-removed liver.

**DISCUSSION**

The present study identifies biochemical alterations within liver adjacent to intrahepatic Morris hepatomas 7795 and 5123D in rats and adjacent to a primary hepatoma and metastatic breast carcinoma in two humans. Throughout the course of these studies, minimal or no necrotic regions appeared grossly or microscopically in either Morris hepatoma, probably attributable to the fact that these tumors were relatively small (~1.3 g) and well perfused. These conditions contrast to those of other studies using larger Morris hepatomas in which diffusible breakdown products of tumor necrosis resulted in impaired cytochrome P-450 activity within the host liver (3, 4).

Several explanations for our observations in liver adjacent to tumor merit consideration. Decreased DNA levels might result from fewer hepatocytes per g of liver due to changes in cell volume; conversely, increased cytochrome P-450 content could result from more hepatocytes per g of liver. However, histological analyses and dry weight determinations suggest that the number of hepatocytes per g of liver was similar for liver far-removed from and adjacent to tumor. In addition, cytochrome c reductase activity within tumor-adjacent liver remained unchanged (Table 1), suggesting that the number of hepatocytes per g of liver within adjacent tissue remained unchanged. Therefore, all these results collectively support neither of the above possibilities as satisfactory explanations of our observations.

An alternative explanation for decreased DNA per g of liver involves an increase in the number of diploid (2N) hepatocytes and a corresponding decrease in the number of tetraploid (4N) hepatocytes. Under normal physiological conditions, in adult rats the liver is primarily composed of mononucleated tetraploid hepatocytes (16). However, under certain conditions, such as partial hepatectomy, the proportion of tetraploid hepatocytes decreases whereas that of diploid hepatocytes increases (17). This change in ploidy could in turn decrease DNA content per g of liver.

Decrease of DNA and increase of cytochrome P-450 within tumor-adjacent liver could result from some unidentified factor(s) originating from the tumor. Initial attempts to detect such a factor(s) by administering the 100,000 X g supernatant of adjacent or tumor tissue to normal rats proved unsuccessful. Nevertheless, our experiments do not exclude this possibility because sufficiently high concentrations may not have been attained in the liver after injection of the unpurified fractions. Moreover, such putative factors may act only at short range or may be inactivated after systemic administration. Alternatively, these biochemical alterations within tumor-adjacent tissue may result from a nonspecific response produced by changes in blood flow or by close proximity to the enlarging mass.

The higher cytochrome P-450 content in intrahepatic than in intramuscular Morris hepatomas may be related to regional differences in blood flow and nutrient supply which in turn could affect rates of synthesis and degradation of cytochrome P-450 within tumors. In addition, multiple types of interactions between tumor cells and adjacent nontumorous hepatocytes may stimulate cytochrome P-450 formation within both tumor and adjacent tissue.

The results in Table 3 suggest that tumor-containing liver in human subjects displays regional changes in cytochrome P-450-mediated monooxygenases similar to those observed in rats. These data also suggest that alterations observed in tissues adjacent to tumors in rats probably do not result from effects associated with the transplantation process itself (such as histocompatibility). Nor does this phenomenon appear to be restricted to primary hepatomas because it occurred in the liver adjacent to a metastasis from a primary mammary carcinoma (Table 3). These regional differences within tumor-containing liver raise practical problems regarding the capacity of liver to biotransform drugs, foreign compounds, and certain endogenous substances. For example, with respect to cyclophosphamide, which requires metabolic activation for antitumor activity, these regional differences in drug-metabolizing activity that exist within the tumor-containing liver could result in several different consequences. Increased antitumor activity might ensue if the high drug-metabolizing activity of the tumor-adjacent liver activated more cyclophosphamide, allowing greater exposure of tumor cells to activated metabolites. Alternatively, less cyclophosphamide might reach the tumor.

### Table 2. Cytochrome P-450 content of intramuscular and intrahepatic Morris hepatomas

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Microsomal protein</th>
<th>nmol/g</th>
<th>nmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>7795</td>
<td>0.39 ± 0.03*</td>
<td>2.86 ± 0.19*</td>
<td></td>
</tr>
<tr>
<td>5123D</td>
<td>0.24 ± 0.02*</td>
<td>1.85 ± 0.14*</td>
<td></td>
</tr>
</tbody>
</table>

* Each value represents the mean ± SEM of four determinations.

### Table 3. Cytochrome P-450-mediated monooxygenases in three regions of liver from two patients with intrahepatic tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Tissue region</th>
<th>Cytochrome P-450</th>
<th>N-demethylase activity</th>
<th>Aniline hydroxylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmol/mg</td>
<td>nmol/g tissue</td>
<td>activity*</td>
</tr>
<tr>
<td></td>
<td>microsomal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma</td>
<td>Tumor</td>
<td>0.14</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Tumor-adjacent</td>
<td>0.55</td>
<td>0.71</td>
<td>2.25</td>
</tr>
<tr>
<td>Mammary</td>
<td>Far-removed</td>
<td>0.24</td>
<td>4.44</td>
<td>0.90</td>
</tr>
<tr>
<td>carcinoma</td>
<td>Tumor</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Tumor-adjacent</td>
<td>0.25</td>
<td>2.11</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Far-removed</td>
<td>0.19</td>
<td>1.88</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* Shown as nmol product/mg microsomal protein per min.

† Obtained by surgical resection, from 67-year-old woman (R.C.).

‡ Obtained at autopsy at 4 hr after death; patient was 59-year-old woman (K.K.).
because more might be metabolized and bound in nontumorous adjacent liver, thereby reducing the antitumor activity of cyclophosphamide. In the same liver, some parts of a tumor might receive more drug whereas other parts might simultaneously receive less drug as a consequence of these regional differences in the activity of cytochrome P-450-mediated monooxygenases.

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