Angiotensinogen-Producing Hepatocellular Carcinoma

NAOTO UENO, KAORU YOSHIDA, SHIGEHISA HIROSE, HISAMITSU YOKOYAMA, HIDEO UEHARA, AND KAZUO MURAKAMI

SUMMARY This paper describes the first case of an angiotensinogen-producing tumor. The tumor obtained from a hypertensive patient was examined for its renin and angiotensinogen contents. Renin activity was undetectable; however, the angiotensinogen level was extremely high compared with the levels in the tissue surrounding the hepatoma. The presence of angiotensinogen immunoreactivity in the tumor cells was demonstrated by immunohistochemical staining with an angiotensinogen antisem. The plasma level of angiotensinogen was also markedly elevated. These results strongly suggest that the hepatoma was an angiotensinogen-producing tumor. (Hypertension 6: 931-933, 1984)

KEY WORDS • angiotensinogen • hepatoma • hormone-producing tumor

It is now widely accepted that neoplasms can give rise to autonomous overproduction of hormone or specific antigen. Hepatocellular carcinoma produces an extraordinary amount of alpha-fetoprotein (AFP), well known as a useful tumor marker. Some investigators have reported that liver cell carcinomas are capable of producing not only AFP but also normal serum proteins, including albumin, transferrin, and alpha-1-antitrypsin. Angiotensinogen (renin substrate) is also a plasma protein of hepatic origin. It is an integral component of the renin-angiotensin system which plays an important role in the regulation of blood pressure and fluid and electrolyte balance. The encounter of a hypertensive patient with a hepatoma and high plasma concentrations of angiotensinogen, therefore, prompted us to measure the angiotensinogen content in the hepatoma tissue.

Case Report

Examination

A 36-year-old man presented with upper-abdominal pain and backache; his blood pressure was 170/96 mm Hg. Investigations revealed slight hypokalemia (serum potassium level was 3.6 mEq/liter), high plasma renin activity (PRA, 36.7 ng angiotensin [ANG] I/ml/hr), high plasma renin concentration (44.1 ng ANG I/ml/hr), and high plasma aldosterone concentration (418.7 pg/ml). The plasma AFP concentration was very high (74 x 1Cng/ml), and a hepatic angiogram showed a major and several minor space-occupying lesions in the left lobe of the liver. A diagnosis of hepatoma was entertained.

Postmortem Examination

After half a year, the patient fell into hepatic coma and died. Sectioning the tumor, removed at autopsy, showed a trabecular hepatocellular carcinoma without cirrhosis. Ectopic production of renin by the hepatoma was considered, as the PRA was high throughout the clinical course. However, renin was absent from the tumor, and the renin concentration in the patient’s kidney was in the normal range (0.75 /xg angiotensin I/mg protein/hr).

Blood from the patient had been collected 2 weeks before death, and the plasma, separated by centrifugation, was kept frozen at -60° C. The hepatoma tissue and nonneoplastic tissue surrounding the tumor were obtained at autopsy within 1 hour of death and frozen immediately at —60° C. Plasma samples taken from five healthy males (aged 22-27 years) on a free diet and livers obtained from the cadavers of traffic accident victims were used as controls.

Angiotensinogen Assay

Angiotensinogen levels in tissue extracts and in plasma were quantitated as the maximum amount of ANG I enzymatically cleaved by exhaustive incuba-
tion of samples at 37° C with purified human renin (0.4 GU). The ANG I released was determined by radioimmunoassay (RIA). To abolish the degradation of ANG I by angiotensinase, 20 mM EDTA and 20 mM phenylmethylsulfonylfluoride (PMSF) were added to the reaction mixture.

**Measurements of Plasma Renin Activity and Plasma Renin Concentration**

These measurements were performed as described previously.

**Immunostaining**

An avidin-biotin-peroxidase complex (ABC) method that uses a Vectastain ABC kit (Vector Laboratories, California) was applied to deparaffinized sections of the hepatoma and surrounding normal liver tissue. The sections were reacted with the primary antiangiotensinogen antiserum at a 1:1,000 dilution for 24 hours at 4° C. (The antiserum was kindly supplied by Dr. T. Ito, Department of Clinical Research, Japanese Defense Forces Central Hospital, Japan.) After reaction with the primary antiserum and buffer washes, biotinylated goat antirabbit IgG diluted 1:200 was allowed to react with the sections for 30 minutes, followed by avidin-HRP (horseradish peroxidase) for 1 hour, and then dianimobenzidine (Wako Chemicals, Tokyo, Japan). The sections were washed in distilled water and counterstained with hematoxylin. As a control, nonimmunized rabbit serum diluted 1:1000 was reacted with tumor tissue instead of antiangiotensinogen antiserum.

**Results and Discussion**

The angiotensinogen content of the patient’s plasma and of three separate samples from the peripheral, intermediate, and central portions of the tumor is shown in Table 1. Plasma angiotensinogen concentration in the patient was high (4 to 5-fold) as compared to that of normal subjects. The angiotensinogen level in the tumor is more than 100 times higher (2930 ng ANG I/ml) than that in the nonneoplastic tissue (18 ng ANG I/ml) surrounding the tumor or in the control livers (8-25 ng ANG I/ml). These results suggested that a continuous unregulated production of angiotensinogen in the hepatoma resulted in the increased plasma levels.

To confirm angiotensinogen production by the tumor cells, we immunostained sections from hepatoma and surrounding normal liver tissue using antiserum specific to human angiotensinogen and compared the distribution of the antigen. The hepatoma tissue reacted strongly with the angiotensinogen antiserum (Figure 1 top), but not with nonimmunized control serum. These results clearly demonstrated high angiotensinogen content in the tumor cells. In the surrounding nonneoplastic liver tissue, the angiotensinogen immunostaining was much less conspicuous (Figure 1 bottom).

**TABLE 1. Angiotensinogen Contents in Tissue Extracts and Plasma**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Angiotensinogen concentration (ng ANG I ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue extracts</td>
<td></td>
</tr>
<tr>
<td>Hepatoma (center)</td>
<td>1488</td>
</tr>
<tr>
<td>Hepatoma (intermediate)</td>
<td>1396</td>
</tr>
<tr>
<td>Hepatoma (peripheral)</td>
<td>2930</td>
</tr>
<tr>
<td>Surrounding liver</td>
<td>18</td>
</tr>
<tr>
<td>Control liver 1</td>
<td>8</td>
</tr>
<tr>
<td>Control liver 2</td>
<td>25</td>
</tr>
<tr>
<td>Plasma samples</td>
<td></td>
</tr>
<tr>
<td>Hepatoma patient</td>
<td>8340</td>
</tr>
<tr>
<td>Normal subjects (n = 5)</td>
<td>1833±311</td>
</tr>
</tbody>
</table>

Angiotensinogen concentration was expressed as the maximum amount of angiotensin (ANG I) generated during incubation of samples with purified renin. For normal subjects the concentration value is the mean ± SD.

A sample (2 g) of each tissue was extracted in 4 ml of 20 mM imidazole buffer, pH 6.0.

Morris et al. successfully demonstrated localization of angiotensinogen in normal rat liver using the peroxidase-antiperoxidase (PAP) method. Our failure to immunostain angiotensinogen in the normal liver surrounding the tumor may be due to the species difference, the sensitivity of antibodies used, or feedback inhibition of the high angiotensinogen production by the tumor to suppress the production from normal liver.

Using perfused rat liver, Nasjletti and Masson confirmed that the liver is the origin of angiotensinogen. Some investigators have suggested production of normal serum proteins by hepatomas. Therefore, it would not be surprising that a hepatoma, as in this case, was capable of producing angiotensinogen autonomously. Such an analogous hepatic tumor might have arisen without being recognized until now, because the estimation of angiotensinogen is not generally carried out in routine clinical assays. Recently developed direct radioimmunoassays of angiotensinogen may help to detect similar hepatic tumors.

Heterogeneity of the angiotensinogen molecule has been demonstrated in the plasma of pregnant or menstruating women, and high molecular weight species of angiotensinogen has been observed in addition to the predominant form (60,000 daltons). Plasma from the patient and a mixture of plasmas from healthy volunteers were subjected to gel filtration on Ultrogel AcA 44 to estimate molecular weights of angiotensinogens by determining their Stokes radii. Both angiotensinogens were eluted in a single symmetrical peak corresponding to a molecular weight of 60,000. This observation suggests that the oncodevelopmental molecule is similar, if not identical, to the naturally occurring one, although it remains to be seen whether
posttranslational glycosylation of the tumoral angiotensinogen occurs in the normal manner.

An angiotensinogen-producing tumor is a new entity in disorders of the renin-angiotensin system, although the contribution of the tumoral angiotensinogen to the system is not clear. We suggest that the high PRA in this patient was due to both the high plasma angiotensinogen concentration and the high plasma renin concentration. It is hoped that the description of this case will increase awareness of not only orthotopic but also ectopic angiotensinogen-producing tumor and will help to elucidate the clinical features of tumor-associated hormone changes.

Acknowledgments
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References
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FIGURE 1. Immunocytochemical detection of angiotensinogen. The tumor tissue (top) and surrounding normal liver tissue (bottom) were immunostained with antiangiotensinogen antisera and then counterstained with hematoxylin (X 200).
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