Decrease of Cytochrome P-450 Having Arylhydrocarbon Hydroxylase and Increase of UDP-Glucuronyltransferase Glucuronizing Phenicn Xenobiotics in Rat Liver Nodule

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ABSTRACT. Microsomal arylhydrocarbon (benzo[a]pyrene) hydroxylase activity in hyperplastic nodules in the livers of rats was decreased by feeding 2-acetylaminofluorene in their diet to 10% of that without 2-acetylaminofluorene feeding. By immunoblotting analysis with the antibodies raised against P-450/B[a]P, which is a form of cytochrome P-450 catalyzing benzo[a]pyrene hydroxylation in liver microsomes of untreated rats, it was shown that P-450/B[a]P content was decreased in the microsomes prepared from the nodular tissues. Microsomal UDP-glucuronyltransferase activity toward phenicn xenobiotics such as 1-naphthol and 4-nitrophenol was increased by 4.5-5.0-fold in the nodular tissues. This increase was inhibited by the addition of antibodies raised against GT-1, a form of UDP-glucuronyltransferase glucuronizing phenicn xenobiotics. Immunoblotting analysis with the antibodies against GT-1 showed that a protein band corresponding to GT-1 was increased in the microsomes from nodular tissues. The increased UDP-glucuronyltransferase also had about 4,000 daltons "high mannos" oligosaccharide(s) like GT-1. The decreases of P-450/B[a]P and increases of GT-1 were observed mainly in nodular foci of the liver tissues. These results indicate that in liver nodules, decreased cytochrome P-450-dependent benzo[a]pyrene hydroxylase activity and increased UDP-glucuronyltransferase activity toward phenicn xenobiotics result from the decrease of the P-450 corresponding to P-450/B[a]P and the increase of the GT corresponding to GT-1. —KEY WORDS: cytochrome P-450, liver nodule, UDP-glucuronyltransferase, 2-acetylaminofluorene.

The development of hyperplastic nodules in the livers of rats which received 2-acetylaminofluorene in their diet is an interesting model system for studying the process of chemical carcinogenesis [6, 7]. It has been shown that hyperplastic nodules are relatively resistant to the cytotoxic action of carcinogens and other hepatotoxins in vivo as well as in vitro [8, 13]. During the promotion stage in hyperplastic liver nodules, the changes of drug metabolizing enzymes are well known [1, 4, 28]. Decreased activity of cytochrome P-450-dependent monoxygenase [4], and increased activities of drug-detoxification enzymes such as epoxide hydase [5, 15] glutathione S-transferase [11] and UDP-glucuronyltransferase [3] have been reported. Arylhydrocarbone (benzo[a]pyrene) hydroxylase activity was decreased in many microsomal monoxygenase activities in the liver nodules [1, 4]; however, it is unknown how cytochrome P-450s alters in the liver nodules. Epoxide hydase, which increased in hyperplastic liver nodules, was purified and immunochemically identified with preneoplastic antigen [15]. Alteration in the molecular forms of glutathione S-transferase has been reported [5, 11]. A form of UDP-glucuronyltransferase, corresponding to GT1 which was purified from 3-methylcholanthrene-treated rat liver microsomes, was found to be enhanced in nodular tissues by the method of rocket immunoelectrophoresis [3]; however, further properties of the increased UDP-glucuronyltransferase in nodules are unknown. We recently purified a form of cytochrome P-450, which catalyzed benzo[a]pyrene hydroxylation and was named "P-450/B[a]P", from liver microsomes of untreated rats [24], and a form of UDP-glucuronyltransferase, which glucuronized some xenobiotics and was named "GT-1", from liver microsomes of 3-methylcholanthrene-treated rats [35]. Herein we report our finding that in nodular tissues, the reduction of benzo[a]pyrene hydroxylase activity accounted for the decrease of the P-450 corresponding to P-450/B[a]P, and the increase of UDP-glucuronyltransferase activity was dependent on the increase of the GT corresponding to GT-1.

MATERIALS AND METHODS

Materials: Male Fisher rats (F344/N snc) were obtained from the Shizuoka Laboratory Animal Center, Shizuoka, Japan. Benzo[a]pyrene, 2-acetylaminofluorene and cholic acid were purchased from Wako Pure Chemical Industries, Osaka,
Japan. Cholic acid was further purified and converted to sodium salt as described previously [10]. Nitrocellulose sheets were purchased from Toyo Roshi Co. Anti-rabbit IgG-peroxidase conjugate was from Jackson Immuno-Research, and endo-β-N-acetylglucosaminidase H (Endo H) was from Seikagaku Kogyo Co., Ltd. Tokyo, Japan.

**Treatment of animals and preparation of nodular tissues:** Male Fisher rats (6–7 weeks old) were used. 2-Acetamidinofluorene was dietarily administered to the rats. A diet containing 0.05% (w/w) 2-acetamidinofluorene was fed to the experimental animals for 3 months with a week interval and hyperplastic nodules were formed in the liver of the 2-acetamidinofluorene-treated rat. These rats were killed 3 weeks after the final administration. Rat liver was perfused with 0.15 M KCl solution. Hyperplastic nodular tissue, which was macroscopically distinguishable, was carefully dissected from the surrounding tissue, and these tissues were immediately frozen in liquid nitrogen, and stored at −80°C until used for following experiments. Control livers from rats administered with commercial diet for the same period were prepared.

**Preparation of microsomes:** The nodular tissues prepared from livers described above were histologically identified by hematoxylin-eosin staining. The tissues were minced and homogenated with 4 volumes of the 0.15 M KCl solution. The homogenate was centrifuged for 15 min at 9,000 × g. The supernatant fraction was further centrifuged at 105,000 × g for 60 min to obtain microsomes.

**Purification of P-450 and GT-1:** P-450/B[a]P was purified from liver microsomes of untreated rat by monitoring benzo[a]pyrene hydroxylase activity as previously described [24]. P-450c (corresponds to “IA1” named by Nebert et al. [21]) was purified from liver microsomes of 3-methylcholanthrene-treated rat as described by Ryan et al. [22]. UDP-glucuronyltransferase (named GT-1) was purified from 3-methylcholanthrene-treated rat liver microsomes as previously described [35].

**Preparation of antibodies:** The purified enzyme (80 µg) was emulsified with Freund’s complete adjuvant and injected into a rabbit (2.0 kg) at multiple intracutaneous sites 3 times at a week intervals. The immunoglobulin was prepared from the serum of the rabbit by the method of ammonium sulfate fractionation.

**Inhibition of UDP-glucuronoyltransferase activity by antibodies:** Microsomes suspended (5 mg/ml) in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1 mM DTT were completely solubilized with 1.0% sodium cholate. The 105,000 × g supernatant was incubated overnight at 4°C with various amounts of anti-GT-1 immunoglobulin as indicated in 20 mM potassium phosphate buffer (pH 7.4) containing 0.12% sodium cholate, 0.1 mM DTT, and the enzyme activity in the mixture was determined.

**SDS-polyacrylamide gel electrophoresis and immunoblotting:** SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli [12]. Proteins were transferred to nitrocellulose sheets by the method of Howe and Hershey [9] except that transfer buffer contained 348 mM glycine, 50 mM Tris base, and 20% (v/v) methanol. The transfer was carried out at 200 mA (5–6 V) for 5 hr. Nonspecific binding sites on the nitrocellulose sheets were blocked overnight in 1.0% bovine serum albumin dissolved in 10 mM phosphate buffered saline (PBS), pH 7.5. The blocked sheets were incubated with primary antibodies in PBS containing 1.0% bovine serum albumin and 0.1% Triton X-100 (AB buffer) for 3 hr at room temperature. The sheets were washed with AB buffer and PBS. The washed sheets were subsequently incubated with secondary antibodies (goat anti-rabbit IgG-peroxidase conjugate) diluted 1:1000 in AB buffer for 3 hr at room temperature. Immunoblotts were stained using 0.12% diaminobenzidine as a substrate of peroxidase in 0.05 M Tris-HCl (pH 7.6) containing 0.004% H2O2.

**Treatment with endo-β-N-acetylglucosaminidase H:** Five units of endo-β-N-acetylglucosaminidase H (Endo H) was added to the microsomes (2 µg) dissolved in 200 µl of 0.1 M citrate-phosphate buffer (pH 5.0). The mixture was incubated for 20 hr at 37°C. SDS-polyacrylamide gel electrophoresis and immunoblotting of the treated samples were performed as described above.

**Analytical procedure:** Benzo[a]pyrene hydroxylase activity in microsomes was assayed by the method of Nebert and Gelboin [20]. The activities for N-demethylation of aminopyrine and benzphetamine were assayed by determining the product of formaldehyde as described by Nash [19]. O-deethylase activity of 7-ethoxycoumarin was assayed as described by Ullrich and Weber [30]. UDP-glucuronyltransferase activities toward various substrates were assayed at following concentrations of aglycone and by the methods described in

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respective references: 0.5 mM 1-naphthol [18]; 0.5 mM 4-nitrophenol [36]; 0.5 mM 4-hydroxybiphenyl [2]; 1.0 mM 5-hydroxytryptamine [14]; and 0.1 mM bilirubin [31]. Cytochrome P-450 in microsomes was estimated from the CO difference spectrum of a dithionite reduced sample as described by Omura and Sato [25]. NADPH-cytochrome P-450 reductase was determined by the method of Omura et al. [26] using cytochrome c as an electron acceptor. Protein was determined by the method of Lowry et al. [17], using bovine serum albumin as the standard.

RESULTS

Effects of acetylaminoﬂuorenone: In the liver microsomes of rats fed a diet containing 0.05% acetylaminoﬂuorenone for 3 months, γ-glutamyl transpeptidase activity was increased, and cytochrome P-450 content was gradually decreased as compared with that of rats fed a control diet (Table 1), as previously described [1, 4]. Micromosomal UDP-glucuronosyltransferase activity toward 1-naphthol was gradually increased by feeding a diet containing 2-acetylaminoﬂuorenone (Table 1), as previously described [1, 3, 32].

Decrease of benzo[a]pyrene hydroxylase activity: Liver nodular foci formed by feeding the diet containing 0.05% acetylaminoﬂuorenone for 3 months could be macroscopically observed and histochemically identiﬁed. Micromosomal monoxygenase activities and cytochrome P-450 content in the nodular and surrounding tissues of the nodular foci are shown in Table 2. The content of cytochrome P-450 in some monoxygenase activities catalyzed by cytochromes such as aminopyrine N-demethylase, ethoxycoumarin O-deethylase and benzphetamine N-demethylase in the liver microsomes of the nodules were moderately reduced to 31–38% of the control. Benzo[a]pyrene hydroxylase activity was greatly reduced to 10% of the control as previously described [1, 4]. On the other hand, in the microsomes of the surrounding tissues, cytochrome P-450 content was decreased in 57% and all monoxygenase activities tested were slightly decreased to 90% of control (Table 2). NADPH-cytochrome P-450 reductase activity in both types of tissues were not

Table 1. Effects of 2-acetylaminoﬂuorenone on enzyme activities of γ-glutamyl transpeptidase (γ-GTP), UDP-glucuronosyltransferase (GT) of 1-naphthol glucuronidation, and cytochrome P-450 (P-450) contents in the liver microsomes of the rats. The microsomes were prepared from liver of rats administrated with control diet (None) and rats administrated with 0.05% (W/W) 2-acetylaminoﬂuorenone diet (AAF) for the corresponding periods. Enzyme activities were assayed by the methods described in “MATERIALS AND METHODS”. Values represent the mean ± SD for 3–5 animals.

<table>
<thead>
<tr>
<th>Day of treatment</th>
<th>γ-GTP (nmole/min/mg of protein)</th>
<th>P-450 (nmole/mg of protein)</th>
<th>GT (nmole/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>AAF</td>
<td>None</td>
</tr>
<tr>
<td>0</td>
<td>8.80±0.25.31</td>
<td>1.39±0.11</td>
<td>0.65±0.13</td>
</tr>
<tr>
<td>50</td>
<td>8.83±0.53</td>
<td>13.9±1.1</td>
<td>0.87±0.015</td>
</tr>
<tr>
<td>90</td>
<td>9.40±0.28</td>
<td>42.4±10.2</td>
<td>1.07±0.13</td>
</tr>
</tbody>
</table>

Table 2. Microsomal cytochrome P-450 content and monoxygenase activities in liver microsomes from nodules and surrounding tissues. The microsomes were prepared from livers of rats administrated with basal diet without 2-acetylaminoﬂuorenone (Control) and of rats administrated with 0.05% (W/W) 2-acetylaminoﬂuorenone diet for 3 month as described in “MATERIALS AND METHODS”.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>NADPH-Cytochrome P-450 reductase</th>
<th>Cytochrome P-450 content</th>
<th>Monoxygenase activities (nmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmole/min/mg of protein)</td>
<td>(nmole/mg of protein)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.051±0.002</td>
<td>1.07±0.13</td>
<td>0.238±0.018</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Nodule[9]</td>
<td>0.064±0.002</td>
<td>0.33±0.03</td>
<td>0.023±0.003</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(31)</td>
<td>(10)</td>
</tr>
<tr>
<td>Surrounding[9]</td>
<td>0.050±0.001</td>
<td>0.61±0.15</td>
<td>0.221±0.015</td>
</tr>
<tr>
<td></td>
<td>(94)</td>
<td>(57)</td>
<td>(95)</td>
</tr>
</tbody>
</table>

a) μ mole/min/mg of micromosomal protein. b) amol/mg of micromosomal protein. c) These microsomes were prepared from the tissues which were collected from the hyperplastic nodule foci (Nodule) and from surrounding the foci (Surrounding) of 2-acetylaminoﬂuorenone-treated rat livers. Values represent the mean±SD for 3–5 animals.
significantly altered (Table 2). We have recently shown that P-450/B[a]P highly catalyzes benzo[a]pyrene hydroxylation in liver microsomes of untreated rats, and obtained specific anti-P-450/B[a]P antibodies which inhibited benzo[a]pyrene hydroxylase activity in the liver microsomes of untreated rats [24]. The data of immunoblotting analysis of liver microsomes from the nodular and surrounding tissues with the anti-P-450/B[a]P antibodies and anti-rat liver NADPH-cytochrome P-450 reductase antibodies are shown in Fig. 1. Both antibodies bound only to corresponding protein bands. The protein level of reductase was unchanged not only in the surrounding tissues but also in the nodular tissues. The decrease of protein level of P-450/B[a]P is clearly shown in Fig. 1. In nodular microsomes (Fig. 1A, lane 3), an unknown band, which had a lower molecular size (50 K daltons) than P-450/B[a]P (51 K daltons) was detected. It is unknown whether the 50 K band was newly induced in the nodules or originally exists in the microsomes of untreated rat liver. The same immunoblotting data of anti-P-450c antibodies are shown in Fig. 1B. P-450c, which showed high catalytic activity in the hydroxylation of benzo[a]pyrene and 2-acetylaminofluorene [22, 24], could not be detected in microsomes from the control, or in the nodular and surrounding tissues (Fig. 1B). These antibodies against P-450c could cross react with P-450d (corresponds, to “IA2” named by Nebert et al. [21]), which could catalyze the hydroxylation of some arylhydrocarbons such as 2-acetylaminofluorene [24]. P-450d, which was detected in microsomes from 3-methylcholanthrene-treated rat liver by immunoblotting analysis with this antibody, was undetectable in microsomes from both nodular and surrounding tissues (Fig. 1B). P-450b (corresponds to “IIIb” named by Nebert et al. [21]), which catalyzed the hydroxylation of other xenobiotics such as benzphetamine [24], was also not observed in the microsomes from these tissues by the same analysis (data not shown).

Increase of microsomal UDP-glucuronyltransferase: UDP-glucuronyltransferase activity towards various substrates in microsomes prepared from nodular foci and surrounding tissues are shown...
Table 3. UDP-glucuronyltransferase activities toward various substrates in rat liver microsomes from hyperplastic nodules and surrounding tissues. Enzyme activities were determined in liver microsomes, fully activated by the addition of sodium cholate (final concentration: 0.025%). The microsomes were prepared from livers of rats administered with control diet (Control) and of rats administered with 0.05% (W/W) 2-acetylaminofluorene diet for 3 month.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>1-Naphthol</th>
<th>4-Nitrophenol</th>
<th>5-Hydroxytryptamine</th>
<th>4-Hydroxybiphenyl</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.9±4.6</td>
<td>26.8±1.3</td>
<td>14.2±1.5</td>
<td>26.5±2.0</td>
<td>0.92±0.09</td>
</tr>
<tr>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Nodule a)</td>
<td>72.6±11.8</td>
<td>122.0±10.5</td>
<td>65.2±2.4</td>
<td>50.8±8.7</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>(332)</td>
<td>(456)</td>
<td>(459)</td>
<td>(192)</td>
<td>(77)</td>
<td></td>
</tr>
<tr>
<td>Surrounding b)</td>
<td>38.8±4.6</td>
<td>43.4±13.9</td>
<td>25.4±2.3</td>
<td>37.4±7.6</td>
<td>0.98±0.08</td>
</tr>
<tr>
<td>(177)</td>
<td>(162)</td>
<td>(179)</td>
<td>(141)</td>
<td>(107)</td>
<td></td>
</tr>
</tbody>
</table>

a) These microsomes were prepared as described in Table 2. Values represent the mean ±SD for 3–5 animals.

Fig. 2. Inhibition of microsomal UDP-glucuronyltransferase activity in hyperplastic liver nodules by the antibodies raised against GT-1. Microsomes prepared from liver nodules of 2-acetylaminofluorene-treated rat were solubilized by 0.6% sodium cholate and incubated with various amounts of the antibodies for 10 hrs at 4°C. Enzyme activities of UDP-glucuronyltransferase toward 4-nitrophenol (C) and 1-naphthol (●) were assayed as described in “Materials and Methods”.

in Table 3. All transferase activities except bilirubin glucuronidation in the nodular foci, were higher than those in the surrounding tissues (Table 3). The glucuronidation activity towards 1-naphthol, 4-nitrophenol and 5-hydroxytryptamine, which could be conjugated by GT-1 purified from 3-methylcholanthrene-treated rat liver [35] were markedly increased by 3.3–4.6-fold in the microsomes of the nodular foci (Table 3). The transferase activity toward 4-hydroxybiphenyl was increased only by 2-fold in the microsomes (Table 3). Adverse effects from the results above described were observed in the activity of bilirubin glucuronidation (Table 3).

Immunochromic properties of increased UDP-glucuronyltransferase: We have obtained polyclonal antibodies against GT-1 [34]. Effect of antibodies raised against GT-1 on UDP-glucuronyltransferase activity in the microsomes of hyperplastic liver nodules is shown in Fig. 2. Inhibitory effects of the antibodies on UDP-glucuronyltransferase activity toward 1-naphthol and 4-nitrophenol were observed (Fig. 2). On Ouchterlony double-diffusion analysis with the antibodies, a single immunoprecipitate line was observed between the microsomal protein of hyperplastic liver nodules and the antibodies, and this precipitate line completely fused with the precipitate line formed with the microsomal protein from 3-methylcholanthrene-treated rat liver and the antibodies against GT-1 (data not shown). These results indicate that glucuronidation of 1-naphthol, 4-nitrophenol and 5-hydroxytryptamine, which were enhanced in the microsomes of hyperplastic liver nodules, were catalyzed by form(s) of UDP-glucuronyltransferase having the same immunochromic properties as GT-1. Further analysis of the properties of increased UDP-glucuronyltransferase in hyperplastic nodules by Western Blotting is shown in Fig. 3. Results demonstrated that an immunoreactive protein with the antibodies raised against GT-1 was increased in the microsomes of hyperplastic liver nodules. Furthermore, amount of the immunoreactive UDP-glucuronyltransferase protein in the nodular foci was greater than that in the surrounding tissues, as shown by the activity level (Table 3), and the increased immunoreactive band in the nodular tissues had the same molecular
weight of 54,000 daltons as GT-1. Since the activity of UDP-glucuronosyltransferase toward 1-naphthol solubilized from the microsomal membrane of hyperplastic liver nodules was absorbed to Con A-Sepharose (data not shown) as 3-hydroxy-3-methylglutaryl-CoA reductase [16], the effects of treatment with Endo H of the microsomes from hyperplastic liver nodules on the oligosaccharide chain(s) linked with the increased UDP-glucuronosyltransferase were studied and result is shown in Fig. 4. The molecular size of the increased UDP-glucuronosyltransferase was reduced by the treatment with Endo H (Fig. 4). These results indicate that UDP-glucuronosyltransferase corresponding to GT-1 increases in the microsomes of nodular tissues without any alterations in the immunochromatographic properties or nature of the oligosaccharide chains linked with it.

DISCUSSION

One possible reason why hyperplastic nodules are relatively resistant in vivo and in vitro to the cytotoxic action of carcinogens and other hepatotoxins [8, 13] is the lower metabolic activation of the precarcinogens caused by the low level of drug-metabolizing activities [1]. Hyperplastic liver nodules contained less cytochrome P-450 and lower activity of various monooxygenases than the control as previously described [4, 27], and the surrounding tissues of the nodular foci (Tables 1 and 2). Decrease of arylhydrocarbon (benzo[a]pyrene) hydroxylase activity in nodular tissues accounted for the reduction of the protein level of P-450/B[a]P which catalyzes hydroxylation in liver microsomes of untreated rats without the participation of P-450c, P-450d and P-450b, which are known to catalyze hydroxylation of benzo[a]pyrene and many other xenobiotics such as 2-acetylaminofluorene and benzophenine [22, 24]. Any other forms of P-450 would also be decreased in nodules, because aminopyrine N-demethylase and ethoxycoumarin O-deethylase activities were reduced to 30–40% of the control. Recently we obtained data showing that 2-acetylaminofluorene was highly metabolically activated by P-450/B[a]P using a new test system for screening potential DNA-damaging agents, namely
the umu test developed by Oda et al. [23]. The process of P-450/B[a]P reduction by its substrate, 2-acetylaminofluorene, will be interesting for analysis of the promotion of liver nodules.

UDP-glucuronyltransferase, which is one of the detoxication enzyme of chemical carcinogens, was markedly increased in the liver with the promotion of the nodules by 2-acetylaminofluorene [1]. The activities of late fetal stage and 3-methylcholanthrene-inducible UDP-glucuronyltransferase are increased in hyperplastic nodules [32]. It has been shown that GT1 protein purified from 3-methylcholanthrene-treated rat liver by Bock et al. is enhanced in nodular tissues by the method of rocket immunoelectrophoresis [3]. We obtained data from immunoblotting analysis that the enhancement of UDP-glucuronyltransferase activity towards phenolic xenobiotics in hyperplastic liver nodules was due to the increase of a form of UDP-glucuronyltransferase, named GT-1, conjugating corresponding substrates (Tables 2 and 3, and Figs. 2 and 3) [35]. Our results also demonstrated that a single form of UDP-glucuronyltransferase immunoreactive with anti-GT-1 antibodies and having a monomeric molecular size of 54,000 daltons was increased in nodular tissues from the inhibition data of increased UDP-glucuronyltransferase activities by the antibodies against GT-1 (Fig. 2) and immunoblotting analysis (Fig. 3). GT-1 contained "high mannose type" oligosaccharide chain(s), which could be released from the protein portion by Endo H-treatment [33, 35]. The sugar chain(s) of the increased UDP-glucuronyltransferase in the microsomes of liver nodules was also N-linked "high mannose" oligosaccharide, because the chain(s) was released from the protein portion by Endo H-treatment (Fig. 4). It is known that during the promotion of hyperplastic liver nodules, γ-glutamyl transpeptidase of plasma membrane was markedly increased and also altered in its sugar portion [29]. Glutathione S-transferase of cytosol protein increased and was altered in its protein portion during the formation of hyperplastic liver nodules [11]. In contrast, a form of UDP-glucuronyltransferase binding in endoplasmic reticulum and catalyzing activities of various xenobiotics were markedly increased, but the properties of the molecular form (immunochemical properties and molecular size) and the sugar chain(s) linked with it were not significantly altered during the formation of hyperplastic nodules.

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