Cell Proliferation Induced by Triiodothyronine in Rat Liver Is Associated with Nodule Regression and Reduction of Hepatocellular Carcinomas

Giovanna M. Ledda-Columbano, Andrea Perra, Roberto Loi, Hisashi Shinozuka, and Amedeo Columbano

Department of Toxicology, Oncology and Molecular Pathology Unit, University of Cagliari, 09124 Cagliari, Italy [G. M. L.-C., A. P., R. L., A. C. I.]; and Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261 [H. S.]

ABSTRACT

Previous studies have demonstrated that short-term treatment with peroxisome proliferators decreased the size and number of γ-glutamyl transpeptidase or placental glutathione S-transferase (GSTP)-positive hepatic hyperplastic lesions. In this study, we have examined the effect of the hormone triiodothyronine (T3), which, similarly to peroxisome proliferators, is a strong liver mitogen and a ligand of nuclear receptors, on the growth of GSTP-positive nodules generated by the resistant hepatocyte model and on the development of hepatocellular carcinoma. Hepatic hyperplastic nodules were induced in male Fischer rats by a single dose (150 mg/kg) of diethylnitrosamine, followed by a 2-week exposure of the animals to 2-acetylaminoﬂuorene and partial hepatectomy. Nine weeks after diethylnitrosamine administration, rats were switched to a diet containing 4 mg/kg T3 for 1 week (experiment 1) and sacrificed during T3 feeding or were exposed to seven cycles of T3-supplemented diet (1 week/month per 7 months), and sacrificed 6 months after the last cycle (experiment 2). Results showed that T3 treatment for 1 week caused a 70% reduction in the number of GSTP-positive nodules (14/cm² in T3-fed rats versus 44/cm² of control animals), as well as GSTP-positive area (12% versus 43% of controls). Reduction in the number of GSTP-positive nodules observed 1 week after T3 feeding was associated with a strong increase in the labeling index of enzyme-altered nodules compared with that of controls (labeling index was 64 and 31%, respectively). No signiﬁcant differences in the apoptotic index were observed between the two groups. Results from experiment 2 did reveal that although rats treated with diethylnitrosamine + 2-acetylaminoﬂuorene developed 100% hepatocellular carcinoma and 33% of them showed lung metastasis, only 50% of rats exposed to repeated cycles of triiodothyronine developed hepatocellular carcinoma with no lung metastasis. This study indicates that cell proliferation per se might not necessarily represent a promoting condition for putative preneoplastic lesions and demonstrates an anticarcinogenic effect of T3.

INTRODUCTION

Liver cell proliferation is considered to play an important role in the several steps of carcinogenic process, initiation, promotion, and progression (1). Although the exact mechanism by which cell proliferation plays a role in initiation is not known, its involvement in events such as fixation of a miscoding lesion in the newly made DNA has been entertained (2–5). A second site at which cell proliferation exerts a critical effect is the promotion of carcinogen-initiated cells. Increased incidence of preneoplastic lesions and tumors has been observed when carcinogen treatment was followed by compensatory regeneration induced by repeated PH or multiple treatment with necrogenic agents (6–8). On the contrary, the effect of agents that cause liver hyperplasia without previous cell loss/death (primary mitogens) on the growth of carcinogen-induced preneoplastic hepatoctyes is far less clear. Indeed, although long-term treatment with PPs3 gives rise to HCC (9–11), a short-term treatment with PPs results in a decreased number and size of preneoplastic lesions induced by genotoxic carcinogens and identified by either adenosine triphosphate, γ-glutamyl transpeptidase, or GSTP staining (12–14). The latter effect has been attributed to the inhibitory action of these agents on the marker enzyme used to identify preneoplastic lesion (14) and not to a real growth-inhibitory effect. However, Chen et al. (15) have shown that a short-term exposure to the PP ciprofibrate reduced from 40% to <5% the L.I. of preneoplastic nodules generated by the R-H protocol (16), and this effect was associated with a rapid decrease in area and number of the nodules. This raises the important issue as to whether induction of liver cell proliferation is always a promoting condition for preneoplastic lesions or whether the nature of the proliferative stimulus is important in determining the type of response of carcinogen altered cells.

The hormone T3 resembles PPs in that: (a) it is a ligand of a nuclear receptor (TRs) of the same superfamily of steroid hormone nuclear receptors (17); (b) it is an inducer of peroxisome proliferation (18); and (c) it is a potent hepatomitogen (19, 20). However, the effect of T3 on the hepatocarcinogenic process is not known. Recently, evidence was obtained in our laboratory that a short exposure to a mitogenic dose of T3 exerts an inhibitory effect on the number of DENA-induced GSTP-positive hepatocytes. To further investigate the effect of hepatocyte proliferation induced by primary mitogens on the growth of carcinogen-induced putative preneoplastic lesions, we have examined the effect of T3 on the progression of hepatic nodules, induced in rats by the R-H model, to HCCs.

The results demonstrate that T3 administration for 1 week, despite its powerful mitogenic capacity, resulted in a 70% reduction of the number of GSTP-positive lesions with no increase in the size of the remaining nodules. In addition, repeated exposures of nodule-bearing rats to T3 caused a 50% reduction in the incidence of HCCs and 100% inhibition of lung metastasis. Our data add further support to the notion that the growth response of preneoplastic lesions is dependent upon the nature of the proliferative stimulus and indicate that T3 possesses anticarcinogenic effect in the liver.

MATERIALS AND METHODS

Animals. Male Fischer F-344 (100–125 g) purchased from Charles River (Milano, Italy) were maintained on a laboratory diet (Ditta Mucedola, Milano, Italy). The animals were given food and water ad libitum with a 12-h light/dark daily cycle and were acclimatized for 1 week before the start of the experiment.

Guidelines for the Care and Use of Laboratory Animals were followed during the investigation. DENA, T3, and 2-AAF were purchased from Sigma Chemical Co. (St. Louis, MO).

Experimental Protocol I. Rats were injected i.p. with a single dose of diethylnitrosamine (DENA), dissolved in saline, at the dose of 150 mg/Kg body weight (Fig. 1). After a 2-week recovery period, rats were placed on a

Received 8/17/99; accepted 12/2/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 Supported in part by funds from Associazione Italiana Ricerca sul Cancro (Milano, Italy) and Ministero dell’Università e della Ricerca Scientiﬁca e Tecnologica (MURST Coin ex-40% and 60%), Rome, Italy.

To whom requests for reprints should be addressed, at Dipartimento di Toxicologia, Sezione di Oncologia e Patologia Molecolare, Via Porcella 4, 09124 Cagliari, Italy. Phone: 011-39-070-6758345; Fax: 011-39-070-666062.

3 The abbreviations used are: PP, peroxisome proliferator; T3, 3,5,3'-triiodothyronine; GSTP, glutathione S-transferase; DENA, diethylnitrosamine; 2-AAF, 2-acetylaminofluorene; R-H, resistant hepatocyte; BrUrd, bromodeoxyuridine; L.I., labeling index; A.I., apoptotic index; PH, partial hepatectomy; HCC, hepatocellular carcinoma.

Unpublished data.

603
INHIBITORY EFFECT OF T3 ON RAT LIVER CANCER DEVELOPMENT

DENA

2-AAF

PH

T3

Kill

Fig. 1. Schematic representation of the experimental protocol 1. Rats were injected i.p. with a single dose of DENA (150 mg/kg). After 2 weeks, rats were placed on a diet containing 0.02% 2-AAF for 2 weeks and after the first week were subjected to two-thirds PH. Five weeks after the end of 2-AAF feeding, rats were either maintained on a basal diet or switched to a diet containing 4 mg/kg of T3. All animals were sacrificed 7 days after starting the T3 diet.

DENA

2-AAF

PH

T3

Kill

Fig. 2. Schematic representation of the experimental protocol 2. Rats were subjected to the initiation-promotion regimen as described in experimental protocol 1. Five weeks after release from 2-AAF diet, rats were either maintained on a basal diet or exposed to seven cycles of T3-supplemented diet (1 week/month/7 months). Rats were then placed on a basal diet and sacrificed 6 months later (16 months after initiation).

RESULTS

Previous studies have described a mitogenic activity of a single dose of T3 in rat liver (19). Preliminary experiments performed with various T3 concentrations revealed that feeding a diet supplemented with 4 mg/kg of T3 for 1 week induced a 10-fold increase in synthetic activity of hepatic DNA (data not shown). Subsequent immunohistochemical studies showed that hepatocyte L.I., following 1-week exposure to this concentration of T3, was approximately 31% versus 4% of controls (Fig. 3). No evidence of liver cell damage could be observed throughout the experimental period. The concentration of 4 mg/kg of T3 was selected for all future experiments.

Effect of T3 on the Number and Size of GSTP-positive Nodules.

In agreement with previous data (23), livers from rats exposed to DENA + AAF + PH and sacrificed 6 weeks after release of 2-AAF with 2 N HCl for 1 h and incubated with trypsin 0.1% for 20 min and then with normal goat serum for 20 min at room temperature. The sections were then incubated for 2 h with an anti-BrdUrd monoclonal antibody (1:200) and for 30 min with peroxidase goat antimouse IgG. The sites of peroxidase binding were detected with diaminobenzidine. A segment of duodenum, an organ with a high rate of cell proliferation, was included from each rat to confirm delivery of the DNA precursor. The location of GSTP in the liver was determined by using an antirat GSTP polyclonal antibody (Biotrin, Dublin, Ireland) and Dako Envision alkaline phosphatase goat antirabbit (K4018; Dako Corp.). The sites of phosphatase binding were detected with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate system (K598, Dako Corp.).

Measurement of GSTP-positive Nodules. GSTP-positive foci were measured with a computer-assisted image processor, programmed for the three-dimensional calculation by Campbell et al. (21). Only foci >76 μm in diameter were measured.

Determination of Labeling Index. Random microscopic fields were scored for BrdUrd-positive hepatocytes within GSTP-positive lesions. The L.I. was calculated as BrdUrd-positive hepatocyte nuclei/100 hepatocyte nuclei. At least 2500 hepatocytes/rat were scored. The same procedure was used to obtain the L.I. for surrounding GSTP-negative hepatocytes.

Classification of Liver Tumors. Histological typing of liver tumors was performed according to the classification proposed in “Histological Typing of Liver Tumors of the Rat” (22).

Statistical Analysis. Comparison between treated and control group was performed by Student’s t test.

with 2 N HCl for 1 h and incubated with trypsin 0.1% for 20 min and then with normal goat serum for 20 min at room temperature. The sections were then incubated for 2 h with an anti-BrdUrd monoclonal antibody (1:200) and for 30 min with peroxidase goat antimouse IgG. The sites of peroxidase binding were detected with diaminobenzidine. A segment of duodenum, an organ with a high rate of cell proliferation, was included from each rat to confirm delivery of the DNA precursor. The location of GSTP in the liver was determined by using an antirat GSTP polyclonal antibody (Biotrin, Dublin, Ireland) and Dako Envision alkaline phosphatase goat antirabbit (K4018; Dako Corp.). The sites of phosphatase binding were detected with 5-bromo-4-chloro-3-indolyl phosphatase/nitro blue tetrazolium substrate system (K598, Dako Corp.).

Measurement of GSTP-positive Nodules. GSTP-positive foci were measured with a computer-assisted image processor, programmed for the three-dimensional calculation by Campbell et al. (21). Only foci >76 μm in diameter were measured.

Determination of Labeling Index. Random microscopic fields were scored for BrdUrd-positive hepatocytes within GSTP-positive lesions. The L.I. was calculated as BrdUrd-positive hepatocyte nuclei/100 hepatocyte nuclei. At least 2500 hepatocytes/rat were scored. The same procedure was used to obtain the L.I. for surrounding GSTP-negative hepatocytes.

Classification of Liver Tumors. Histological typing of liver tumors was performed according to the classification proposed in “Histological Typing of Liver Tumors of the Rat” (22).

Statistical Analysis. Comparison between treated and control group was performed by Student’s t test.

Fig. 2. Schematic representation of the experimental protocol 2. Rats were subjected to the initiation-promotion regimen as described in experimental protocol 1. Five weeks after release from 2-AAF diet, rats were either maintained on a basal diet or exposed to seven cycles of T3-supplemented diet (1 week/month/7 months). Rats were then placed on a basal diet and sacrificed 6 months later (16 months after initiation).

with 2 N HCl for 1 h and incubated with trypsin 0.1% for 20 min and then with normal goat serum for 20 min at room temperature. The sections were then incubated for 2 h with an anti-BrdUrd monoclonal antibody (1:200) and for 30 min with peroxidase goat antimouse IgG. The sites of peroxidase binding were detected with diaminobenzidine. A segment of duodenum, an organ with a high rate of cell proliferation, was included from each rat to confirm delivery of the DNA precursor. The location of GSTP in the liver was determined by using an antirat GSTP polyclonal antibody (Biotrin, Dublin, Ireland) and Dako Envision alkaline phosphatase goat antirabbit (K4018; Dako Corp.). The sites of phosphatase binding were detected with 5-bromo-4-chloro-3-indolyl phosphatase/nitro blue tetrazolium substrate system (K598, Dako Corp.).

Measurement of GSTP-positive Nodules. GSTP-positive foci were measured with a computer-assisted image processor, programmed for the three-dimensional calculation by Campbell et al. (21). Only foci >76 μm in diameter were measured.

Determination of Labeling Index. Random microscopic fields were scored for BrdUrd-positive hepatocytes within GSTP-positive lesions. The L.I. was calculated as BrdUrd-positive hepatocyte nuclei/100 hepatocyte nuclei. At least 2500 hepatocytes/rat were scored. The same procedure was used to obtain the L.I. for surrounding GSTP-negative hepatocytes.

Classification of Liver Tumors. Histological typing of liver tumors was performed according to the classification proposed in “Histological Typing of Liver Tumors of the Rat” (22).

Statistical Analysis. Comparison between treated and control group was performed by Student’s t test.

Fig. 3. L.I. of rat hepatocytes after 1 week exposure to a T3-supplemented diet. All rats were given BrdUrd (1 mg/ml) in drinking water for 7 days. At least 5000 hepatocyte nuclei/rat were scored. L.I. was expressed as the number of BrdUrd-positive hepatocyte nuclei/100 nuclei. Results are expressed as means of five animals/group; bars, SE. Significantly different from controls at P < 0.001.
showed the presence of several macroscopically evident, white nodules merging from the surface. Immunohistochemically, two types of nodules could be easily identified: those characterized by a uniform GSTP staining and therefore classified as persistent nodules; and those showing a progressive loss of GSTP staining (remodeling nodules, 22 per cm²). The total number of GSTP-positive nodules was 41 per cm² (Table 1). Feeding T3 for 1 week caused a dramatic change in the macroscopic appearance of the liver. Indeed, most of T3-fed rats exhibited a liver characterized by a smooth surface, with only a few protruding nodules. Accordingly, histological observation of liver sections stained with H&E did reveal the presence of very few nodules in T3-fed rats, in contrast with the large number found in DENA + 2-AAF + PH group. Quantification of the number of GSTP-positive nodules in T3-treated rats showed a 3-fold reduction in their number (from 41 to 14; Table 1). Reduction of the number of GSTP-positive nodules was accompanied by a decrease in the percentage of area occupied by GSTP-positive hepatocytes (Fig. 4 and Table 1).

Interestingly, the reduction in the number of GSTP-positive nodules by T3 was associated with an increased proliferative activity of hepatocytes both in the residual GSTP-positive nodules (L.I., 64% versus 42% of controls) as well as in surrounding liver (L.I., 31% versus 7% of controls). Size distribution of the nodules in the two groups (Fig. 5) showed that no selective growth of some subpopulations of putative preneoplastic nodules had occurred in T3-treated rats.

**Effect of T3 on L.I. of GSTP-positive Nodules.** The above results clearly show that T3 feeding for 1 week, despite its mitogenic potency, exerted an inhibitory effect on the number of GSTP-positive nodules. To determine whether the disappearance of the vast majority of the nodules observed in T3-fed rats could have occurred as a consequence of an initial inhibition of hepatocyte proliferation within the nodules, rats treated as described in experimental protocol 1 were given BrdUrd in drinking water (24) and sacrificed 2, 4, and 7 days after starting of T3 diet. As shown in Table 2, although T3 feeding for 1 week exerted a strong inhibitory effect on the number of GSTP-positive nodules, confirming the results presented in Table 1, treatment with T3 for only 2 or 4 days did not cause any significant change in number and/or size of GSTP-positive nodules. L.I. of hepatocytes was determined by analyzing the number of BrdUrd-positive hepatocyte nuclei within the GSTP-positive nodules, using a double immunohistochemical technique. As shown in Fig. 6 and Table 3, feeding of T3 for 2 days caused a striking increase in L.I. of GSTP-positive nodules compared with that of nodules from rats maintained on a basal diet (51 and 12%, respectively); the range of L.I. was 47–57% in nodules from T3-treated rats versus 10–24% of controls. An increased L.I. in T3-liver nodules was also observed at 4 days (55% versus 21% of controls). As mentioned above, despite the increased L.I., no difference in the percentage of GSTP-positive area, either in the mean area of GSTP-positive nodules between T3-fed rats or the control group, was observed at 2 and 4 days. Moreover, when distribution analysis of nodule size was performed, no indication of a shift of T3 nodules to higher classes was observed (Fig. 7).

**Effect of T3 on A.I. of GSTP-positive Nodules.** On the basis of the findings that liver nodules generated by several promoting proto-

### Table 1. Effect of T3 on the number, size, and DNA synthesis of GSTP-positive nodules in rat liver initiated by DENA

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of GSTP⁺ nodules/cm²</th>
<th>Mean area GSTP⁺ nodules (mm²)</th>
<th>% area GSTP⁺</th>
<th>L.I. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAF + PH</td>
<td>41.3 ± 3.2a</td>
<td>1.57 ± 0.23</td>
<td>43.6</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>AAF + PH + T3</td>
<td>14.4 ± 2.7b</td>
<td>1.20 ± 0.36</td>
<td>12.5</td>
<td>31 ± 4b</td>
</tr>
</tbody>
</table>

*a* Mean ± SE of six animals/group of one representative experiment. Similar results were obtained in three different experiments. BrdUrd was given for the last 7 days using a Minipump delivery system. For determination of L.I., at least 4000 hepatocytes/rat were counted. 

*b* Significantly different from AAF + PH, P < 0.001.

*c* Significantly different from AAF + PH, P < 0.050.
INHIBITORY EFFECT OF T3 ON RAT LIVER CANCER DEVELOPMENT

Increased proliferative activity, liver weight in T3-treated rats was lower than controls (3.7 g/100 g body weight versus 4.7 g/100 g of controls), most probably as a consequence of the profound depletion of glycogen caused by the hormone and leading to an overall decrease in cellular size.

Effect of T3 on the Incidence of HCCs. To determine whether the inhibitory effect of T3 on GSTP-positive nodules could result in a decreased incidence of HCC, nodule-bearing rats were exposed to seven cycles of T3 (1 week/month for 7 months) and sacrificed 16 days after starting the T3 diet. Rats were sacrificed 2, 4, and 7 days after starting the T3 diet.

Table 2. Number and size of GSTP-positive nodules in rat liver 2, 4, and 7 days after administration of T3 or basal diet, following the R-H Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Persistent GSTP⁺ nodules (no/cm²)</th>
<th>Mean area of GSTP⁺ nodules (mm²)</th>
<th>% GSTP⁺ area</th>
</tr>
</thead>
<tbody>
<tr>
<td>DENA + AAF + PH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>47 ± 3.9a</td>
<td>0.87 ± 0.24</td>
<td>47</td>
</tr>
<tr>
<td>4 days</td>
<td>35 ± 6.3</td>
<td>0.95 ± 0.14</td>
<td>42</td>
</tr>
<tr>
<td>7 days</td>
<td>35 ± 6.0</td>
<td>1.14 ± 0.05</td>
<td>49</td>
</tr>
<tr>
<td>DENA + AAF + PH + T3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>55 ± 6.3</td>
<td>0.78 ± 0.12</td>
<td>61</td>
</tr>
<tr>
<td>4 days</td>
<td>31 ± 5.9</td>
<td>0.87 ± 0.13</td>
<td>32</td>
</tr>
<tr>
<td>7 days</td>
<td>8 ± 2.1e</td>
<td>0.82 ± 0.30</td>
<td>9</td>
</tr>
</tbody>
</table>

*Mean ± SE of at least four rats/group.

**Significantly different from AAF + PH; P < 0.001.
methionine, potent inhibitors of carcinogenesis, appear to exert their anticarcinogenic effect by inducing an increased apoptotic incidence in preneoplastic lesions (42–44). Our results, however, did not sup-
port a significant role for apoptosis in the regression of GSTP-positive
nodules induced by T3. Indeed, although it is conceivable that the higher A.I. observed 2 days after T3 might in part explain the lack of increase in nodule size at that particular time point (compared with controls), no difference in A.I. between T3-fed and control rats was observed at later time points.

Although, at the present, it is difficult to draw any conclusion, at least two possibilities can be envisaged to explain the dramatic re-
duction in the number of nodules observed in T3-fed rats, despite the concomitant increase in hepatocyte proliferation: (a) the loss of GSTP-positive foci is attributable to inhibition of GSTP expression by T3, similarly to what was proposed for PPs (14), and not a to a real

Table 3 Effect of T3 on L.I. and A.I. of GSTP-positive nodules after T3 administration

Five weeks after 2-AAF release, rats were fed T3 for 1 week and given BrdUrd (1 mg/ml) in drinking water. Rats were sacrificed 2, 4, and 7 days after starting the T3 diet. For determination of L.I. and A.I., at least 2500 and 3500 nodule hepatocytes/rat, respectively, were counted.

<table>
<thead>
<tr>
<th>Group</th>
<th>L.I. %</th>
<th>A.I. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>DENA + AAF + PH</td>
<td>12 ± 4</td>
<td>2.38 ± 0.28</td>
</tr>
<tr>
<td>2 days</td>
<td>21 ± 2</td>
<td>2.04 ± 0.19</td>
</tr>
<tr>
<td>4 days</td>
<td>31 ± 5</td>
<td>3.18 ± 0.85</td>
</tr>
<tr>
<td>7 days</td>
<td>59 ± 7</td>
<td>2.19 ± 0.75</td>
</tr>
<tr>
<td>DENA + AAF + PH + T3</td>
<td>2 days</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>4 days</td>
<td>55 ± 5</td>
<td>2.05 ± 0.22</td>
</tr>
<tr>
<td>7 days</td>
<td>68 ± 4</td>
<td>1.48 ± 0.29</td>
</tr>
</tbody>
</table>

Table 4 Effect of repeated dietary T3 treatments on the incidence of HCC and lung metastasis

Rats initiated with DENA were then subjected to the R-H model to generate GSTP-positive nodules. Five weeks after 2-AAF release (9 weeks after DENA), rats were subjected to cycles of T3 (1 week/month for 7 months). Rats were then placed on basal diet and sacrificed 6 months later.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>HCC %</th>
<th>Lung metastasis %</th>
</tr>
</thead>
<tbody>
<tr>
<td>DENA + AAF + PH</td>
<td>15</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>DENA + AAF + PH + T3</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>
reduction in nodule number; (b) T3, in addition to its mitogenic capacity, might also interfere with the differentiation program by inducing retrodifferentiation of the nodular hepatocytes to normal-appearing cells via loss of their “resistant” phenotype, a rearrangement to single-cell plates, and an integration into the organizational pattern of the surrounding liver (23). The former possibility (direct inhibition by T3 on \( GSTP \) gene expression) appears very unlikely, in virtue of the following: (a) reduction in nodules number was confirmed by analysis of sections stained with H&E; (b) macroscopic observation revealed that livers from T3-fed rats were characterized by a smooth surface, with only a few protruding nodules; and (c) short-term T3 administration did not exert any inhibitory effect on GSTP mRNA or protein levels induced in rat liver by the known GSTP-inducer lead nitrate.4

Thus, remodeling (or regression) induced through a redifferentiation program appears to be the most likely explanation for the loss of hepatic nodules caused by T3. T3 is known to possess a nuclear receptor (TRs) of the same superfamily of receptors of PPs (peroxisome proliferator activated receptors), and retinoic acids (retinoic acid receptors and retinoid X receptors), and these receptors have been shown to exert a profound effect on cellular differentiation (45–47).

The observation that the histological appearance of HCCs seen in rats treated with T3 was different from that of rats without T3 also suggests that this hormone, via interaction with and activation of its receptor, may have effects on differentiation of putative preneoplastic hepatocytes.

A second and even more important result achieved by this study was the reduction of HCC development and inhibition of metastases to the lung in rats exposed to repeated cycles of T3. To our knowledge, it has been extremely difficult thus far to reduce the progression of hepatic nodules generated by genotoxic agents to HCC. The fact that the anticarcinogenic effect played by T3 is associated with several cycles of hepatocyte proliferation further supports the notion that cell proliferation \textit{per se} may not necessarily represent a carcinogenic and/or promoting condition. On the other hand, our data suggest that certain proliferative stimuli may play an anticarcinogenic effect, probably by causing a redifferentiation of preneoplastic cells.

**REFERENCES**

INHIBITORY EFFECT OF T3 ON RAT LIVER CANCER DEVELOPMENT


Cell Proliferation Induced by Triiodothyronine in Rat Liver Is Associated with Nodule Regression and Reduction of Hepatocellular Carcinomas

Giovanna M. Ledda-Columbano, Andrea Perra, Roberto Loi, et al.

Cancer Res 2000;60:603-609.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/3/603

Cited articles
This article cites 44 articles, 30 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/3/603.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/60/3/603.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.