Increase in the Active Form of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in Human Hepatocellular Carcinoma: Possible Mechanism for Alteration of Cholesterol Biosynthesis

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ABSTRACT

3-Hydroxy-3-methylglutaryl coenzyme A reductase activity and the rate of sterol biosynthesis are positively correlated with DNA synthesis and proliferation of mammalian cells. The total (active plus latent) activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase and the activity of its active form in hepatocellular carcinoma (HCC) from seven patients were measured and compared with those in liver tissue from five control subjects. The activity of the active form in HCC was 61 ± 21 (SD) pmol/min/mg microsomal protein, while it was only 17 ± 9.8 pmol/min/mg in the liver tissue from the controls; the difference was significant (P < 0.005). The total activity of the reductase was also higher in HCC although the difference was not significant. The microsomal contents of the enzyme protein also were not significantly different. The rate of cholesterol biosynthesis was 307 ± 81 pmol/h/mg tissue in HCC and 79.6 ± 52 in normal liver tissue, indicating a significant increase in the rate in HCC (P < 0.001). Thus, enhanced synthesis of cholesterol in human HCC seems to result partly from an increase in the active form of the reductase.

INTRODUCTION

HMG-CoA reductase [mevalonate:NADP oxidoreductase (CoA-acylating), EC 1.1.1.34] is the major rate-limiting enzyme in cholesterol biosynthesis in mammalian cells (1–3). HMG-CoA reductase activity and the rate of sterol synthesis have been shown to be positively correlated with DNA synthesis and proliferation of cells (4, 5). Extensive data have been acquired which suggest that the enzymic activity of HMG-CoA reductase and cholesterol biosynthesis are regulated through three distinct basic mechanisms: (a) long-term control which involves the modulation of enzymic activity by changes in HMG-CoA reductase concentration through transcriptional control as well as by enzyme degradation (6–9); (b) modulation of HMG-CoA reductase activity or degradation by alterations in the composition and fluidity of the microsomal membrane (10, 11); and (c) short-term regulation by a biologic cascade system involving reversible phosphorylation of both HMG-CoA reductase and reductase kinase (12–15).

In the liver, HMG-CoA reductase activity is suppressed by dietary cholesterol that enters hepatocytes as a result of the receptor-mediated uptake of chylomicron remnants. The cholesterol negative feedback system is known to be absent from liver tumor (16). The cholesterol feedback defect in malignant cells has been located at the site of HMG-CoA reductase action (16–18). However, Brown et al. (19) reported that HMG-CoA reductase isolated from tumor cells has the same cold sensitivity, as well as kinetic and structural properties identical to those of the HMG-CoA reductase isolated from normal cells. In addition, the enzyme from tumors appears to be antigenically very similar to that from the liver (20).

It has been suggested recently that interconversion of HMG-CoA reductase between active and latent forms through phosphorylation-dephosphorylation could be associated with changes in the rate of cellular proliferation in cell cultures (21). It would be of interest to know how the interconversion of the reductase between the active and latent forms and cholesterol biosynthesis are regulated in relation to autonomous cell proliferation in malignant cells. Feingold et al. (22) have demonstrated that the activation state of HMG-CoA reductase in three minimal deviation hepatomas of rat is consistently higher than that observed in normal liver. However, it is not known whether this altered activation state of the reductase in malignant tumors is correlated with the biosynthesis rate of cholesterol.

In addition to serving as a precursor for the structural cholesterol requirements of cell proliferation, mevalonic acid production by HMG-CoA reductase seems to regulate cell growth, independent of cholesterogenesis, by playing a direct role in DNA synthesis (23). Thus, regulation of the altered activation state of the reductase in HCC may encourage a novel approach for suppressing tumor growth through a decrease in the rate of cholesterol biosynthesis and inhibition of DNA synthesis.

Regulation of HMG-CoA reductase by phosphorylation-dephosphorylation in human HCC has not been studied. To clarify the relationship between the interconversion of the reductase and cholesterol biosynthesis, we measured the total activity of the reductase, its active form activity, and the amount of the enzyme protein immunoquantitated by dot-blotting in human HCC and liver tissue in comparison with the rate of cholesterol biosynthesis.

MATERIALS AND METHODS

Samples of HCC and Liver. Samples of HCC and liver were obtained from seven patients at hepatectomy. These patients, who did not show hypercholesterolemia, had not undergone any treatment such as transcatheter arterial embolization before surgery. The histology of the tumors was well-differentiated HCC in three cases and moderately differentiated HCC for the other four. Histological examination for underlying liver disease showed chronic hepatitis in four patients and hepatic cirrhosis in three. HCC and liver tissue resected were quickly separated with scissors. Part of the homogeneous HCC was placed immediately on ice and used for the isolation of microsomes within 4 h of surgery as described by Beg et al. (24). The other part of the same HCC nodule was immediately frozen and stored at −80°C until use for assay of cholesterol biosynthesis from acetate. Liver tissue resected from the seven patients was handled in the same way. Normal liver tissues from five patients without HCC were used as normal controls and prepared in the same way.

Preparation of Microsomes. Fresh HCC and liver tissue (0.3 g/ml) were homogenized in 50 mm potassium phosphate buffer (pH 7.4) containing 10 mm EDTA and 5 mm DTT in the presence of 50 mm NaF or NaCl. The homogenate was centrifuged at 10,000 × g for 20 min. The supernatant was centrifuged at 105,000 × g for 1 h to obtain the microsomal fraction.

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1To whom requests for reprints should be addressed.

2The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HCC, hepatocellular carcinoma.
min and then the supernatant was further centrifuged at 105,000 × g for 60 min to obtain microsomes as described previously (25–27), except that phosphate buffer containing 50 mM NaF or NaCl was used as the homogenate buffer. The microsomal pellets were washed once in the same buffer and stored frozen at −80°C.

Assay of HMG-CoA Reductase Activity. The activity of HMG-CoA reductase was assayed by a modification of the method of Shapiro et al. (28). Aliquots of the microsomal suspension (200–400 μg of protein) were diluted to 100 μl with 50 mM potassium phosphate buffer (pH 7.4) containing 10 mM EDTA and 5 mM diethiothreitol in the presence of 50 mM NaF or NaCl and preincubated in 1.5-ml Eppendorf tubes for 5 min at 37°C. The assay was conducted by addition of 50 μl of the cofactor-substrate cocktail containing 4.5 μmol of glucose 6-phosphate, 0.3 IU of glucose-6-phosphate dehydrogenase, 450 nmol of NADPH, 50 nmol of D,L-hydroxymethyl-3-[14C]glutaryl-CoA, and 20,000 dpm D,L-[2-3H]mevalonolactone. The incubation was continued for 15 min and then terminated by the addition of 10 μl of 6 N HCl. Labeled mevalonolactone was separated by thin layer chromatography (Whatman LK5D plate) in acetonitrile:toluene (1:1). The [3H]-plus [14C]mevalonolactone was counted in 4 ml of liquid scintillator. All incubations were carried out in duplicate. The values described represent the average of the duplicate assays.

Assay of Cholesterol Synthesis from [14C]Acetate. Tissue slices of HCC and liver tissue approximately 1 mm thick were prepared in chilled Krebs-Ringer bicarbonate buffer (pH 7.4). The rate of cholesterol synthesis from [14C]acetate was assayed as cholesterol digitonide according to the method described by Siperstein and Fagan (29, 30). Lipids were extracted from HCC and liver tissue approximately 1 mm thick were prepared in phosphate-buffered saline/0.5% (w/v) low fat, dried skimmed milk/0.2% (v/v) Triton X-100 containing 0.1% sodium azolylbenzene solution in toluene, and the 14C content was determined by gas-liquid chromatography as described previously (32).

Statistical Analysis. Statistical analyses of the data were done with the Student t test. Chemicals and Reagents. D,L-Hydroxymethyl-3-[14C]glutaryl-CoA, D,L-[2-3H]mevalonolactone, and [2-14C]acetate were purchased from New England Nuclear. D,L-[2-3H]labeled donkey anti-rabbit IgG was purchased from Amersham Corp. Mevalonic acid, HMG-CoA, and NADPH were obtained from Sigma. Anti-reductase antiserum was a generous gift from Professor Ishaiahu Shechter from the Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel. All other chemicals were of the best grade available commercially.

RESULTS

Cholesterol Content in HCC and Liver Tissue. The cholesterol content was 3.85 ± 0.66 (SD) mg/g tissue in HCC, but 1.87 ± 0.62 in the liver tissue from the patients and 2.22 ± 0.76 in the tissue from the controls, indicating that the content is higher in HCC (P < 0.001 and < 0.005, respectively).

Activation State of HMG-CoA Reductase in HCC and Liver Tissue. The HMG-CoA reductase activity in the microsomes isolated in sodium fluoride is representative of the actual enzyme activity in vivo (active form of the reductase), while the activity in the microsomes isolated in sodium chloride represents an index of the total tissue reductase enzyme content (total HMG-CoA reductase activity). The total activity of the reductase in the microsomes from HCC was 124 ± 53 pmol/min/mg microsomal protein, while that from the liver tissue was 55 ± 24 in the patients and 73 ± 39 in the controls (Table 1). Thus, the total activity was higher in HCC but not significantly different between HCC and normal liver tissue. The activity of the form in the microsomes from HCC was 61 ± 21 pmol/min/mg microsomal protein, while that from the liver tissue was 12 ± 6.5 in the patients and 17 ± 9.8 in the controls, indicating a significant increase of the active form in HCC compared to that in liver tissue from the patients and the controls (P < 0.001 and < 0.01, respectively). The ratio of the active form to the total reductase activity was 0.52 ± 0.11 in HCC, while that from the liver tissue was 0.23 ± 0.09 in the patients and 0.23 ± 0.19 in the controls, indicating a significant increase in the ratio in HCC compared to that in liver tissue from the patients and the controls (P < 0.001 and < 0.01, respectively). These data showed that the active form of the reductase significantly increased in HCC.

DISCUSSION

Short-term regulation of the enzymic activity of human HMG-CoA reductase involves a bicyclic cascade system of phosphorylation-dephosphorylation of the reductase and reductase kinase. Beg et al. (13) reported the presence of human hepatic HMG-CoA reductase in active and latent forms and the in vitro modulation of its enzyme activity by reversible phosphorylation. The ratio of the active form to the total reductase activity in liver tissue from the five control subjects in this study was similar to that found by Beg et al. (13). This indicates that the function of the cascade system of phosphorylation-dephosphorylation of the reductase was reliably assessed in the liver tissue.

The mean value of the total activity of the reductase in the liver tissue from the controls was 73 pmol/min/mg microsomal protein (range, 32–122), which is similar to those in human
Table 1 Activation state of HMG-CoA reductase in hepatocellular carcinoma and liver tissue

<table>
<thead>
<tr>
<th>HMG-CoA reductase activity (pmol/min/mg microsomal protein)</th>
<th>Hepatocellular carcinoma</th>
<th>Liver tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+NaF*</td>
<td>+NaF*</td>
</tr>
<tr>
<td>Case 1</td>
<td>59</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>189</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>135</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>171</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>162</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>61 ± 21*</td>
<td>124 ± 53*</td>
</tr>
</tbody>
</table>

| Controls                                               |                          |              |            |
|--------------------------------------------------------|--------------------------|--------------|
|                                                         | +NaF                     | +NaCl*       | NaF/NaCl*  |
| Case 1                                                  | 8.4                      | 56           | 0.15       |
| 2                                                      | 23                       | 48           | 0.42       |
| 3                                                      | 9.8                      | 122          | 0.08       |
| 4                                                      | 31                       | 107          | 0.29       |
| 5                                                      | 12                       | 32           | 0.38       |
| Mean ± SD                                              | 17 ± 9.8                 | 73 ± 39      | 0.23 ± 0.19 |

* Active form of HMG-CoA reductase.
* Total HMG-CoA reductase activity.
* Ratio of the active form to the total activity.
* P < 0.001 (hepatocellular carcinoma versus liver tissue from patients).
* P < 0.005 (hepatocellular carcinoma versus liver tissue from patients).
* P < 0.01 (hepatocellular carcinoma versus liver tissue from patients).
* Not significant (hepatocellular carcinoma versus liver tissue from controls).
* P < 0.01 (hepatocellular carcinoma versus liver tissue from controls).

We measured total and active form activities of the reductase in human HCC in comparison with those in the liver tissue. Resected HCC samples often contain necrotized tissue even if the tumors have not been treated with anticancer drugs or transcatheter arterial embolization before surgery. In this study, relatively little HCC was used to avoid contamination by necrotized tissue. Since the activity of HMG-CoA reductase and the rate of cholesterol biosynthesis appear to be different among tumor nodules even from the same patient (data not shown), we measured the activities and the rate using homogeneous HCC tissue.

The active form of the reductase significantly increased with the rising ratio of the active form to the total activity in HCC from the seven patients. No significant differences in total activity or the microsomal content of the enzyme protein were noted between HCC and normal liver tissue. This altered activation state of the reductase appears to be characteristic of HCC since the enzyme from fetal liver and rapidly regenerating liver cells showed an activation state similar to that of the enzyme from normal liver (20). On the other hand, the rate of cholesterol biosynthesis increased in HCC in comparison with that in the liver from the patients and the controls. These observations suggested that the enhanced cholesterol biosynthesis in HCC corresponded to the increase in the active form of the reductase rather than the total activity. Thus, the alteration of cholesterol biosynthesis in HCC seems to result partly from an increase in the active form of the reductase. This may be a fundamental difference between the HMG-CoA reductase of human HCC and that of normal liver tissue.

It has been suggested that HMG-CoA reductase activity and cholesterol biosynthesis decline with increasing cell density.
(21). This reduction of the cholesterol biosynthesis in relation to an increase in the latent form of the reductase seems to be related to growth restriction due to confluence of the cells. However, malignant tumors rapidly and autonomously proliferate without contact inhibition. Thus, malignant cells may express enhanced cholesterol biosynthesis in relation to an increase in the active form of the reductase, which, in turn, is related to rapid and autonomous growth.

The mechanism for the increase in the active form of HCC remains to be determined. One possibility is that the HMG-CoA reductase kinase or phosphatase system may be affected. On the other hand, HCC seems to lose dietary cholesterol feedback control because of the reduced numbers of cell membrane receptors that recognize chylomicron remnants (35, 40). However, measurements of the total enzyme activity and immunotitration with antibodies indicated that dietary cholesterol suppresses hepatic HMG-CoA reductase primarily by reducing the amount of enzyme protein rather than by affecting phosphorylation (1, 41, 42). Thus, the altered activation state of the reductase in human HCC does not seem to result from the reduced numbers of lipoprotein receptors (41).

In summary, more of the active form of HMG-CoA reductase was found with an elevated ratio of the active form to the total activity in HCC from the seven patients. Enhanced biosynthesis of cholesterol in HCC seems to result partly from an increase in the active form of HMG-CoA reductase, which is related to rapid and autonomous growth.

REFERENCES

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