Short Communication

Biosynthesis of Sterols and Dolichol in Human Hepatomas*

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Changes in membrane fluidity have been reported for cells from human tumor tissue and much interest has been focused on cholesterol and phospholipids in hepatomas, for which a disturbance in cholesterol metabolism has been observed.[1] The inhibition of HMG–CoA reductase activity, which is normally regulated via receptor-mediated uptake of LDL-cholesterol,[2] is lost in hepatoma-carrying animals. LDL also suppresses squalene synthetase,[3] which in part regulates the level of farnesyl pyrophosphate, while this intermediate in turn influences the levels of cholesterol, ubiquinone and dolichol.

Dolichol, which is found in high concentrations in human tissues, has been demonstrated to destabilize the lipid bilayer structure in model membranes.[4] Cellular concentrations of sterols and dolichols are therefore of great importance, since these substances influence the behaviour of cells and membranes. In the present study we have collected a number of highly differentiated human hepatocellular carcinomas directly after transplantation surgery.

Microsomes from normal human liver tissue and those isolated from the hepatocellular carcinomas were similar in many respects, since the highly differentiated malignant cells resemble control hepatocytes.[5]

In primary hepatocellular carcinomas the total dolichol content was clearly reduced relative to control hepatocytes, whereas the total cholesterol content demonstrated a clear increase (Table 1). This high cholesterol content is in agreement with earlier studies with hepatoma-bearing animals;[8] there are also reports of high serum levels of cholesterol and squalene in humans with hepatoma.[9] The level of ubiquinone is also drastically changed in the hepatomas, being less than half of the control value (Table 1).

These different alterations in the content of sterols and dolichols indicate that the levels of these compounds are independently regulated. In

Table 1. Dolichol, ubiquinone and cholesterol content in human liver and hepatomas. The values are the means of 5 experiments.

	Dolichol/μg g^{-1}	Ubiquinone/μg g^{-1}	Cholesterol/μg g^{-1}
Control	510	48	2050
Hepatocellular carcinoma	76	22	3600

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Table 2. HMG-CoA reductase activity in microsomal fractions from control human liver and from hepatocellular carcinomas. The values are the means of 4 experiments.

Microsomes	HMG-CoA reductase activity/pmol min^{-1} mg^{-1}
Control liver	40 (100 %)
Hepatocellular carcinoma	70 (175 %)

the case of the major regulatory enzyme of cholesterol synthesis, HMG–CoA reductase, the increased enzyme activity observed in the microsomal fraction from human hepatomas (Table 2) is in agreement with earlier findings on spontaneous mouse hepatomas,[8] and this increased activity partially explains the high cholesterol level. The divergent changes in sterol and dolichol levels indicate that the regulation in these cases occurs at a site more distal than the HMG–CoA reductase. Squalene synthetase has been suggested to be a regulatory enzyme in this respect,[2,3] and it is very probable that this enzyme, which is normally inhibited by LDL-cholesterol, is less inhibited in the tumor than in the control tissue. The increased rate of cholesterol synthesis, in contrast to the decreased rate of synthesis of dolichol and ubiquinone, is consistent with this hypothesis (Table 3).

In order to explain the low dolichol and ubiquinone levels and the relative differences in their rates of synthesis, one must propose a decrease in the content of farnesyl pyrophosphate and a flux of metabolites in the direction towards cholesterol, and/or altered enzyme activity in the dolichol and ubiquinone pathways distal to farnesyl pyrophosphate. The results clearly demonstrate independent regulation of the pathways for sterol and dolichol biosynthesis and it is suggested that loss of enzyme inhibition in hepatoma tissue is not restricted only to HMG–CoA reductase, but also occurs with enzymes more distal to this regulatory site.

Experimental

A portion of each liver tissue was subjected to histological examination and the remaining tissue was used immediately or frozen to −70 °C. The microsomal fractions obtained were characterized routinely using enzymatic and morphological methods.

Dolichol levels were measured by high performance liquid chromatography (HPLC), as described previously.[6,7]

After homogenisation and lipid extraction, cholesterol and ubiquinone were isolated on a C18 SEP-PAK® column, after which the lipid fraction was injected onto a Hypersil® column with a particle size of 3 μm (Hewlett Packard). The lipids were then separated by HPLC. In all cases appropriate synthetic compounds were used as internal standards to demonstrate that the observed peaks corresponded only to the expected lipids and to correct for incomplete recovery.

To measure the rates of cholesterol, ubiquinone and dolichol synthesis, homogenates of control liver and hepatocellular carcinomas were incubated with [^{3}H]-mevalonic acid at 37 °C for 45 min. The lipids were then extracted and isolated as above, and determined by HPLC and by scintillation counting.

In order to assay HMG–CoA reductase activity, the microsomal fraction was incubated with labelled HMG–CoA and the product mevalonolactone was subsequently extracted, isolated by

Table 3. Rates of cholesterol, ubiquinone and dolichol synthesis in homogenates of control liver and hepatocellular carcinomas. The values are the means of 5 experiments.

Tissue	Synthesis of: Cholesterol	Ubiquinone (cpm/mg protein)	Dolichol
Control liver	240 (100 %)	40 (100 %)	40 (100 %)
Hepatocellular carcinoma	600 (250 %)	21.2 (53 %)	4 (10 %)

thin-layer chromatography and determined by scintillation counting.

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References

1. Siperstein, M. D. and Fagan, V. *Cancer Res. 24* (1964) 1108.
2. Brown, M. S. and Goldstein, J. L. *J. Lipid Res. 21* (1980) 505.
3. Faust, J. R., Goldstein, J. L. and Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A. 76* (1979) 5018.
4. Valtersson, C., van Duyn, G., Verkleij, A. J., Chojnacki, T., de Kruijff, B. and Dallner, G. *J. Biol. Chem. 260* (1985) 2742.
5. Eriksson, L. C., Torndahl, U.-B. and Andersson, G. N. *Cancer Res. 43* (1983) 3335.
6. Eggens, I., Chojnacki, T., Kenne, L. and Dallner, G. *Biochim. Biophys. Acta 751* (1983) 355.
7. Eggens, I. and Elmberger, G. *Acta Chem. Scand., Ser. B 39* (1985) 326.
8. Chen, H. W., Kandutsch, A. A. and Heiniger, H. J. *Prog. Exp. Tumor Res. 22* (1978) 275.
9. Hirayama, C., Yamanishi, Y. and Irisaj, T. *Clin. Chim. Acta 91* (1979) 53.

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