Short Communication

Regulation of the Level of Dolichyl Phosphate in Human Hepatomas*

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It has been well documented that several human hepatocellular carcinoma cell lines are capable of synthesizing and secreting most of the plasma glycoproteins. However, some of these secreted proteins show defects in glycosylation.[1] Since glycoprotein levels and structure are of importance for cellular behaviour, it is of great interest to study the various steps in glycoprotein biosynthesis.

Phosphorylated dolichol participates in the synthesis of oligosaccharide chains linked through *N*-glycosidic bonds to proteins and it has been suggested that the level of this compound may be rate-limiting for protein glycosylation under certain conditions.[2,3] The detailed mechanisms involved in the regulation of the level of dolichyl phosphate (dolichyl-P) have, however, not yet been made clear. It has been reported that during the early stages of embryonic development, the process providing the main source of dolichyl-P is intracellular phosphorylation of the free alcohol.[4]

Under certain conditions the level of dolichyl-P is apparently regulated by the CTP-dependent dolichol kinase and by dolichol phosphatase.[4] In normal adult rat liver the major portion of the newly synthesized dolichyl-P was found to be formed by *de novo* synthesis.[5,8] In a recent study on primary cultures of rat hepatocytes, it was concluded that about 10 % of the newly formed dolichyl-P was produced by phosphorylation of the free alcohol.[6]

The purpose of the present study has been to compare the level and biosynthesis of dolichyl-P in highly differentiated human hepatomas and control livers. Microsomal fractions were also prepared from both control and hepatoma tissue. Since organelles in hepatomas may be altered in some fashion, the possibility that fragmented organelles might contaminate the microsomal fraction was considered. The microsomal fractions obtained were therefore examined by electron microscopy and analyzed with respect to protein and marker enzymes.[11] No major differences between the control tissue and hepatomas could be observed in the electron microscope or when lysosomal, plasma membrane, mitochondrial and microsomal enzyme activities were measured.

In homogenates, the dolichyl-P level of hepatomas did not differ from that of control livers,

Table 1. Dolichyl phosphate levels in healthy human liver and in human hepatoma tissue. The values are the means of 8 experiments.

Tissue		Dolichyl phosphate
Normal liver	homogenate[a]	15(1)
	microsomes[b]	0.12(1)
Hepatoma	homogenate[a]	15(1)
	microsomes[b]	0.23(2)

[a]μg per g wet weight. [b]μg per mg protein.

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Table 2. Rates of dolichyl phosphate synthesis in homogenates of healthy human liver and of hepatocellular carcinomas. The values are the means of 8 experiments.

Sample	Dolichyl monophosphate synthesis/ cpm per g wet weight per 40 min
Normal liver	$1.8(2)\times10^3$
Hepatoma	$9(2)\times10^1$

whereas the level of dolichyl-P in hepatoma microsomes was almost twice the level in the controls (Table 1). This finding indicates an altered subcellular distribution of the polyisoprenoid compounds in hepatoma tissue.

The rate of biosynthesis of dolichyl-P was measured by monitoring the incorporation of [^{3}H]mevalonic acid into the polyisoprenoid compounds, and was surprisingly low in the hepatomas (Tab. 2). When comparing the incorporation of [^{3}H]mevalonic acid in control tissue and hepatomas, the possibility of differences in the sizes of the mevalonate pool was considered. For this reason all experiments were performed using in creasing concentrations of unlabelled mevalonate. Under these conditions the differences be tween control tissue and hepatomas persisted, indicating that the results obtained did not reflect differences in pool size.

When incorporation of [^{3}H]mevalonic acid into the free alcohol was monitored, a similar decrease in biosynthesis was observed.[12] This finding explains, at least in part, the low level of dolichol in hepatomas reported previously.[3,7] Since dietary uptake and excretion[10] of polyisoprenols seem to be of minor importance in determining the total intracellular levels of these compounds, the data thus indicate that some mechanism(s) other than altered *de novo* synthesis may determine the level of dolichyl-P. The similar dolichol phosphatase activities in hepatoma and control microsomes (Table 3) suggest that the rate of dephosphorylation is unaltered in the tumor tissue. The relatively high dolichol kinase activity in hepatoma microsomes (about 65 % above control values) may, however, partially explain the increased amount of dolichyl-P in this subcellular fraction from hepatomas (Table 3).

Under normal conditions, *de novo* synthesis is the major pathway for maintaining the levels of dolichyl-P and dolichol.[6,8] Under special circumstances, in particular during tumor growth, the importance of maintaining or perhaps even increasing the rate of protein glycosylation requires that tumor cells maintain appropriate levels of dolichyl-P. This might be achieved by increasing the rate of phosphorylation of the free alcohol, which is probably quantitatively more important than *de novo* synthesis under these conditions.

Experimental

Biopsies from 4 highly differentiated hepatomas and 4 healthy livers were collected during surgery and analyzed immediately. Liver samples (0.5 g tissue per ml) were homogenized using an Ultra Turrax blender. The microsomal fractions were prepared using procedures[3,11] described previously, and the fractions used were characterized using microsomal, mitochondrial, lysosomal and plasma membrane marker enzymes,[11] as well as by electron microscopy.

Following tissue homogenization and extraction, acid and alkaline hydrolyses were performed, after which dolichyl-P was isolated on a Silica gel SEP-PAK® column.[7] Dolichyl-P levels

Table 3. Dolichol kinase and dolichol monophosphatase activities in microsomal fractions from healthy normal liver and from hepatoma tissue. The values are the means of 8 experiments.

Sample	Enzyme activities	
	Dolichol monophosphatase dolichol formed/cpm per mg protein per 10 min	Dolichol kinase dolichyl monophosphate formed/cpm per mg protein per min
Healthy liver	$3.9(3)\times10^3$	$4.8(3)\times10^3$
Hepatoma	$4.1(4)\times10^3$	$7.8(6)\times10^3$

were then determined by high performance liquid chromatography (HPLC) as described in a recent study.[6]

In order to measure the rates of dolichyl-P synthesis, homogenates of healthy livers and hepatomas were incubated with 125 μCi of [^{3}H]mevalonic acid (0.5 Ci mmol^{-1}) at 30 °C for 40 min. Control experiments with incubation mixtures containing a constant amount of [^{3}H]mevalonic acid and increasing concentrations of unlabelled mevalonate were also performed. Dolichyl-P was extracted and its amount and degree of labelling determined by HPLC[7] and scintillation counting.

In order to measure the activities of dolichol kinase and dolichol phosphatase, microsomal fractions were incubated with labelled dolichol-15 (75 carbon chain length) and labelled dolichyl-19-P (95 carbon chain length), respectively.[3] The labelled products, dolichyl-15-P and dolichol-19, were then extracted, isolated by thin-layer chromatography and determined by scintillation counting.[3]

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