Dolichol biosynthesis in human malignant cells

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Cholesterol, ubiquinone and dolichol biosynthesis from mevalonic acid was measured in non-malignant and malignant cultured human lymphocytes, freshly isolated human mononuclear leucocytes and in cultured human hepatoma cells. The relative flux of mevalonate into ubiquinone, dolichol and cholesterol was not significantly different between malignant and non-malignant cells, although the extent of labelling of each product was an order of magnitude greater in the malignant cultured cells. The most prominent dolichol isolated from total cellular lipid and synthesized in short-term labelling of cultured leukaemic cells had a chain length one isoprene unit shorter than that observed in normal human cells. Cultured human hepatoma cells and mononuclear leucocytes isolated from the peripheral blood of individuals with lymphoblastic and myelogenic leukaemia similarly synthesized shorter-chain dolichols. The dolichols made in cultured non-tumorigenic cells, freshly isolated mononuclear leucocytes from a normal individual or a patient with non-haematological malignancy had normal chain length.

INTRODUCTION

Biosynthetic pathways of the isoprenoid lipids, cholesterol, dolichol and the side chain of ubiquinone have common early steps in animal cells. Regulation of cholesterologenesis in non-malignant cells occurs via several mechanisms, including transcriptional control, enzyme degradation, allosteric regulation and covalent modification of key regulatory enzymes. An early enzyme in this pathway, hydroxymethylglutaryl-CoA reductase, has been the focus of many studies on regulation, although several other enzymes, including squalene synthase and farnesyl diphosphate synthase, are subject to regulation in other test systems [1–5].

Studies with whole cells and cell-free systems describe the tight control hydroxymethylglutaryl-CoA reductase imposes over the flow of carbon into the post-mevalonate section of the cholesterologenic pathway [3,6]. In normal cells the rate of sterol synthesis is under negative control by the intracellular concentration of cholesterol or a metabolite of cholesterol. In malignant cells, however, this negative-feedback mechanism is defective or absent [7–9].

The effect of changes in the flux of carbon to cholesterol on the branch pathways to ubiquinone and dolichol has been studied by others in non-malignant tissue from non-human sources [10–14]. However, the present study was directed toward cultured non-malignant and leukaemic human lymphocytes and freshly isolated mononuclear leucocytes (MNLs) from a normal individual, a patient with non-haematological malignancy and patients with different forms of leukaemia.

The relative flux of mevalonate into ubiquinone, dolichol and cholesterol was not appreciably different between malignant and non-malignant cells, although the level of radiolabel incorporated into each product was an order of magnitude greater in the malignant cultured cells. A novel finding from these studies is that the chain length of the dolichols isolated from malignant cells was one isoprene shorter than normal.

EXPERIMENTAL

Tissue culture

Epstein–Barr-virus-transformed non-malignant human B-cell

lymphoblasts (IM-9) and malignant T-cell lymphocytes (MOLT-4) cells were obtained from Dr. N. Lawrence Edwards, Department of Medicine, University of Florida, Gainesville, FL, U.S.A. The human erythroleukaemic cells SPI-801 and 802 [15] were obtained from Dr. H. Nick, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, U.S.A. The malignant myelocytes K562 (A.T.C.C. CCL243) and HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Each cell line was grown in suspension culture at 37 °C in air/CO₂ (19:1) in 200 ml of medium consisting of RPMI-1640 (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% (v/v) fetal bovine serum (Hyclone, Logon, UT, U.S.A.) and antibiotics [16]. When cell growth reached (3–4) × 10⁵ cells/ml, one quarter of the suspension was diluted to 200 ml with fresh medium to maintain cell growth. The remainder of the cells was used for experimentation. Cell number was counted with a haemocytometer and viability was monitored by Trypan Blue exclusion. HepG2 cells were grown as a monolayer in 75 cm² tissue-culture flasks in Eagle's Minimal Essential Medium (Gibco) supplemented with 10% (v/v) calf serum (Hyclone).

MNLs

Dr. W. D. Noyes and Dr. R. L. Marsh of the Shands Teaching Hospital, University of Florida, Gainesville, FL, U.S.A., provided peripheral venous blood taken from patients with acute lymphoblastic leukaemia (ALL), acute myeloblastic leukaemia (AML), chronic lymphocytic leukaemia (CLL) and chronic myelocytic leukaemia (CML), and non-leukaemic volunteers. The MNLs were isolated by previously published methods [17]. These studies were approved by the Institutional Review Board on Human Subjects Research, University of Florida, Gainesville, FL, U.S.A.

Metabolic labelling of isoprenoid lipids

The same general incubation protocol was adopted for cells grown in suspension or in monolayer culture and for freshly isolated MNLs. Cells [(1–2) × 10⁶] were incubated in 1 ml of growth medium supplemented with 10 μCi of [5-³H]mevalonic

Abbreviations used: MNLs, mononuclear leucocytes; ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myelocytic leukaemia; C₉₀(D18), C₉₅(D19), C₁₀₀(D20) etc. represent dolichols with 18, 19 or 20 isoprene units (etc.) respectively.

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acid lactone (1.26 Ci/mmol; Amersham International, Arlington Heights, IL, U.S.A.), in 25 cm² tissue-culture dishes gently agitated on a rocking platform at 37 °C in an ambient atmosphere for 6 h. MNLs were incubated in the supplemented RPMI-1640 medium, described above, for 1 h before adding radiolabelled substrate. Incorporation of radiolabel into product in IM-9 cells was examined over a 24 h period; it reached a plateau after 6–8 h of incubation. With HepG2 cells, the monolayer of cells in the tissue-culture flask was harvested after treatment with 0.25 % trypsin and 2 mM-EDTA, and the isoprenoid lipids were isolated as described below.

Isolation of isoprenoid lipids

Cultured cells or MNLs were harvested from the incubation medium and washed free of excess radiolabel by centrifugation and resuspension three times with iso-osmotic saline at room temperature. The cell pellet was extracted three times with 3 ml of chloroform/methanol (2:1, v/v). In each case 1 ml of methanol was added first, the sample mixed and 2 ml of chloroform was added before final vortex-mixing. Samples (20 µg each) of unlabelled cholesterol, dolichol and ubiquinone-10 (Sigma Chemical Co., St. Louis, MO, U.S.A.) were added as carriers to the pooled extract. The lipid extract was dried under N₂ and resuspended in 3 ml of 20 % KOH and 0.08 % pyrogallol in methanol. Mild alkaline hydrolysis was achieved by refluxing the mixture for 1 h at 80 °C under an N₂ atmosphere. The non-saponifiable lipids were isolated from the saponification mixture by a second total lipid extraction with 3 ml of chloroform/methanol (2:1, v/v) as described above. The non-saponifiable

lipids were purified on a C₁₈ reverse-phase Sep-Pak mini-column (Waters, Milford, MA, U.S.A.) previously equilibrated with methanol. Cholesterol was eluted with 10 ml of methanol, and ubiquinone and dolichol were eluted with 10 ml of ethanol. The methanol and ethanol eluates were collected separately and the solvent was removed under vacuum. The residual non-saponifiable material was redissolved in a known volume of 0.1 % propan-2-ol in hexane before analysis. An assessment of the incorporation of radiolabel and sample recovery were determined by radiochemical analysis of aliquots of the total non-

Table 1. Incorporation of [³H]mevalonic acid into non-saponifiable lipids in cultured malignant and non-malignant leucocytes

[³H]Mevalonic acid was incubated with the different cells, and non-saponifiable lipids were isolated as described in the Experimental section. Results are means ± s.d. Only one experiment with K-562 cells was performed.

Cell type	Cell line	Incorporation of [³ H]mevalonic acid into non-saponifiable lipids over a 6 h period (d.p.m./10 ⁶ cells)
Freshly isolated MNLs (non-malignant) (n = 3)	–	1308 ± 191
Cultured non-malignant lymphoblasts (n = 6)	IM-9	6325 ± 1041
Cultured malignant T-lymphocytes (n = 12)	MOLT-4	65 552 ± 20 393
Cultured malignant myelocytes	K-562	109 278

Table 2. Incorporation of [³H]mevalonate into isoprenoids in freshly isolated mononuclear cells and cultured human cells

[³H]Mevalonic acid was incubated with different cells, and the non-saponifiable lipids were isolated and analysed by h.p.l.c. as described in the Experimental section. Results are means ± s.d., except where n = 1. The numbers in parentheses represent the ratio of radiolabel incorporated relative to ubiquinone.

Product...	Incorporation (d.p.m./10 ⁶ cells)			
	Non-saponifiable lipids	Ubiquinone	Dolichol	Cholesterol
Cultured cells				
Cultured non-malignant B-lymphocytes (IM-9) (n = 4)	6325 ± 1041	783 ± 456 (1)	305 ± 167 (0.4)	1357 ± 633 (1.7)
Cultured malignant T-lymphocytes (MOLT-4) (n = 12)	65 552 ± 20 393	7117 ± 4263	7976 ± 3476 (1.1)	26379 ± 11 627 (3.7)
Cultured malignant myelocytes (K562)	109 278	13 532 (1)	22017 (1.6)	35 382 (2.6)
Cultured erythroleukaemia SPI 801	217 370	30 371 (1)	26 765 (0.9)	153 324 (5)
Cultured erythroleukaemia SPI 802	215 385	20 141 (1)	18 440 (0.9)	171 270 (8.5)
Cultured human hepatoma (HepG2)	109 905	5217 (1)	17 715 (3.4)	99 970 (19)
Freshly isolated MNLs				
Non-malignant MNL	4065	548 (1)	331 (0.6)	1436 (2.6)
MNL from breast-cancer patient	11 313	1551 (1)	1433 (0.9)	4308 (2.7)
Malignant MNL (T-cell leukaemia) (ALL patient)	5498	1276 (1)	541 (0.4)	2173 (1.7)
Malignant MNL (AML patient)	5314	275 (1)	265 (1.0)	2148 (7)
Malignant MNL (CLL patient)	5352	449 (1)	354 (0.8)	1459 (3.3)
Malignant MNL (CML patient)	1142	188 (1)	306 (1.6)	588 (3.1)

saponifiable material and both methanol and ethanol Sep-Pak eluates. Developmental work utilizing a known quantity of radiolabel from both [^3H]cholesterol and [^3H]dolichol consistently showed a 80–90% recovery of non-saponifiable radioactivity from broken cells by these methods.

Straight-phase h.p.l.c.

Analysis of ubiquinone, dolichol and cholesterol was achieved by separate analysis of the methanol and ethanol eluates on straight-phase h.p.l.c. by the method of Palmer *et al.* [18]. A Series 4 h.p.l.c. apparatus (Perkin–Elmer, Norwalk, CT, U.S.A.) was used with an LC-75 Perkin–Elmer variable-wavelength detector and a 10 μm -particle-size cyanopropyl (250 mm $\times$ 4.9 mm) column (Brownlee Laboratories, Santa Clara, CA, USA). A mobile phase of 0.1% propan-2-ol in hexane was pumped at a constant rate of 1 ml/min. The u.v. absorbance of the column eluate was monitored at 210 nm. This system gave baseline separation of ubiquinone (elution volume 7.5 ml), dolichol (9.7 ml) and cholesterol (13.7 ml). When analysed for radio-

labelled products, the column eluates were collected in 500 μl fractions, the solvent was removed under a stream of N_2 and the residue was assayed for ^3H in 4 ml of toluene-based scintillation fluid. Incorporation of [^3H]mevalonate into each product was expressed as d.p.m./ 10^6 cells. Recovery of radioactivity from the h.p.l.c. column was routinely > 90%.

Radiolabel in the non-saponifiable fractions which is not found in the dolichol, ubiquinone or cholesterol fractions may be accounted for by procedural losses or by the formation of products which were not identified and quantified. For example, squalene, which was a component of the methanol (cholesterol) fraction after Sep-Pak chromatography, was eluted among several minor radiolabelled products before cholesterol on h.p.l.c., but it was not identified or quantified. Dolichyl phosphate was not measured in the present study and would not have been recovered during elution of products from the Sep-Pak column.

Reverse-phase h.p.l.c.

The dolichol fraction, isolated from straight-phase h.p.l.c., was

Fig. 1. Metabolically labelled dolichols from freshly isolated MNLs incubated with [^3H]mevalonate

MNLs were isolated from peripheral blood and incubated in the presence of [^3H]mevalonate for 6 h. Dolichols were extracted and isolated by reverse-phase h.p.l.c. as described in the Experimental section. The dotted line represents standard porcine liver dolichols detected by their u.v. absorbance at 210 nm. The continuous line represents the radioactivity present in each h.p.l.c. fraction.

Fig. 2. Metabolically labelled dolichols from cultured cells incubated with [^3H]mevalonate

Cells were cultured in the presence of [^3H]mevalonate for 6 h, and the dolichols were extracted and isolated by reverse-phase h.p.l.c. as described in the Experimental section. The dotted line represents standard porcine liver dolichols detected by their u.v. absorbance at 210 nm. The continuous line represents the radioactivity present in each h.p.l.c. fraction.

Table 3. Chain length of dolichols synthesized in freshly isolated MNLs from healthy subjects or patients with malignancy or in cultured tumour-cell lines

[^3H]Mevalonic acid was incubated with different cells, the non-saponifiable lipids isolated, the dolichols separated by normal-phase h.p.l.c. and analysed by reverse-phase h.p.l.c. as described in the Experimental section. The radiolabel associated with each dolichol isoprenologue was determined by summing the net activity in the h.p.l.c. fractions co-eluted with the standard peak and one fraction on either side and is represented as a percentage of the total dolichol. Abbreviation: N.D., none detected.

Cell type	Dolichol ...	Incorporation of [^3H]mevalonate into isoprenologues of dolichol (d.p.m./ 10^6 cells) (percentage of total dolichol)			
		D18	D19	D20	D21
Freshly isolated MNLs (control)	77 (20 %)	92 (24 %)	123 (32 %)	92 (24 %)	
Freshly isolated MNLs (breast-cancer patient)	N.D.	239 (32 %)	292 (39 %)	216 (28 %)	
MOLT-4 cultured human leukaemic T-lymphocytes	1050 (31 %)	1290 (38 %)	880 (26 %)	220 (5 %)	
SP1 801 cultured human erythroleukaemic cells	1371 (31 %)	1687 (39 %)	1272 (29 %)	N.D.	
SP1 802 cultured human erythroleukaemic cells	1858 (28 %)	2498 (38 %)	1711 (26 %)	556 (8 %)	
HepG2 cultured human hepatoma	8390 (29 %)	9850 (35 %)	6240 (22 %)	3761 (13 %)	
K562 cultured human myelogenous cells	2867 (27 %)	4356 (41 %)	2577 (24 %)	835 (8 %)	
Freshly isolated MNLs, T-cell ALL patient	150 (33 %)	180 (40 %)	122 (27 %)	N.D.	
Freshly isolated MNLs, AML patient	222 (29 %)	293 (38 %)	196 (25 %)	66 (8 %)	
Freshly isolated MNLs, CML patient	38 (26 %)	58 (40 %)	34 (23 %)	16 (10 %)	
Freshly isolated MNLs, CLL patient	59 (29 %)	80 (39 %)	49 (24 %)	15 (7 %)	

separated into its different-chain-length isoprenologues by modification of the method of Ekstrom *et al.* [19], using reverse-phase h.p.l.c. on a 10 μ m Perkin-Elmer C₁₈ ODS column (250 mm $\times$ 4.9 mm) with an isocratic A/B (4:1, v/v) solvent mixture at a flow rate of 1 ml/min. Solvent A was methanol/propan-2-ol/water (12:8:1, by vol.) and solvent B was hexane/propan-2-ol (7:3, v/v).

RESULTS

Metabolic labelling of isoprenoid lipids

Cultured MOLT-4 cells showed an approx. 10-fold higher incorporation of [³H]mevalonic acid into the non-saponifiable lipid fraction compared with non-malignant B-lymphoblasts (IM-9) (Table 1). These differences in labelling of the non-saponifiable fractions were also apparent in the individually separated lipids, cholesterol, dolichol and ubiquinone (Table 2). The relative incorporation of mevalonate into cholesterol in the cultured leukaemic cells (MOLT-4) was higher than into ubiquinone and dolichol but, comparatively, much lower than in the human hepatoma (HepG2) cells. By contrast, mevalonic acid incorporation into non-saponifiable lipid and individual isoprenoid lipids of non-malignant MNLs, and MNLs from leukaemic patients, was similar to that observed in non-malignant cultured (IM-9) cells. Similarly, there was no difference in the relative distribution of mevalonic acid into the isoprenoid end products between malignant and non-malignant leucocytes.

Dolichol chain length

Each dolichol fraction separated by straight-phase h.p.l.c. was subjected to reverse-phase h.p.l.c. to separate the different isoprenologues, each of which was analysed for the amount of radiolabel. The predominant isoprenologue of dolichol synthesized *in vivo* in peripheral MNLs from a normal individual and a patient with non-haematological malignancy was C₁₀₀ (D20), with smaller amounts of C₉₅ (D19) and C₁₀₅ (D21) (see Figs. 1a and 1b).

In contrast, freshly isolated MNLs from leukaemic patients with ALL, AML, CLL and CML (Figs. 1c–1f) and cultured leukaemic cells (MOLT-4, K562 and SPI) (Figs. 2a–2c) showed a dolichol-labelling pattern one isoprene unit shorter than that observed in non-malignant cells. C₉₅ dolichol (D19) was the predominant product, with smaller amounts of C₉₀ (D18) and C₁₀₀ (D20). A similar one isoprene-chain-length shift was seen in dolichols synthesized in cultured human hepatoma cells (HepG2) (Fig. 2d). A summary of the relative percentages of the different dolichol isoprenologues synthesized from these cells is presented in Table 3. Endogenous dolichol isolated from MOLT-4 cells showed the same distribution of dolichols as that seen in the dolichols synthesized in the short-term-labelling experiment (Fig. 3a). Similarly, endogenous dolichol isolated from IM-9 cells showed the same dolichol pattern as that seen in short-term-labelling experiments with MNLs from a normal individual and a patient with non-haematological malignancy (Fig. 3b).

The degree of unsaturation in the α -isoprene unit was assessed for the C₉₅ dolichol (D19) fraction taken from the MOLT-4 cells. Since C₉₅ polyprenol is not well separated from the α -saturated C₉₅ dolichol by reverse-phase chromatography [19,20], this C₉₅ dolichol (D19) fraction was subjected to straight-phase h.p.l.c. on silica in 1% ethanol in hexane by a modification of the method of Adair & Keller [20]. No polyprenol was detected in this MOLT-4 dolichol fraction. Commercially available C₉₅ polyprenol and C₉₅ dolichol were shown to chromatograph separately by this method (results not shown).

Fig. 3. Dolichols isolated from human MOLT-4 T-lymphocytes and IM-9 B-lymphoblasts by reverse-phase h.p.l.c.

Endogenous dolichols were isolated from (a) MOLT-4 lymphocytes (3×10^9 cells) and (b) IM-9 lymphoblasts (5×10^9 cells) analysed by reverse-phase h.p.l.c. as described in the Experimental section. Standard porcine liver dolichols (D18, D19 and D20 markers) were chromatographed before application of the sample to verify the elution volumes of the dolichol isoprenologues. The endogenous dolichol sample was injected at an elution volume of 0 ml, and column eluates were monitored at 210 nm.

DISCUSSION

Metabolic labelling of isoprenoid lipids

The use of mevalonic acid as the metabolic probe permitted localization of any differences in isoprenoid labelling, due to metabolic state and tissue type, to steps in the isoprenoid-biosynthetic pathway beyond the hydroxymethylglutaryl-CoA reductase-catalysed step. Although the extent of labelling of cholesterol, ubiquinone and dolichol was an order of magnitude greater in the malignant cultured cells, the relative flux of mevalonate into these products was not appreciably different between malignant and non-malignant cells. Since the specific activity of mevalonate was not determined here, absolute rates of synthesis of these products cannot be calculated, but increased cholesterol synthesis in malignant cells [21], including leukaemic mouse [22] and guinea-pig leucocytes [23], have been well documented. An increased rate of cholesterol synthesis in MOLT 4 cells is also consistent with recent observations that the specific activity of hydroxymethylglutaryl-CoA reductase in these cells is 100-fold higher than in freshly isolated MNLs from normal individuals and nearly 6-fold higher than in IM-9 cells [24].

The relative incorporation of mevalonate into cholesterol, compared with ubiquinone and dolichol, in both malignant and non-malignant lymphocytic cells, was remarkably lower than

similar comparisons in the hepatoma cell lines (HepG2), normal rat liver [25] or in a variety of non-malignant non-haematological cells [26]. This difference is thought to be due to a defect in one or more of the microsomal enzymes converting squalene into cholesterol and to a deficiency of cytosolic sterol-carrier protein(s) [27].

Dolichol chain length

Dolichol chain length is known to vary in the same tissue from different species and in different tissues within a single species [28,29]. The effect of disease on dolichol chain length and the effect of dolichol chain length on cell function are not as well understood. Individuals with non-malignant liver disease (cirrhosis) showed the same dolichol-chain-length distribution as seen in normal liver [28], whereas earlier studies on rats treated with a phthalate ester carcinogen had shown an increase in liver dolichol chain length [30].

Eggens *et al.* [31] noted that the chain length of dolichols isolated at autopsy from patients with hepatocarcinoma was shorter by one isoprene unit from those observed in normal individuals. They reported the most prominent dolichol in normal liver as C₉₅ (D19), whereas tumours showed a relative increase in the C₉₀ (D18) and C₈₅ (D17) dolichols with a decrease in C₁₀₀ (D20) and C₁₀₅ (D21).

Our experiments with malignant MNLs from patients with four types of leukaemia and in malignant cultured cells revealed families of dolichol with chain lengths shorter than normal, similarly to the observations of Eggens *et al.* [31]. The chain-length distribution of the dolichols synthesized in the labelling *in vivo* of the MOLT-4 cells was not an artefact of the experimental design, since the same dolichol-chain-length distribution was observed on analysis of the bulk endogenous dolichols from these cells. Dolichol-chain-length shortening was also not a general feature of cultured cells, since endogenous IM-9-cell dolichols showed the same chain-length distribution as dolichols from MNLs of a normal individual and a patient with a non-haematological malignancy. Such chain-length shortening, however, is not seen in all malignant cells, as shown by the presence of normal-chain-length dolichols in the human T-cell leukaemia, CEM-C7 [32] and in highly differentiated human hepatomas [33].

The isoprenoid-chain-length distribution in the dolichyl phosphates was not examined in the present study. Most reported comparisons of the chain-length distribution between dolichols and dolichyl phosphates isolated from the same tissue show no differences among normal tissue [31,34–36], neoplastic tissue [28,31] and the newly synthesized products of metabolic labelling *in vitro* [19]. Differences in the chain length between dolichyl phosphate and dolichol have, however, been seen in mouse kidney [37].

The factors that determine dolichol chain length *in vivo* are not well understood. It is probable that chain shortening occurs as a result of changes in substrate availability of enzyme function. For example, studies by Ekstrom *et al.* [38], who used rat liver homogenates, showed that an increase in mevalonate concentration resulted in a shift of the dolichol pattern towards longer-chain-length products. Eggens *et al.* [31] suggested that the shorter chain length of the dolichols in the hepatocarcinoma cells, which synthesize cholesterol at a high rate relative to dolichol and ubiquinone, was caused by preferential use of farnesyl diphosphate by the cholesterol pathway and hence reduced the farnesyl diphosphate available for dolichol biosynthesis. In contrast, Sagami *et al.* [39] proposed that formation of the shorter-chain-length product was due to a limiting concentration of the polymerizing monomeric substrate isopentenyl diphosphate. Their studies *in vitro* with rat liver dehydrololichyl diphosphate synthase demonstrated that increasing the isopent-

enyl diphosphate concentration resulted in an increase of the dehydrololichol chain length by one isoprene unit. These studies are consistent with the favoured hypothesis that a depletion of isopentenyl diphosphate due to a high rate of cholesterol biosynthesis leads to shorter dolichol chain length because of the lack of available C₅ units. Recent studies to establish determinants of isoprenoid side-chain length for ubiquinone biosynthesis in rat heart mitochondria support this hypothesis, namely that isopentenyl diphosphate is limiting for isoprenoid chain elongation [40].

Alternative explanations could account for the shorter-chain-length isoprenoids. For example, the function or specificity of enzymes of the dolichol biosynthetic pathway in leukaemic cells could have led to the synthesis of shorter-chain-length polyprenols with little or no dolichol synthesis. Stoll & Krag [41] have described a mutant Chinese-hamster-ovary-cell line that synthesizes glycoproteins with reduced glycosylation. This was attributed by Stoll *et al.* [42] to a decreased level of dolichyl phosphate with compensatory accumulation of polyprenol and its phosphorylated derivatives. These polyprenol derivatives apparently function less effectively in the glycosylation reactions. Furthermore, Ekstrom *et al.* [38] suggested that the addition of the last (α) isoprene unit in dolichol synthesis occurs by a reductive mechanism independent of polyprenol biosynthesis. Failure to add a reduced isoprene unit would result in accumulation of polyprenols one isoprene unit shorter than the normal dolichol distribution. Explanations such as these are not a possibility with the leukaemic cells studied here, because our analysis of the C₉₅ fraction proved that these biosynthetic products were dolichols.

Another possible explanation for dolichol-chain-length shortening in tumour cells might be an alteration in the character of the cellular membranes. Leukaemic cells are reported to have a lower cholesterol/phospholipid ratio and lower viscosity than normal lymphocytes [21]. Such lipid-bilayer changes have been shown to alter enzyme function and could have similar effects on dolichol-chain elongation. For example, studies *in vitro* by Sagami *et al.* [39] with dehydrololichyl diphosphate synthase indicated that addition of Triton X-100 to rat liver microsomes leads to the biosynthesis of dolichols of shorter chain length than those found in the absence of detergent.

Dolichol-chain shortening may have adverse effects on cell function. Although chain-length differences in naturally occurring dolichols appear not to have a significant effect on their function as activated sugar acceptors [43], the role of dolichol may reach beyond its function in glycoprotein biosynthesis. The effect of chain shortening on dolichol function in malignant cells bears further examination.

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