GENETICS

Lipid Spectrum in Fractions of Liver Cell Chromatin from Mice after Partial Hepatectomy

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 1, pp. 85-88, January, 2006
Original article submitted February 3, 2005

The qualitative and quantitative differences in lipids composition were found in chromatin fractions differing by attachment to the nuclear matrix and transcriptional activity. During liver regeneration after partial hepatectomy Changes in the lipid spectrum of chromatin fractions in G_1- and S-phases are associated with transcriptional activity and their primary involvement in replication.

Key Words: *hepatectomy; chromatin; transcription; replication; lipids*

Much attention is now paid to the role of lipids in genetic processes. Previous studies identified site-specific DNA-bound lipids and lipids of chromosomes, chromatin, and nuclear matrix [3]. The effect of lipids on genetic processes can be related to modulation of DNA conformation, DNA interaction with regulatory proteins (repressors and transcriptional factors) and enzymes, and activation of protein kinase C phosphorylating RNA and DNA polymerases [5,6]. The existence of specific nuclear domain suggests that the regulation of lipid metabolism in the nucleus is directed toward modulation of genetic processes [4].

In the present work variability of chromatin lipids isolated from the liver of albino mice and differing by attachment to the nuclear matrix and transcriptional activity was studied under conditions of regeneration after partial hepatectomy.

MATERIALS AND METHODS

Experiments were performed on albino mice weighing 20-30 g. Liver resection was performed under light ether anesthesia; the surgery took ~15 min and was performed in the morning (9.00-10.00). The mice were decapitated. Cell nuclei were isolated in a medium containing 0.05 M Tris-HCl (pH 9.0), 0.25 M sucrose, 5 mM $CaCl_2$, and 20 mM NH_4Cl at 4°C, purified in the medium with 0.25% Triton X-100, and pelleted with 1 M sucrose at 1000*g*. Endogenous Ca^{2+}/Mg^{2+} DNase was activated with

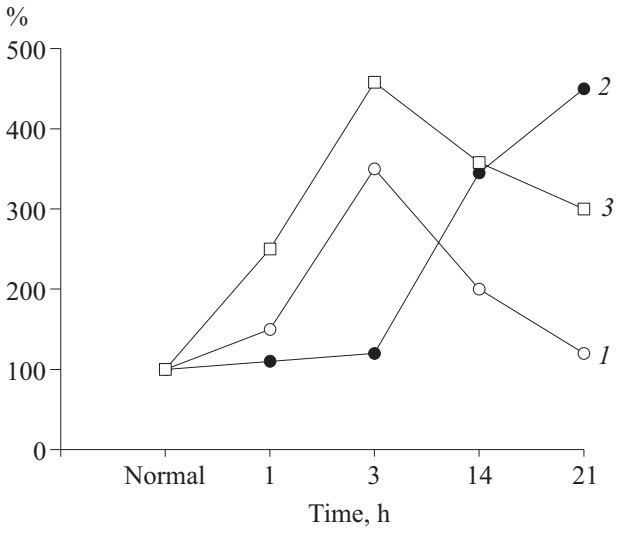

Fig. 1. Content of RNA (*1*), DNA (*2*), and protein (*3*) in the liver of mice after partial hepatectomy.

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0007-4888/06/1411-0077

0.05 M Tris-HCl (pH 8.0) containing 0.25 M sucrose, 10 mM $MgCl_2$, and 1 mM $CaCl_2$ at 30°C for 15 min [1]. Fraction 1 of soluble chromatin (Chr-1) was extracted with 10 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM $MgCl_2$. Fraction 2 of soluble chromatin (Chr-2) was obtained by repeated extraction of nuclei in 10 mM Tris-HCl (pH 7.5) containing 0.2 mM $MgCl_2$ at 30°C for 20 min. High-salt Chr (Chr-HS) was extracted with 10 mM Tris-HCl (pH 7.5) containing 2 M NaCl. The pellet contained nuclear matrix-attached chromatin (Chr-NM). Lipids were extracted with a chloroform-methanol mixture (1:2) and fractionated in thin layers of silica gel (Merck) [2]. Phospholipids were bidimensionally separated in the following mixtures of solvents: chloroform, methanol, 25% ammonia, and water (ratio 90:54:5:8); and chloroform, methanol, glacial acetic acid, and water (ratio 90:40:10:4). Neutral lipids were separated in a mixture of heptane, ether, and acetic acid (60:40:2). Phospholipid content was estimated by the method of Vas'kovskii. The concentration of neutral lipids was measured densitometrically. Protein content was determined by the method of Bradford. The amount of DNA and RNA was measured with diphenylamine and orcinol. The significance of intergroup differences was evaluated by Student's *t* test.

RESULTS

The isolated fractions of chromatin were analyzed by measuring the spectrum of proteins, DNA fragments (electrophoretic study), and lipids (chromatographic study, Tables 1 and 2). Euchromatin (Chr-1 and Chr-2) included high concentrations of cholesterol, cardiolipin, phosphatidylinositol, and lysophospholipids. The presence of acid phospholipids in high concentration can be associated with decon-

TABLE 1. Composition of Neutral Lipids in Chromatin Fractions under Conditions of Liver Regeneration after Partial Hepatectomy (% of total content, *M*±*m*)

Chromatin fraction	Lipids	Normal	G_1-phase	S-phase
Chr-1	Cholesterol esters	20.00±0.56	20.40±0.35	18.10±0.56**
Chr-2		26.50±0.23	15.90±0.23***	23.50±0.23**
Chr-HS		19.20±0.45	8.70±0.34***	23.50±0.25***
Chr-NM		20.80±0.46	17.00±0.79***	16.70±0.45**
Chr-1	Triacylglycerols	2.60±0.53	2.80±0.24	Trace
Chr-2		9.10±0.23	Trace	Trace
Chr-HS		4.10±0.23	Trace	Trace
Chr-NM		4.20±0.47	5.40±0.25**	16.60±0.67***
Chr-1	Free fatty acids	10.80±0.45	7.60±0.34***	8.90±0.23*
Chr-2		9.50±0.83	18.40±0.29**	7.10±0.26***
Chr-HS		11.40±0.23	13.90±0.63***	12.50±0.45**
Chr-NM		16.70±0.36	20.20±0.23***	16.10±0.43
Chr-1	Diacylglycerols	Trace	4.70±0.63	Trace
Chr-2		Trace	Trace	Trace
Chr-HS		Trace	Trace	Trace
Chr-NM		2.90±0.76	Trace	1.80±0.46
Chr-1	Cholesterol	40.20±0.27	42.50±0.23***	23.90±0.46**
Chr-2		27.50±0.66	41.10±0.24***	39.40±0.43***
Chr-HS		34.40±0.76	56.00±0.26***	19.50±0.34**
Chr-NM		33.10±0.23	32.70±0.26*	24.90±0.73***
Chr-1	Total phospholipids	26.90±0.19	19.40±0.22***	18.10±0.56***
Chr-2		27.10±0.37	24.70±0.24***	30.00±0.53***
Chr-HS		31.50±0.26	21.80±0.63**	44.50±0.53**
Chr-NM		22.30±0.45	25.60±0.79**	23.80±0.63*
Chr-1	Total lipids+	110.10±1.34	172.20±2.6***	99.10±0.57***
Chr-2		120.20±1.56	151.10±1.76***	50.20±0.24*
Chr-HS		500.30±2.45	183.30±1.34***	1050.90±3.93***
Chr-NM		150.10±1.56	151.60±1.23**	720.10±2.56***

Note. +μg lipids/mg DNA. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to normal.

TABLE 2. Composition of Phospholipids in Chromatin Fractions under Conditions of Liver Regeneration after Partial Hepatectomy (% of total content, *M*±*m*)

Chromatin fraction	Lipids	Normal	G_1-phase	S-phase
Chr-1	Phosphatidylcholine	27.10±0.43	39.10±0.43***	33.70±0.22**
Chr-2		33.10±0.89	32.10±0.45***	18.90±0.28***
Chr-HS		24.20±0.34	22.50±0.56***	17.70±0.79***
Chr-NM		30.10±0.34	30.70±0.45*	12.70±0.34***
Chr-1	Phosphatidylserine	Trace	3.10±0.34	Trace
Chr-2		3.00+0.23	4.60±0.40***	5.300±0.345***
Chr-HS		Trace	4.80±0.23	Trace
Chr-NM		7.10±0.34	6.50±0.24**	5.100±0.435***
Chr-1	Phosphatidylinositol	15.20±0.78	Trace	17.30±0.23
Chr-2		13.10±0.34	4.40±0.23***	8.50±0.78***
Chr-HS		7.50±0.45	10.00±0.73***	21.30±0.73***
Chr-NM		5.70±0.83	6.50±0.23	13.10±0.34**
Chr-1	Sphingomyelin	6.20±0.78	1.80±0.63***	4.20±0.56***
Chr-2		18.10±0.93	19.70±0.93***	26.20±0.31***
Chr-HS		17.10±0.45	30.20±0.34**	31.10±0.34***
Chr-NM		17.50±0.67	21.10±0.12*	30.30±0.53***
Chr-1	Phosphatidyl-	22.20±0.78	29.20±0.26***	29.20±0.76***
Chr-2	ethanolamine	11.20±0.34	27.7±0.7***	11.90±0.17***
Chr-HS		38.20±0.45	21.60±0.23***	21.60±0.23*
Chr-NM		27.10±0.43	31.90±0.25	17.10±0.57***
Chr-1	Cardiolipin	16.10±0.34	0.90±0.34*	6.1±0.3**
Chr-2		9.10±0.43	1.30±0.45**	22.40±0.12***
Chr-HS		12.10±0.34	4.50±0.76***	8.10±0.23**
Chr-NM		6.80±0.53	2.30±0.12***	15.20±0.43***
Chr-1	Lysophospholipids	13.20±0.76	18.50±0.64***	9.60±0.36***
Chr-2		8.50±0.78	5.10±0.23*	7.80±0.56
Chr-HS		0.80±0.06	6.40±0.45***	0.60±0.01**
Chr-NM		4.60±0.76	1.80±0.56***	7.00±0.78**

densation of chromatin and activation of RNA polymerase [8]. Heterochromatin (Chr-HS) was enriched with phosphatidylethanolamine, cholesterol, and phosphatidylserine, but included only trace amounts of lysophospholipids. Chr-NM was characterized by transcriptional activity and primarily containing phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, cholesterol, cholesterol esters, and free fatty acids.

In the dynamics of regeneration process (1-3 h after partial hepatectomy) we observed an increase in transcriptional function of chromatin (G_1-phase of the cell cycle). However, DNA synthesis was observed 14-21 h after partial hepatectomy (S-phase, Fig. 1).

During G_1-phase lipid content increased in Chr-1 and Chr-2, but decreased in Chr-HS and Chr-NM (Tables 1 and 2). The amount of lipids increased in Chr-NM, but decreased in Chr-1 and Chr-2 during S-phase. In the period of postresection active transcription the lipid spectrum of euchromatin (Chr-1, Chr-2, and Chr-NM) was characterized by accumulation of phosphatidylethanolamine and decrease in the contents of cardiolipin and total phospholipids (Tables 1 and 2). The concentrations of phosphatidylserine and cholesterol increased, while the content of phosphatidylinositol decreased in Chr-1 and Chr-2. We also revealed specific changes in the lipid spectrum: the concentrations of phosphatidylcholine, lysophospholipids, and diacylglycerols increased, while the ratio of sphingomyelin decreased in Chr-1. Chr-2 was characterized by accumulation of free fatty acids and decrease in the concentrations of triacylglycerols and cholesterol

ethers. The ratio of cardiolipin and triacylglycerols decreased, while the concentrations of sphingomyelin, phosphatidylserine, and lysophospholipids increased in Chr-HS. The concentrations of cholesterol esters and lysophospholipids decreased, while the content of sphingomyelin decreased in Chr-NM.

During S-phase the ratio of triacylglycerols, sphingomyelin, cardiolipin, cholesterol, and total phospholipids decreased, while the concentrations of phosphatidylethanolamine, phosphatidylcholine, and cholesterol esters increased in Chr-1. The lipid spectrum of Chr-2 was characterized by a decrease in the ratio of phosphatidylcholine and phosphatidylinositol and accumulation of cholesterol, cardiolipin, sphingomyelin, and phosphatidylserine. During S-phase the lipid spectrum of Chr-HS was characterized by a decrease in the ratio of phosphatidylcholine, phosphatidylethanolamine, cholesterol, and diacylglycerols and increase in the concentrations of sphingomyelin and phosphatidylinositol.

Specific changes in the lipid spectrum of Chr-NM include an increase in the concentrations of cardiolipin, triacylglycerols, and lysophospholipids.

Our results show that chromatin fractions differ by qualitative and quantitative composition of lipids. Genetic reconstructions associated with transcription and replication also include variability of chromatin lipids. These data reflect differences in the ratio of lipids involved in initiation and regulation of genetic processes. Sphingomyelin plays a role in the attachment of DNA to the nuclear matrix during replication and serves as a substrate in the sphingomyelin cycle [7]. Cardiolipin and free fatty acids regulate activity of DNA and RNA polymerases. Cardiolipin in concentrations of 2-5 μg activates RNA polymerases, but in higher concentrations it inhibits transcription [9]. Cardiolipin regulates replication by modulating activity and binding of replication, inductor to the corresponding DNA site [10].

It can be hypothesized that small molecules of lipids exist in numerous molecular forms and determine the formation of specific nuclear regions. Qualitative and quantitative variations in the lipid spectrum contribute to changes in physicochemical characteristics of DNA sites, interaction with regulatory proteins, and activity of genetic processes.

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