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Transgenic mice expressing nuclear sterol regulatory element–binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis

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Abstract

Nonalcoholic steatohepatitis (NASH) is one of the life-threatening hepatic diseases associated with insulin resistance. Here we report that nuclear sterol regulatory element–binding protein 1c (nSREBP-1c) transgenic mice, an inherited lipodystrophic model with severe insulin resistance, spontaneously develop steatohepatitis. The animal had marked fatty liver accompanied by hyperglycemia, hypoleptinemia, and hypoadiponectinemia. Liver histology similar to NASH, that is, mononuclear cell infiltration, pericellular fibrosis, ballooning degeneration, and Mallory hyaline body formation were seen in the livers from transgenic mice 20 weeks or older. In contrast, no liver histologic abnormalities were noted in wild-type mice aged 30 weeks. Immunoreactive 8-hydroxy-2′-deoxyguanosine was observed in the nuclei of livers from transgenic mice, suggesting that in addition to insulin resistance, oxidative stress may be involved in the development of the NASH-like lesion. Thus, the nSREBP-1c transgenic mouse may serve as a unique model of spontaneously occurring NASH.

1. Introduction

Nonalcoholic hepatic steatosis in obese subjects had been considered a benign condition. However, it can progress to nonalcoholic steatohepatitis (NASH), which is histologically characterized by macrovesicular steatosis, hepatocellular ballooning, and mild intralobular inflammation with scattered polymorphonuclear leukocytes and monocytes [1-4]. Furthermore, NASH may deteriorate to either liver cirrhosis or hepatocellular carcinoma. Although the molecular mechanism for the transformation from steatosis to NASH is still unclear, it is obvious that insulin resistance plays a key role in the pathogenesis of NASH [5-7]. Main features of the metabolic syndrome such as obesity, diabetes mellitus, insulin resistance, and hyperlipidemia have been reported as risk factors of NASH [7-9]. Furthermore, increased oxidative stress has been implicated in the development of NASH as a second hit [10].

Generalized lipodystrophy is characterized by diminished adipose tissue and ectopic triglyceride accumulation. Despite the marked difference in fat distribution, obesity and lipodystrophy share common metabolic disorders including insulin resistance and liver steatosis, potentially leading to NASH [11-14]. Some recent studies have shown that liver biopsy specimens from lipodystrophic patients frequently meet histologic criteria for NASH [15,16].

Sterol regulatory element–binding protein 1c (SREBP-1c) is a transcription factor that is involved in adipocyte differentiation [17,18]. Transgenic mice overexpressing nuclear SREBP-1c (nSREBP-1c) in adipose tissue under the control of the adipocyte-specific aP2 enhancer/promoter developed lipodystrophy accompanied by insulin resistance and hyperglycemia [19]. The transgenic mice had severe fatty liver from birth, although the natural course of liver pathology has not been reported. In this study we histologically examined the liver from nSREBP-1c transgenic mice up to 50 weeks of age and found that most animals developed inflammatory changes together with pericellular fibrosis characteristic to NASH.

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2. Materials and methods

2.1. Animals

The experimental protocol has been approved by the Ethics Review Committee for Animal Experimentation of Kurume University School of Medicine, Fukuoka, Japan. Transgenic mice (C57BL/6 background) expressing nSREBP-1c in adipose tissue [19] were purchased from The Jackson Laboratory (Bar Harbor, ME). They were bred in our laboratory, mating to wild-type C57BL/6 mice (Jackson), in plastic cages with wood chip bedding at a temperature of 18°C to 22°C, moisture of 40% to 60%, and on a 12-hour light/dark cycle. They were supplied with regular mouse chow (1450 kJ/100 g; protein, 24.9 g/100 g; fat, 4.6 g/100 g; Nippon CLEA, Shizuoka, Japan) and water ad libitum. The transgenic mice were bled and humanely killed at the age of 10, 20, 30, and 50 weeks. Wild-type C57BL/6 mice aged 10, 20, or 30 weeks served as controls.

We identified nSREBP-1c transgenic mice by amplifying genomic DNA isolated from tails by polymerase chain reaction using a forward primer 5′-CTACATTCGC-TTTCTGCAAC-3′ and a reverse primer 5′ -ATAGAAG-GACACCTAGTCAG-3′, and used heterozygous transgenic mice in the following studies.

2.2. Glucose tolerance and insulin sensitivity

For glucose tolerance test, mice were fasted overnight and given intraperitoneal injections of 10% glucose solution at a dose of 1 g/kg body weight. Blood samples were taken from the tail vein, and glucose concentration was measured by the glucose dehydrogenase method at 0, 30, 60, 90, and 120 minutes. Insulin sensitivity was assessed by the reduction of plasma glucose after an intraperitoneal injection of 0.25 U/kg body weight human insulin (Eli Lilly, Indianapolis, IN).

2.3. Biochemical assays

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, and cholesterol levels were measured by spectrophotometric enzyme assays using peroxidase, lipoprotein lipase, and cholesterol oxidase, respectively (Wako, Osaka, Japan). Plasma levels of mouse insulin (Shibayagi, Gunma, Japan), leptin (R&D, Oxon, UK), and adiponectin (AdipoGen, Seoul, Korea) were measured with enzyme-linked immunosorbent assay kits.

2.4. Histologic studies

Paraffin-embedded sections of the liver were stained with either hematoxylin-eosin (HE) for standard microscopy or Azan-Mallory stain to observe the location of extracellular matrix. The specimens were reviewed by one hepatopathologist. Each specimen was assigned to one of the following histologic subgroups for the purpose of comparative analysis: type 1, fatty liver that is predominantly macrovesicular more than 33% of the lobules alone; type 2, fat accumulation and lobular inflammation; type 3, fat accumulation and ballooning hepatocytes; type 4, fat accumulation, ballooning hepatocytes, and either Mallory hyaline bodies or fibrosis. We dealt with types 3 and 4 as NASH, as described previously by Matteoni et al [3]. Immunoreactivity of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, in the liver was examined as described previously [20]. Briefly, paraffin sections were incubated with 5 μg/mL of mouse monoclonal anti-8-OHdG (Japan Institute for the Control of Aging, Fukuroi, Japan) overnight followed by incubation with alkaline phosphatase–labeled horse antimouse IgG (Vector, Burlingame, CA) and visualization by diaminobenzidine.

2.5. Statistical analysis.

Numerical data were expressed as means ± SD. Student *t* test was performed to assess statistical significance between nSREBP-1c transgenic mice and wild-type mice. *P* values less than .05 were considered significant.

3. Results

To confirm the metabolic characteristics of lipodystrophic nSREBP-1c transgenic mice used in this study, we performed a glucose tolerance test at the age of 20 weeks (Fig. 1A). The transgenic mice had higher plasma glucose levels than wild-type mice before and 120 minutes after

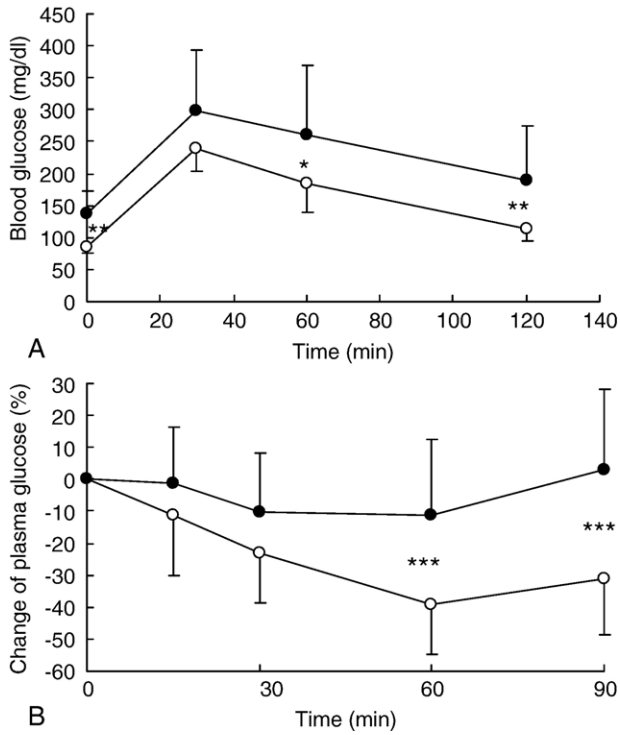

Fig. 1. Glucose tolerance (A) and insulin sensitivity (B) of nSREBP-1c transgenic mice (solid circles) and wild-type mice (open circles). Glucose of 1 g/kg weight was injected intraperitoneally at the age of 20 weeks (A). Human insulin of 0.25 U/kg weight was administered intraperitoneally at the age of 15 weeks (B). Data are means ± S.D. n = 11 each. *$P < .05$, **$P < .01$, ***$P < .005$.

Table 1
Plasma levels of transaminases, lipids, leptin, and adiponectin of nSREBP-1c transgenic mice and wild-type mice at the age of 20 weeks

	nSREBP-1c	Wild-type	*P*
AST (U/L)	183 ± 150	45 ± 5	.044
ALT (U/L)	60 ± 86	29 ± 3	NS
Total cholesterol (mg/dL)	119 ± 33	83 ± 4	.019
Triglyceride (mg/dL)	72 ± 31	32 ± 21	.022
Leptin (ng/mL)	1.6 ± 0.3	13 ± 2.3	<.00001
Adiponectin (μg/mL)	3.7 ± 1.4	48 ± 4.3	<.00001

Means ± SD, n=8 each group.

glucose loading. Intraperitoneal insulin tolerance tests (0.25 U/kg weight) performed at 15 weeks of age showed that the reduction rate of plasma glucose was significantly lower in transgenic mice than in wild-type mice at 60 and 90 minutes, indicating marked insulin resistance of lipodystrophic mice (Fig. 1B). Serum levels of AST, cholesterol, and triglyceride were significantly higher in 20-week-old transgenic mice than in age-matched wild-type mice (Table 1). nSREBP-1c transgenic mice had significantly lower levels of leptin and adiponectin than wild-type mice.

The livers of nSREBP-1c transgenic mice were significantly heavier than those of wild-type mice at the ages of 10 weeks (1.34 ± 0.1 vs 0.95 ± 0.07 g, $P = .002$) and 30 weeks (2.54 ± 0.60 vs 1.27 ± 0.13 g, $P = .0015$). The body weights of nSREBP-1c transgenic mice and wild-type mice were not significantly different at the ages 10 weeks (23.3 ± 1.4 vs 23.5 ± 2.2 g) and 30 weeks (31.7 ± 2.2 vs 31.0 ± 4.1 g).

Ten-week-old nSREBP-1c transgenic mice, but not wild-type mice, had fatty droplets in their livers (Table 2). Intralobular necroinfiltration could be observed in 3 of 6 transgenic mice at this age. However, morphological changes similar to NASH according to the criteria by Brunt et al [2], that is, fibrosis, ballooning, lipogranuroma, and Mallory hyaline bodies, were seen only in the livers from transgenic mice 20 weeks or older, and the incidences of such lesions increased with age (Table 2, Fig. 2A-E). By contrast, no liver histologic abnormalities were noted in wild-type mice aged 30 weeks (Fig. 2F, G). We examined the time course of liver morphology up to the age of 50 weeks, when the transgenic mice remained lipodystrophic. Although 50-week-old transgenic mice showed no apparent nodular regeneration or hepatocellular carcinomas in their livers, all the mice examined had marked liver fibrosis (Table 2, Fig. 2D, E). Immunoreactivity of 8-OHdG was observed in hepatocyte nuclei from all 8 transgenic mice at the age of 30 weeks, whereas no 8-OHdG immunoreactivity was detected in any of the livers from 4 wild-type mice aged 30 weeks, suggesting that oxidative DNA damage may be involved in the development of the NASH-like lesions (Fig. 3).

4. Discussion

In this study we found that lipodystrophic mice expressing nSREBP-1c in the adipose tissue developed NASH-like lesions in the liver. The transgenic mice showed insulin resistance and hyperglycemia accompanied by hyperlipidemia and marked fatty liver. Examination of HE-stained sections demonstrated macrovesicular steatosis in transgenic mice humanely killed at the age of 10, 20, 30, or 50 weeks. Most of the 30-week-old mice exhibited intralobular inflammation with ballooning degeneration and spotty focal necrosis. Mallory hyaline bodies were observed in hepatocytes near inflammatory foci. Azan-Mallory staining showed marked pericellular fibrosis in the intralobular spaces. Intralobular inflammation, which is one of the histologic characteristics of NASH, was also observed in approximately one half of 20-week-old mice. These morphological changes were more marked at the age of 50 weeks. Although we did not observe nodular regeneration or tumor in the liver, the possibility remains that cirrhotic or neoplastic lesions might develop at older ages.

A number of animal models have been used to investigate the pathogenesis and prevention of NASH. A methionine- and choline-deficient diet has been widely used to induce NASH-like liver injury in animals [21-24]. However, it may not be a suitable model for the common form of NASH because the methionine- and choline-deficient diet–induced NASH is not associated with insulin resistance. Another nutritional animal model is inherited hyperlipidemic mice (LDLR −/− and ApoE −/−) fed with cholesterol- and fat-enriched diets [25]. Several genetically engineered animal models have been reported to spontaneously develop NASH-like liver injury. Transgenic mice expressing retinoic acid receptor α dominant negative form in hepatocytes by albumin promoter and enhancer develop NASH probably through the up-regulation of oxidative stresses [26]. Liver-specific inactivation of the *Nrf1* gene or the *Pten* gene leads to NASH and hepatic neoplasia [27].

Table 2
Morphologic changes in livers of transgenic mice by age

	Age (wk)											
	10 (n = 6)			20 (n = 13)			30 (n = 14)			50 (n = 6)		
	Negative	Positive	%	Negative	Positive	%	Negative	Positive	%	Negative	Positive	%
Fatty droplet	0	6	100	3	10	77	7	7	50	0	6	100
Inflammation	3	3	50	6	7	54	2	12	86	0	6	100
Ballooning	6	0	0	7	6	46	2	12	86	0	6	100
Mallory body	6	0	0	10	3	23	2	12	86	0	6	100
Lipogranuloma	6	0	0	12	1	8	5	9	64	0	6	100
Fibrosis	6	0	0	6	7	54	1	13	93	0	6	100

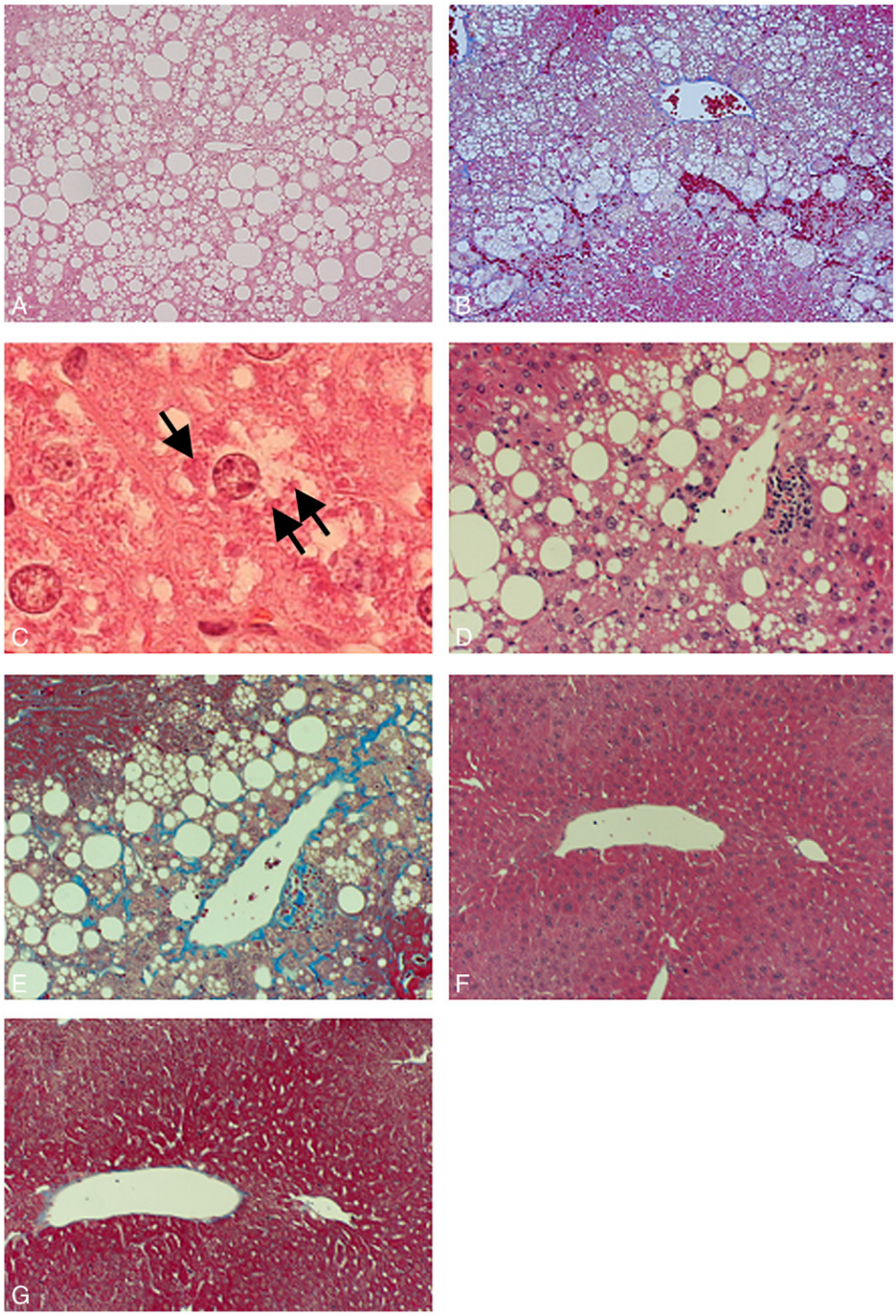

Fig. 2. A, Representative photomicrograph (HE, original magnification ×100) showing hepatic macrosteatosis of a 30-week-old nSREBP-1c transgenic mouse. Lipid droplets were observed around the perivenular zone. B, Azan-Mallory stain of the liver from a 30-week-old nSREBP-1c transgenic mouse (original magnification ×100) highlights perivenular and pericellular fibrosis. C, Ballooned hepatocytes with Mallory hyaline bodies (arrows, original magnification ×800) of a transgenic mouse aged 30 weeks. HE stain (D, F) and Azan-Mallory stain (E, G) of the liver from a 50-week-old nSREBP-1c transgenic mouse (D, E) or a 30-week-old wild-type mouse (F, G) (original magnification ×100).

Furthermore, a low dose of lipopolysaccharide was used to induce hepatitis in the presence of steatosis to mimic steatohepatitis [28]. All these animal models have improved our understanding of the pathogenesis of human NASH.

Unlike the abovementioned genetically engineered animals, hepatic gene expression was not directly altered in nSREBP-1c transgenic mice because the nSREBP-1c gene was exclusively expressed in adipose tissue. Histologic

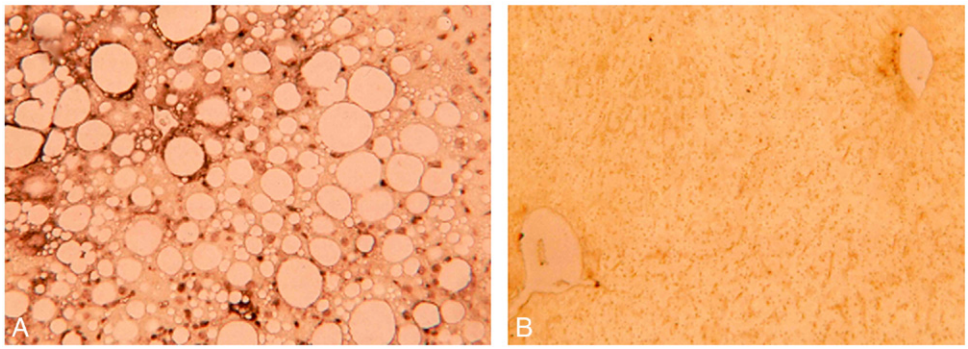

Fig. 3. A, Representative photograph of 8-OHdG immunostaining in the liver from an nSREBP-1c transgenic mouse aged 30 weeks. Immunoreactive 8-OHdG was observed in the nuclei of hepatocytes and infiltrating mononuclear cells. B, No 8-OHdG immunoreactivity was detected in the livers from wild-type mice.

changes in the liver were induced by metabolic disorders such as in the common form of human NASH. The molecular mechanism involved in the development of NASH in the nSREBP-1c transgenic mice remains unknown. However, insulin resistance is likely associated with not only the development of fatty liver, but also the progression to NASH [7-10]. Positive immunostaining of 8-OHdG in nuclei of the liver from nSREBP1c transgenic mice suggests the involvement of oxidative stress in the development of steatohepatitis. It should be noted that plasma leptin of the transgenic mice could participate in the progression to NASH, despite low concentration, because leptin signal through short-form receptors has been shown to play an important role in the pathogenesis of NASH [29,30]. Limitations in this animal model are lack of obesity and low level of leptin. The nSREBP-1c transgenic mouse may serve as a unique model of NASH, not only associated with lipodystrophy but also with obesity and metabolic syndrome. This spontaneous model requiring no special nutrition may be a convenient tool for investigating the mechanism of onset and searching novel therapies for NASH.

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