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Proto-oncogene c-*jun* and c-*fos* messenger RNAs increase in the liver of carnitine-deficient juvenile visceral steatosis (jvs) mice

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We determined the mRNA levels of c-*jun* and c-*fos* in the liver of C3H-H-2° jvs mice. Both were higher in jvs mice than in normal mice. The level of c-*jun* mRNA increased gradually after birth, but in the control mice there was almost no change. In addition, α-fetoprotein and aldolase A mRNA levels were also higher than in normal littermates. These results suggest that the pattern of the gene expression in jvs mice partly resembles the one that occurs in undifferentiated hepatocytes and/or hepatocellular carcinoma.

Fatty liver; c-*fos*; c-*jun*; α-Fetoprotein; Aldolase A; Carnitine deficiency

1. INTRODUCTION

Juvenile visceral steatosis (jvs) is an autosomal recessive mutation associated with fatty liver, hyperammonemia and hypoglycemia. Most jvs mice die within 5–6 weeks of birth [1–3]. These mice have systemic carnitine deficiency [4] and carnitine treatment is very effective in prolonging life [5]. It thus seems that the primary defect may be related to carnitine metabolism (manuscript in preparation).

We have reported that the gene expression of some but not all liver-specific and/or liver-enriched proteins, for example, urea cycle enzymes, serine dehydratase and albumin, which are known to be expressed in differentiated hepatocytes, was suppressed in the liver of jvs mice. On the other hand, two glycolytic enzymes tested, lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, which are expressed in many tissues, were increased in the liver of jvs mice [3,6]. The pattern of these enzyme expressions resembles the one observed in undifferentiated and/or de-differentiated hepatocytes [7–12]. Therefore, jvs mice may be a useful animal model to study the regulation of gene expression in the liver during development and the possible effect of lipid accumulation on liver function. The proto-oncogenes, c-*jun* and c-*fos*, coding for nuclear proteins regulate cell growth and differentiation by controlling gene transcription through AP-1 enhancer element [13,14]. To further study abnormal gene expression in the liver of jvs mice, we determined the levels of c-*jun*, c-*fos*, α-fetoprotein and aldolase A mRNAs which are expressed highly in undifferentiated or de-differentiated hepatocytes [10,11,15–19].

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2. EXPERIMENTAL

Homozygous mutants designed jvs/jvs and normal littermates as control (+/?) were identified as described previously [6]. Concentration of mRNAs was measured by Northern blot or slot blot hybridization as described [6]. Human c-*fos* clone was supplied by Dr. S. Taniguchi [20]; rat c-*jun* clone by Dr. M. Muramatsu [15]; mouse α-fetoprotein clone by Dr. T. Tamaoki [21], and rat aldolase A clone by Dr. T. Mukai [22].

3. RESULTS

Fig. 1A shows c-*jun* and c-*fos* mRNA levels demonstrated by Northern blot analysis in the liver of jvs mice and control littermates at 25 days after birth. Both proto-oncogene transcripts were higher in jvs mice. Slot blot analysis revealed that c-*jun* and c-*fos* mRNA levels were about 3 times and 2.5 times higher in jvs mice than in control mice (Fig. 1B).

We next examined the change of c-*jun* mRNA level from 5 days of birth in the liver of jvs and normal mice, and compared the c-*jun* mRNA level with that of carbamoylphosphate synthetase (CPS) (Fig. 2). As previously reported, CPS mRNA expression during development was suppressed in jvs mice [6]. In contrast, c-*jun* mRNA expression was stimulated. The expression patterns of c-*fos* mRNA during development could not be compared because the expression was too low to quantify.

α-Fetoprotein, a major fetus serum protein, and aldolase A, a muscle-type isozyme of aldolase are known to be highly expressed in fetal liver and even in adult liver during hepatocarcinogenesis [10,11,18,19]. We

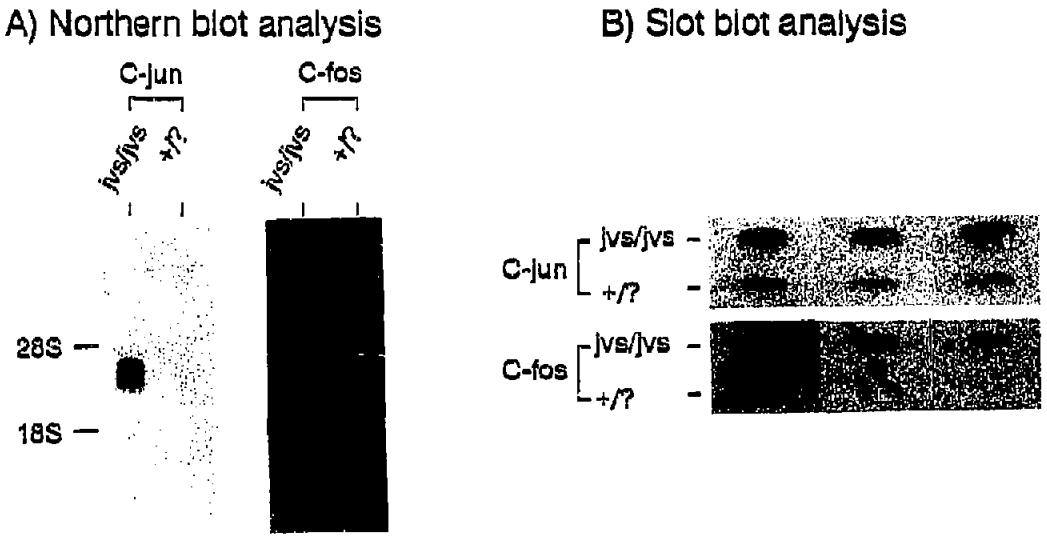

Fig. 1. c-*jun* and c-*fos* mRNA levels in the liver of jvs and normal mice. Total RNA isolated from liver at the age of 25 days was analyzed by Northern blot (A) and slot blot (B) hybridization with c-*jun* and c-*fos* cDNA probes.

measured mRNA concentrations for α-fetoprotein and aldolase A at 25 days compared with albumin and aldolase B mRNAs. As shown in Fig. 3, aldolase A mRNA level was about 2.5 times higher in jvs mice than in control mice. On the other hand, α-fetoprotein mRNA level was not detectable in control mice while it was clearly detectable in jvs mice. Albumin mRNA was much lower in jvs than in control mice, while aldolase B did not vary significantly, as observed previously [6].

4. DISCUSSION

For maturation and maintenance of liver function, various genes need to be expressed under the control of a complex network. In jvs mice, a group of mRNAs for liver-specific and/or liver-enriched proteins are lower

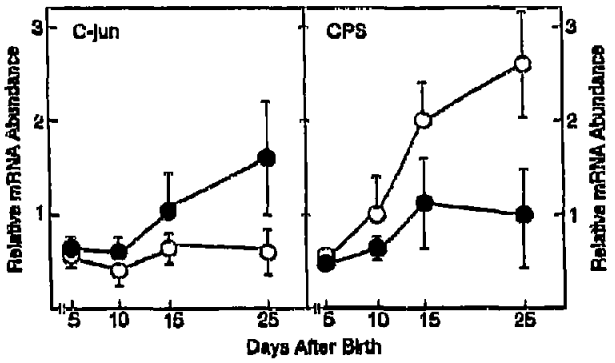

Fig. 2. Developmental profiles of c-*jun* and CPS mRNA accumulation. Slot blot hybridization was performed on 10 μg of total RNA isolated from the liver of jvs (●—●) and control (○—○) mice at indicated stages after birth. The intensities of hybridization signal were measured densitometrically. Each point for mRNA concentration represents the mean ± S.D. (n = 4).

than in normal mice. Such disordered gene expression cannot be caused by general liver damage because other proteins and/or mRNAs are normal [3,6]. We consider that the disordered gene expression results from a defect in some specific transcriptional regulation system. As shown in section 3, we observed that mRNA levels of proto-oncogenes, c-*jun* and c-*fos*, are high in jvs mice. α-Fetoprotein and aldolase A mRNA levels are also high. Elevated c-*jun* and c-*fos* mRNA levels may affect gene expression in the liver. In fact, upstream of the 5′-regulatory region of α-fetoprotein gene has an AP-1-like element regulated by c-*jun*/*fos* [23]. Aldolase A gene also contains an Ap-1 motif in the enhancer region [24]. At present the mechanism governing the reduced expression of liver-specific and/or liver-enriched genes including urea cycle enzymes in the liver of jvs mice is not clear. It should be kept in mind that all mRNA reductions in the liver of jvs mice are known to be induced by glucocorticoid [25–27]. Recently it was reported that AP-1 interacts with glucocorticoid receptor and inhibits glucocorticoid function [28,29]. It is possible that elevated proto-oncogene products (AP-1) counteract glucocorticoid function. Now we are investigating glucocorticoid signal transduction in jvs mice and the trans-acting factor concerning liver-specific expression using gel shift analysis.

There are a few reports that proto-oncogenes increase in diseases other than cancer, e.g. c-*jun* and c-*fos* together with fetal type of some genes in heart hypertrophy [30,31]. The precise mechanism governing what mediates gene activation of c-*jun* and c-*fos* is still not known.

In jvs mice, increased c-*jun* and c-*fos* mRNAs may be caused by activated protein kinase C (PKC). In a recent report by Shinomura et al. free fatty acids activated

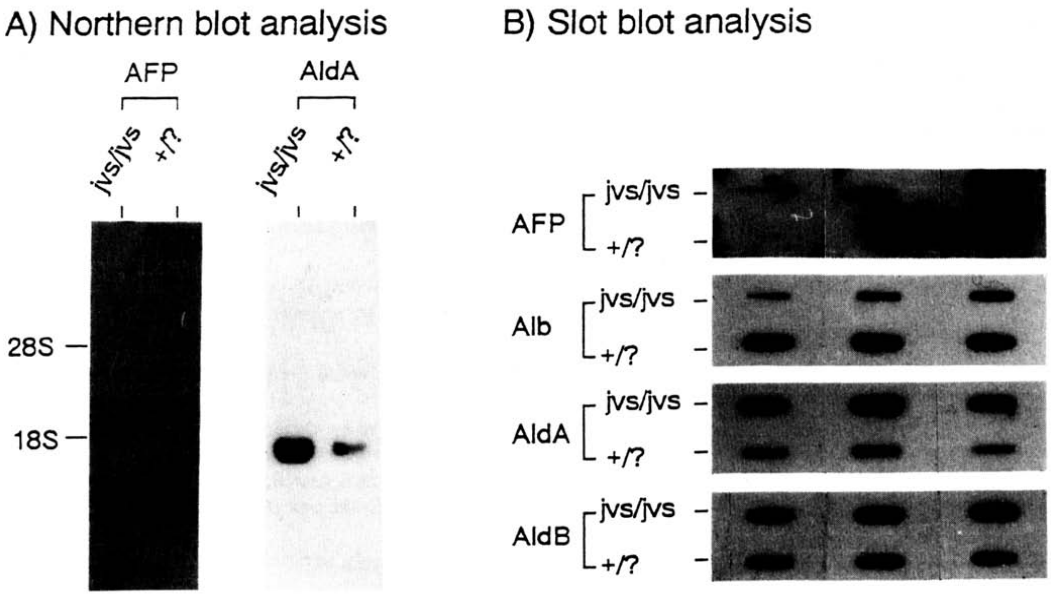

Fig. 3. α-Fetoprotein (AFP), albumin (Alb), aldolase A (AldA) and aldolase B (AldB) mRNA levels in the liver of jvs and normal mice. Total RNA isolated from liver at the age of 25 days was analyzed by Northern blot (A) and slot blot (B) hybridization with each cDNA probe.

PKC activity synergistically with diacyl glycerol at low Ca^{2+} concentrations [32]. Therefore, high expression of c-*jun* and c-*fos* in the liver of jvs mice may result from pathological conditions such as fatty liver. In any case, this is the first paper showing that proto-oncogene c-*jun* and c-*fos* mRNAs increase in metabolic disease and jvs mice should offer insights into how gene regulation is disturbed in pathological conditions caused by carnitine deficiency.

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