JPP Journal of Pharmacy and Pharmacology

JPP 2010, 62: 477–484

Received April 14, 2009
Accepted January 11, 2010
DOI 10.1211/jpp/62.04.0010
ISSN 0022-3573

Research Paper

Up-regulation of the lysyl hydroxylase 2 gene by acetaminophen and isoniazid is modulated by transcription factor c-Myb

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Abstract

Objectives Lysyl hydroxylase 2 (LH2), an isoform of hydroxylase, catalyses the hydroxylation of lysine residues in the telopeptide of collagen to form stable and irreversible cross-linkages in collagen. Increased activity of this enzyme in activated stellate cells in human liver has been proposed to relate to the promotion of hepatic fibrosis. In the present study, we examined the regulation of LH2 expression in drug-induced liver injury in order to clarify the mechanisms behind the hepatic fibrosis caused by certain drugs.

Methods The mRNA and protein expression of the target gene were detected by real-time reverse transcription-polymerase chain reaction (RT-PCR) with specific primers and Western blotting with a specific antibody, respectively.

Key findings The expression of LH2 was increased in HepG2 cells incubated with acetaminophen and isoniazid. This increase was accompanied by an increase in the expression of c-myeloblastosis viral oncogene homolog (Myb) mRNA. Over-expression of c-Myb in cells transfected with a c-Myb expression plasmid, pMbm I, caused an increase in the expression of LH2 mRNA. Mutation of the Myb-binding site in the promoter region of the LH2 gene resulted in a loss of transcriptional activation in the reporter gene assay.

Conclusions These results suggest that c-Myb modulates the expression of the LH2 gene in HepG2 cells incubated with drugs causing hepatic fibrosis

Keywords acetaminophen; c-Myb; hepatotic fibrosis; HepG2; isoniazid; lysyl hydroxylase 2

Introduction

Certain drugs, such as acetaminophen, isoniazid and methotrexate, are known to damage the liver. The disruption of hepatocyte function and secretion of bile caused by these drugs and their metabolites results in hepatic impairment. The mechanism of the impairment is intricate and still under investigation. Although hepatic impairment by drugs is classified differently depending on the prospective damage, it is divided into two types, allergic and addictive, based on the pathogenic mechanism involved.[1] It is difficult to predict the onset of allergic hepatic impairment because of the many factors involved in allergies and the complicated nature of allergic reactions. However, the mechanism of addictive hepatic impairment can be clarified since this type is thought to occur reproducibly, in proportion to the dosage administered, independently of constitutional differences. It therefore seems possible to predict the onset of addictive hepatic impairment and, if so, take precautions against it.

Many factors within the body are understood to be involved in the induction of hepatic impairment by drugs.[1] Although researchers have reported on the pathogenic mechanism of addictive hepatic impairment, problems at the genetic, protein and cellular signalling levels have not been solved. We have previously reported that mRNA levels of lysyl hydroxylase 2 (LH2), thymidine synthetase and several genes involved in the mechanism of hepatic fibrosis are increased or decreased in HepG2 cells treated with acetaminophen, one of the drugs inducing hepatic impairment.[2] Solving common problems in hepatic fibrosis caused by drugs would be useful not only for the prevention of hepatic fibrosis in clinical therapy but also for developing a drug in the pre-clinical stage. Mitchell *et al.*

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reported that acetaminophen hepatic impairment was due to its biotransformation to a reactive metabolite, N-acetyl-*p*-benzoquinone imine, by cytochrome P450.[3] However, Schiodt *et al*. found, in the case of hepatotoxicity induced by acetaminophen, high levels of non-metabolized acetaminophen in the serum of the patients.[4] We therefore used HepG2 cells in which metabolic activation of drugs was reported to be generally weaker than that in normal liver cells[5] and it was previously found that the expression level of LH2 mRNA increased in HepG2 cells treated with acetaminophen.[2]

Lysyl hydroxylase activity has been observed to increase in the liver of rats treated with carbon tetrachloride and dimethylnitrosoamine as an inducer of hepatic fibrosis, and liver fibrosis developed as the hepatic damage progressed.[6–8] It has been speculated that LH2 participates in the development of fibrosis since increased expression of this enzyme was observed in human hepatic stellate cells when the liver fibrosis occurred.[9] We have identified a binding domain for a transcription factor, myeloblastosis viral oncogene homolog (Myb), in the promoter region of the LH2 gene. Since the factor was reported to regulate the gene expression of collagen type I and α-smooth muscle actin, we concluded that it participated in the transcription of the LH2 gene.[10,11] In this study, we demonstrate that Myb is involved in the up-regulation of LH2 gene expression in HepG2 cells treated with acetaminophen and isoniazid.

Materials and Methods

Materials

The human c-Myb expression plasmid, pMbm I, and human B-Myb expression plasmid, pCMV-BMYB, were kindly provided by Dr Edward V. Prochownik and Dr Arturo Sala, respectively.[12,13] Acetaminophen, isoniazid, methotrexate and carbon tetrachloride were purchased from Merck Hoei (Osaka, Japan), Daiichi Pharmaceutical (Tokyo, Japan), Wako Pure Chemical (Osaka, Japan) and Kishida Chemical (Osaka, Japan), respectively. TRIzol, the Oligo $(dT)_{12–18}$ Primer and SuperScript III reverse transcriptase were obtained from Invitrogen Corp. (Carlsbad, CA, USA). TaKaRa EX Taq, Syber Premix Ex Taq and TaKaRa Taq, and FuGene6 were purchased from Takara Bio Inc. (Osaka, Japan) and Roche Diagnostics K. K. (Tokyo, Japan), respectively. The Dual-Luciferase Reporter Assay System, the GeneEditor in-vitro Site-Directed Mutagenesis System and other DNA-manipulating enzymes were acquired from Promega Corp. (Madison, WI, USA). Anti-c-Myb antibody and Anti-β-actin antibody were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA) and Sigma (St Louis, MI, USA). All other chemicals were of analytical grade and purchased commercially.

Cell culture and treatment with compounds

Human hepatic HepG2 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) under a humidified atmosphere with 5% CO_2 at 37°C. Cells were seeded on 96-well plates and 35-mm dishes at a density of 8×10^3 and 2×10^5 cells/well, respectively, and incubated in a CO_2 incubator for 24 h. Cells were washed once with phosphate-buffered saline (PBS) and treated with acetaminophen, isoniazid and other compounds dissolved in DMEM containing 10% FCS.

RNA isolation and reverse transcriptase polymerase chain reaction

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The extracted RNA was dissolved in diethylpyrocarbonate-treated water and quantified by measuring absorbance at 260 nm. Aliquots of 5 μg of total RNA were used to synthesize the first-strand cDNA with SuperScript III (Invitrogen). Real-time monitoring of reactions was performed using the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) with the SYBR Premix Ex Taq (Takara Bio Inc.). At the end of the polymerase chain reaction (PCR), a dissociation curve analysis was performed to examine the specificity of the product. The PCR was performed under the following conditions: denaturation at 95°C for 5 s, annealing at each temperature for 20 s and extension at 72°C for 15 s. The specific primers, annealing temperature, and number of PCR cycles are listed in Table 1. The β-actin housekeeping gene was used for normalization of LH2 mRNA expression.

Table 1 Sequences of oligonucleotide primers for RT-PCR and real-time RT-PCR

Gene name		Sequence	Temperature[a] (°C)	Cycles[b]
β-actin	Forward	5′-AGTAACAGCCCACGGTGTTC-3′	60	35
	Reverse	5′-GTTGGAAAGTCCAAGCCGTA-3′		
Lysyl hydroxylase 2	Forward	5′-ACCTGAAAGCCTGGGATTCT-3′	63	35
	Reverse	5′-TCCTGAGGGAAACCTTTGTG-3′		
c-myb	Forward	5′-GCCAGAAATCGCAAAGCTAC-3′	60	30
	Reverse	5′-CAGGGAGTTGAGCTGTAGGC-3′		
B-myb	Forward	5′-CTGCCTTACAAGTGGGTGGT-3′	57	28
	Reverse	5′-AGATGGTTCCTCAGGGAGGT-3′		

[a]Annealing temperature. [b]Number of PCR cycles.

Plasmid construction

Human genomic DNA from HepG2 cells was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. A DNA fragment containing the regulatory region –1559 to +155 5′ upstream of LH2's transcription initiation site was prepared by PCR with a forward primer containing a *Kpn* I recognition site, 5′-TAGGTACCGCCAA AACACCAGCCATTCCTAGC-3′, and a reverse primer containing a *Sac* I recognition site, 5′-TGAGCTCACTTTCT GGCCCCCTCCAATACT-3′. The PCR product was ligated into the multiple cloning site of a pGL3 basic reporter vector after digestion with *Kpn* I and *Sac* I and the reporter plasmid obtained was named 1559LH2pGL3. The PCR product was digested with restriction enzymes, *Hind* III-*Sac* I and *Nco* I-*Sac* I. The fragments obtained were ligated into a pGL3 plasmid vector and the reporter plasmids prepared were designated as 926LH2pGL3 and 505LH2pGL3, respectively.

Site-directed mutagenesis

Site-directed mutagenesis was performed using the GeneEditor in-vitro Site-Directed Mutagenesis System (Promega) according to the manufacturer's instructions. Five micrograms of 505LH2pGL3 as a template DNA and two oligonucleotides, 5′CAGCGCTTCTGGCTGGCCAGGGGAAGCCTGCAC-3′ and 5′ -CCCCACGTCTGGACTTGGTGCTCAGCGCGGAC G-3′, for the mutagenesis of putative Myb-binding sites, bps –259 to –255 (far) and –182 to –178 (near) of the 5′-flanking region of the LH2 gene, were used. Plasmids which were altered at the far site, the near site and both sites, were named 505LH2mut1pGL3, 505LH2mut2pGL3 and 505LH2mut3p GL3, respectively.

Reporter gene assay

HepG2 cells were seeded at a density of 4×10^4 cells/well into a 24-well culture plate. Twenty-four hours later, the cells were transfected using FuGene6 reagent according to the manufacturer's instructions with 0.32 μg of the reporter plasmid prepared above and 0.16 ng of pRL-CMV as a reference plasmid containing the *Renilla* luciferase gene in a CO_2 incubator. Cells were co-transfected with pMbm I (0.06 μg) and pCMV-BMyb (0.06 μg), together with the reporter plasmid and pRL-CMV. Forty-eight hours later, cell lysates were prepared and levels of firefly and *Renilla* luciferase activity were measured using the Dual-luciferase reporter assay system according to the manufacturer's instructions.

Western blot analysis

HepG2 cells were harvested with cold phosphate-buffered saline (PBS), suspended in extraction buffer, 20 mM Hepes-NaOH, pH 7.4, containing 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM sodium vanadate, 5 mM sodium fluoride, 1% Triton X-100, 2 mM dithiothreitol, 3 μg/ml leupeptin and 2 μg/ml aprotinin, and lysed by five cycles of freezing and thawing. After protein concentration was quantified by Bradford assay,[14] 12 μg of sample was separated by SDS-PAGE (7.5%), and transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking with 1% bovine serum albumin (BSA) in PBS containing 0.1% Tween-20 for 1 h at room temperature, the membrane was incubated with anti-c-Myb antibody overnight at 4°C. After washing three times with PBS containing 0.1% Tween-20, the membrane was incubated with the secondary antibody, conjugated horseradish peroxidase, for 1 h at room temperature. Immunoreactive bands were detected using the ECL system (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Statistical analysis

The significance of differences between two groups was calculated with Student's *t*-test, and the significance of differences among the groups was assessed with a one-way analysis of variance followed by Dunnett's test.

Results

Effect of drugs on the expression of LH2 mRNA

We first examined whether the expression of LH2 mRNA in HepG2 cells was affected by treatment with acetaminophen, isoniazid, methotrexate and carbon tetrachloride, known to be inducers of hepatic fibrosis. The viability of HepG2 cells was unaffected on treatment with these drugs at the concentrations indicated except if treated with 10 mM acetaminophen. The viability was estimated as about 60% at this concentration of acetaminophen. As shown in Figure 1, an increase in the expression was observed in cells cultured with more than 2.5 mM acetaminophen and 7.5 mM isoniazid for 48 h. Methotrexate and carbon tetrachloride, of which the metabolites exhibited hepatotoxicity and resulted in hepatic fibrosis, had no effect.[1] Because drug-metabolizing enzymes in HepG2 cells were expressed less than in normal hepatocytes,[5] sufficient of those metabolites may not be produced under experimental conditions.

Identification of a transcription factor for the LH2 gene's regulation by acetaminophen and isoniazid

Several binding sites for a transcriptional regulatory factor, myeloblastosis viral oncogene homolog (Myb), which has been reported to participate in the transcriptional regulation of genes promoting liver fibrosis,[15–18] were found in the promoter region of the LH2 gene from bp –1559 to +155 5′-upstream of the transcription initiation site as a result of searching the MOTIF Search database. We therefore examined the effect of acetaminophen and isoniazid on the expression of Myb mRNAs in HepG2 cells in order to clarify whether this factor was related to the transcriptional control of the LH2 gene and increased expression of LH2 by these drugs. As shown in Figure 2a, the expression of c-Myb mRNAs in HepG2 cells was increased significantly on treatment with 2.5 and 5 mM acetaminophen and 10 and 20 mM isoniazid, but that of B-Myb mRNA was not significant because of variable experimental data. The suppression of c-Myb mRNA expression was observed on treatment with 10 mM acetaminophen. This may be due to depression of cell viability at this high concentration of acetaminophen as previously mentioned. To investigate further whether the increased expression of LH2 mRNA caused by acetaminophen and isoniazid was due to transcriptional regulation, LH2's promoter region, from

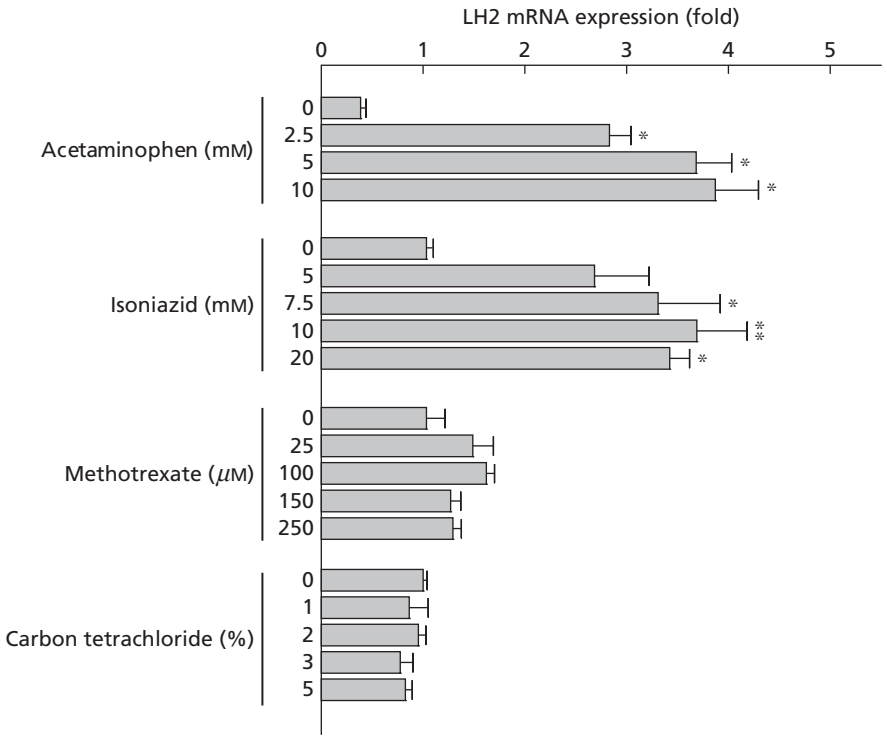

Figure 1 Effect of drugs inducing liver injury on expression of lysyl hydroxylase 2 mRNA in hepatic HepG2 cells. HepG2 cells were incubated with various concentrations of acetaminophen, isoniazid, methotrexate and carbon tetrachloride for 48 h. Total RNA was isolated from the cells, and first-strand cDNA was prepared and subjected to quantitative real-time RT-PCR with specific primers for lysyl hydroxylase 2 (LH2). The results were normalized with the β-actin mRNA levels and the mRNA level of the control was taken as 100%. Data show the mean ± SD from five experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control

bp –1559 to +155 5′-upstream of the transcription initiation site, was ligated into the luciferase reporter vector pGL3, and this construct, 1559LH2pGL3, was introduced into HepG2 cells. As shown in Figure 2b, both luciferase activities significantly increased, to about twice the control level, on treatment with 5 mM acetaminophen and 10 mM isoniazid for 48 h. There seems to be a discrepancy between, on the one hand, the two-fold transcriptional activation and the 10-fold level of c-Myb mRNA with 10 mM isoniazid and, on the other, the two-fold transcriptional activation and the 2.5-fold level of c-Myb mRNA with 5 mM acetaminophen. This may mean that the transcriptional activity of c-Myb for the LH2 gene is regulated in some fashion dependent on the drug used.

Western blotting for c-Myb was performed to confirm whether the protein expression in HepG2 cells increased on treatment with acetaminophen and isoniazid. As shown in Figure 3, the level of c-Myb increased significantly on treatment with 10 mM isoniazid for 3 days. This increase in c-Myb expression seemed to parallel the increase in c-Myb mRNA on treatment with the same concentration of isoniazid. These results suggest that the signalling evoked by these drugs is responsible for the quantitative control of c-Myb and these factors are responsible for regulating the increased expression of LH2 caused by these drugs.

Transcription of the LH2 gene regulated by Myb

Since it was suggested that B-Myb and c-Myb regulate the transcription of the LH2 gene, we examined the responsiveness of these factors to the expression of LH2 by transiently transfecting HepG2 cells with the Myb-expression plasmids, pCMV-BMYB and pMbm for B-Myb I and c-Myb, respectively. The increased expression of B-Myb and c-Myb mRNAs in the transfected cells was confirmed by the RT-PCR method (Figures 4a and 4b). An increase in expression of LH2 mRNA was found in cells transfected with pMbm I, but not with pCMV-BMYB (Figure 4c). The increase with pMbm I was estimated to be approximately 1.7-fold higher than that with the control plasmid without the c-Myb gene. The proliferation of the cells transfected with pCMV-BMYB and pMbm I was observed to be the same as that of the cells transfected with the control plasmid (data not shown).

That over-expression of c-Myb resulted in increased expression of LH2 mRNA seems to imply that this transcription factor is responsible for the transcriptional regulation of the LH2 gene. We therefore further examined whether or not the promoter activity of the LH2 gene was affected by the over-expression of c-Myb. Plasmids containing three different lengths of the promoter region of the LH2 gene, –1559LH2p GL3, –926LH2pGL3 and –505LH2pGL3, were introduced with the Myb expression plasmids, pCMV-BMYB and pMbm I, into HepG2 cells. As shown in Figure 5a, levels of luciferase activity were significantly increased by co-transfection with the three reporter plasmids and pMbm I, but did not change on co-transfection with pCMV-BMYB. Since we found two putative binding sites of Myb in the promoter region bp –505 to +155 5′-upstream of the transcription

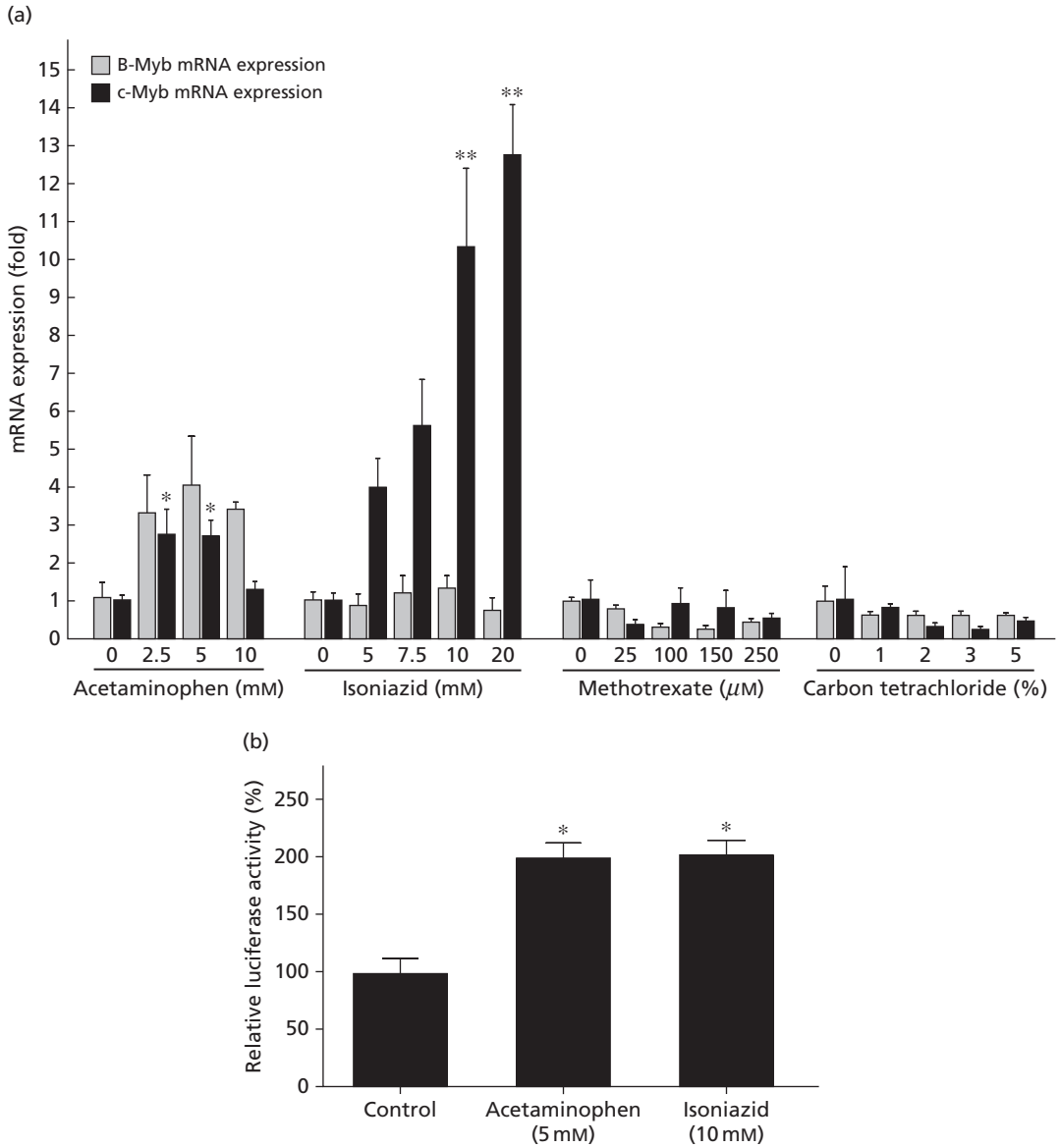

Figure 2 Transcriptional regulation of the lysyl hydroxylase 2 gene evoked by acetaminophen and isoniazid. (a) HepG2 cells were incubated with indicated concentrations of compounds for 24 h. Total RNA was isolated from the cells, and first-strand cDNA was prepared and subjected to quantitative real-time RT-PCR with specific primers for B-Myb and c-Myb. The results were normalized with the β-actin mRNA levels and the mRNA level of the control was taken as 100%. Data show the mean ± SD from five experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control. (b) HepG2 cells were transiently transfected with 1559LH2pGL3 (0.32 μg) and pRL-CMV (0.16 ng) as a reference plasmid. After transfection for 24 h, the cells were incubated with indicated concentrations of acetaminophen and isoniazid for 48 h in a CO_2 incubator. Luciferase activities were measured using the Dual-Luciferase Reporter Assay System. Firefly luciferase activity was normalized to *Renilla* luciferase activity and is expressed relative to the control treated without the drugs. Data represent the mean ± SD from four experiments. $^{*}P < 0.05$ vs control

initiation site of the LH2 gene, we performed site-directed mutagenesis to eliminate these sites. The mutated plasmids, 505LH2mut1pGL3, 505LH2mut2pGL3 and 505LH2mut3-pGL3, were used to test whether these sites took part in the transcriptional regulation of the LH2 gene. As shown in Figure 5b, the luciferase activities decreased due to the mutation of the Myb-binding sites. These results suggest that the putative binding sites for c-Myb respond to the transcription of the LH2 gene.

Discussion

Chronic damage to the liver sometimes causes liver fibrosis, with excessive accumulation of extracellular matrix (ECM) proteins, including collagen, which is a characteristic of most types of chronic liver diseases.[19] Advanced liver fibrosis results in cirrhosis, liver failure and potential hypertension. Activated stellate cells, portal fibroblasts and myofibroblasts of bone marrow origin have been reported to be major

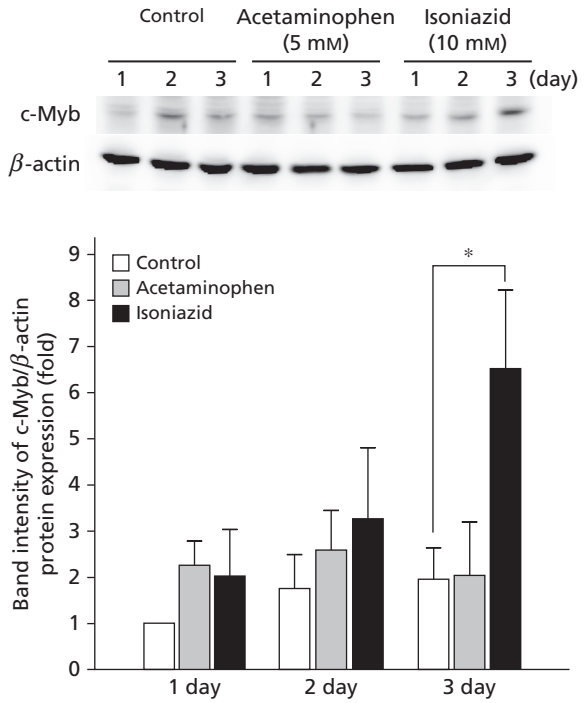

Figure 3 Up-regulation of the c-Myb protein evoked by acetaminophen and isoniazid. HepG2 cells were treated with 5 mM acetaminophen or 10 mM isoniazid for 1–3 days. After incubation, the cells were collected with cold PBS and lysed by five cycles of freezing and thawing. The lysates were subjected to western blotting using anti-c-Myb or anti-β-actin antibody. Data represent the mean ± SD from three experiments. $^{*}P < 0.05$ vs control

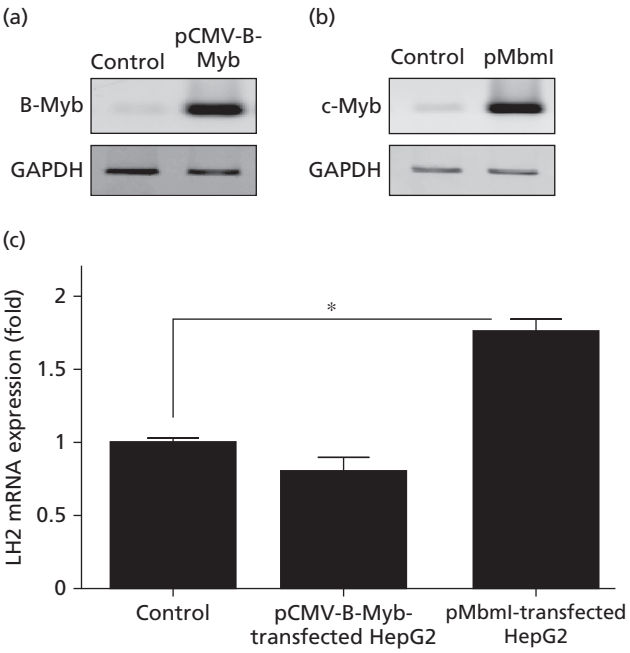

Figure 4 Effects of Myb over-expression on expression of lysyl hydroxylase 2 mRNA and proliferation of transfected HepG2 cells. HepG2 cells were seeded at a density of 2×10^5 cells/well into 35-mm dishes and cultured in a CO_2 incubator for 24 h. HepG2 cells were transfected with 1 μg/ml of pCMV-BMYB and 1 μg/ml of pMbm I for the over-expression of B-Myb and c-Myb, respectively, using FuGene 6 transfection reagent and incubated for an additional 48 h. Total RNA was isolated from the cells, and first-strand cDNA was prepared and subjected to RT-PCR for (a) B-Myb and (b) c-Myb and quantitative real-time RT-PCR for (c) lysyl hydroxylase 2 (LH2). The results were normalized with the β-actin mRNA levels and the mRNA level of the control was taken as 100%. Data show the mean ± SD from three experiments. $^{*}P < 0.01$ vs control

collagen-producing cells in the injured liver. Inflammatory cells, either lymphocytes or polymorphonuclear cells, activate hepatic stellate cells to secrete collagen. Collagen synthesis in hepatic stellate cells is regulated at the transcriptional and post-transcriptional levels. Quiescent stellate cells express markers that are characteristic of adipocytes (PPARγ, SREBP-1c and leptin), while the activated cells express myogenic markers (α smooth muscle actin, c-Myb and myocyte enhancer factor-2).[1,10]

Lysyl hydroxylase was reported to catalyse the post-translational hydroxylation of lysine residues in the pro-α-chain of the collagen precursor.[20–22] The triple-strand helix structure of collagen is stabilized by hydrogen bonds coupled by hydroxyl groups, which are formed by the hydroxylation of lysine and proline residues, catalysed by lysyl hydroxylase and prolyl hydroxylase, respectively.[9,23] The oxidation of lysine residues in collagen is also important to form and stabilize cross-links as well as for the hydroxylation by hydroxylases.[20] Van der Slot *et al.* reported a genetic defect in two Bruck syndrome families, a mutation in procollagen-lysine 2-oxoglutarate 5-dioxygenase 1 (PLOD2), showing that PLOD2 encodes telopeptide lysyl hydroxylase (TLH).[9,23] The importance of TLH in fibrotic processes was demonstrated by its markedly increased expression in fibroblasts cultured from the fibrotic skin of systemic sclerosis (SSc) patients. The activation of lysyl hydroxylase was observed in model animals with liver injury induced by the administration of carbon tetrachloride and dimethyl nitrosoamine.[6–8] An increase in the expression of LH2 was observed in hepatic stellate cells with the progression of liver fibrosis.[9] Long-term and low-dose treatment with acetaminophen was reported to induce liver fibrosis in humans,[24,25] thus LH2 is suggested to participate in the development of liver fibrosis.

The transcription factor Myb is a sequence-specific DNA-binding protein with the ability to transactivate promoters with the specific consensus sequence 5′-(C/T)AAC(G/T)G-3′.[26] Myb is reported to be responsible for hepatic fibrosis and expression of the Myb gene increases on treatment with compounds, causing hepatic impairment.[15–18] Increased expression of the c-Myb gene in murine hepatic stellate cells is reported on liver fibrosis induced by carbon tetrachloride.[10] Collagen Iα2 deposits in fibrotic tissue and Myb are reported to take part in the expression of the collagen Iα2 gene.[27] In this study, we found two Myb-binding sites at bp –505 to +155 in the 5′-flanking region of the LH2 gene. Transcriptional activation of the LH2 gene triggered by acetaminophen and isoniazid was suppressed on the mutation of these binding sites and induced by over-expression of c-Myb. This observation suggests that the up-regulation of LH2 expression is mediated by c-Myb expression increased

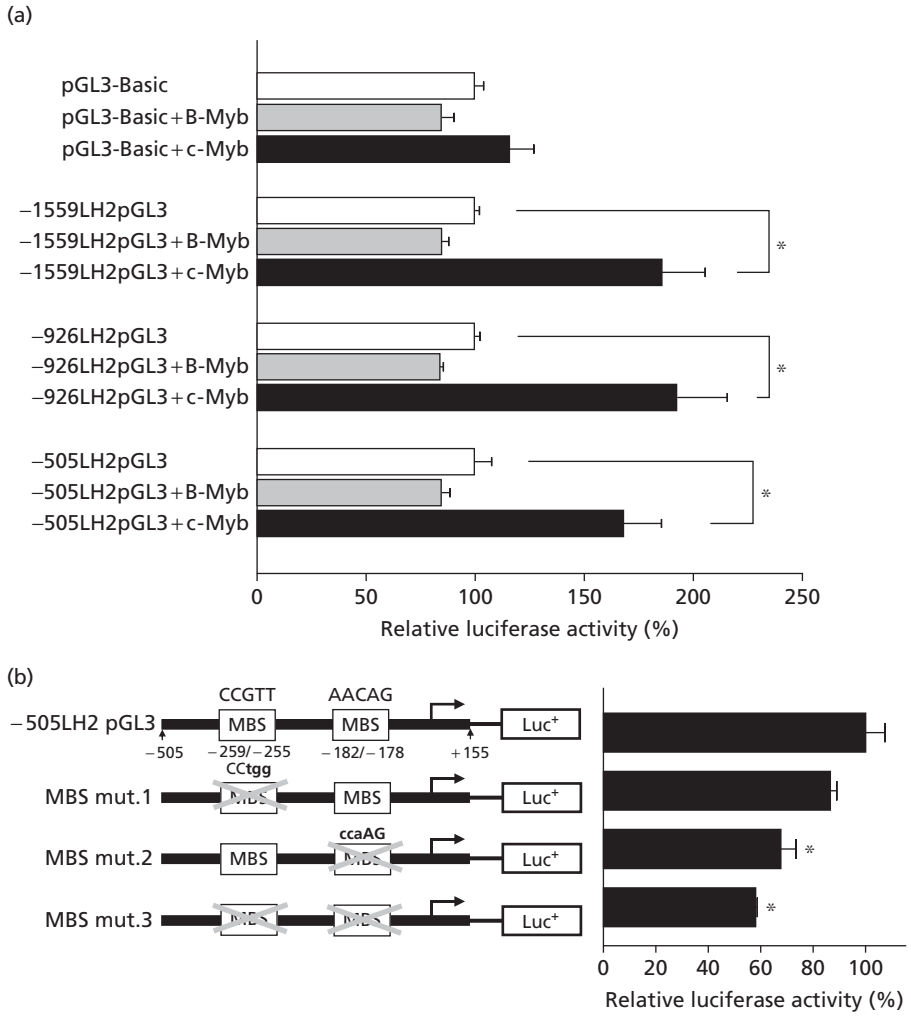

Figure 5 Transcriptional regulation of the lysyl hydroxylase (LH2) gene by c-Myb. (a) HepG2 cells were simultaneously transfected with a reporter vector containing the promoter region of LH2, the reference vector pRL-CMV, and pCMV-BMYB and pMbm I for over-expression of B-Myb and c-Myb, respectively. (b) Wild-type and mutant constructs of the LH2 gene promoter (bp –505 to +155) were ligated to a luciferase reporter vector. Three base pairs were mutated in the Myb-binding sites. HepG2 cells were simultaneously transfected with a plasmid containing the consensus sequence of Myb or a mutated sequence, pRL-CMV and pMbm I. The transfectants were cultured for 48 h in a CO_2 incubator. Cell lysates were prepared and luciferase activity was measured with a Dual-Luciferase Reporter Assay System. Firefly luciferase activity was normalized with *Renilla* luciferase activity and is expressed relative to the control. Data represent the mean ± SD from three experiments. $^{*}P < 0.01$ vs control

by these drugs. Since Myb was shown to be one of the transcriptional regulators for the collagen and elastin genes,[28–30] our finding that the increased expression of LH2 induced by acetaminophen and isoniazid is modulated by c-Myb may help to show how liver injury results in fibrosis.

Conclusions

We previously reported an increase in the expression of LH2 mRNA in HepG2 cells treated with acetaminophen.[2] In the present study, we demonstrated that c-Myb modulated the expression of the LH2 gene in HepG2 cells incubated with acetaminophen and isoniazid, inducing hepatic fibrosis. The findings suggest that these drugs promote the transcription of the LH2 gene through the quantitative induction of c-Myb expression and induce liver fibrosis based on the accumulation of collagen synthesized with LH2. Prolyl hydroxylase, catalysing the hydroxylation of proline residues in collagen, is used as a serum marker for evaluating liver fibrosis.[31] An inhibitor for prolyl 4-hydroxylase has been reported to prevent the activation of Ito cells, reducing procollagen gene expression in rat liver fibrosis.[32,33] LH2 is also considered a target for developing a therapeutic medication against liver fibrosis.

Declarations

Conflict of interest

The Authors(s) declare that they have no conflicts of interest to disclose.

Funding

This work was supported in part by a Grant-in-aid for Scientific Research (No. 16590115) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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