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Regulation of SREBP1c Expression by mTOR Signaling in Hepatocytes

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The transcription factor sterol regulatory element–binding protein 1c (SREBP1c) plays an important role in the regulation of fatty acid metabolism in the liver. Although the importance of phosphoinositide 3-kinase in the regulation of SREBP1c expression is widely accepted, the role of mammalian target of rapamycin (mTOR) in such regulation has remained unclear. We have now shown that the insulin-induced increase in the abundance of SREBP1c mRNA in cultured AML12 mouse hepatocytes was largely abolished by LY294002, an inhibitor of phosphoinositide 3-kinase, but was reduced only slightly by rapamycin, an inhibitor of mTOR. Forced expression of a constitutively active form of Akt containing a myristoylation signal sequence (MyrAkt) in these cells with the use of an adenoviral vector resulted in the phosphorylation of p70 S6 kinase, a downstream target of mTOR signaling, and this effect was inhibited by rapamycin. MyrAkt also increased the abundance of SREBP1c mRNA and protein as well as the expression of the SREBP1c target genes for fatty acid synthase and stearoyl-CoA desaturase 1. These effects of MyrAkt were also markedly inhibited by LY294002 and by rapamycin. These results thus suggest that mTOR signaling plays a major role in Akt-mediated up-regulation of SREBP1c expression but that it plays only a minor role in insulin-induced expression of this transcription factor.

The liver is the major site of fatty acid metabolism. The transcription factor sterol regulatory element–binding protein 1c (SREBP1c) plays a key role in hepatic fatty acid metabolism by regulating the expression of various genes that contribute to fatty acid synthesis (22). The observations that the expression of SREBP1c in the liver is up-regulated in mouse models of obesity (24) and that transgenic overexpression of SREBP1c in the liver induces hypertriglyceridemia or hepatic steatosis (21) suggest that an increase in the abundance of SREBP1c in the liver contributes to the pathogenesis of these diseases. The mechanism underlying the increase in hepatic expression of SREBP1c in obese animals has remained unknown, however.

Insulin stimulates the expression of SREBP1c in hepatocytes (23). Moreover, the hepatic expression of SREBP1c is reduced in mice with insulin deficiency (23) or with liver-specific disruption of the insulin receptor gene (16), suggesting that insulin is an important physiological regulator of SREBP1c expression. Phosphoinositide 3-kinase (PI3K) (17), 3′-phosphoinositide–dependent kinase 1 (PDK1) (18), and the atypical λ isoform of protein kinase C (14) play important roles downstream of the insulin receptor in the regulation

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SREBP1c expression in the liver. However, insulin appears not to contribute to the exaggerated hepatic expression of SREBP1c associated with obesity, given that the liver of obese animals is resistant to insulin (8).

Mammalian target of rapamycin (mTOR) is a protein kinase implicated in a variety of biological processes (3). The mTOR signaling pathway has been thought to be independent of the regulation of SREBP1c, given that insulin-induced SREBP1c expression in cultured hepatocytes is not substantially affected by rapamycin (4), a specific inhibitor of mTOR. However, the expression of SREBP1c induced by a constitutively active form of Akt, a downstream kinase in insulin signaling, was recently shown to be markedly inhibited by rapamycin in retinal pigment epithelial cells (20). We have therefore now investigated the role of the mTOR pathway in the expression of SREBP1c induced either by insulin or by an active form of Akt in cultured hepatocytes. Our results suggest that, although Akt is thought to play a major role in the metabolic actions of insulin (19), insulin and Akt activate different signaling pathways in the regulation of SREBP1c expression.

MATERIALS AND METHODS

Cells, adenoviral vectors, antibodies, and reagents

AML12 mouse hepatocytes (26) were obtained from American Type Culture Collection. Adenoviral vectors encoding a constitutively active form of Akt (AxCAMyrAkt) were described previously (10). Cells were subjected to immunoblot or gene expression analyses after infection with adenoviral vectors for 24 h. Antibodies to Akt, to the Ser^{473}-phosphorylated form of Akt, to p70 S6 kinase (p70S6K), to the Ser^{389}-phosphorylated form of p70S6K, or to β-tubulin were obtained from Cell Signaling Technology (Beverly, MA, USA), and those to SREBP1c were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). LY294002 and rapamycin were obtained from Calbiochem (San Diego, CA, USA).

Gene expression analysis

Gene expression was evaluated by reverse transcription (RT) and real-time polymerase chain reaction (PCR) analysis with 36B4 mRNA as the invariant control, as described (6). The primers for mouse SREBP1c, fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD1) were as described previously (13, 18).

Statistical analysis

Data are presented as means ± SEM and were compared between or among groups by analysis of variance (ANOVA). A P value of <0.05 was considered statistically significant.

RESULTS

Effects of LY294002 and rapamycin on insulin-induced expression of SREBP1c

Quantitative RT-PCR analysis revealed that insulin induced a time-dependent increase in SREBP1c gene expression in cultured AML12 mouse hepatocytes, with an approximately threefold increase in the abundance of SREBP1c mRNA being apparent at 6 h (Fig. 1A). LY294002, an inhibitor of PI3K, almost completely blocked the insulin-induced increase in the amount of SREBP1c mRNA, whereas rapamycin, a specific inhibitor of mTOR, attenuated this effect of insulin only slightly (Fig. 1B). These results are essentially consistent with those of a previous study (4). Insulin also induced an approximately twofold increase in the expression of the SCD1 gene (Fig. 1B), which is regulated by SREBP1c (22). LY294002 prevented this effect of insulin, whereas rapamycin had no such effect (Fig. 1B).These results thus suggested that PI3K signaling plays a major role in insulin-induced expression of SREBP1c as well as in that of the SREBP1c target gene for SCD1, whereas

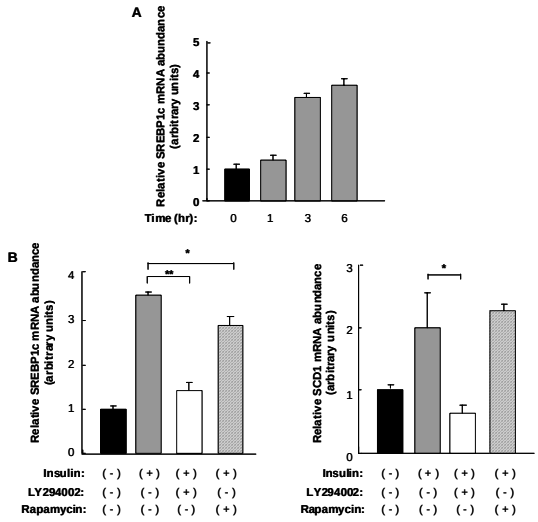

Figure1 Effects of LY294002 and rapamaycin on insulin-induced expression of SREBP1c and SCD1 genes. (A) AML12 cells were incubated for the indicated times with 100 nM insulin, after which the abundance of SREBP1c mRNA was determined by quantitative RT-PCR analysis. (B, C) AML12 cells were incubated for 6 h in the absence or presence of 100 nM insulin, LY294002 (30 μM), or rapamycin (100 nM), as indicated, after which the abundance of mRNAs for SREBP1c (left panel) or SCD1 (right panel) was determined by quantitative RT-PCR analysis. Data are expressed relative to the corresponding value for time 0 (A) or for cells incubated without addition (B) and are means ± SEM of values from 6 independent experiments. *P < 0.05, **P < 0.01 for the indicated comparisons.

mTOR signaling contributes minimally to the insulin-induced expression of the SREBP1c and SCD1 genes.

Effects of LY294002 and rapamycin on Akt-induced expression of SREBP1c

We next investigated the effect of a constitutively active form of Akt on the expression of SREBP1c. This form of Akt (MyrAkt), which contains a myristoylation signal sequence, has been shown to be active in quiescent cells and to mimic certain of the biological activities of insulin (10, 11). Infection of AML12 cells with an adenoviral vector encoding MyrAkt (AxCAMyrAkt) resulted in a dose-dependent increase in the abundance of the MyrAkt protein (Fig. 2A). Immunoblot analysis also revealed that Akt was phosphorylated on Ser^{473} in the infected cells (Fig. 2A), with phosphorylation of this residue having been shown to correlate with Akt activity (1, 15). p70S6K is phosphorylated and activated by mTOR (3). Although infection of AML12 cells with AxCAMyrAkt did not affect the abundance of p70S6K, it induced a shift in the electrophoretic mobility of this protein that was likely attributable to its phosphorylation. Immunoblot analysis with antibodies to the Ser^{389}-phosphorylated form of p70S6K revealed that the protein was indeed phosphorylated at this residue and was therefore activated (1) in cells expressing MyrAkt (Fig. 2A). Infection of cells with AxCAMyrAkt also increased the abundance of mRNAs for SREBP1c, SCD1, and FAS (Fig. 2B, D), the latter of which is also encoded by a target gene of SREBP1c (22). Immunoblot analysis further revealed that expression of MyrAkt resulted in an increase in the amount of SREBP1c protein (Fig. 2C).

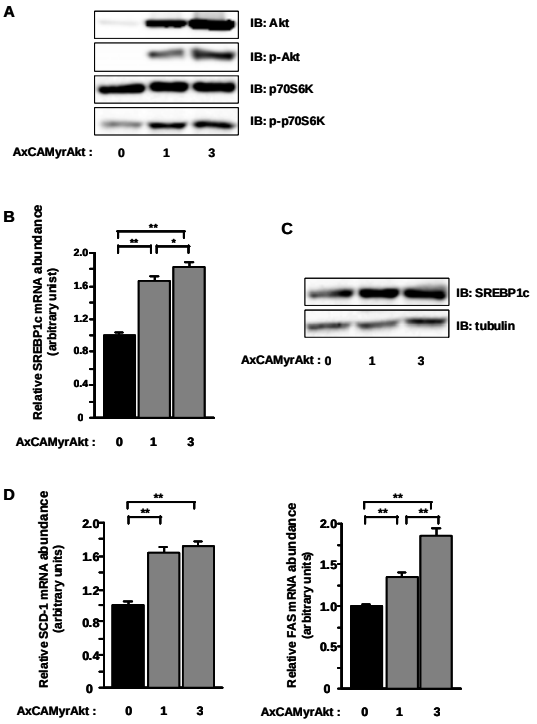

Figure2 Effects of a constitutively active form of Akt on the expression of SREBP1c and genes for lipogenic enzymes. AML12 cells were infected with AxCAMyrAkt at a multiplicity of infection (MOI) of 0, 1, or 3 plaque-forming units (PFU) per cell for 24 h, after which the cells were subjected to immunoblot (IB) analysis with antibodies specific for phosphorylated (p-) or total forms of the indicated proteins (**A**, **C**) or to quantitative RT-PCR analysis of the indicated mRNAs (**B**, **D**). RT-PCR data are expressed relative to the corresponding value for noninfected cells and are means ± SEM of values from 3 independent experiments. *P < 0.05, **P < 0.01 for the indicated comparisons.

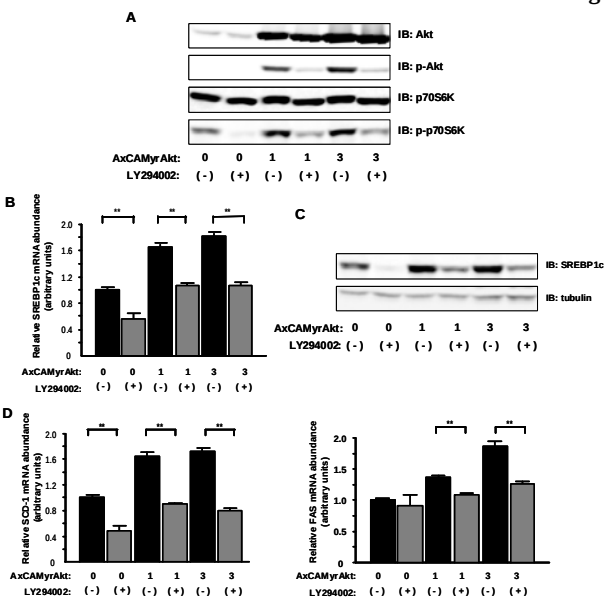

Figure3 Effects of LY294002 on the expression of SREBP1c and genes for lipogenic enzymes induced by a constitutively active form of Akt. AML12 cells were infected with AxCAMyrAkt at an MOI of 0, 1, or 3 PFU/cell for 24 h and then incubated in the absence or presence of LY294002 (30 μM) for 3 h, after which the cells were subjected to immunoblot analysis with antibodies specific for the indicated proteins (A, C) or to quantitative RT-PCR analysis of the indicated mRNAs (B, D). RT-PCR data are expressed relative to the corresponding value for noninfected cells and are means ± SEM of values from 3 independent experiments. **P < 0.01 for the indicated comparisons.

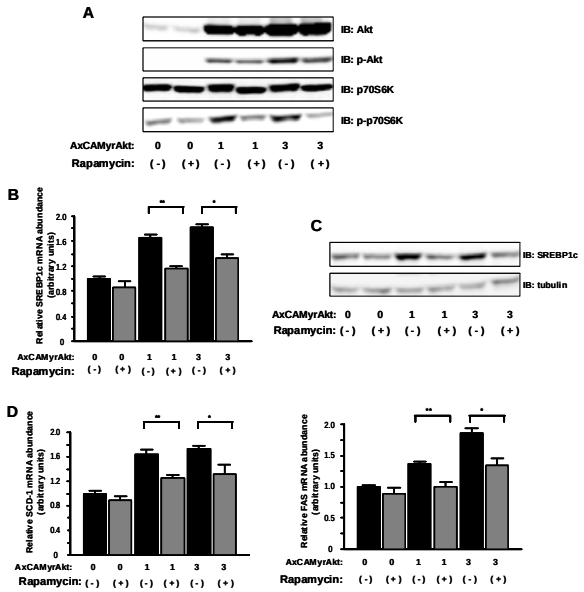

Figure4 Effects of rapamycin on the expression of SREBP1c and genes for lipogenic enzymes induced by a constitutively active form of Akt. AML12 cells were infected with AxCAMyrAkt at an MOI of 0, 1, or 3 PFU/cell for 24 h and then incubated in the absence or presence of rapamycin (100 nM) for 3 h, after which the cells were subjected to immunoblot analysis with antibodies specific for the indicated proteins (A, C) or to quantitative RT-PCR analysis of the indicated mRNAs (B, D). RT-PCR data are expressed relative to the corresponding value for noninfected cells and are means ± SEM of values from 3 independent experiments. *P < 0.05, **P < 0.01 for the indicated comparisons.

LY294002 prevented the phosphorylation of Akt as well as the mobility shift and phosphorylation of p70S6K in AML12 cells infected with AxCAMyrAkt (Fig. 3A), consistent with the notion that the activity of these kinases is regulated by PI3K signaling (14). The MyrAkt-induced increases in the amounts of SREBP1c mRNA (Fig. 3B) and protein (Fig. 3C) as well as in those of SCD1 and FAS mRNAs (Fig. 3D) were also markedly inhibited by LY294002. In contrast, rapamycin inhibited the mobility shift and phosphorylation of p70S6K but did not substantially affect the phosphorylation of Akt in cells infected with AxCAMyrAkt (Fig. 4A). The MyrAkt-induced increases in the amounts of SREBP1c mRNA and protein (Fig. 4B, C) as well as in those of SCD1 and FAS mRNAs (Fig. 4D) were inhibited by rapamycin to an extent similar to that observed with LY294002. These results thus indicated that signaling by PI3K as well as that by mTOR contribute to the up-regulation of SREBP1c expression by MyrAkt.

DISCUSSION

The regulation of lipid metabolism in the liver is a promising therapeutic target in metabolic diseases. For example, statins and fibrates, the most widely prescribed cholesterol- and triglyceride-lowering drugs, respectively, exert their effects by influencing lipid metabolism in the liver. Given that SREBP1c plays an important role in fatty acid synthesis in the liver, this transcription factor and the signaling pathways that modulate its expression are potential therapeutic targets in conditions associated with up-regulation of fatty acid synthesis, such as hypertriglyceridemia and hepatic steatosis. We have now shown that the expression of SREBP1c induced by a constitutively active form of Akt (MyrAkt) was markedly inhibited by rapamycin, a specific inhibitor of mTOR, indicating that mTOR regulates SREBP1c expression. Although recent evidence suggests that the mTOR pathway regulates the expression of SREBP1c in retinal pigment epithelial cells and thereby

contributes to regulation of the proliferation of these cells (3), our data show for the first time that mTOR participates in the regulation of SREBP1c expression in hepatocytes. We found that the abundance of mRNAs for SCD1 and FAS, key enzymes in fatty acid synthesis, was also increased by MyrAkt in a manner sensitive to rapamycin, consistent with the notion that mTOR regulates hepatic fatty acid synthesis by increasing the expression of SREBP1c.

Evidence suggests that Akt plays a key role in various metabolic actions of insulin (19). Expression of constitutively active mutants of Akt containing either a myristoylation signal sequence (10, 11) or a CAAX motif (4) has thus been shown to mimic certain actions of insulin. Although rapamycin markedly inhibited the expression of SREBP1c induced by MyrAkt, it had only a minimal effect on that induced by insulin, indicating that insulin and MyrAkt activate different signaling pathways to achieve up-regulation of SREBP1c expression. This conclusion is consistent with our previous observation that insulin-induced expression of SREBP1c was not prevented by a dominant negative mutant of Akt (13). Although constitutively active mutants of signaling molecules can be informative as to whether such molecules are capable of inducing a particular biological response, overexpression of hyperactive enzymes may induce biological effects that are not mediated by endogenous enzymes under physiological conditions.

The conditions under which the mTOR pathway contributes to the induction of SREBP1c expression in living animals remain to be determined. In addition to insulin, branched-chain amino acids stimulate the activity of mTOR (3). The plasma concentrations of such amino acids are increased in obese humans or animal models of obesity (2, 25), and mTOR signaling in the liver is increased in a rodent model of obesity (9). It is thus possible that mTOR signaling, likely activated by the increase in the plasma concentrations of branched-chain amino acids, is responsible for the increased expression of SREBP1c in the liver of obese animals.

In summary, we have shown that mTOR regulates SREBP1c expression in hepatocytes. Our results suggest that inhibition of mTOR signaling in the liver may ameliorate hypertriglyceridemia or hepatic steatosis. Rapamycin is administered as an immunosuppressant to prevent organ rejection in transplants patients. However, individuals treated with rapamycin or a rapamycin analog have been shown to manifest an increase, rather than a decrease, in the plasma triglyceride concentration (5, 7, 12). Given that the mTOR pathway appears to participate in energy metabolism in multiple organs (3), such an adverse effect might be attributable to actions of these drugs in organs other than the liver. Further characterization of the functions of mTOR in living animals is required before clinical application of mTOR inhibitors to down-regulate hepatic SREBP1c expression.

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