Synopsis of Original Research Paper

Regulation of fatty acid and cholesterol metabolism in human fibroblast cells

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In an attempt to identify unknown target genes for SREBP-1, total RNA from a stable CHO cell line (CHO-487) expressing a mature form of human SREBP-1a (amino acids 1 to 487) with a LacSwitch Inducible Mammalian Expression System was subjected to a PCR subtraction method. One of the fragments was found to have 90 and 86% homology with rat and human ATP citrate-lyase (ACL) cDNA, respectively. When Hep G2 cells are cultured under either sterol-loaded or -depleted conditions, expression of the gene is induced approximately 2 to 3-fold by sterol depletion. To investigate the direct effect of SREBP-1a on transcription, luciferase assays using the promoter of the human ACL gene were performed. These deletion studies indicated that a minimum 160-base pair segment contains the information required for the transcriptional regulation brought about by enforced expression of SREBP-1a. Luciferase assays using mutant reporter genes revealed that SREBP-dependent transcriptional regulation is mediated by two nearby motifs, the SREBP-binding site (a TCAGGCTAG sequence) and the NF-Y-binding site (a CCAAT box). It was confirmed by gel mobility shift assays that recombint SREBP-1a binds to the sequence. Data from studies with transgenic mice and reporter assays show that the ACL gene promoter is activated by SREBP-1a more strongly than SREBP-2 in contrast to the HMG CoA synthase and LDL receptor gene promoters, which exhibit the same preference for the two factors . Therefore, SREBPs transcriptionally regulates ACL enzyme activity, which generates the cytosolic acetyl CoA required for both cholesterol and fatty acid synthesis.