IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells

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Abstract The mRNA level for cytosolic NADP-dependent isocitrate dehydrogenase (IDH1) increases 2.3-fold, and enzyme activity of NADP-isocitrate dehydrogenase (IDH) 63%, in sterol-deprived HepG2 cells. The mRNA levels of the NADP- and NAD-dependent mitochondrial enzymes show limited or lack of regulation under the same conditions. Nucleotide sequences that are required, and sufficient, for the sterol regulation of transcription are located within a 67 bp region of an IDH1-secreted alkaline phosphatase promoter-reporter gene. The IDH1 promoter is fully activated by the expression of SREBP-1a in the cells and, to a lesser degree, by that of SREBP-2. A 5’-end truncation of 23 bp containing a CAAT and a GC-Box results in 6.5% residual activity. The promoter region involved in the activation by the sterol regulatory element binding proteins (SREBPs) is located at nucleotides −44 to −25. Mutagenesis analysis identified within this region the IDH1-SRE sequence element GTGGGCTGAG, which binds the SREBPs. Similar to the promoter activation, electrophoretic mobility shifts of probes containing the IDH1-SRE element exhibit preferential binding to SREBP-1a, as compared with SREBP-2. These results indicate that IDH1 activity is coordinately regulated with the cholesterol and fatty acid biosynthetic pathways and suggest that it is the source for the cytosolic NADPH required by these pathways.—Shechter, I., P. Dai, L. Huo, and G. Guan. IDH1 gene transcription is sterol regulated and activated by SREBP-1a and SREBP-2 in human hepatoma HepG2 cells: evidence that IDH1 may regulate lipogenesis in hepatic cells. J. Lipid Res. 2003. 44: 2169–2180.

Supplementary key words cytosolic NADP-dependent IDH • sterol regulatory element binding protein • fatty acid synthesis • transcriptional regulation • cholesterogenesis

Eukaryotic cells express three different forms of isocitrate dehydrogenase (IDH). These enzymes catalyze the oxidative decarboxylation of isocitrate into α-ketoglutarate utilizing either NAD or NADP as cosubstrates (1, 2). The role of the mitochondrial NAD-dependent IDH (IDH3) is well known, as it is the IDH that catalyzes a step in the tricarboxylic acid cycle (3). In contrast, the biochemical role of the two NADP-dependent IDHs (E.C. 1.1.1.42) is not entirely clear. These latter forms of the enzyme are encoded in the nucleus and function as homodimers (4, 5). One NADP-IDH is localized in the mitochondria (IDH2), but gene disruption studies in yeast have shown that it cannot replace the function of the IDH3 (6). The human cytoplasmic NADP-specific enzyme (IDH1) is encoded by the IDH1 gene, and its mRNA sequence was reported (7). The subcellular localization of the enzyme’s protein was shown to be in both peroxisomes and the cytoplasm (8). Interestingly, the rat enzyme was shown to localize exclusively in peroxisomes (9). The subcellular localization of IDH1 in peroxisomes is consistent with the presence of the peroxisomal targeting PST-1 sequence (Ala-Lys-Leu-COOH).

An additional physiological role for peroxisomal IDH1 may be to provide cytosolic NADPH for several NADPH-dependent enzymes, such as 3-hydroxy-3-methylglutaryl-CoA reductase (10–13), acyl-CoA reductase (14), and 2,4-dienoyl-CoA reductase (15, 16). The activity of IDH1 is the only known source for peroxisomal NADPH.

Another physiological role of peroxisomal IDH1 is to provide α-ketoglutarate, which is required as a cosubstrate for the phytanoyl-CoA α-hydroxylase reaction (17–20).

Abbreviations: HSS, human squalene synthase; IDH, isocitrate dehydrogenase; IDH1, cytosolic NADP-dependent IDH; IDH2, mitochondrial NADP-dependent IDH; IDH3, mitochondrial NAD-dependent IDH; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SCAP, SREBP cleavage-activating protein; SEAP, secreted alkaline phosphatase; SQS, squalene synthase; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein.

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This enzyme catalyzes a step in the degradation of phytanoic acid, a by-product of the plant-derived phytol. Several metabolic functions for phytic acid have been reported: regulation of glucose metabolism in primary rat hepatocytes by acting as an agonist to peroxisome proliferator-activated receptors (PPARs) (21, 22) and retinoid X receptor (RXR) (23); white adipocyte differentiation (24); and activation of uncoupling protein-1 gene transcription and brown adipocyte differentiation (25). In the autosomal disorder Refsum’s disease, α-oxidation of phytic acid is affected in some, but not all, cases, and accumulation of this acid is observed in various tissues (26).

The likely function of the cytoplasmic IDH1 is to produce NADPH for reductive reactions (27). Although the hexose monophosphate shunt is considered to be the major source of NADPH in the cytoplasm, genetic defects in glucose-6-phosphate dehydrogenase (G6PD), which catalyzes the first step in the shunt, do not affect sterol or fatty acid metabolism in humans (28). Population studies provide no evidence that any disorders other than hemolytic anemia are associated with G6PD deficiency (29), and both Chinese hamster ovary cells and human fibroblasts with less than 5 percent of normal G6PD activity grow normally (30, 31). This result suggests that the contribution of IDH1 to NADPH production may be significant. There are growing indications that IDH1 participates in cytosolic NADPH production and in fatty acid biosynthesis (32, 33). In support of this idea, IDH1 was also identified as a major source of cytosolic NADPH needed for the regeneration of reduced glutathione, critically important in cellular defense against oxidative damage (32, 34). Finally, an interesting and unexpected role for IDH1 was shown in bovine eyes, where the enzyme protein fulfills the criteria for a corneal epithelial crystallin, which may be involved in maintaining corneal epithelial transparency (35). Because the activity of IDH1 serves as a major source for nonmitochondrial NADPH required in multiple metabolic pathways, it is important to understand how IDH1 activity is regulated. The regulation in the peroxisomes and cytoplasm is not known.

Cholesterol and fatty acid synthesis is regulated mainly by coordinated transcription involving sterol regulatory elements (SREs), which are the targets of three basic-helix-loop-helix leucine zipper transcription factors called sterol regulatory element binding proteins (SREBPs) (36). These proteins are synthesized as membrane-bound endoplasmic reticulum (ER) precursors (M-SREBPs) with two membrane-spanning domains (37). After synthesis, the SREBPs are bound to an SREBP cleavage-activating protein (SCAP). The NH2-terminal region of SCAP contains eight membrane-spanning helices that serve as the sterol-sensing domain; the COOH-terminal region contains several copies of a WD40 sequence that binds to the SREBPs (37–41). At low membrane cholesterol levels, SCAP escorts the SREBPs to the Golgi compartment (42), where the SREBPs are processed by site-1 and site-2 pro tease. Proteolytic cleavage releases the C-terminal SREBPs and the soluble transcriptionally active N-terminal SREBPs (N-SREBPs) into the cytosol. The latter can then enter the nucleus and activate sterol and fatty acid synthesis. The sterol-regulated movement of the SCAP-SREBP complex from the ER to the Golgi is a pivotal event that controls cholesterol homeostasis in eukaryotic cells (37, 43–45).

Recently, two additional ER-bound proteins, INSIG-1 (46) and INSIG-2 (47), were shown to be involved in the regulated transport of the SCAP-SREBP complex to the Golgi. These two proteins have 59% identity with five putative transmembrane domains. In the presence of high membrane cholesterol content, the INSIG proteins bind to the sterol-sensing domain of SCAP, causing its retention in the ER and preventing SREBP-SCAP complex transport to the Golgi. At low cholesterol levels, the INSIGs dissociate from the SCAP, allowing the migration of the SREBP-SCAP complex to the ER budding region for transport to the Golgi (46–49). We have recently demonstrated that the ER-to-Golgi transport of the SREBP-SCAP complex is inhibited at 20°C and that this inhibition in transport can be overcome by the overexpression of SCAP (50).

The N-SREBPs display differential activation of gene transcription. It was first reported that various cis elements in the human hepatic squalene synthase (HSS) promoter differentially bind N-SREBP-1a and N-SREBP-2. Based on that, distinct functional specificity for the two transcription factors was predicted (51, 52). A subsequent study by Goldstein and colleagues (53) established the differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in Chinese hamster ovary cells expressing the individual SREBPs. There again, the mRNA for squalene synthase (SQS) was differentially expressed by SREBP-2 and not by the two isoforms of SREBP-1. These observations, together with results from studies in genetically manipulated mice (54), indicate that SREBP-1 is selectively involved in the activation of genes associated with fatty acid metabolism while SREBP-2 is more specific in cholesterol homeostasis, primarily through its selective activation of the SQS gene. The number of genes found to be activated by the SREBPs is constantly increasing, and includes enzymes involved in cellular cholesterol homeostasis and fatty acid synthesis (55–57). Our study describes the promoter for the IDH1 gene, its transcriptional regulation, and the relationship between this regulation and the regulation of fatty acid and sterol biosynthesis.

**EXPERIMENTAL PROCEDURES**

**Cell cultures and transient transfections**

Human hepatoma HepG2 cells were cultured in Eagle’s minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 1 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37°C under 5% CO2 atmosphere. Transient transfections were conducted in 12-well plates. Used for each transfection were 0.5 g of a given IDH1-SEAP reporter plasmid and 0.1 μg of pCMV-β-galactosidase (as a control for transfection efficiency) plus 25 ng of one of the plasmids pCDNA3.1 (as pCMV vector control), pCMV-CSA10 (con-
nucleotide located 3' to the promoter-reporter for the human IDH1 gene. The sequences of the two primers used are: 5'-GGCTGGGGAGGTGGCTGAGGA-3' and 5'-GAGGCGTGTCGGGGGCG-3'. The amplified DNA fragment is in the region −962 to +170 relative to the IDH1 gene transcription initiation site (see below). The DNA was then ligated into the pKpn cloning vector (Clontech) to produce the SEAP-reporter plasmids of various lengths. The sequences were determined.

Preparation of IDH1 promoter-reporter constructs

The 5' flanking region of the IDH1 gene was amplified by PCR from HepG2 genomic DNA using a pair of primers set for the desired region, based on sequence information of the human chromosome 2 (GenBank accession number AC016697). The sequences of the two primers used are: 5'-GGCTGGGGAGGTGGCTGAGGA-3' and 5'-GAGGCGTGTCGGGGGCG-3'. The amplified DNA fragment is in the region −962 to +170 relative to the IDH1 gene transcription initiation site (see below). The DNA was then ligated into the pKpn cloning vector (Clontech) to produce the SEAP-reporter plasmids of various lengths. The sequences were determined.

Preparation of poly(A+) RNA and Northern blot analysis

HepG2 cells (1 × 10^6) were plated onto 150 mm plates and grown for 2 days at 37°C then treated as described above. Two sets of plates were treated. One set was kept at 37°C, and the other was transferred and kept at 20°C. At the end of the 24 h treatment period, the cells were harvested and total RNA was prepared using Trizol Reagent (Invitrogen). Poly(A+) RNA was extracted from total RNA using the FastTrack® 2.0 Kit (Invitrogen). For Northern blots, 4 μg poly(A+) RNA from each sample was separated on a 1.2% agarose gel, transferred onto nylon membranes, and probed with different 32P-labeled cDNA probes. IDH1 and IDH2 cDNA probes were generated by RT-PCR based on known mRNA sequences, and the probes for IDH3-β and IDH3-γ were excised from clones containing corresponding cDNA sequences obtained from American Type Culture Collection. Quantitation of specific signal intensities was performed by densitometry using ImageQuant software (Molecular Dynamics).

Site-directed mutagenesis of IDH1 promoter

Replacement mutation of the IDH1-SRE-1 element in the human IDH1 promoter was obtained using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene). The primers used in this procedure were the pair of primers used in the electrophoretic mobility shift assay to produce the IDH1−40/−5 m5 probe (see Fig. 7B), in which the sequence TGAT of the proposed IDH1-SRE element was replaced by the sequence GTTT. This mutation procedure produced the IDH1-67m5-SEAP mutant promoter-reporter construct.

Determination of transcription initiation sites

To determine the transcription initiation site(s) of the IDH1 gene, a 5' rapid amplification of cDNA ends (RACE) was performed (58) using the 5'RACE System kit from Invitrogen. The gene-specific primers GSP1 (5'-TTCTCAACCCTTCTCTCATC-3') and GSP2 (5'-TTCTGTCATTCACTCTCTCTC-3') were used at +42 and +234 3' to the AUG translation initiation site. The various RACE clones isolated were sequenced, and the most 5' end sequences were determined.

Preparation of poly(A+) RNA and Northern blot analysis

HepG2 cells (1 × 10^6) were plated onto 150 mm plates and grown for 2 days at 37°C then treated as described above. Two sets of plates were treated. One set was kept at 37°C, and the other was transferred and kept at 20°C. At the end of the 24 h treatment period, the cells were harvested and total RNA was prepared using Trizol Reagent (Invitrogen). Poly(A+) RNA was extracted from total RNA using the FastTrack® 2.0 Kit (Invitrogen). For Northern blots, 4 μg poly(A+) RNA from each sample was separated on a 1.2% agarose gel, transferred onto nylon membranes, and probed with different 32P-labeled cDNA probes. IDH1 and IDH2 cDNA probes were generated by RT-PCR based on known mRNA sequences, and the probes for IDH3-β and IDH3-γ were excised from clones containing corresponding cDNA sequences obtained from American Type Culture Collection. Quantitation of specific signal intensities was performed by densitometry using ImageQuant software (Molecular Dynamics).

Enzyme activity is expressed in μmol increase of NADPH per minute.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was carried out as described previously (60). Briefly, annealed oligo probes were end-labeled with γ-32P-ATP using T4 polynucleotide kinase. For the binding reaction, 8 × 10^5 dpm of each probe was incubated with ~50 ng of purified SREBP protein (60) in a buffer containing 25 mM Tris-HCl (pH 7.9), and 1 mM EDTA, followed by centrifugation to obtain clear extracts. NADPH-dependent IDH enzymatic activity assays were performed at 25°C using the Farrell procedure (59). Enzyme activity is expressed in μmol increase of NADPH per minute.

RESULTS

Sterol-mediated regulation of IDH1 mRNA level

The possible regulatory role of sterols in IDH1 expression was investigated by the addition of sterols to HepG2 cells, and analysis of IDH1 mRNA level. At 37°C, a 2.3-fold induction of the IDH1 mRNA level was observed after 24 h in steroid-depleted cells, as compared with steroid-loaded

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cells (Fig. 1). Under the same conditions, the sterol regulation of the NADP-dependent mitochondrial IDH2 was only 1.3-fold, and no sterol regulation was observed for the mRNA levels of either the β- or the γ-subunits of the NAD-dependent mitochondrial IDH3. We have recently demonstrated that sterol regulation of enzymes in the cholesterol and fatty acid biosynthetic pathways is diminished or completely absent in cells maintained at 20°C, due to lack of processing of the SREBPs at this temperature (50). It appears that a similar low-temperature effect is observed for the regulation of IDH1. The sterol-regulated increase in IDH1 mRNA level is inhibited in sterol-deprived cells maintained at 20°C, and the mRNA levels are lower than those observed in cells at 37°C, regardless of the presence of sterols. The limited sterol regulation of IDH2 is also absent at 20°C, but the level of mRNA for this enzyme is still rather substantial. No temperature effect or sterol regulation was observed for the mRNA of either the β- or the γ-subunits of IDH3 (Fig. 1). These observations, together with our earlier studies (50), indicate a coordinated regulation between cholesterol and fatty acid synthesis and the expression of the IDH1 gene. They also point to SREBPs as the likely common regulatory transcription factors for all of these genes. Lack of, or limited, sterol regulation of the mRNA for the other IDH genes may suggest that IDH1 is the major source for NADPH for sterol and fatty acid biosynthesis.

**Sterol regulation of NADP-dependent IDH enzyme activity**

The 24 h sterol effect on the NADP-dependent IDH activity in HepG2 extracts is shown in Fig. 2. Sterol deprivation of cells for this period of time results in a 63% increase in NADP-dependent IDH activity compared with the activity in cells grown in the presence of sterols. Because this enzyme assay cannot distinguish between the activities of IDH1 and IDH2, it is not possible to assess the contribution of each to the observed activity and the participation of either in the sterol regulation. Nevertheless, a significant sterol effect on the NADP-dependent activity in total cell extract is observed.

**Analysis of gene structure and the cloning of the promoter for IDH1**

Blast analysis of GenBank was performed using different sequences of the cDNA structure for IDH1 (accession number NM005896). All sequences of the cDNA were identified in a 172,304 bp BAC clone, RP11-140C4 (accession number AC016697). The intron-exon organization for the IDH1 gene was determined by comparing the cDNA sequence with that of the BAC clone, applying the GT-AG rule for intron splicing (61). Figure 3 depicts the intron-exon organization and the codons at the various splice sites. The first two 5’ exons (black boxes) are located at the 5’ untranslated region (see transcription start site below). The largest exon is located at the very 3’ end of the cDNA (941 bp) and contains the polyA tail (Fig. 3A).

Five distinct transcription start sites were determined by RACE analysis at 122, 157, 167, 180, and 211 bp 5’ to the ATG codon (Fig. 3B). The major site, at 167 bp 5’ to the ATG codon, was also identified by primer extension analysis, as well as a minor site at 122 bp (data not shown). The sequences of all the RACE-generated clones fully matched sequences in the genomic RP11-140C4 BAC clone, indicating genuine transcription sequences. In separate experiments, some clones at the size indicated below were not found. However, in a single experiment in which 11 clones were sequenced, four different isolated RACE clones with 5’ untranslated sequences of 122, 157, 180, and 211 bp were identified, whereas seven identical clones
with a 5’ untranslated sequence of 167 bp were found. Thus, it appears that clones with a 5’ untranslated sequence of 167 bp are at approximately \( \times 7 \) frequency. The most 5’ adenosine of the longest clone (211 bp 5’ to the ATG) was designated 1. Based on the above, the sequence of \(-962\) to +170 was amplified for the construction of the pIDH1-962-SEAP promoter-reporter.

**Sterol regulation of pIDH1-962-SEAP promoter-reporter activity**

The IDH1 promoter activity is enhanced 2-fold in cells grown in media containing 10% LDS, as compared with the activity in cells grown in the presence of 10% FBS (Fig. 4). The promoter activity in cells grown in the presence of FBS is suppressed by the addition of sterols to the media. Addition of sterols completely diminishes the activation by LDS, and the IDH1 promoter activity is suppressed regardless of the type of serum in the media. As expected for a sterol-mediated regulation, addition of lovastatin to the growth media greatly enhanced the IDH1 promoter activity. A 5.4-fold enhancement in activity is observed in lipid-deprived cells in which the promoter is fully activated (LDS + lovastatin), compared with suppressed conditions (LDS + sterols). Thus, the regulation of the IDH1 promoter activity is sterol mediated, similar to the observed variations in IDH1 mRNA levels seen in Fig. 1.

**Regulation of pIDH1-962-SEAP promoter-reporter activity by SREBP**

Because the SREBP transcription factors are involved in sterol-mediated regulation of genes involved in cholesterol and fatty acid biosynthesis, it is anticipated that activation of the sterol-responsive IDH1 promoter is similarly regulated by these transcription factors. Indeed, the activity of the pIDH1-962-SEAP promoter increases in re-
the loss of promoter activity in this reporter gene may be due to a lack of sequences for the binding of NF1/NFY, or to an SP1 transcription factor, or both. A truncation of additional 19 nts resulted in an almost complete loss of promoter activity. This latter truncation (IDH1-25-SEAP) was so designed in order to cleave at an inverted SRE-1-like element, designated IDH1-SRE-1 sequence. Relatively weak signals in the mobility shift assay indicate that the IDH1-SRE-1 sequence element at its core, and its binding to the SREBP-1a protein. This probe contains the SRE-1 element in the HSS promoter (HSS-SRE-1) (52). SREBP-1a and SREBP-2 similarly retard the electrophoretic mobility shift assays, and the results were compared with the binding of the SREBPs to a probe containing the SRE-1 element in the HSS promoter (HSS-SRE-1) (52). SREBP-1a and SREBP-2 similarly retard the HSS-SRE-1 probe, resulting in radioactive signals of approximately the same intensity (Fig. 7A). A significantly weaker binding of SREBP-1a to the probe IDH1-71/21 is observed. This probe contains at its very 3' end the IDH1-SRE-1 sequence. Relatively weak signals in the mobility shift assay indicate that the IDH1-SRE-1 sequence does not bind to the SREBP-2 protein in a significant way. The IDH1-49/ +1 probe displays a much stronger binding to the SREBP-1a protein. This probe contains the IDH1-SRE-1 sequence element at its core, and its binding to SREBP-1a is comparable in signal intensity to that of
the HSS-SRE-1 probe. Only a weak retardation signal is observed for the IDH1-49/+1 probe with the SREBP-2 protein, indicating a weaker interaction with this protein compared with that with SREBP-1a (Fig. 7A).

On the basis of these results, the IDH1-40/−5 synthetic probe was designed for mutation analysis of the interaction of the SREBPs with sequence elements in the IDH1 promoter. This shorter probe also contains the IDH1-SRE sequence element at its core and displays retardation by the SREBPs similar to the retardation observed for the longer IDH1-49/+1 probe (Fig. 7B). A successive 4 nt scanning mutation analysis indicates that the eight 5' bp (mutations m1 and m2) and the sixteen 3' bp (mutations m6 to m9) of the probe are not essential for the retardation and are therefore not involved in the binding to the SREBPs. However, mutations m3, m4, and m5 of the IDH1-40/−5 probe completely abolish the mobility shift. These three mutations are within the sequence...
The sequence, the region of the probes, and the three potential sterol-regulated promoter sequence of IDH1 (IDH1-67-SEAP) promoter that binds SREBPs. Various 32P-end-labeled synthetic oligonucleotide probes outside of the IDH1-SRE element display high affinities to SREBP-1a as compared with SREBP-2. The spanning regions of the IDH1-SRE element, fail to bind SREBPs. All other mutant probes, as well as the wild-type IDH1-40/-5 probe, bind SREBP-1a preferentially.

GTGGGCTGAG (or the antisense sequence 5′-CTCAGG-CCAC), designated the IDH1-SRE element. Interestingly, retardation signals of the IDH1-40/-5 and all its mutants outside of the IDH1-SRE element display high affinity to SREBP-1a and a much weaker signal for the binding to SREBP-2.

Functional analysis of the IDH1-SRE element

To verify the function of the IDH1-SRE element within the context of the IDH1 promoter, a replacement mutation was introduced into this sequence element in the IDH1-67-SEAP reporter. We have introduced the m5 mutation, because it was demonstrated to hinder binding of the SREBPs to the IDH1-40/-5 probe containing the IDH1-SRE element (Fig. 7B). The activity of the resulting mutant promoter-reporter, IDH1-67m5-SEAP, expressed in HepG2 cells, is very low and is only 4.6% that of the wild-type promoter in sterol-deprived cells (Fig. 8). As anticipated, the SREBPs also fail to activate the IDH1-67m5-SEAP promoter significantly. Only 5.5% and 6.6% of the activation of the IDH1-67-SEAP promoter by SREBP-1a and SREBP-2, respectively, are observed for the IDH1-67m5-SEAP promoter.

DISCUSSION

The results in this report suggest an involvement of IDH1 in lipogenesis and provide a possible mechanistic rationale for this involvement, using HepG2 cells as a model. The enzyme’s specific role in supplying NADPH for the reduction reactions in the cholesterol and fatty acid biosynthetic pathways is suggested by the sterol-mediated regulation of the IDH1 gene transcription, which is...
coordinated with the transcription of all other regulated genes in these pathways. This coordinated regulation is achieved through the SREBP transcription factors, which are long recognized to have a pivotal role in the lipid-mediated regulation of the lipogenic pathways (37, 43–45).

The biochemical source of the cytosolic NADPH required for the cholesterol and fatty acid biosynthetic reactions has not been firmly established. Recently, it was suggested that the reactions catalyzed by the three cytosolic enzymes malic enzyme, G6PD, and 6-phosphogluconate dehydrogenase supply the necessary NADPH (43). However, there is no evidence to suggest that the regulation of any of these enzymes is linked to the production of sterols and fatty acids. In fact, there are several reports suggesting that G6PD, or the reactions catalyzed by the hexose monophosphate shunt, may not be the source of the NADPH needed to drive lipogenesis in humans or in cultured cells (28–31). Because the exact cellular function of IDH1 has not been established, its potential in generating significant amounts of NADPH in the cytosol may be underestimated. The significant contribution of IDH1 in producing cytosolic NADPH was actually demonstrated in one early comparative study in which IDH1 in rat liver was shown to be 16- and 18-fold more active in producing NADPH than were G6PD and malic enzyme, respectively (62).

The regulation of IDH1 gene transcription by sterols is now demonstrated in HepG2 cells (Fig. 1). The magnitude of the regulation of the IDH1 mRNA level by sterols exceeds by far that of the mitochondrial dehydrogenase IDH2 mRNA, which displays limited response, or that of the β- and γ-subunits of IDH3 mRNA, which do not display any response to sterols. The regulatory response to sterols is also demonstrated in the modulation of enzyme activity (Fig. 2). Because this enzyme assay, performed with total-cell extracts, cannot distinguish between the activities of IDH1 and IDH2, the level of the sterol regulation of IDH1 cannot be determined precisely, because of a background contribution of activity by IDH2. However, based on the data shown in Fig. 1, the activity of IDH2 is not likely to be highly regulated and, thus, the sterol response could be attributed mostly to IDH1. Future production of IDH1-specific antibodies should resolve this issue. The 20°C suppression of IDH1 mRNA level and its
sterol regulation (Fig. 1) provided the first evidence that this regulation, similar to the sterol-mediated regulation of all other enzymes in the cholesterol biosynthetic pathway, is effected through the SREBPs (50). The function of SREBPs in the sterol regulation of the IDH1 gene transcription is demonstrated by the activation of the IDH1 promoter-reporter genes by the SREBPs (Figs. 4–6). In all of these experiments, consistently, the activation by SREBP-1a is significantly higher than that obtained by the expression of SREBP-2. For IDH1-67-SEAP, the shortest sterol-responsive reporter gene, the activation by SREBP-1a is more than 24fold higher than that obtained with SREBP-2 (Fig. 6B).

On the basis of mutation analyses of electrophoretic mobility shift (Fig. 7B) and promoter activity (Fig. 8), the cis-regulatory element in the IDH1 promoter targeted by the SREBPs is most likely the IDH1-SRE sequence [−30]5′-GTGGGCTGAG[−21] (or the antisense sequence 5′-CTCAGCCCAC), which resembles (but in a reverse orientation to) the SRE-1 element in the LDL receptor gene (63). Unlike the HSS-SRE-1 sequence element in the SQS gene, this sequence element preferentially binds to SREBP-1a, as compared with a much weaker binding to SRRBP-2 (Fig. 7). This difference in binding may explain the differential activation of the IDH1 promoter by the two SREBPs (Figs. 4–6). The most pronounced difference in sequence between the consensus SRE-1 and the IDH1-SRE elements is an AT to CT change in the most 5′ end of the latter (Fig. 6A). Yet, this seemingly minute change in sequence uniquely results in a preferential binding of the IDH1-SRE sequence to SREBP-1a. In an earlier report, we have demonstrated that the sequence 5′-TCCACCCCAC, present in the HSS promoter [originally designated HSS-SRE(8/10)], exclusively binds to SREBP-2 and not to SREBP-1a (52). Here again, the most pronounced difference in the sequence is at the 5′ end, where a TC replaces the AT in the consensus SRE-1 element. Thus, it appears that the specificity in binding to (and presumably activation by) the various SREBPs resides, in part, in the two 5′ nucleotides of the SRE motif. This observation, which appears to be correct for the HSS, IDH1, and LDL receptor promoters, will have to be further examined by mobility shift assays of variant SRE motifs. If correct, specific response in transcription to the various SREBPs may be predicted by promoter sequence analysis. Whatever the explanation, the activation of the IDH1 promoter is effected in lipid-depleted cells mostly by SREBP-1a and SREBP-1c (data not shown for the latter).

The shortest IDH1 promoter-reporter gene that displays sterol regulation is IDH1-67-SEAP (Fig. 6). IDH1-44-SEAP, which is a 23 bp shorter promoter-reporter gene, still contains the intact IDH1-SRE element but lacks the CAAT and the GC-Box elements at its 5′ end. These elements are known to bind the NFY/NF1 and SP1 transcription factors, respectively. The IDH1-44-SEAP promoter-reporter activity is very low, is no longer responsive to sterols, and in the presence of SREBPs, is only a fraction of the activity of the longer promoters. Thus, the earlier observation that activation of gene transcription by the SREBPs requires the coactivators NFY/NF1 or SP1 (51, 64–67) is also correct for the IDH1 gene, which further supports the idea that, functionally, this gene is a member of the SREBP-activated gene family.

The preferential activation of the IDH1 promoter by SREBP-1a compared with SREBP-2 may indicate that the regulation of IDH1 activity may be coordinated more with fatty acid biosynthesis than with sterol production (43). Thus, it is expected that activation of IDH1 is a response to a shortage in hepatic fatty acid supply. The subcellular localization of IDH1 in both cytosol and peroxisomes ideally situates it to respond to the lipogenic requirement of the cell. The coproduct of the reaction, α-ketoglutarate, which is required as a cosubstrate for the phytanoyl-CoA α-hydroxylase reaction (17–20), is required for the catalytic removal of phytic acid, a known agonist to PPARα (21, 22). PPARα is highly expressed in heart, muscle, kidney, and endothelial cells, but mostly in liver (27, 34), where its function is in the catabolism of fatty acids through the regulation of genes encoding mitochondrial and peroxisomal enzymes important for β-oxidation (68). In addition, phytic acid was also found to bind and activate the RXR (23), which forms a functional heterodimer with PPARα. Therefore, an increase in IDH1 activity will have a compounded effect on the production and maintenance of the hepatic fatty acid pool, by first supplying NADPH, which will enhance fatty acid biosynthesis, and by then lowering the phytic acid levels, which, in turn, will diminish the PPARα/RXR effect on the β-oxidative degradation process (Fig. 9). Indeed, the expression of IDH1 in rats is primarily in liver, where the above processes are likely to occur (27, 34).

The available data, and the results presented herein, strongly suggest that the activity of IDH1 is required as a major source for cytosolic NADPH, which is specifically targeted as a substrate in the biosynthesis of cellular fatty acids and cholesterol. This activity also produces α-ketoglutarate, which indirectly regulates the β-oxidation of fatty acids through the degradation of the PPARα/RXR agonist phytic acid. Thus, IDH1 activity is involved in maintaining cellular cholesterol and fatty acid homeostasis through synthesis and degradation. Additional data, from studies using gene overexpression and knockout methodologies in cultured cells and animals, are needed to assess the specific function of IDH1 under various lipogenic conditions.

If the activity of IDH1 is proven to be the major biochemical NADPH-producing reaction for these processes, it may be worthwhile to consider IDH1 as a target enzyme for lipid-lowering pharmacological strategies.

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