UDP-glucose dehydrogenase (UGDH) is a key enzyme of the unique pathway for the synthesis of UDP-glucuronate, the substrate for the numerous glucuronosyl transferases, which act on the synthesis of glycosaminoglycans and glucuronidation reaction of xenobiotics. Using the bacterial artificial chromosome approach, we have cloned and characterized the human UGDH promoter. The core promoter contains −644 nucleotides conferring reporter gene activity in transient transfection assay of a variety of cell types, including MRC5 fibroblasts and the HepG2 hepatoma cell line. The minimal promoter of −100 nucleotides contains a functional inverted TATA box. No consensus CAAT sequence was found up to −2133 nucleotides. The expression of UGDH was up- and down-regulated by transforming growth factor (TGF)-β and hypoxia, respectively. TGF-β enhanced the activity of all the deletion constructs, except the minimal promoter. Hypoxia slightly increased the activity of the short promoter-containing constructs but decreased that of the −374 nucleotides and core promoter constructs. The core promoter contained numerous GC-rich sequences for the binding of Sp1 transcription factor. Bisanthracycline, an anti-Sp1 compound, decreased UGDH mRNA expression and inhibited the core promoter constructs activity. Gel mobility shift and supershift assays after TGF-β stimulation demonstrated an increased DNA binding of the nuclear extract proteins to the two Sp1 sequences located in the −374-bp promoter. By contrast, nuclear extract proteins from hypoxia-treated cells demonstrated a decreased binding of the consensus Sp1 sequence. These results indicate that numerous Sp1 cis-acting sequences of the UGDH core promoter are responsible for up- and down-regulation of the gene after TGF-β stimulation and in hypoxic conditions, respectively.

UDP-glucose dehydrogenase (UGDH, EC 1.1.1.22) is a key enzyme in the synthesis of UDP-glucuronate from glucose. It constitutes the unique pathway for glucuronate formation (1). Because glucuronate is a component of glycosaminoglycans, the mutation inactivation of UGDH (sugarless) abolishes glycosaminoglycan assembly and, consequently, abolishes GAG-dependent growth factor signaling. For example, such a mutation was shown to induce cardiac valve malformation in zebrafish (2) or the “wingless” and “no white eyes” phenotypes in Drosophila (3, 4). The primary structure of the mammalian enzyme was obtained from the protein sequence of bovine UGDH (5). The human gene, recently cloned in our laboratory (6), was assigned to chromosome 4p15.1 by radiation hybrid mapping (7). It contains 12 exons, extends over 26 kb, and has one major transcription start site (8). The structure of the enzyme is well conserved between the species and phyla. The cloned mammalian proteins from different species showed overall 97% identity. The human sequence has 27% identity with the Escherichia coli ortholog, with 100 and 60% identity of the NAD+ binding and the catalytic sites, respectively (8).

Glycosaminoglycan chains of proteoglycans and hyaluronan are ubiquitous components of extracellular matrix and pericellular spaces. There is a growing body of information on the implication of GAGs in cell behavior, including signal transduction, cell proliferation, spreading, migration, and cancer growth and metastasis (9–11). GAG synthesis is influenced by cytokines and growth factors. TGF-β is the most potent stimulator of proteoglycan and glycosaminoglycan synthesis, including that of hyaluronan. Its action, however, depends on the cell type (12). The synthesis of GAGs is also modulated by oxygen cell status. Hypoxic endothelial cells and lung fibroblasts enhanced heparan sulfate/chondroitin sulfate ratio, which led to an increase of basic fibroblast growth factor reactivity on the cell surface (13, 14). It was also shown that the level of intracellular UDP-glucuronate could influence GAG synthesis (1).

The knowledge of the cytokine regulation of UGDH expression is scanty. The human UGDH was shown to be an early response gene after interleukin-1β treatment of ocular fibroblasts (15), as well as an early androgen response gene in breast cancer (16). It was also up-regulated by fetal serum (5, 17). TGF-β signaling in the cells is initiated through receptor-dependent Ser/Thr kinase phosphorylation of SMAD3 (18), which forms a heterodimer complex with SMAD4 (19). The complex is
translocated into the nucleus where it activates its target genes (20). SMAD proteins, however, cooperate with other transcription factors to activate the promoters of different genes. In several systems, Sp1 is a powerful intermediate of the TGF-β signaling cascade. For example, Sp1 drives TGF-β activation of promoters such as the collagen I(21) and α2(1) chains (22, 23), plasminogen activator inhibitor-1 (24), SMAD? (25), TGF-β receptor II, and TGF-β itself (26). The involvement of Sp1 in the TGF-β-mediated induction of different genes is mediated by its interaction with the canonical GC-rich sequences, implicating the formation of a complex with phosphorylated SMAD3. The methylation inhibition of Sp1 expression (27, 28), or mutation of GC-rich binding sequences (24, 25, 29, 30), completely suppressed TGF-β action, indicating similar roles of SMAD and Sp1 systems in signaling cascades. Several genes are activated through Sp1 after TGF-β treatment and export proteins, and it was proposed that Sp1 is a major regulator of the expression of extracellular proteins (31). In TATA-less promoters, Sp1 can also drive the basal activity of the gene (32).

In the present study, we characterized the regulation of UGDH expression. The expression of its mRNA was studied in different cell strains of human origin. We cloned the human UGDH promoter and defined its minimal sequence and basal activity. We showed that the core promoter of 650 bp is activated under TGF-β treatment through numerous Sp1 sites. On the other hand, in HepG2 cells, which harbored a high constitutive level of UGDH activity, we demonstrated that hypoxic conditions led to the down-regulation of this enzyme by nuclear depletion/inactivation of the same Sp1 protein. We thus proposed that Sp1 transcription factor may be a universal modulator of UGDH expression and, by modulating the enzyme level, may control the UDP-glucorone level in different metabolic pathways, including GAG synthesis.

EXPERIMENTAL PROCEDURES

Cell Culture—The following cells were obtained from American Type Culture Collection: HepG2 (ATCC number HB-8065, hepatocellular carcinoma); MCF7 (ATCC number HTB-22, adenocarcinoma cell line); K562r and K562s (resistant or sensitive to doxorubicin treatment, respectively) derived from K562 (ATCC number CCL-243, leukemia, chronic myeloid) (33); HT1080 (ATCC number CRL-1427, osteosarcoma cell line); and MRC5 (ATCC number CCL-171, normal lung fibroblasts). Human umbilical vein endothelial cells were obtained from Promocell (Heidelberg, Germany). NCTC2544 cells (ICLC number HL97002, skin keratinocytes cell line) were obtained from Interlab Cell Line Collection. Cells were cultured in the media recommended by the supplier, supplemented with 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Before TGF-β stimulation, confluent MRC5 cells were preincubated for 18 h in a medium containing 0.5% FCS, and the stimulation was performed as indicated in the figure legends. For studies involving hypoxic conditions, HepG2 cells were cultured to confluence, then the medium was replaced with fresh medium supplemented with 10% FCS. The cells were pureed with 100% N2 for 15 min and then incubated with 0.1–20% O2/5% CO2/balance N2 for 24 h. Oxygen concentration was monitored with an Oxypocket oxymeter (Bioblock Scientific, Illkirch, France).

Northern Blot—Total RNA from different cell lines were extracted by the guanidine thiocyanate method and separated on 1.1% denaturing gel, transferred onto a membrane, and hybridized at 60°C with random priming labeled AAATAGGG/5′ primer containing the 496-bp insert containing a part of human open reading frame UGDH (BACPAC Resource Center at the Children’s Hospital, Oakland Research Institute, Oakland, CA) was digested with EcoRI restriction enzyme, and the fragments were separated on a 0.8% agarose gel followed by a Southern blot. The blot was hybridized with a 165-bp probe containing the 5′ portion of UGDH cDNA (b). A 3-kb fragment, which gave a weak hybridization band with the probe, was excised from the gel, subcloned into a Bluescript plasmid, and fully sequenced. The resulting sequence was analyzed with the Transfac data base (36) and MatInspector algorithms (37), using the Transcription Element Search System (available at www.chib.upenn.edu/less). A 2.133-kb EcoRI/SacI fragment containing 50 bp from exon 1 and 2.083 kb from the 5′ untranslated region was subcloned into pGL2-basic (Promega, Charbonnieres, France) and named the −2133 construct. Additional constructions were made using restriction endonucleases: HincII/SacI for the −644 construct and PstII/SacI for the −100 construct, respectively. Other constructs were made using PCR-amplified pBluescript containing the 3-kb fragment with forward primers containing KpnI site (for −249 construct, 5′-TTAGTGATCCTGAGCTGGCCAAAGGGGAATCC-3′) and reverse primer containing the HindII site (for −374 construct, 5′-TGGCGACCCACGCGTACGAC-3′). The amplimers were digested with KpnI/SacI or by HindII/SacI and subcloned into pGL2-basic. Mutation in the inverted TATA box was introduced using the QuikChange site-directed mutagenesis kit (Stratagene, Amstderdam Zuidest, the Netherlands) with the synthetic oligonucleotides 5′-TCGAGCGGAAAATTCGAGGTCGTCAGC-3′ and 5′-GCGCTGAGCCGGATTTGGGAGGTCGTCGAGG-3′. The PCR was performed on the −100 construct according to the manufacturer’s instruction. All plasmids and site-directed mutagenesis constructs were sequenced to confirm the intended structure.
Transient Transfections and Luciferase Assay—UGDH promoter-luciferase reporter gene constructs (3 and 1 ng for MRC5 and HepG2 cells, respectively) were co-transfected into MRC5 cells or HepG2 cells with 25 ng of pRL-TK vector, containing the herpes simplex virus thymidine kinase promoter upstream of Renilla luciferase gene using the Fu-gene-6 transient transfection kit according to the manufacturer’s protocol. After 24 h of incubation in the appropriate media, MRC5 and HepG2 cells were harvested and lysates were prepared. Firefly luciferase activities were assayed with a dual luciferase reporter assay system (Promega) in a Lumi-One luminometer (Bioscan, Washington, D. C.) and normalized to Renilla luciferase activity.

Nuclear Extracts and Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared from confluent flasks of TGF-β-treated or untreated MRC5 or from HepG2 cells incubated under normal aerobic conditions or under 1% O2 (see hypoxia experiments above), as described by Dignan et al. (38). Final protein concentrations were determined by using the Bio-Rad protein assay kit. A double-stranded oligonucleotide containing consen-sus Sp1 site (underlined: 5'-CCCTTGTTGGGGCCGCTATTGC-3') and an His-mutated counterpart (5'-CCCTTGTTGGGGCCGCTAAAGCT-3') were purchased from Geneka, Montreal, Canada. The oligonucleotides corresponding to three potential Sp1 sites on UGDH promoter were as follows: Sp1–253 (5'-CCCGTAAAGCGGGAAGCGCTGA); Sp1–276 (5'-CAGGAGGCGCAGCGCG); and Sp1–312 (5'-GGGCGCCAGCGCCGAGCGCG). The complementary oligonucleotides were annealed overnight and end-labeled with [32P]-32P]ATP using T4 kinase (New England BioLabs). Nuclear proteins (7.5 μg/assay) were incubated at room temperature, in a reaction solution containing 4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 50 μCi of poly(dl-dC)poly(dl-dC). For competition experiments, a 100× molar excess of non-labeled competitor oligonucleotides was added, as indicated in the figures legends. After 15 min, the 32P-labeled duplex oligonucleotide (1.75 fmol) was added, and the incubation was followed by another 30 min at room temperature, in a total volume of 20 μl.

DNA-protein complexes were separated on 6% non-denaturing polyacrylamide gels in Tris borate/EDTA buffer, pH 8.0, at room temperature and 120 V for 4 h. The gels were dried, and complexes were revealed by autoradiography.

For supershift assay, the nuclear extracts were preincubated with 1 μl of antiserum (0.2 mg/ml) at room temperature for 1 h before analysis.
by EMSA as described above. Human anti-Sp1 monoclonal antibody was obtained from Santa Cruz Biotechnology (SC-420) and from Geneka. Human anti-Sp3 polyclonal immunoserum was from Santa Cruz Biotechnology (SC-644). The Sp1 anti-sera specifically detect the presence of the Sp1 transcription factor, whereas the Sp3 anti-serum lightly cross-reacted with the Sp1 transcription factor (verified by Western blot, data not shown). Nonspecific IgGs were incubated with the nuclear extracts as described and used as negative control. Nuclear extract from HeLa cells (Geneka) were used as positive control.

RESULTS

Basal Expression of UGDH mRNA, Effects of TGF-β, and Hypoxia—It was previously shown that UGDH is ubiquitously expressed in all analyzed tissues (8, 15). We used a panel of different human cells in culture to establish the basal expression of the enzyme. All the tested cells expressed a double transcript of UGDH mRNA of 2.8 and 3.4 kb (Fig. 1), with a slightly different proportion of those two transcripts. The lowest mRNA expression was observed in umbilical vein endothelial cells and in monocytes, whereas HepG2 hepatoma cells displayed the highest level. The mRNA abundance suggests that the transformation of these epithelial cells of hepatic origin had little or no influence on UGDH expression. Skin fibroblasts and MRC5 embryonic pulmonary fibroblasts expressed an intermediate level of UGDH mRNA. In dermal fibroblasts, we observed some variability of the expression, depending on the age of donor, biopsy site, and cell passage number. Consequently, we decided to use MRC5 fibroblasts, which give more constant results as a model of low UGDH expression, to analyze the TGF-β stimulatory effects, and the HepG2 hepatoma cells as a model of high UGDH expression, to analyze the hypoxia inhibitory effects.

Because TGF-β is a major growth factor able to influence GAG synthesis, we studied its effects on UGDH expression by MRC-5 cells. TGF-β increased the incorporation of [35S]sulfate into GAG fraction in a dose-dependent manner (Fig. 2A). In parallel, TGF-β enhanced the steady-state level of UGDH mRNA. Both transcripts were equally affected (Fig. 2B). The effect was time-dependent, with pronounced expression after 8 h of stimulation (Fig. 2C). The expression was maximal after 12-h stimulation and persisted for 24 h. Cycloheximide had an additive effect (Fig. 2D), indicating that the stimulation did not need new protein synthesis and that UGDH is a direct response gene after TGF-β treatment. The late response of UGDH suggests, however, that the mechanism of stimulation may be complex and involve the recruitment of different transcription factors.

Hypoxia is another factor capable of influencing GAG synthesis (13). For that reason, we studied the effect of oxygen status on the UGDH mRNA expression in HepG2 cells. Twenty-four hours of hypoxia inhibited the steady-state UGDH mRNA level in a dose-dependent manner (Fig. 3A). Hypoxia also reduced the incorporation of [35S]sulfate into GAG in HepG2 cells cultures (Fig. 3B). The effect was similar in MRC5 cells (not shown).

Characterization of UGDH Promoter—To investigate the mechanism of the regulation of UGDH expression by TGF-β and hypoxia, the 3-kb genomic fragment, including the exon 1 was cloned from a BAC containing a part of the human chromosome 4p (6). From this construction, a subcloned fragment of 2183 bp, limited by EcoRI and SacI, was fully sequenced (Fig. 4). It contained 50 bases of the previously determined 5’ untranslated region. A detailed computer analysis using the Transfac (36) and MatInspector (37) algorithms revealed a high probability score for the presence of the promoter. The inverted TATA box was located 22 bases upstream from the transcription start site, and no consensus CAAT sequence was found. The proximal fragment of 374 bp contained seven consensus sequences for Sp1 transcription factor and one consensus sequence for hypoxia-inducible factor-1 (39).

To ascertain the transcriptional promoter activity, a panel of constructions containing different lengths of the 5’-flanking region coupled to firefly luciferase reporter gene was generated (Fig. 5A). The transient transfection of the constructs drove the luciferase activity resulting from the promoter activity in both MRC5 and HepG2 cells (Fig. 5B). The luciferase level was always higher in HepG2 cells compared with MRC5 fibroblasts, even for the shortest constructs. Increasing the length of the promoter up to −644 bp enhanced the basal expression of the reporter gene. The longest construct, containing 2133 bp of the promoter, displayed about 30% of the activity compared with the −644-bp construct, suggesting the presence of inhibitory elements. The shortest construct (−100 bp) showed low, but measurable, promoter activity. This sequence contained only one Sp1 consensus site and an inverted TATA box. Mutation of this box decreased the reporter gene activity to the background level (Fig. 5C), indicating that −100 bp may be defined as a
minimal promoter for the UGDH gene and that the inverted TATA box is necessary for its activity.

Influence of TGF-β and Hypoxia on Promoter Activity—The constructs containing different lengths of the UGDH promoter were transiently transfected to MRC5 cells and HepG2 cells to study the mechanisms of TGF-β/H9252 and hypoxia effects, respectively. TGF-β/H9252 (10 ng/ml) had no effect on minimal and 2133-bp promoters but enhanced the activity of all the constructs up to 644 bp (Fig. 6A). The strongest stimulation was observed for the constructs of 374 and 644 bp. These fragments contain six and eleven additional Sp1 consensus sequences, respectively (Fig. 4). When the plasmids were transiently transfected into the HepG2 cells maintained in 1% O2, the activity of the constructs 165 and 196 bp were enhanced about 2-fold in comparison to the cells cultured in 20% O2. The constructs containing 374, 644, and 2133 bp were strongly inhibited in hypoxic conditions suggesting that the hypoxia inhibitory site was located between 249 and 374 bp. This portion of the promoter contains three Sp1-binding sequences.

Implication of SP1 in UGDH Promoter Activity—Recently, it was shown that bisanthracine (WP-631) acts as a selective inhibitor of Sp1 transcription factor (40). We used this compound to further study the implication of Sp1 in the activity of UGDH promoter. WP-631 at a concentration of 1 μM slightly inhibited basal UGDH mRNA level in MRC5 fibroblasts and completely abolished the stimulatory effect of TGF-β/H9252 (Fig. 7A). In MRC5 fibroblasts, WP-631 did not influence the activity of 100-bp and 165-bp constructs, indicating that the Sp1 cis-acting elements of these sequences are not involved in the regulation of UGDH promoter. In keeping with the mRNA results, WP-631 inhibited all the longer promoter constructs and suppressed the stimulatory effect of TGF-β/H9252 (Fig. 7B). More intense inhibition was obtained when WP-631 was added to the cultures of HepG2 cells. At the concentration of 1 μM, WP-631 significantly inhibited UGDH mRNA level (Fig. 8A). Moreover, in this cell line, WP-631 inhibited all the constructs activity in a dose-dependent manner (Fig. 8B).

The role of Sp1 was further investigated by EMSA studies with nuclear extracts, using a consensus Sp1 sequence DNA fragment. The nuclear extracts of MRC5 cells and HepG2 cells incubated with labeled probe showed on the gel three delayed complexes (Fig. 9A, first lane). Using a 100-excess of non-labeled Sp1 fragment (specific competition) (Fig. 9A, third lane), we observed a disappearance of the bands corresponding to Sp1 and Sp3, showing that the lowest one corresponded to nonspecific fixation, the weak one to...
the binding of SP3 factor (41), and the highest, which was also the more labeled, to SP1. In contrast to the specific competition experiment, a 100-fold molar excess of the AP1 consensus sequence had no effect on the intensity of the fixation (Fig. 9A, fourth lane). TGF-β enhanced the fixation of the DNA consensus binding to Sp1 and Sp3 (Fig. 9A, second lane). The nuclear extracts obtained from HepG2 cells incubated in the presence of 1% O2 showed weaker fixation of Sp1 factor as compared with normoxic cells (Fig. 9B).

Results from Fig. 6A showed that the main TGF-β enhancement effect was obtained with the –374 bp, construct (1.6- and 4.1-fold stimulation for the –249-bp and –374-bp constructs, respectively). The fragment of promoter –374/–249 contains three putative Sp1 binding sites (Fig. 4). To identify which potential Sp1 binding site from this fragment is involved in the TGF-β stimulation, we used three oligonucleotide probes from this region and a commercially available Sp1 consensus oligonucleotide as a control in EMSA experiments. Incubation of nuclear extracts from control and TGF-β-treated MRC5 cells with the different probes revealed an increase of Sp1-DNA binding under TGF-β treatment with Sp1 sequences located at –253 and –276 bp but not with the Sp1–312 probe (Fig. 10). The main binding effect was obtained with the Sp1–276 probe (Fig. 10, lane 6). The specificity of binding was checked using 100-fold molar excess of unlabelled double-strand wild type consensus Sp1 sequence or site-directed Sp1 oligonucleotide mutant. The non-labeled wild type probe, but not the mutant probe, suppressed Sp1- and Sp3-shifted bands (Fig. 10B), indicating the specificity of binding. These results were further confirmed by supershift experiments (Fig. 11). By using both the commercial and the Sp1–276 probes, we demonstrated that the increased Sp1 binding activity present in TGF-β-treated MRC5 cells was actually due to the Sp1 transcription factor. Two supershifted bands were observed when using the commercial anti-Sp1 antibodies. It is not clear at present whether these doublings correspond to partial fixation of another transcription factor or the formation of higher order complexes. Specificity of the Sp1-supershifted band was confirmed using (i) control nuclear extract from HeLa cells (Fig. 11) and with an anti-Sp1 antiserum from another supplier and (ii) supershift experiments with the anti-Sp3 antiserum (data not shown). Anti-Sp3 antiserum slightly cross-reacted with the
Sp1 transcription factor and was probably directed against the DNA-binding site, leading to a decrease in the complexes formation as shown by other authors (42). All these results strongly suggest that Sp1 is a universal modulator of UGDH promoter activity and that, according to its concentration/phosphorylation status and supply to the nucleus, it might increase or decrease UGDH mRNA expression.

**DISCUSSION**

UDP-glucuronate is an intermediate metabolite and the substrate for numerous glycosyltransferases, including those implicated in sulfated GAGs and hyaluronan synthesis. The synthesis of UDP-glucuronate is catalyzed by the unique pathway of UGDH in animals, and it was shown that mutation inactivation of the enzyme impairs embryonic growth by lack of GAGs. There is a body of evidence that the enzyme has a regulatory role in the cell. First, the relative concentration of UDP-glucuronate in the mesenchymal cells was estimated between 10^{-4} and 10^{-5} M (43, 44), a value similar to the $K_m$ of the downstream acting glycosyltransferases. Second, the expression of UGDH mRNA is higher in the cells and tissues with increased UDP-glucuronate metabolism as, for example, liver, or kidney (Ref. 8 and Fig. 1). Third, UGDH is a direct response gene as demonstrated by time-course experiments (Fig. 2C).

![Diagram](image_url)
and by the cycloheximide-enhanced response to TGF-β stimulation (Fig. 2D). Similar results were previously reported with interleukin-1 (15).

To investigate the molecular regulation of UGDH, we cloned its 5'-upstream region that drove the promoter activity of luciferase reporter gene in transient transfection assays. Present work is the first report of the characterization of UGDH promoter. The minimal promoter (Fig. 5) contains an inverted TATA box, as it was shown for several mammalian genes (45–48), and no CAAT box was detected in the promoter up to −2133 bp. The core promoter (Fig. 4) contains numerous GC-rich sequences, characteristic of TATA-less and CAAT-less promoters of housekeeping genes (49). In such genes, Sp1 consensus sites were shown to take the function of TATA boxes for polymerase II assembly (50). Here, the inverted TATA box of UGDH promoter drove the basal activity of the reporter gene (Fig. 5), a feature characteristic of "atypical" housekeeping genes that show a low level of basal expression but are regulated by different extracellular factors, including cytokines. This feature of UGDH promoter is distinct from other sugar or alcohol dehydrogenases (51), including the enzymes of the glycolytic pathway (52, 53). The UGDH core promoter contains twelve Sp1 consensus sequences. The successive deletion analysis of different fragments showed a decreased activity of the reporter gene proportional to the promoter length, up to −644 bp. The full cloned sequence of −2133 bp was much less active, indicating the presence of putative inhibitor sequence. The core promoter of about 650 bp was responsible for the increased activity in HepG2 cells compared with MRC5 fibroblasts, indicating that the major transcription regulatory cis-elements are located in this region from these two cell types. The increase of the promoter activity correlated with its length and its down-regulation by bisanthracycline suggest that several of the Sp1 sites are functional in defining HepG2 cell activity. The Sp1 transcription factor can act as an initiator for assembling polymerase II complex (50, 54), but often it acts as an enhancer/modulator for different kinds of signals (55). Expression of UGDH mRNA is up-regulated by serum (8, 17). TGF-β is one of the major factors in the serum that enhances GAG synthesis. TGF-β increased UGDH mRNA abundance rapidly.
and in a dose-dependent manner, with a sustained effect after 24-h treatment. Cycloheximide had an additive effect, indicating the direct action of TGF-β. In mammals, the signaling of TGF-β is transduced via phosphorylated SMAD proteins, which bind to the target genes by its Smad binding element (56). However, this binding is of low affinity and always requires the interaction with additional transcription factors to become functional. There is a body of evidence showing that Sp1 cooperates in TGF-β signal transduction. Here, we showed that the promoter of human UGDH does not contain any consensus Smad binding element (Fig. 4) but includes many Sp1 sites in its sequence. On the other hand, all the constructs except that of −100 bp showed enhanced reporter gene expression after TGF-β treatment of MRC5 cells (Fig. 6). The most efficient were the constructs containing −374 and −644 bp of the UGDH promoter. These constructs have three and eight additional Sp1 binding motifs, respectively, compared with the −249-bp construct. Moreover, the specific Sp1 inhibitor, WP-631, completely suppressed the TGF-β stimulation of UGDH mRNA expression and blocked the activity of all the constructs in HepG2 cells. There was a specific fixation of nuclear proteins on Sp1 consensus sequence oligonucleotide as revealed by EMSA and supershift assays. Accordingly, we show that two Sp1 sites located at −253 and −276 bp upstream from the transcription start site are involved in the enhanced binding after TGF-β stimulation, the −276 bp being the most active (Fig. 10). All these results strongly support the hypothesis that TGF-β acts on the human UGDH promoter through numerous Sp1 sites. The differences in the susceptibility of MRC5 cells and HepG2 cells to WP-631 treatment are correlated with the differences in their basal expression of UGDH mRNA. The higher susceptibility of HepG2 cells to WP-631 inhibition may be ascribed for enhanced metabolism and enhanced growth of this hepatocarcinoma cell line. Either the pool of Sp1 factor might be used to stimulate multiple gene expression, or the chromatin might be less protected and more attractive to Sp1.

Another factor influencing UGDH expression is hypoxia. In the human body, an acute hypoxic state may be acquired in rapidly growing tumor tissue with poor vasculature and in wound healing, when the blood vessels are damaged, or, in physiological state, in working muscular tissue. The hypoxia is created when the oxygen/energy consumption surpass its delivery. Hypoxia impairs GAG synthesis with preferential inhibition of chondroitin/dermatan sulfate production (57). Under hypoxic conditions, the cell metabolism, including energy production, is switched to anaerobic phosphorylation, which produces a large quantity of lactate and acidifies the cell environment. It was shown that, in hypoxic conditions, at least 9 among the 11 glycolytic enzymes are up-regulated (58). The
reaction catalyzed by UGDH consumes 2 mol of NAD\(^+\) for each mole of UGDH-glucuronate synthesized. In hypoxic conditions, this NAD\(^+\) is no more available for the oxidative phosphorylation pathway. It is therefore conceivable that UGDH synthesis may be down-regulated when oxygen supply is low. The inhibition is relatively rapid, and the dose-dependent decrease of UGDH mRNA abundance is observed after 24-h exposure (Fig. 3). The down-regulation of the reporter gene was observed in transient transfection experiments with the constructs containing the −374-bp and −644-bp core promoters (Fig. 6). The inhibition of UGDH promoter activity was, at least in part, mediated by the Sp1 factor in HepG2 cells. Indeed, WP-631 treatment mimics hypoxic conditions, and the EMSA assay showed a decreased capacity for Sp1 fixation on its consensus sequence in the nucleus from hypoxic cells. All these results indicate that the Sp1 transcription factor can play a determinant role in the promoter activity of UGDH. Sp1 can either increase the UGDH expression after TGF-\(\beta\) stimulation or inhibits its expression in hypoxic conditions. Accordingly, the enzyme concentration could control the UGDH-glucuronate supply for glycosaminoglycan synthesis and glucuronon conjugate synthesis.

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REFERENCES
1. Toyoda, H., Kinoshita-Toyoda, A., Fox, B., and Selleck, S. B. (2000) J. Biol. Chem. 275, 21856–21861
2. Walsh, E. C., and Stainier, D. Y. (2001) J. Biol. Chem. 276, 30810–30816
3. Binari, R. C., Staveley, B. E., Johnson, W. A., Godavarti, R., Sasisekharan, R., Biochem. Biophys. Res. Commun. 275, 3045–3050
4. Iozzo, R. V., and San Antonio, J. D. (2001) J. Clin. Invest. 107, 169–178
5. Vangenderen, J. B., Rounds, S., and Farber, H. W. (1992) J. Biol. Chem. 267, 15335–15340
6. Massague, J. (2000) Nat. Rev. Mol. Cell. Biol. 1, 169–178
7. Cutroneo, K. R. (2000) Mol. Biol. Rep. 27, 191–194
8. Inagaki, Y., Truter, S., and Ramirez, F. (1994) J. Biol. Chem. 269, 14828–14834
9. Poncelet, A. C., and Schnaper, H. W. (2001) J. Biol. Chem. 276, 6983–6992
10. Datta, F. K., Blake, M. C., and Moses, H. L. (2000) J. Biol. Chem. 275, 49014–49019
11. Brodin, G., Ahgren, A., and Friedman, S. L. (1998) J. Biol. Chem. 273, 32529–32537
12. Spicer, A. P., Kaback, L. A., Smith, T. J., and Seldin, M. F. (1998) J. Biol. Chem. 273, 25117–25124
13. Karlinsky, J. B., Rounds, S., and Farber, H. W. (1992) J. Biol. Chem. 267, 15335–15340
14. Periyasamy, S., Ammanamanchi, S., Tillekeratne, M. P., and Brattain, M. G. (2000) Oncogene 19, 4660–4667
15. Toole, B. P., Wight, T. N., and Tammi, M. I. (2002) J. Biol. Chem. 277, 789–795
Specific Protein-1 Is a Universal Regulator of UDP-glucose Dehydrogenase Expression: ITS POSITIVE INVOLVEMENT IN TRANSFORMING GROWTH FACTOR-β SIGNALING AND INHIBITION IN HYPOXIA
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