Ectopic SOX9 Mediates Extracellular Matrix Deposition Characteristic of Organ Fibrosis

Received for publication, September 4, 2007, and in revised form, February 22, 2008. Published, JBC Papers in Press, February 22, 2008, DOI 10.1074/jbc.M707390200

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Appropriate temporospatial expression of the transcription factor SOX9 is important for normal development of a wide range of organs. Here, we show that when SOX9 is expressed ectopically, target genes become expressed that are associated with disease. Histone deacetylase inhibitors in clinical trials for cancer therapy induced SOX9 expression via enhanced recruitment of nuclear factor Y (NF-Y) to CCAAT elements in the SOX9 proximal promoter. The effect of histone deacetylase inhibitors could be elicited in cells that normally lack SOX9, such as hepatocytes. In human fetal hepatocytes, this aberrant induction of SOX9 protein caused ectopic expression of COL2A1 and COMP1 that encode extracellular matrix (ECM) components normally associated with chondrogenesis. Previously, ectopic expression of this “chondrogenic” profile has been implicated in vascular calcification. More broadly, inappropriate ECM deposition is a hallmark of fibrosis. We demonstrated that induction of SOX9 expression also occurred during activation of fibrogenic cells from the adult liver when the transcription factor was responsible for expression of the major component of fibrotic ECM, type 1 collagen. These combined data identify new aspects in the regulation of SOX9 expression. They support a role for SOX9 beyond normal development as a transcriptional regulator in the pathology of fibrosis.

The transcription factor SOX9 (sex-determining region Y-box 9) is responsible in chondrocytes for the deposition of extracellular matrix (ECM), predominantly composed of type 2 collagen (COL2) (1). SOX9 directly activates transcription of the COL2A1 gene via a binding site in the first intron (2). It is also responsible in chondrocytes for expression of COMP1 (collagen oligomeric protein-1) (3). In humans, inactivating mutations of the SOX9 gene cause campomelic dysplasia, a condition characterized by failure of chondrogenesis (4, 5). Studying the wider phenotype of campomelic dysplasia, along with models of Sox9 function in other species, has demonstrated the importance of the transcription factor for the development of other cell types and organs, such as neural crest (6, 7), heart valves (8, 9), intestinal Paneth cells (10, 11), Sertoli cells of the testis (12–14), and pancreatic progenitor cells (15–17).

Regulation of transcription from the SOX9 gene is complex. Mutations and chromosomal rearrangements up to 1 megabase upstream of the SOX9 locus can cause campomelic dysplasia, implying the existence of distant enhancer elements (18). By analyzing conservation across widely divergent species, several elements have been identified (19) that are capable of driving reporter gene expression to appropriate sites of ECM deposition. They support a role for SOX9 beyond normal development as a transcriptional regulator in the pathology of fibrosis.

§ The work described in this manuscript has been filed as patent application 0709506.0. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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SOX9, ECM Deposition, and Fibrosis

**EXPERIMENTAL PROCEDURES**

**Chemicals, Reagents, and Vectors**—Trichostatin A (TSA) was purchased from Sigma, TGF-β was purchased from R&D Systems, and FK228 was a gift from Professor Graham Packham (University of Southampton). All other chemicals were purchased from Sigma or Thermo Fisher Scientific. Promoter constructs were cloned into pGL3 Basic (Promega) and numbered relative to the transcriptional start site. Mutations within the SOX9 p–192 promoter construct were generated by PCR with the oligonucleotides shown in supplemental Table 1.

**Immortalized and Primary Cell Culture**—PANC-1 and HeLa cell lines were purchased from the European Collection of Cell Cultures (Salisbury, UK). Human LX2 cells were a gift from Dr. Scott Friedman (Mount Sinai School of Medicine, New York, NY) (26). Human fetal material was collected from first trimester termination of pregnancy with informed consent and following ethical approval from the Southampton & South West Hampshire Local Research Ethics Committee under guidelines issued by the Polkinghorne Committee (27). Human fetal hepatocytes were prepared by mechanical disaggregation. Cells other than rat HSCs were grown in a monolayer at 5% CO₂ and 37 °C in complete medium (Dulbecco’s modified Eagle’s medium + 1-glutamine containing antibiotics supplemented with 10% fetal bovine serum) (PAA Laboratories). For TSA-induced chromatin immunoprecipitation assay, cells were stimulated in the complete medium for 2 consecutive days, following which cells were washed and fixed in 4% paraformaldehyde. Primary rat HSCs were grown in Dulbecco’s modified Eagle’s medium containing 16% fetal bovine serum. For TSA stimulation and/or transient transfection using Transfast (Promega) or gene silencing using HiPerFect (Qiagen), all cell types were plated at ~60–70% density the day prior to treatment. For immunocytochemistry, cells were plated onto glass slides coated with fibronectin (5 μg/cm²; Sigma-Aldrich). To assess the effect of TGF-β on SOX9 expression, activated HSCs and LX2 cells were cultured for 48 and 24 h, respectively, in 0 or 0.5% serum. TGF-β was then added for the next 24 h under the same low serum conditions.

**CCL₄ Liver Injury Model and the Preparation of Rat Hepatic Stellate Cells**—Liver fibrosis was induced by 5-week treatment of adult male Sprague-Dawley rats with carbon tetrachloride (CCL₄). This methodology, along with tissue fixation and processing, has been described previously (28). Quiescent HSCs were isolated from the livers of untreated rats and plated onto tissue culture plastic as described previously (28). In this in vitro setting, HSCs replicate in vivo activation over the course of a week or so, becoming positive for α-smooth muscle actin (α-SMA) and adopting a profibrotic phenotype secreting COL1 (29).

**Gene Expression Analyses**—Antibodies used are listed in supplemental Table 2. Tissue preparation, immunohistochemistry, immunofluorescence, and immunoblotting were performed as described previously (27, 30). Quantification of the protein band intensity after chemiluminescent detection of the immunoblotting was conducted using Quantity One software (Bio-Rad). For reverse transcription-PCR, RNA was extracted using TriReagent (Sigma), and cDNA was synthesized with SuperScript III (Invitrogen). PCR used 2 μl of cDNA with intron-spanning primers wherever possible (supplemental Table 3).

**Luciferase, Electrophoretic Mobility Shift, and Chromatin Immunoprecipitation Assays**—The methodology for the luciferase assays and the electrophoretic mobility shift assay has been described previously (31). Mutations of the proximal and distal CCAAT elements are shown in supplemental Table 1. Chromatin immunoprecipitation assays were carried out according to the manufacturer’s instructions (Active Motif Europe) followed by PCR amplification of the SOX9 proximal promoter from ~163 bp to the transcriptional start site.

**RNA Interference**—SOX9 expression was silenced using commercial RNA oligonucleotides (supplemental Table 4) by introduction into human fetal hepatocytes according to the manufacturer’s instructions (Qiagen) or into the rat HSCs via “nucleofection” (Amaza Biosystems GmbH). Briefly, 2.5 × 10⁶ HSCs were resuspended in 100 μl of T-solution and combined with 2 μl of 20 μm siRNA. Cells were transfected using the U-25 pulsing parameter and immediately transferred to tubes containing 37 °C prewarmed culture medium. Cells were cultured for 48 h on 12-well plates prior to protein extraction.

**Statistical Analysis**—Statistical significance was determined using one-way analysis of variance with a Newman-Keuls post hoc test or a two-tailed Student’s t test.

**RESULTS**

**SOX9 Expression in Derivatives of the Distal Human Foregut**—The liver and pancreas arise from a common pool of precursor cells according to the balance of fibroblast growth factor signal-
**SOX9, ECM Deposition, and Fibrosis**

**A**

![Immunocytochemistry for SOX9 in PANC-1 and HeLa cells](image)

**B**

**Human SOX9 5' flanking region**

| Mutations in p-192 | Lanes 1–3 | Lanes 4–6 |
|--------------------|-----------|-----------|
| mut dpCCAAT        | 25        | 25        |
| mut pCCAAT         | 25        | 25        |
| mut dCCAAT         | 25        | 25        |
| CREB CCAAT         | 25        | 25        |

**Fold induction**

| Dose (ng/ml) | Fold induction |
|--------------|---------------|
| 0            | 1             |
| 5            | 5             |
| 10           | 10            |
| 25           | 25            |

**C**

**Human SOX9 5' flanking region**

| Mutations in p-192 | Lanes 1–3 | Lanes 4–6 |
|--------------------|-----------|-----------|
| mut dpCCAAT        | 25        | 25        |
| mut pCCAAT         | 25        | 25        |
| mut dCCAAT         | 25        | 25        |
| CREB CCAAT         | 25        | 25        |

**Fold induction**

| Dose (ng/ml) | Fold induction |
|--------------|---------------|
| 0            | 1             |
| 5            | 5             |
| 10           | 10            |
| 25           | 25            |

**D**

**TSA, ChIP antibody**

| ChIP antibody | Lanes 1–3 | Lanes 4–6 |
|---------------|-----------|-----------|
| TFIIIB        | 25        | 25        |
| SOX9          | 25        | 25        |
| NF-Y          | 25        | 25        |
| IgG           | 25        | 25        |

**E**

**TSA, SOX9, NF-Y, β-actin**

| Lanes 1–3 | Lanes 4–6 |
|-----------|-----------|
| 0         | 0         |
| 25        | 25        |
| 50        | 50        |
| 100       | 100       |
| 0         | 0         |
| 2         | 2         |
| 4         | 4         |
| 8         | 8         |
| 12        | 12        |

**FIGURE 3. HDAC inhibitors induce SOX9 expression via NF-Y.**

A. Immunocytochemistry for SOX9 in PANC-1 (positive) and HeLa (negative) cells counterstained with 4',6-diamidino-2-phenylindole. Scale bar represents 25 μm. B and C, effect of HDAC inhibitors on the SOX9 promoter. Lengths of the 5' flanking region and/or mutations (mut or X) are shown to the left with corresponding fold induction of luciferase (luc) activity induced by TSA and/or FK228 shown to the right (mean from at least three separate experiments with error bars showing S.E.). CREB, cAMP-response element-binding protein motif. B, PANC-1 cells. EV, empty vector. C, HeLa cells. D, chromatin immunoprecipitation (ChIP) analysis from HeLa cells in the absence (−) and presence (+) of TSA (25 ng/ml) for the proximal SOX9 promoter. IgG and input controls are shown. E, immunoblotting for SOX9 of HeLa cell protein extract following treatment with TSA for 48 h (dose response) or 25 ng/ml (time course).

**FIGURE 2. Analysis of the SOX9 promoter in PANC-1 cells.** A, lengths of the 5' flanking region or mutations (mut or X) of the distal (d) and/or proximal (p) CCAAT elements in the p-192 SOX9 promoter construct shown to the left with corresponding luciferase (luc) values relative to the p-192 construct to the right (mean from at least three separate experiments with error bars showing S.E.). CREB, cAMP-response element-binding protein motif. EV, empty vector. B, alignment of the proximal promoter with nucleotides conserved across all species shown in black, numbered according to the human transcriptional start site. C, electrophoretic mobility shift assay of nuclear extract from PANC-1 cells binding to the distal and proximal CCAAT elements. Lanes 1–3, wild-type (wt) proximal CCAAT motif; lanes 4–6, wild-type distal CCAAT motif; lanes 7 and 8, mutated distal CCAAT motif. Lanes 1, 4, and 7 contain free probe. In lanes 2 and 5, complexes A, B, and C were retarded. In lanes 3 and 6, complex A was "supershifted" by the inclusion of anti-NF-Y antiserum. Complex A was no longer retarded when the distal or proximal CCAAT element was mutated (lane 8 and data not shown, respectively). D, chromatin immunoprecipitation assay of NF-Y binding to the CCAAT elements(s) in the SOX9 proximal promoter in PANC-1 cells with IgG, water, and input (diluted 10-fold) controls.
whereas conversely, HDAC inhibitors relieve this repressed state of the basal promoter to favor gene expression (34). Wild-type SOX9 promoter-luciferase constructs containing at least one CCAAT site were activated ~15–20-fold over the corresponding basal levels shown in Fig. 2A by TSA, an inhibitor of Class I and Class II HDACs (Fig. 3B). The effect was replicated with the Class I compound FK228 (also known as depsipeptide) and abolished by the loss of functional CCAAT elements via either sequential truncation of the 5’ flanking region or discrete mutations within a larger construct (Fig. 3B).

**HDAC-induced Ectopic SOX9 Expression in HeLa Cells—** PANC-1 cells express nuclear SOX9 protein (Fig. 3A). Activation of the SOX9 promoter in PANC-1 cells via CCAAT elements and the ubiquitous presence of NF-Y led us to question whether we could induce SOX9 in cells that do not normally express it, such as the immortalized HeLa cell line (Fig. 3A). TSA induced wild-type SOX9 promoter activity in HeLa cells by 150–180-fold when both CCAAT elements were present (Fig. 3C). By mutating both sites within the context of the p−192 construct (mut dpCCAAT), TSA lost the vast majority of its ability to induce reporter gene expression. Thus, the HDAC effect on SOX9 expression required intact NF-Y-binding sites. In line with these in vitro findings, chromatin immunoprecipitation assay of TSA-treated HeLa cells demonstrated recruitment of transcription factor IIB at the proximal SOX9 promoter (Fig. 3D). Transcription factor IIB is critical for the assembly of multiple factors including RNA polymerase II for the initiation of gene expression. Although cellular levels of NF-Y protein were unaltered (supplemental Fig. 1A), TSA increased NF-Y that was associated with the endogenous SOX9 promoter (Fig. 3D). These events at the proximal promoter correlated with a dose- and time-dependent accumulation of SOX9 protein in TSA-treated HeLa cells (Fig. 3E). The very large fold induction of SOX9 reporter gene expression in this cell line compared with PANC-1 cells was most likely explained by the virtual absence of SOX9 expression in untreated HeLa cells. Taken together, these data demonstrate a mechanism by which HDAC inhibitors induce SOX9 expression in cells where the gene is normally silenced.

**Ectopic SOX9 in Human Fetal Hepatocytes—** We hypothesized that if SOX9 were induced similarly in human fetal hepatocytes, cell phenotype might be altered, potentially prompting transdifferentiation toward a pancreatic phenotype. As in HeLa cells, TSA induced dose-dependent SOX9 expression in primary fetal hepatocytes that at the outset were SOX9-negative (Figs. 4A and 1E). Maximal stimulation by TSA occurred at 25 ng/ml. Induction directly in epithelial hepatocytes was confirmed by observing SOX9 in the nucleus of cells that expressed cytoplasmic α-fetoprotein (Fig. 4B). To investigate cell phenotype following TSA, we analyzed the expression of selected markers indicative of other SOX9-positive developmental cell types and their progeny. Neither pancreatic progenitor nor neuroprogenitor cell markers were detected following SOX9 up-regulation by TSA treatment (supplemental Fig. 1B). However, by reverse transcription-PCR, COL2A1 and COMP1, both regulated by SOX9 in chondrocytes (2, 3), were induced in fetal hepatocytes (Fig. 5A). The increase in COL2A1 transcripts was mirrored by dose-dependent increases in COL2 protein visible by immunofluorescence and immunoblotting in primary cultures of monolayer hepatocytes (Fig. 5, B and C). At

![FIGURE 4. SOX9 induction by TSA in human fetal hepatocytes. A, immunoblotting for SOX9 from the protein extracts of human fetal hepatocytes following treatment with TSA. B, immunocytochemistry for SOX9 (red) and α-fetoprotein (AFP, green) following treatment for 48 h with TSA (25 ng/ml) counterstained with 4’,6-diamidino-2-phenylindole (DAPI; blue).](image)

![FIGURE 5. SOX9 induction of ECM genes in human fetal hepatocytes. A, reverse transcription-PCR for COL2A1 and COMP1 in the absence or presence (+) of TSA. HPRT, hypoxanthine-guanine phosphoribosyltransferase. B, immunocytochemistry for COL2 in the absence or presence of TSA. Scale bar represents 25 μm. C, induction of COL2 expression quantified by immunoblotting. Fold induction has been normalized to β-actin and standardized in each experiment against control cells incubated without TSA. D, siRNA abrogation of SOX9 in human fetal hepatocytes following TSA induction. Expression, quantified by immunoblotting, has been normalized to β-actin and standardized against control cells transfected with scrambled siRNA. *, p < 0.001 compared with the corresponding control. E and D comprise five separate experiments with error bars showing the S.E.](image)
25 ng/ml TSA, when SOX9 was maximally stimulated (Fig. 4A), COL2 was increased ∼5-fold (Fig. 5C). To determine a causal effect of SOX9 upon the COL2A1 expression, we knocked down SOX9 by RNA interference during the addition of TSA to the culture medium. SOX9 abrogation decreased COL2 protein levels commensurate with the level of reduced SOX9 (Fig. 5D). Similar decreases were observed using oligonucleotides targeted to one other region of the SOX9 transcript (data not shown).

SOX9 Expression in Hepatic Stellate Cells—The aberrant expression of ECM components by primary hepatocytes as a consequence of reactivated SOX9 expression led us to speculate that inappropriate SOX9 could also be involved in fibrosis. The hallmark of fibrotic ECM deposition is COL1 (29). Neither COL1A2 transcripts nor COL1 protein was significantly altered in the fetal hepatocytes following induction of SOX9 by TSA (data not shown). However, the broad alteration of gene expression induced by TSA includes a direct negative effect on COL1A2 expression, which would thus obscure a potential induction by SOX9 (35, 36). In the rat CCl4 model of liver fibrosis, α-SMA marks activated HSCs with COL1-rich fibrotic tracts disrupting the normal tissue architecture (29, 37). In serial sections for both α-SMA (Fig. 6, A and B) and COL1 (Fig. 6, C and D) staining, nuclear SOX9 was detected in the same region. We isolated quiescent HSCs, negative for α-SMA and

![Figure 6. SOX9 expression in fibrotic rat liver following repeated CCl4 injection. A and B, consecutive 5-μm tissue sections stained for α-SMA and SOX9, respectively. C and D, consecutive 5-μm tissue sections stained for COL1 and SOX9, respectively. For A and B and for C and D, note similarly located staining. Scale bars represent 25 μm.](image)

![Figure 7. SOX9 expression in activated hepatic stellate cells. A, immunoblotting of quiescent (Q) and activated (A; 10 days of culture) rat HSCs (rHSC). B, quantified induction of SOX9 and collagen subtypes following immunoblotting at time points during the activation of HSCs in culture. Fold induction has been normalized to β-actin and standardized in each experiment against protein levels in quiescent cells. Error bars show the S.E. of induction from three separate HSC preparations. C, fluorescence immunocytochemistry showing nuclear SOX9 (red) and cytoplasmic α-SMA (green) in activated HSCs counterstained by 4′,6-diamidino-2-phenylindole (DAPI; blue). Scale bar represents 25 μm.](image)
not secreting COL1, and activated the cells by culturing on tissue culture plastic in serum in vitro (28). The starting quiescent HSCs did not express SOX9 or COL1, whereas fully activated HSCs robustly expressed both proteins as well as some COL2 (Fig. 7A). After 7 days of the activation process, SOX9 protein had doubled and was further increased to nearly 20-fold greater than quiescent levels after 10 days (Fig. 7B). In these activated COL1-expressing HSCs, SOX9 localized to the nucleus surrounded by an α-SMA-positive cytoplasm, confirming the presence of the transcription factor in activated myofibroblast-like HSCs (Fig. 7C). SOX5 and SOX6 transcripts were not detected in these cells by reverse transcription-PCR (data not shown). This method of in vitro HSC activation is spontaneous. In vivo, TGF-β signaling is a major profibrotic stimulus leading to HSC activation and COL1 deposition (29).

To study the effect of TGF-β on SOX9 expression, we placed activated HSCs in low serum conditions. TGF-β increased SOX9 expression ~3-fold in rat primary HSCs. Under similar conditions, SOX9 was induced ~2.5-fold in a human model of HSCs, the LX2 cell line (Fig. 8A). In activated rat HSCs, we abrogated SOX9 by RNA interference (Fig. 8B). Following reduction of SOX9 protein levels by ~60%, COL1 was lowered by a similar magnitude, confirming its direct role for SOX9 in COL1 expression in activated HSCs (Fig. 8B). The unexpected presence of COL2 in activated HSCs was also abrogated by lowered levels of SOX9. Similar results were obtained by targeting one other region of the SOX9 transcript (data not shown).

**DISCUSSION**

The shared developmental origin of liver and ventral pancreas underlines the potential that the two cell types can be interconverted (32, 38, 39). SOX9 was robustly expressed in both motifs markedly attenuated the induction of promoter activity. The remaining promoter activity, seen particularly in HeLa cells in the context of the p−192 construct (Fig. 3C), presumably reflected a minor contribution from other cis-elements, such as the cAMP-response element-binding protein motif. The second point of interest relates to the ability of NF-Y bound to CCAAT sites to mediate transcriptional repression as well as activation (34): although the induction of SOX9 expression in HeLa cells was associated with greater detection of DNA-bound NF-Y, some was apparent in the absence of TSA when SOX9 was not expressed (Fig. 3, A and D).

The induction of SOX9 expression in cell lines was matched by HDAC inhibitors in primary fetal hepatocytes. However, an obvious pancreatic phenotype was not induced, indicating that SOX9 alone is inadequate at inducing such transdifferentiation and that, presumably, other key transcription factors, such as PDX1, are required. However, COL2A1 and COMP, both direct targets of SOX9 in chondrocytes (2, 3), became expressed. An identical ectopic expression profile of SOX9 and COL2A1 has been observed in calcified blood vessels, leading to the hypothesis that aberrant recapitulation of endochondral bone formation is the causative mechanism underlying vascular calcification (23). Here, we demonstrated causality between ectopic SOX9 induced by TSA and expression of its target gene, COL2A1. HDAC inhibitors, including FK228, are in advanced patient trials to treat a range of malignancies (40). Our data suggest potential side effects from unforeseen SOX9 expression in abnormal sites.

COL1 is the major collagen subtype deposited in organ fibrosis. It is composed of two α1 chains and one α2 chain, encoded by two distinct genes, COL1A1 and COL1A2, respectively. Transcriptional regulation of these genes is interrelated and has
been shown previously to rely on Sp1, Sp3, and NF-Y transcription factors among others (41, 42). Here, we questioned whether ectopic SOX9 expression, as we observed in human fetal hepatocytes, might be involved. HDAC inhibitors exert a direct negative effect on COL1 expression (36). Therefore, we chose established in vivo and in vitro models of rat liver fibrosis. The in vivo origin of the α-SMA–positive cells remains debated, probably reflecting heterogeneity, but it certainly includes the perisinusoidal HSC, which is responsible for COL1 deposition (29). In tissue sections, SOX9 localized to regions that were positively stained for α-SMA and COL1. In vitro, the transcription factor also became expressed and localized to the nucleus during activation of HSCs. In activated cells it was required for maximal COL1 expression. The pathology of fibrosis is similar across many soft tissue organs, suggesting that aberrant SOX9 expression might be a common underlying mechanism. Recent association data are supportive: bioinformatic studies have shown an association between SOX9 and COL1A2 in mesenchymal stem cells (43), and microarray analysis of keloid found increased SOX9 transcripts compared with normal skin (24). SOX9 is also overexpressed in glomerulosclerosis, a pathologic thickening of the basement membrane of the glomerulus, which causes renal failure (44). The latter destruction of the kidney’s filtration apparatus is caused by deposition of type IV collagen (COL4) rather than COL1. However, a broad effect of SOX9 on collagen expression seems apparent. SOX9 was recently demonstrated to increase COL1A2 expression in mouse mesangial cells via a distal enhancer element (25). Our finding that SOX9 regulates COL1 expression now warrants similar molecular analysis to identify the responsible cis-element(s) in the genes encoding the different COL1 chains.

Our data fit into existing signaling mechanisms of fibrosis. TGF-β signaling is a major influence in promoting HSC activation and subsequent COL1 expression; fibroblast growth factor 2 augments ECM deposition (29, 45, 46). We showed that TGF-β increased SOX9 expression in two HSC models, similar to observations in developing chondrocytes (47); previously, fibroblast growth factor 2 increased both SOX9 transcripts and protein in a chondrosarcoma cell line (48). Moreover, both TGF-β and fibroblast growth factor 2 promote epithelial-to-mesenchymal transition (EMT), whereby quiescent epithelial cells morph into myofibroblast-like cells characterized by α-SMA. In development, this process induces SOX9 expression during the differentiation of chondrocytes and astrocytes (49). However, epithelial-to-mesenchymal transition is also a foreunner of organ fibrosis when the resultant mesenchymal cells express abundant COL1 as part of the fibrotic matrix (49). Recent data have provided evidence for epithelial-to-mesenchymal transition as part of renal, hepatic, and cardiac fibrosis (45, 50, 51). Although the origin of HSCs remains unclear, in their activated state, marked by α-SMA expression, COL1 deposition, and the transcriptional regulator SOX9, their similarity to the mesenchymal cells that arise post-epithelial-to-mesenchymal transition is striking.

In summary, our data demonstrate that SOX9 can be induced in cells that, under normal conditions, do not express the gene, demonstrating redeployment of a normal developmental mechanism to an abnormal setting. The consequence was ECM production including that characteristic of organ fibrosis. Lessening SOX9 levels lowered expression of the major fibrotic collagen, COL1. As organ fibrosis and related disorders, such as glomerulosclerosis, result in profound morbidity and mortality, ultimately requiring transplantation (45), targeted reduction of SOX9 offers potential therapeutic application to ameliorate fibrosis and related conditions in the liver and other soft tissue organs.

Acknowledgments—We thank Anne Chad and colleagues at the Princess Anne Hospital for the collection of human fetal material.

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