Stearoyl-CoA desaturase 1 (**Scd1**) gene overexpression is associated with genetic predisposition to hepatocarcinogenesis in mice and rats

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The stearoyl-CoA desaturase 1 (**Scd1**) gene is involved in the synthesis and regulation of unsaturated fatty acids. Its expression is increased by several treatments/conditions that are associated with hepatocarcinogenesis (peroxisome proliferators, iron overload, dichloroacetic acid). We found that the **Scd1** gene is differentially expressed, showing >10-fold higher mRNA levels in the normal liver tissue of **C3H/He** mice, which are genetically susceptible to hepatocarcinogenesis, than of **BALB/c** mice, which are resistant. Similarly, **Scd1** mRNA expression was ~4-fold higher in the normal liver of **F344** rats, which are susceptible to hepatocarcinogenesis, than in **Brown Norway** (**BN**) rats, which are resistant. The chromosomal location of the **Scd1** locus, both in mice and rats, excludes **Scd1** candidacy as a hepatocellular tumor-modifier gene, as the **Scd1** locus did not show allele-specific effects in a **BALB/c** × **C3H/He** intercross or in a **BN** × **F344** backcross and intercross. No **Scd1** coding polymorphisms were detected in the mouse and the rat strains showing elevated **Scd1** expression. These results suggest that the **Scd1** gene represents a downstream target of hepatocellular tumor-modifier loci in two rodent species.

Introduction

Hepatocellular carcinoma is one of the most common cancers in the world. The disease has a poor prognosis, and few tools for early diagnosis and therapy are available. Familial aggregation of hepatocellular cancer indicates that complex genetic factors, together with environmental risk factors (e.g. aflatoxin exposure, cigarette smoking, heavy alcohol consumption, inorganic arsenic ingestion, radioactive thorium dioxide exposure, iron overload, use of oral contraceptives and anabolic steroids) may confer a high risk of developing hepatocellular cancer (1–3). The difficulties in directly dissecting the genetic risk factors in humans make the use of experimental models attractive.

Inbred strains of mice and rats differ in their susceptibility to chemically induced hepatocarcinogenesis. Some strains show a high propensity to the development of these tumors, while others are almost completely resistant. Genetic analysis of different crosses has allowed the mapping of several loci that play a role in genetic predisposition or resistance to hepatocellular cancer, providing evidence for the polygenic control of inherited susceptibility to hepatocarcinogenesis in rodents (4–6).

The genes responsible for the hepatocellular cancer-modifier functions are not yet known. A mechanism by which the hepatocellular cancer susceptibility (**Hcs**) loci confer a high genetic predisposition to hepatocellular cancer may involve alteration of their mRNA expression levels or alteration of mRNAs expression of target genes involved in liver tumor development.

To determine whether susceptibility to hepatocarcinogenesis is associated with alterations in gene expression in the normal liver, we focused on the stearoyl-CoA desaturase 1 (**Scd1**) gene, shown by mRNA subtraction analysis to be differentially expressed in mice. We found that the normal liver of **mouse** and **rat** strains characterized by high genetic susceptibility to hepatocarcinogenesis contains high **Scd1** transcript levels.

Materials and methods

Animals

**BALB/cJ**, **C3H/HeJ** and **C57BL/6J** inbred mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME). Parental strains were crossed to produce (**BALB/c** × **C3H/He**)_F1_, (**C57BL/6J** × **C3H/He**)_F1_ and (**BALB/c** × **C3H/He**)_F2_ mice. At 1 week of age, the latter group was treated s.c. with urethane (diluted in 0.9% NaCl solution) at a dose of 300 mg/kg body wt. After weaning, mice were kept under observation without any further treatment. A total of 179 male (**BALB/c** × **C3H/He**)_F2_ mice were killed at 38 weeks of age. Liver tumor phenotypes were analyzed quantitatively as described (5). Twelve hepatocellular tumors that developed spontaneously in 12–27-month-old male (**C57BL/6J** × **C3H/He**)_F1_ mice and two normal liver tissues from 12-month-old **F2** mice were used for northern blot analysis.

Male Fisher 344 and **Brown Norway** (**BN**) rats (160–180 g) (Charles River, Calco, Italy) were housed individually. Rat hepatocellular tumors were induced as described, by a single dose (150 mg/kg, i.p.) of diethylnitrosamine followed by a 15-days diet containing 0.02% 2-acetylaminofluorene, with a partial hepatectomy at the midpoint of this treatment (7). Rats were killed at 71 weeks after tumor initiation, and hepatocellular carcinomas were resected and collected.

Animals were given food and tap water ad libitum. Study protocols were in compliance with Italian law and institutional guidelines for use of laboratory animals.

**mRNA subtraction** and northern blot analysis

Total liver RNA was prepared from the frozen livers of male adult mice using the Ultraspec-II RNA kit (Biotecx, Houston, TX). **mRNA subtraction** was performed using the PCR-Select cDNA Subtraction kit (Clontech, Palo Alto, CA) according to the manufacturer’s instructions. Briefly, double-strand cDNAs were prepared from 2 mg of poly(A)⁺ RNA from two normal livers of (**BALB/c** × **C3H/He**)_F1_ (driver population) and **C3H/He** (tester population) mice. The cDNAs were digested with the restriction enzyme **RsaI**. In the first hybridization, excess driver cDNA was hybridized with tester cDNA for 8 h at 68°C. The PCR product of the first hybridization, which corresponds to the gene population overexpressed in the **C3H/He** normal liver, was ([32P]dCTP)-labeled by random primer synthesis with the Decaprime DNA labeling kit (Ambion, Austin, TX), and used to screen 20,000 lambda phage clones of a lambdaZAP cDNA library of **C3H/He** normal liver. Clones showing a differential hybridization signal were purified.
Northern blots were prepared using 25 μg of total RNA for each sample, electrophoresed on 1% agarose denaturing gel, transferred to nylon membranes, and hybridized with cDNA fragments of the candidate clones. The cDNA fragments of the candidate clones and of the housekeeping LLRep3 gene (8) were [α-32P]dCTP-labeled by random primer synthesis as above and hybridized to northern blots, followed by washing at a stringency of 0.5 × SSC, 0.1% SDS at 60°C.

Transcript levels were quantified using the PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Tissue homogenization, RNA extraction, poly(A)+RNA purification, and northern blotting of normal rat liver were performed as described (9). The Scd1 probe consisted of a 2.8 kb fragment of a cDNA clone containing nucleotides 2724–4727 of the mouse Scd1 mRNA sequence (GenBank accession number BC007474). The housekeeping rRNR-18 clone consisted of the human rRNA cDNA clone HHCAS65 (ATCC repository no. 77242). Probes were [α-32P]dCTP-labeled by random primer synthesis. Relative Scd1 and rRNR-18 mRNA levels were estimated by Instant Imager (Packard Instrument Co., Downers Grove, IL).

Nucleotide sequence analysis

PCR primers were designed to amplify ~500-bp DNA fragments covering nucleotides 10–3492 of the mouse Scd1 mRNA (GenBank accession number BC007474) and nucleotides 55–4525 of rat Scd1 mRNA (GenBank accession number J02585). Total RNA samples from liver (4 μg) of BALB/c and C3H/He mice or of BN and F344 rats were retrotranscribed using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen Co., Carlsbad, CA). DNA samples were amplified in a final reaction volume of 25 μl containing 1/10 of cDNA, 100 μM dNTPs, 1.5 mM MgCl2, 1 U AmpliTaq Gold Polymerase (Applied Biosystems, Foster City, CA) and 10 pmol of each specific primer. Aliquots of the PCR products were loaded on an ethidium bromide-stained agarose gel to check the quality of PCR amplifications. Nucleotides were sequenced using an ABI PRISM 377 automatic sequencer (Perkin Elmer), and aligned and compared to identify polymorphisms.

Gene database searches were performed at the National Center for Biotechnology Information (NCBI).

Genetic linkage mapping and statistical analysis

The D19Mit67 marker (Research Genetics, Huntsville, AL), which co-segregates with the Scd1 gene at 43 cM of chromosome 19 (Mouse Genome Database), was amplified by PCR from genomic DNA of 179 male (BALB/c × C3H/He)F2 mice. PCR products were loaded on a 10% agarose gel, and BALB/c and C3H/He alleles were visualized with ethidium bromide. As the liver tumor phenotypes showed a non-normal distribution, the linkage methods were applied to rank-transformed data (10). Analysis of variance (11) was used to evaluate: (i) strain differences in Scd1 mRNA levels, as estimated by quantitative analysis of northern blot results and (ii) genetic linkage between mouse liver tumor phenotypes and the D19Mit67 marker.

Results

mRNA subtraction indicated that Scd1 is differentially expressed in the normal liver of C3H/He and BALB/c strains (genetically susceptible and resistant to hepatocarcinogenesis, respectively) (4). Transcript levels of Scd1 were >10-fold higher in the normal liver of C3H/He mice than BALB/c mice (P < 0.0001, Figure 1A). Scd1 mRNA levels in (BALB/c × C3H/He)F2 hybrids were similar to those observed in the BALB/c parental strain (Figure 1A, compare lanes 7–12 with lanes 1–3). In addition, the same C3H/He mice (Figure 1A, lanes 4–6) showed ~7-fold higher Scd1 mRNA levels as compared with C57BL/6J mice (resistant to hepatocarcinogenesis) and slightly higher (~2-fold) levels than in (C57BL/6J × C3H/He)F1 (susceptible to hepatocarcinogenesis) mice (data not shown). Comparison of Scd1 mRNA levels in normal liver with levels in 12 spontaneous hepatocellular tumors from (C57BL/6J × C3H/He)F1 mice revealed ~2-fold higher levels in four tumors, ~3-fold higher levels in one tumor, and levels comparable with those in normal liver in the remaining seven tumors (data not shown).

The mouse Scd1 cDNA clone was used to probe a northern blot filter containing RNA from normal liver of F344 (hepatocarcinogenesis-susceptible) and BN (resistant) rats (12).

Relative amounts of Scd1 mRNA, calculated after normalization of Instant Imager counts to the rRNR-18 values, were 3–4-fold higher in the F344 rats (P < 0.0001, Figure 1B). Comparison of Scd1 mRNA levels in hepatocellular carcinomas that developed in F344 rats (three tumors) and in BN rats (four tumors) revealed ~1.5–5-fold higher levels in the F344 tumors (data not shown).

Non-neoplastic cells may represent 15–30% of tumor cell population, as shown previously (13) and therefore, the presence of normal cells in tumors would not be responsible for the observed findings indicating a deregulation of Scd1 gene expression in hepatocarcinogenesis.

Nucleotide sequence analysis of the mouse Scd1 gene, including the full-length coding region, revealed no polymorphisms between BALB/c (AF509567) and C3H/He (AF509570) mice; two nucleotide polymorphisms were seen with respect to the BC007474 GenBank sequence (mouse Scd1 mRNA); however, these polymorphisms did not lead to amino acid changes. Similarly, sequence analysis of the rat full-length coding Scd1 and flanking sequences showed no polymorphisms between BN (AF509568) and F344 (AF509569) rats. However, with respect to the J02585 GenBank sequence (Scd1 mRNA from liver of a Long-Evans rat), a T→G polymorphism was found at nucleotide 974 of sequence J02585, leading to a Ser291Ala variation. Additional variations detected in the coding region did not result in amino acid changes as compared with rat sequence J02585; other variations were located in the 3′-untranslated region.

Mouse Scd1 gene maps on chromosome 19 in the same region where we mapped previously the Hcx6 locus in a (C3H/He × Mus spretus) × C57BL/6J cross (5). Thus, we tested whether the Hcx6 locus affects hepatocellular tumor development in a BALB/c × C3H/He F2 cross, in which parental strains show differential Scd1 gene expression. Genotyping of the D19Mit67 markers, which maps in the Hcx6 chromosomal region and co-segregates with the Scd1 gene (43 cM, MGD: http://www.informatics.jax.org/), in 179 (BALB/c × C3H/He)F2 male mice treated with urethane revealed no significant linkage.
with several tumor phenotypes, i.e. multiplicity of liver tumor >2 mm in diameter, tumor volume, liver volume fraction occupied by tumors and total number of tumors/cm³ (data not shown). Thus, in (BALB/c×C3H/He)F₂ mice, the chromosomal region containing the Scd1 locus does not contain a Hes locus. The rat homolog of the mouse Scd1 gene (J02585 nucleotide sequence, marker AI071229, symbol Scd2) maps on distal chromosome 1, at 1224.5 cR in the rat radiation hybrid map, close to the D1Rat78 marker located at 133.5 cM (http://rgd.mcw.edu/). In the BN×F344 backcross and intercross rats (14), linkage analysis indicated the absence of genetic linkage between Scd1 mRNA levels and susceptibility/resistance to hepatocarcinogenesis, at least in these inbred strains and their F₁ crosses (15–18). Scd1 was also overexpressed in a subset of spontaneous mouse hepatocellular tumors. In addition, Scd1 expression was associated with genetic susceptibility to rat hepatocarcinogenesis, with higher transcript levels in the normal liver of hepatocarcinogenesis-susceptible F344 rats than in the resistant BN rats (12). The association of high Scd1 gene expression with genetic predisposition to hepatocarcinogenesis in two species suggests that Scd1 expression may play a functional role in hepatocellular tumor development.

In a BALB/c×C3H/He backcross and in BN×F344 backcross and intercross, Scd1 does not map in hepatocellular cancer-modifier loci (Hcs or Hcr), i.e. the Scd1 locus does not display allele-specific effects. Thus, Scd1 may be the downstream target of cancer-modifier genes responsible for the genetic susceptibility/resistance to hepatocarcinogenesis. The promoter structures of the human and mouse Scd1 genes contain conserved regulatory sequences for the binding of several transcription factors, including the steroid regulatory element binding protein, CCAAT enhancer binding protein-alpha (CEBP-alpha) and nuclear factor-1, which have been shown to transactivate the transcription of the mouse Scd1 gene (19). Thus, the association that we found between high Scd1 mRNA levels in the normal liver and genetic predisposition to hepatocarcinogenesis in two rodent species may rest in differences in binding affinity or in expression levels of the transcription factors that control Scd1 gene expression. Such transcription factors may themselves be candidate hepatocellular tumor-modifier loci (Hcs and Hcr loci) or, alternatively, Hcs and Hcr loci may regulate them. The Cebpa gene, encoding CEBP-alpha, and Tcf1, encoding transcription factor 1, map at a distance of 12–16 cM from the Hes1 and Hes5 loci, respectively (MGD: http://www.informatics.jax.org/), a distance that excludes them as candidate Hcs genes. However, it is also possible that Hcs loci encode as yet unknown transcription factor proteins, which, in addition to known factors, also control Scd1 gene expression.

The enzyme encoded by Scd1 is involved in the synthesis of unsaturated fatty acids, as well as in the regulation of this process. Scd1 is expressed in the adipose tissue and liver. In 3T3-L1 adipocytes, Scd1 expression is induced by insulin and suppressed by TNFa, and it is activated during adipocyte differentiation (20,21). In liver, Scd1 expression is modulated by diet, being inhibited by fasting and induced upon re-feeding (22); it is down-regulated by a diet rich in polyunsaturated fatty acids (23). In mouse liver, Scd1 expression and/or activity is induced by peroxisome proliferators, iron overload, and dichloroacetic acid, i.e. factors that induce hepatocellular carcinoma development or promote hepatocarcinogenesis (24–26). Involvement of Scd1 overexpression in carcinogenesis might not be limited to the liver, as overexpression has also been reported in rat mammary carcinomas (27) and sterculic acid, a Scd1 activity inhibitor, inhibits rat mammary carcinogenesis (28). The possibility of Scd1 involvement in mammalian carcinogenesis is strengthened by a case-control study suggesting a decreased risk of breast cancer in women with low Scd1 enzyme activity (29).

In conclusion, our data indicate Scd1 gene overexpression in the normal liver of mouse and rat strains genetically susceptible to hepatocarcinogenesis. Scd1 overexpression was also detected in a subset of rodent hepatocellular tumors. These findings and other reports of Scd1 overexpression induced by treatments/conditions associated with increased cancer risk indicate the involvement of Scd1 in carcinogenesis.

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References


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