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Regulation of hepatic sulfotransferase (SULT) 1E1 expression and effects on estrogenic activity in cystic fibrosis (CF)☆

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ABSTRACT

Cystic fibrosis (CF) is a major genetic disease in Caucasians affecting 1 in 2500 newborns. Hepatobiliary pathology is a major cause of morbidity and mortality in CF second only to pulmonary disease. SULT1E1 activity is significantly elevated, generally 20–30-fold, in hepatocytes of mouse models of CF. SULT1E1 is responsible for the inactivation of β-estradiol (E2) at physiological concentrations via conjugation with sulfonate. The increase in SULT1E1 activity results in the alteration of E2-regulated protein expression in CF mouse liver. To investigate the mechanism by which the absence of CFTR in human cholangiocytes induces SULT1E1 expression in hepatocytes, a membrane-separated human MMNK-1 cholangiocyte and human HepG2 hepatocyte co-culture system was developed. The cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in bile duct cholangiocytes but not hepatocytes, whereas SULT1E1 is expressed in hepatocytes but not cholangiocytes. CFTR expression in MMNK-1 cells was inhibited with siRNA by >90% as determined by immunoblot and immunohistochemical analysis. Control and CFTR-siRNA-MMNK-1 cells were co-cultured with HepG2 cells in a Transwell membrane-separated system. After 8 h of co-culture, HepG2 cells were removed from exposure to MMNK-1 cells and placed in fresh medium. After 24–48 h, expression of SULT1E1 and selected E2-regulated proteins was analyzed in the HepG2 cells. Results demonstrated that SULT1E1 message and activity were selectively induced in HepG2 cells co-cultured with CFTR-deficient MMNK-1 cells. The expression of E2-regulated proteins (IGF-1, GST-P1 and carbonic anhydrase II) was also altered in response to decreased E2 levels. Thus, the loss of CFTR activity in cholangiocytes stimulates the expression of SULT1E1 in hepatocytes by a paracrine mechanism. SULT1E1 expression in HepG2 cells is inducible by sterol mediated liver-X-receptor (LXR) activation although not by progestins that induce SULT1E1 in the endometrium. SULT1E1 induction in the human cholangiocyte/hepatocyte co-culture system is consistent with and supports the results observed in CF mice. The changes in hepatocyte gene expression affect liver biochemistry and may facilitate the development of CF liver disease.

1. Background

Cystic fibrosis (CF) is a major lethal genetic disease in Caucasians that affects 1 in 2500 newborns. CF is an autosomal recessive disorder caused by disruption of the expression and function of the gene encoding the CF transmembrane receptor (CFTR) protein [1]. CFTR plays a crucial role in chloride (Cl^-) secretion; absent or diminished CFTR activity confers a phenotypic deficit in Cl^- secretion resulting in the clinical manifestations of CF. CFTR is a cAMP-regulated chloride channel located on the apical plasma membrane of most absorptive and secretory cells including cholangiocytes. Its dysfunction impairs Cl^- secretion and therefore Cl^-/HCO_3^- exchange resulting in the inability to maintain a normal secretory response across cellular membranes [2,3].

In humans, the major clinical problems of CF involve a loss of pulmonary function due to changes in mucus structure and associated bacterial infections [4]. Over the last three decades, advances in the pulmonary and nutritional care of CF patients have resulted in an improved life expectancy and quality of life. Consequently, other chronic and systemic manifestations complicating the clinical course of CF have emerged as significant medical issues including pancreatic insufficiency, liver disease (LD) and cirrhosis,

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Table 1
Human cytosolic SULTS.

Isoform and names	Chromosomal loci
SULT1 or phenol SULT family	
SULT1A1: Phenol-sulfating PST, P-PST-1, thermostable-PST	16p11.2–12.1
SULT1A2:	16p11.2–12.1
SULT1A3: Monoamine-sulfating PST, M-PST, thermolabile-PST	16p11.2–12.1
SULT1B1: ST1B2, thyroid hormone-sulfating ST	4q13.3
SULT1C1:	2q11.2
SULT1C2:	2q11.2
SULT1C3:	2q11.2
SULT1E1: Estrogen-ST, EST	4q13.3
SULT2 or hydroxysteroid SULT family	
SULT2A1: Dehydroepiandrosterone-ST, DHEA-ST	19q13.3
SULT2B1a and 2B1b:	19q13.3
SULT4A1: Brain-selective SULT	2p22.3
SULT6A1:	22q13.1

and gastrointestinal problems [4,5]. CFLD affects approximately 15–20% of CF patients and is the second leading cause of mortality and morbidity. The symptoms of CFLD generally occur during adolescence and gradually increase in severity with age, and the occurrence of CFLD appears to be limited to this group of CF patients [6]. CFLD can be life-threatening and result in the necessity for liver transplantation. The pathogenesis of many of these complications of CF is not understood. The underlying causes for the occurrence of severe CFLD, particularly early in life, and its limitation to approximately 20% of the CF population are not known. This pattern of CFLD incidence suggests that one or more genetic modifiers may be involved in the regulation of disease severity.

Retention of bile acids and damage to the biliary system have been proposed as the primary insult in CFLD [7]. Although steatosis is common in CF liver, the major pathological insult is considered to be focal biliary cirrhosis that slowly develops with age to multilobular cirrhosis requiring liver transplantation [6]. The Cl^- transport activity of the CFTR is critical to the maintenance of normal hepatobiliary transport and bile flow. In normal liver CFTR is present in the apical membrane of intrahepatic bile duct epithelial cells or cholangiocytes as well as the gallbladder [2,3]. Loss of CFTR activity results in a viscous bile with an altered bile acid composition.

2. Sulfotransferases

Rozmahel et al. have reported that the severity of intestinal disease in CFTR(−/−) homozygous recessive mice cosegregates with gene loci that contain the genes for hydroxysteroid and estrogen sulfotransferases (SULTs) in mice [8] and humans [9]. Therefore, these SULT genes may serve as candidate modifiers of CF severity, although the mechanism for modulation of this severity is not known. Table 1 shows the names and chromosomal localization of the genes for the human SULT isoforms. The isoforms generally associated with estrogen and hydroxysteroid sulfation are SULTs 1E1, 2A1 and 2B1b [10–12]. SULTs 2A1 and 2B1b structural genes are located at the same chromosomal loci whereas the gene for SULT1E1 is on a separate chromosome.

Sulfation involves the transfer of the sulfonate group of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an acceptor compound to generate a charged hydrophilic product. Fig. 1 shows the sulfation of β-estradiol (E2) and the formation of the charged E2–3-sulfate. In the sulfation of drugs, xenobiotics and small endogenous compounds including steroids, this reaction is catalyzed by a family of cytosolic SULTs [13]. Sulfation has an important role in the synthesis and metabolism of estrogens and hydroxysteroids in humans. Conjugation of steroids with a sulfonate group is primarily an inactivation reaction because steroid sulfates do not bind and activate their appropriate receptors. Although several human SULT isoforms conjugate steroids, SULT1E1 is the major isoform for the inactivation of E2 and the regulation of its activity at physiological concentrations [10,14,15]. SULT1E1 is a member of the SULT1 or phenolic SULT gene family. The enzyme has a Km of 4 nM for the sulfation of E2 and demonstrates substrate inhibition with E2 concentrations above 20 nM [10]. SULT1E1 is expressed at high levels in human liver during the first trimester of development then the levels decrease to low adult levels shortly after birth [16]. SULT1E1 is also expressed in estrogen-responsive tissues including breast, endometrium, prostate and testis [17–20].

SULT2A1 is a member of the SULT2 or hydroxysteroid SULT gene family and is the most abundant SULT isoform expressed in liver [11]. SULT2A1 conjugates both 3α- and 3β-hydroxysteroids, estrogens and aliphatic hydroxyls in drugs and xenobiotics [11,21,22]. SULT2A1 is highly expressed in the fetal adrenal cortex and the reticular layer of the adult adrenal cortex where it is involved in the synthesis of dehydroepiandrosterone (DHEA) sulfate [11]. In contrast, SULT2B1b, the second member of the SULT2 family, is more selective for the sulfation of 3β-hydroxysteroids [12]; however, SULT2B1b is not expressed in human liver. SULT2B1b is expressed in other tissues including breast, prostate, lung and placenta [12,23,24].

3. CFTR-deficient mouse model

Transgenic mice with mutations in the CFTR have been widely used to investigate functional properties of these mutations and their role in the mechanism and pathology of CF. In humans, the most common CFTR mutation is the ΔF508 mutation that is responsible for approximately 60% of human CF and results in a severe form of the disease. The CFTR-ΔF508 protein is not properly trafficked in the cell and accumulates intracellularly preventing the Cl^- transport function of the protein [25,26]. CFTR-knockout (KO) mice have also been generated that do not express the CFTR protein [25].

CFTR(−/−) mice do not demonstrate pulmonary disease although they display many of the manifestations of CF-associated GI and reproductive pathologies [25]. Homozygous CFTR(−/−) mice are generated by breeding heterozygous CFTR(+/−) mice. The CFTR(−/−) mice frequently die shortly after weaning due to meconium ilius unless maintained on a liquid diet. Many of the CFTR(−/−) mice do not gain weight or thrive on a liquid diet mimicking the growth deficits observed in human CF children. In contrast to humans, significant LD is not observed in young CFTR(−/−) mice although Durie et al. [27] report that many CFTR(−/−) mice over a year of age develop CFLD pathologies. Although there are differences in CF in humans and the CFTR(−/−) mouse models, these animals with disease-associated CFTR mutations can be analyzed through genotype/phenotype studies and provide a mechanistic framework with which to study CF disease mechanisms, genotype-based therapeutic approaches and pharmacologic interventions.

4. CFTR mice and SULT1E1 induction

To investigate the possible involvement of the steroid SULTs in CFLD their expression was investigated in the livers of the

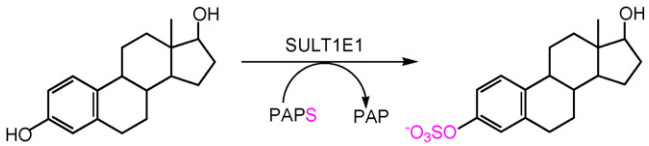

Fig. 1. Formation of β-estradiol 3-sulfate by SULT1E1.

Table 2
SULT1E1 activity in CFTRAF508 mouse tissues. Cytosol was prepared from different mouse tissues and assayed for SULT1E1 activity as described previously [14]. Results are expressed as pmol sulfated product/mg protein/min. ND means that there was no detectable activity.

Tissue	ĘF508(+/+) Female	ĘF508(+/−) Female	ĘF508(−/−) Female	ĘF508(+/+) Male	ĘF508(+/−) Male	ĘF508(−/−) Male
Liver	0.28 ± 0.10	0.15 ± 0.13	20.80 ± 10.4	0.24 ± 0.18	0.15 ± 0.15	13.05 ± 9.1
Lung	0.11 ± 0.03	0.14 ± 0.02	0.13 ± 0.04	0.11 ± 0.04	0.15 ± 0.03	0.12 ± 0.04
Endometrium	0.28 ± 005	0.29 ± 0.03	0.27 ± 0.05	–	–	–
Testis	–	–	–	26 ± 3	25 ± 7	27 ± 5
Intestine	ND	ND	ND	ND	ND	ND
Colon	ND	ND	ND	ND	ND	ND
Kidney	ND	ND	ND	ND	ND	ND
Spleen	ND	ND	ND	ND	ND	ND

CFTR(−/−) mice. Falany et al. [28,29] have reported that SULT1E1 expression is increased up to 100-fold in the livers of both homozygous young adult CFTRΔF508 (−/−) mice and CFTR-KO(−/−) mice. The increase in SULT1E1 expression was not detected in the CFTRΔF508 (−/−) mice before weaning. Wildtype and heterozygous young adult CFTR(+/−) littermates showed no increase in liver SULT1E1 activity. The induction was selective for SULT1E1 activity since neither SULT1A nor SULT2 associated enzyme activities were increased [28]. Also, SULT1E1 activity was increased only in the livers of the CFTR(−/−) mice; SULT1E1 activity in the testes and endometrium of the CFTR(−/−) mice was not increased as compared to wildtype and CFTR(+/−) littermates (Table 2).

Most of the CFTR(−/−) mice did not gain weight normally after weaning and the levels of hepatic SULT1E1 activity were negatively correlated with the loss in body weight although the liver to body weight ratios were not affected [28]. Histopathological examination of the CFTR(−/−) livers as well as determination of plasma levels of enzymes associated with liver damage showed no detectable pathology in the livers of these young adult mice.

To address the possibility that a unique SULT1E1 isoform may be expressed in these CFTR(−/−) livers, the SULT1E isoform induced in ΔF508(−/−) mice was cloned and sequenced for comparison to the published mouse testis SULT1E1 isoform [19]. The sequence of the CF liver isoform was identical to the sequence of the testis isoform indicating that the SULT1E1 isoform is present in CFTR mice. Subsequent examination of the mouse genome has not identified a unique related SULT1E1 gene indicating that a novel SULT1E1-related gene is not being induced.

5. Protein expression in livers of CFTR mice

The large increases in SULT1E1 activity in livers of CFTR(−/−) mice are expected to significantly decrease free E2 levels and thereby alter the expression of estrogen-regulated proteins. Li et al. [29] have reported that the expression of glutathione S-transferase P1 and carbonic anhydrase are down-regulated in the livers of CFTR(−/−) mice consistent with the loss of active E2. CYP2B9 expression was induced in livers of male CFTRΔF508(−/−) mice but not females and the levels of CYP2B9 expression correlated with the increases in SULT1E1 activity. These effects may be related in part to a decrease in the expression of estrogen receptor-α in CFTR(−/−) mice. Fig. 2 shows that the levels of immunoreactive SMAD3 are increased in female ΔF508(−/−) mice. SMAD3 is involved in TGF-β signal transduction pathways and acts as a transcription factor [30,31]. In mice, the pro-fibrotic properties of TGF-β are mediated by SMAD3 and are blocked in SMAD3-KO mice [31]. The association between SULT1E1 and SMAD3 expression has not been reported.

Proteomic analysis of the effects of increased SULT1E1 activity was used to investigate changes in protein expression in both CFTRΔF508 and KO mice. Protein expression in a male CFTR-KO(−/−) mouse and a male ΔF508(−/−) mouse was compared to that of littermate controls. Cytosolic protein (100 μg) from each liver was focused in a Bio-Rad Criterion system using a pH 4–7 gradient. Focused proteins were subsequently resolved in 10–20% pre-cast SDS-PAGE gels. Gels were fixed and stained with Sypro Ruby, destained and imaged with a FX imager then analyzed using PDQuest software. Gels were analyzed for trends in increasing or decreasing intensities of protein spots in the CFTR-KO or ΔF508(−/−) mice compared to the appropriate control. In these experiments each gel possessed 375–400 detectable spots. Using a cut-off of a 2-fold change in spot intensity, PDQuest analysis identified approximately 40 spots in the KO gels and 25 spots in the ΔF508 gels as being significantly altered. Selected spots in each gel were isolated for identification by in-gel trypsin digestion and MALDI-TOF mass spectroscopy (MS). Table 3 shows the change in spot intensity between control and CFTR(−/−) mice as well as the identity of the selected spots. The spots identified in the CFTR(−/−) mice as being down-regulated by MALDI-TOF-MS included GST-P1 and CA that had been previously reported by Li et al. [29].

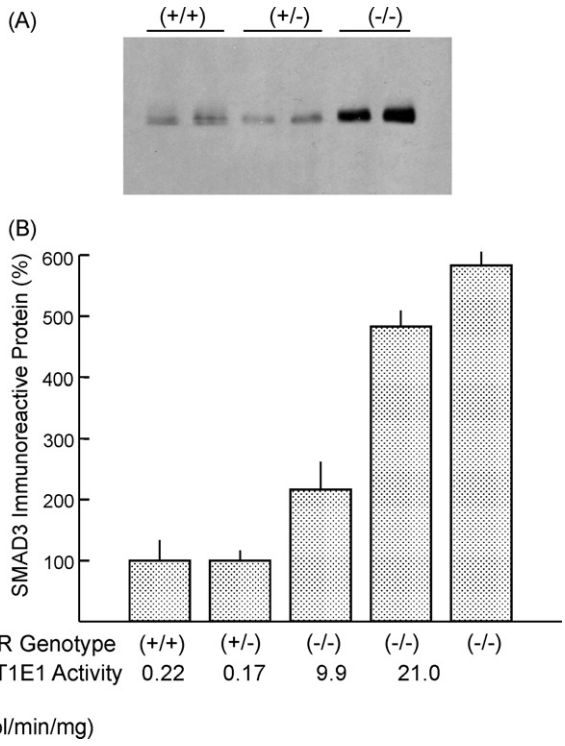

Fig. 2. Immunoblot analysis of SMAD3 expression in liver cytosol from CFTRΔF508 mice. Cytosol (200 mg) prepared from livers of female CFTRΔF508 of each genotype (+/+, +/−, −/−) was resolved in a 12% SDS-polyacrylamide gel, electrotransferred to nitrocellulose membranes, immunoblotted with a rabbit anti-SMAD3 antibody (Calbiochem) and developed with the Pierce West Pico ECL substrate. Panel A shows the immunoreactivity of SMAD3 from two each female CFTRΔF508(+/+, +/−, −/−) mice. Panel B shows the levels of SMAD3 and SULT1E1 activity in liver cytosols of different genotype CFTRΔF508 mice.

Table 3
Proteomic analysis of cytosolic protein changes in male CFTR KO and AF508 mice. Cytosolic protein expression in a male KO(−/−) mouse (SULT1E1 13.74, pmol/min/mg) was compared to that of a littermate control (0.26 pmol/min/mg). In a separate experiment, cytosolic protein expression in a male AF508(−/−) mouse (16.1 pmol/min/mg) was compared to that of a littermate control (0.97 pmol/min/mg). Cytosol (100 μg) from each liver was focused in a Bio-Rad Criterion system using a 4–7 pH gradient and processed as describe above—duplicate gels were run for each sample and analyzed for trends in changing intensities of protein spots comparing the KO or AF508 to its control. In the ΔF508 mice, two control proteins whose intensities were not different were included.

KO Mice			ΔF508 Mice		
Spot	KO/Contrd[@]	Identity	Spot	ΔF508/Contrd[@]	Identity
3401	8.15	Apolipoprotein A IV	0001	0.31	Unknown
5501	5.6	Met Adenosyltrans.	0012	*	Major urinary protein
706	0.24	Albumin	5005	2.0	Adenosine kinase
9201	*	Glutathione S-transferase M1	4001	*	Fatty acid binding protein[#]
1102	0.02	Major urinary protein	3404	⊗	Regucalcin
4706	3.1	Heat shock protein	6001	6.1	Fatty acid binding protein[#]
2504	3.15	ATP synthase	4	*	Apolipoprotein E
2304	0.34	Regucalcin	3202	0.17	ApolipoproteinA1[#]
9203	*	Carbonic anhydrase	3203	0.38	Apolipoprotein A1[#]
10	*	Glutathione S-transferase P1	3713	Control	Heat shock protein
1002	3.13	Paralbumin	3506	Control	Actin

[*] No protein was detectable in the KO or ΔF508 but was detectable in the controls.
[⊗] A protein modification occurred that affected gel migration.
[#] These proteins are related isoforms.

6. Human cystic fibrosis and liver disease

Although the major site of CF pathology in humans is in the pulmonary system, most patients with severe forms of CF are diabetic and display absorptive problems in the GI tract. The CFTR is also important to the maintenance of normal hepatobiliary transport and bile flow. In normal liver, the CFTR is present in the apical membrane of intrahepatic bile duct epithelial cells or cholangiocytes as well as the gallbladder, but is not expressed in hepatocytes [2,3,32]. Disruption of CFTR function in cholangiocytes leads to the production of hyperviscous bile of abnormal composition and ultimately predisposes to hepatobiliary cell damage. It has been suggested that unchecked hepatobiliary damage leads to the emergence of disrupted cholesterol homeostasis and energy metabolism, portal hypertension and the focal biliary fibrosis that herald the onset of CFLD [33,34]. Little is known about the mechanisms of liver parenchymal damage in CF. Liver damage is not simply attributable to cholestasis since treatment with ursodeoxycholate can alleviate many problems with bile flow; however, ursodeoxycholate treatment has no effect on the development or course of CFLD [6].

Approximately 20% of patients with severe CF develop LD that can progress to a degree that liver transplantation is required. This progression in CFLD is not observed in the transgenic CFTR(−/−) mice and represents a fundamental difference from humans in this CF model. Therefore, elucidation of the biochemical and pathological changes in human liver that are associated with the loss of CFTR activity is needed. The biochemical changes in CF liver associated with the development of CFLD may involve the release of proinflammatory cytokines, growth factors, lipid peroxides/reactive oxygen species (ROS) and the activation of hepatic

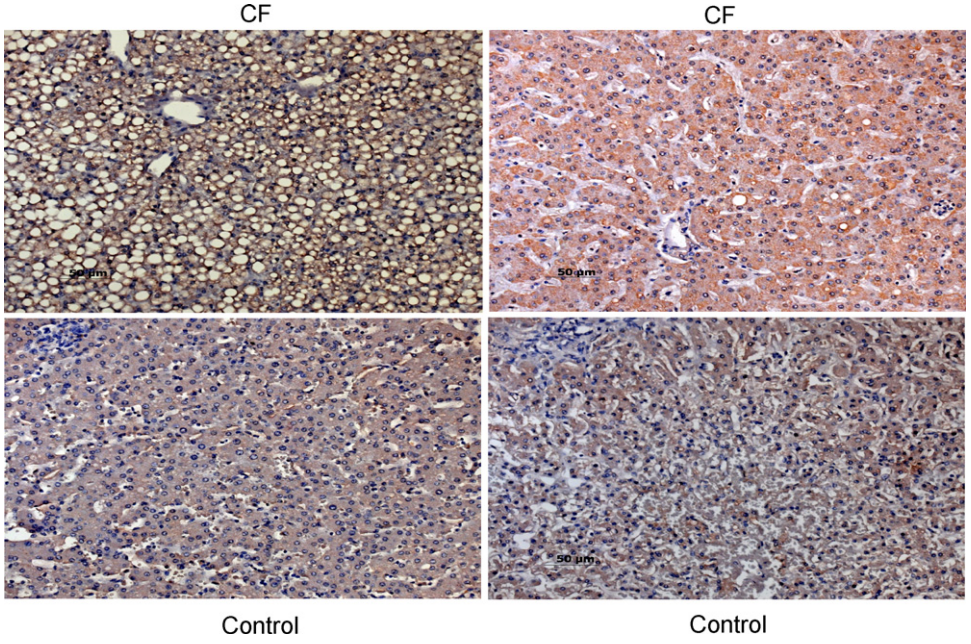

Fig. 3. Immunohistochemical localization of SULT1E1 in human pediatric CF liver. Paraffin sections of liver from children with CF and age-matched controls were treated with a microwave antigen retrieval technique and incubated with rabbit anti-SULT1E1 IgG. After labeling with the biotin–streptavidin complex and counter-stained with hemotoxylin, visualization was carried out with DAB. Stained sections were photographed at 600× magnification. The upper panels are from CF patients and the bottom panels from controls. The upper-left panel (9 mo F) had steatosis and the highest staining, the upper-right panel was from a 4 yo F. The lower panels were from 9 mo M (left) and 4 yo F (right).

stellate cells [3,5,7]. Therefore, the pathogenesis of CFLD may in part involve the disruption of paracrine regulatory mechanisms operating between hepatocytes and cholangiocytes. There is precedent for hepatocyte–cholangiocyte cross-talk in the stringent control of bile production, processing and flow [35–37].

7. SULT1E1 expression in CF liver

The induction of SULT1E1 expression in CFTR(−/−) mouse liver results in alterations in the expression of E2-regulated proteins [29]. In contrast, the understanding of the pathophysiological and histopathological changes in human CF liver is severely limited due to the lack of availability of human CF liver tissue, obtained either during the course of the disease or at death, for scientific research. This has resulted in part from the increased lifespan of CF patients and the ability to diagnose CF genetically that has resulted in a decrease in autopsies. Liver biopsies that represent the best way to diagnose CFLD provide little tissue for research.

To examine whether SULT1E1 is increased in the liver of CF patients, a set of 19 paraffin blocks of liver tissue from children diagnosed with CF prior to 1993 was obtained from The Children's Hospital of Alabama and immunostained for SULT1E1. Three of the 19 specimens showed increased SULT1E1 immunostaining although the presence of CFLD in these patients was not recorded. Fig. 3 shows the increase in SULT1E1 immunostaining in two young CF patients, including one with obvious steatosis. The number of samples showing increased immunostaining for SULT1E1 is consistent with the anticipated percentage of CF patients with CFLD in this small population.

8. Cholangiocyte–hepatocyte co-culture model

The lack of tissue specimens from patients with CFLD and the anticipated interactions of cholangiocytes and hepatocytes during the development of CFLD have led to the use of model systems to study CFLD. To investigate interactions between cholangiocytes and hepatocytes in CF, a co-culture model has been developed [32]. Human MMNK-1 cholangiocytes were cultured with human HepG2 hepatocytes in a membrane-separated Transwell system. MMNK-1 cells are an immortalized human cell line that expresses CFTR. CFTR expression has been inhibited >85% using siRNA to replicate the loss of CFTR function in CF [32]. For these studies, MMNK-1 cells are plated in 60 mm dishes and allowed to grow overnight. HepG2 cells are placed in the Transwell chamber and allowed to attach overnight. The Transwell chamber is then placed in the 60 mm plate and the cells are cultured together for 8 h. The HepG2 cells are then removed and RNA is immediately prepared. For protein expression studies, the Transwell chamber is moved to fresh medium and incubated for 24–48 h to permit translation of synthesized message. Fig. 4 shows the selective expression of SULT1E1 compared to SULTs 1A1 and 2A1. The ability of CFTR repression in MMNK-1 cells to induce SULT1E1 expression in HepG2 cells even when cells are separated by a 0.4 μm membrane suggests involvement of a paracrine factor. Conditioned medium experiments also indicate that a permeable factor secreted by MMNK-1 cells is involved in the induction of SULT1E1 [32].

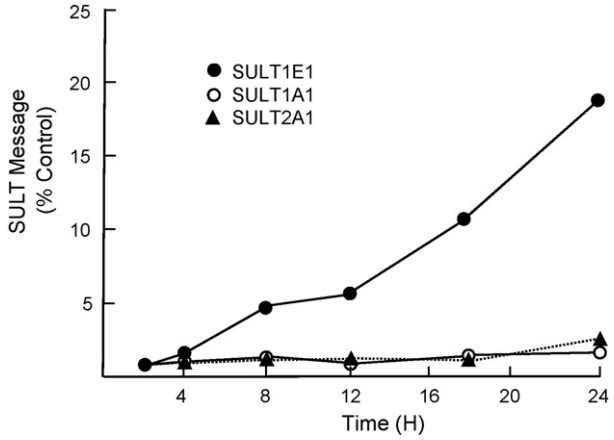

Fig. 4. Quantitative RT-PCR analysis of SULT1E1 expression in HepG2 cells co-cultured with MMNK-1 cells. HepG2 cells were co-cultured with CFTR-siRNA or control-siRNA MMNK-1 cells for increasing times then total RNA was extracted and utilized to synthesize cDNA. Quantitative RT-PCR was performed with TaqMan® Gene Expression Assays for human SULTs 1E1, 2A1 and 1A1. Ribosomal 18S RNA was chosen as the endogenous control for total RNA normalization and mRNA expression levels were calculated using the Ct method, where the calibrators were the samples from normal HepG2 cells without co-culture with MNNK-1. Relative expression of SULT isoforms was presented as fold-change between co-culture with CFTR-siRNA and co-culture with control-siRNA. Each point represents the mean of four separate determinations.

9. Expression profiling of MMNK-1 cells

Information with respect to the transcriptional regulation of human SULT1E1 is limited. Falany and Falany [38] have demonstrated that progesterone regulates SULT1E1 expression in endometrial cancer cells. SULT1E1 expression is also greatly increased in normal human endometrial biopsies during the secretory phase of the menstrual cycle when progesterone levels are high [17]. Although low levels of progesterone receptor can be detected in human HepG2 cells, no effect on SULT1E1 message levels was observed following treatment of HepG2 cells with progesterone or medroxyprogesterone. It has recently been reported that SULT1E1 expression is regulated by liver-X-receptor (LXR) activation in mouse liver [39] and in cultured human breast cells [40].

To investigate the possible mechanisms by which inhibition of CFTR function in cholangiocytes may regulate SULT1E1 expression in hepatocytes, expression profiling of CFTR-siRNA-MMNK-1 cells and control-siRNA MMNK-1 cells was carried out. For expression profiling studies, total RNA was isolated from both control-siRNA and CFTR-siRNA-MMNK-1 cells and used to probe Affymatrix Human Gene 1.0 ST GeneChips. This array represents

Table 4
Pathway analysis of cholesterol and sterol metabolism in Human Gene 1.0 array data. Total RNA was isolated from control and CFTR-siRNA-MMNK-1 cells and used for expression profiling using the Human Gene 1.0 ST array. Both control and siRNA arrays were done in triplicate. Genes showing a significant difference in expression at $p < 0.05$ were used for further analysis. After statistical analysis, the resulting list ($p < 0.05$) was loaded into PathwayArchitect software (Agilent, CA, USA) for Gene Ontology (GO) analysis. Among 1775 probe sets matched to GO database of 90,204 total GO terms, 20 probe sets relate to cholesterol metabolism, e.g., with a p-value of 7.45E−16 when the two ratios of the same term (cholesterol metabolism) were compared.

GO Term	# Genes in selection	Rows in selection	# Genes in database	Total rows in database	p-value
Cholesterol biosynthesis	14	1775	33	90204	7.17 E−16
Cholesterol homeostasis	2	1775	7	90204	0.007
Cholesterol metabolism	20	1775	89	90204	7.45 E−16
Cholesterol transport	2	1775	10	90204	0.016
Sterol biosynthesis	14	1775	49	90204	1.79 E−14
Sterol metabolism	21	1775	100	90204	6.18 E−16

Table 5
Selected genes associated with cholesterol metabolism with altered expression in CFTR-siRNA-MMNK-1 cells.

Gene ST probe set	Mk cont Ave	Mk siRNA Ave	Gene symbol	mRNA Description	*p*-value	Regulation
7945831	195	154	OSBPL5	Oxysterol binding protein-like 5	0.02	Down
7950067	2025	1435	DHCR7	7-Dehydrocholesterol reductase	0.03	Down
7913462	4326	3431	DCHR24	24-Dehydrocholesterol reductase	0.003	Down

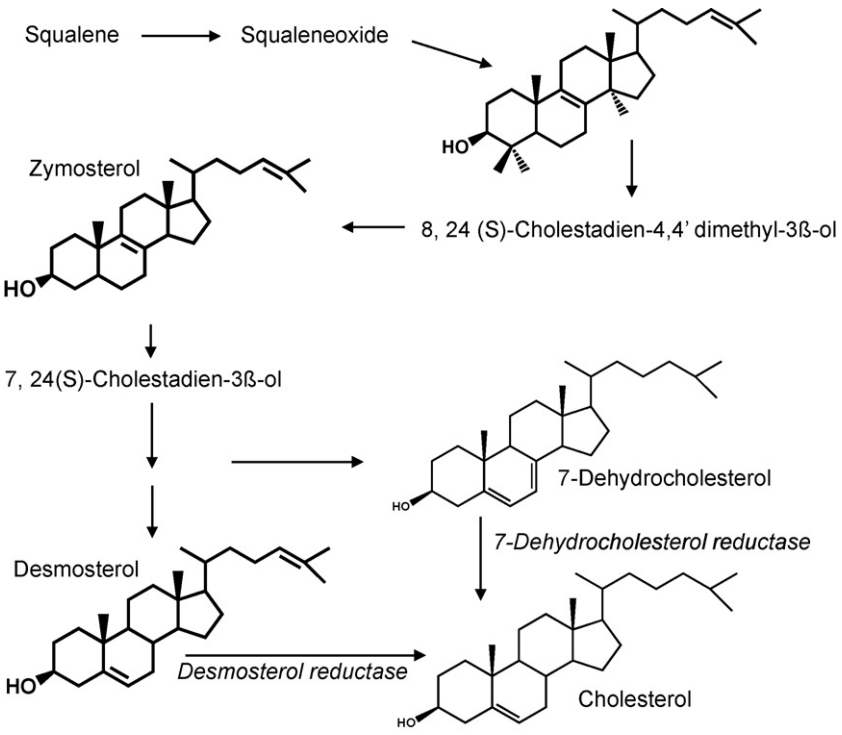

Fig. 5. Overview of cholesterol synthesis showing the roles of 24-dehydrocholesterol (desmosterol) and 7-dehydrocholesterol reductases in final steps to cholesterol formation.

28,869 genes with approximately 26 probes spread across the length of each gene to provide a more complete and more accurate picture of gene expression than 3′ based expression array designs. Table 4 shows the pathway analysis of cholesterol and sterol metabolism in the CFTR-siRNA-MMNK-1 cells. Expression of several genes associated with cholesterol/sterol biosynthesis or metabolism was significantly altered in the CFTR-siRNA-MMNK-1 cells. Included in the cholesterol synthesis pathway was the significant down-regulation of 7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase (Table 5). Fig. 5 demonstrates that 7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase, are responsible for the final steps in cholesterol synthesis. Both desmosterol and 7-dehydrocholesterol, substrates for 7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase, respectively, are activators of the liver-X-receptor (LXR) [41,42]. Fig. 6 shows that desmosterol, 7-dehydrocholesterol, 24-hydroxycholesterol and the synthetic LXR agonist T0901317 are all capable of inducing SULT1E1 expression in HepG2 cells. Neither SULT1A1 or SULT2A1 expression was affected by LXR activation.

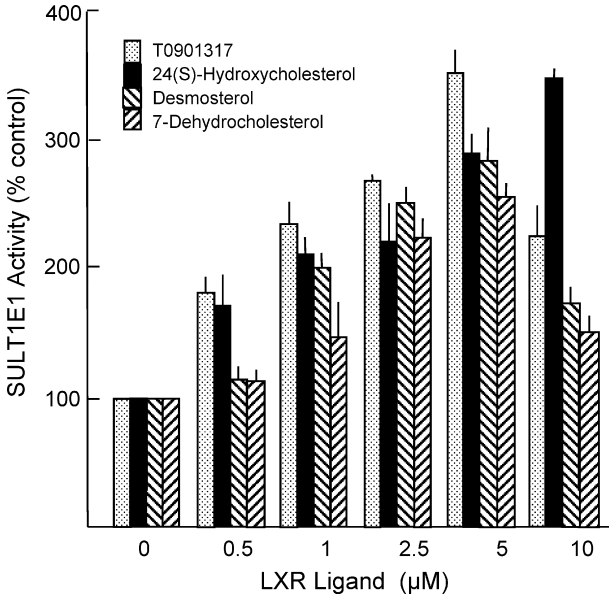

Fig. 6. Induction of SULT1E1 in HepG2 by LXR ligands. HepG2 cells were treated with TO901317, 24(S)-hydroxycholesterol, desmosterol and 7-dehydrocholesterol at varying concentrations for 48 h. Cytosol was prepared from the cells and SULT1E1 and SULT2A1 activities were determined using 20 nm [^{3}H]-E2 or 4 μM [^{3}H]-DHEA as substrate, respectively, with 25 μM PAPS as the sulfate donor as described previously [14]. SULT1A1 activity was assayed with 4 μM p-nitrophenol and [^{35}S]-PAPS as described previously [43].

10. Summary

The gene loci encompassing the human and mouse steroid SULTs have mapped as possible genetic modifiers of CF intestinal disease. The mouse CFTRΔF508 and KO models of CF show selective large increases in liver SULT1E1 activity that alter the expression of estrogen-regulated genes. However, in mouse CF models early onset of CFLD does not occur, although CFLD may occur late in life. Approximately 20% of human CF patients have severe progressive

LD that may ultimately require liver transplantation. The induction of SULT1E1 and potential effects of increased SULT1E1 expression in these patients has not been investigated. To determine whether SULT1E1 is induced in human CF liver, a co-culture model was utilized. Human MMNK-1 cholangiocytes with siRNA-inhibited CFTR expression are capable of inducing SULT1E1 expression in HepG2 hepatocytes that lack CFTR expression in a membrane-separated Transwell co-culture system. The induction appears to be selective for SULT1E1 since SULTs 1A1 and 2A1 as well as CYP3A4 are not induced. Expression profiling of CFTR-siRNA-MMNK-1 cells suggests that oxysterols in the cholesterol biosynthesis pathway may be involved in the paracrine regulation of SULT1E1. Desmosterol and 7-dehydrocholesterol, both immediate precursors in the synthesis of cholesterol, induce SULT1E1 expression in HepG2 cells via activation of LXR. Therefore, LXR activation resulting from changes in cholesterol synthesis in cholangiocytes during CF is a possible mechanism for the selective induction of SULT1E1 in hepatocytes. Results of this study indicate that the pathogenesis of CFLD may involve the disruption of paracrine regulatory mechanisms operating between hepatocytes and cholangiocytes.

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