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Developmental and extrahepatic physiological functions of SREBP pathway genes in mice

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Abstract

Sterol regulatory element-binding proteins (SREBPs), master transcriptional regulators of cholesterol and fatty acid synthesis, have been found to contribute to a diverse array of cellular processes. In this review, we focus on genetically engineered mice in which the activities of six components of the SREBP gene pathway, namely SREBP-1, SREBP-2, Scap, Insig-1, Insig-2, or Site-1 protease have been altered through gene knockout or transgenic approaches. In addition to the expected impacts on lipid metabolism, manipulation of these genes in mice is found to affect a wide array of developmental and physiologic processes ranging from interferon signaling in macrophages to synaptic transmission in the brain. The findings reviewed herein provide a blueprint to guide future studies defining the complex interactions between lipid biology and the physiologic processes of many distinct organ systems.

Keywords

SREBP; cholesterol; lipid; biosynthesis; gene expression; knockout

1. Introduction

Sterol regulatory element-binding proteins (SREBPs) are membrane-bound transcription factors that control lipid biosynthetic gene expression [1]. They are a small family of transcription factors, comprised of three major isoforms, SREBP-1a, SREBP-1c, and SREBP-2 derived from two separate genes. Together, SREBPs control the synthesis of cholesterol, fatty acids, and triglycerides. Whereas SREBP-2 preferentially activates genes encoding enzymes for cholesterol synthesis and SREBP-1c primarily activates genes required for fatty acid (FA) and triglyceride (TG) synthesis, SREBP-1a can activate genes in

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both biosynthetic pathways [2, 3]. In cultured mammalian cells, all three isoforms are subject to end-product feedback regulation by sterols, becoming activated when cellular sterols are depleted and inhibited when cellular sterols accumulate, respectively. SREBPs were first identified in 1993 [4, 5] and over the next 9 years, elegant studies from the Brown and Goldstein laboratory identified five additional proteins – Scap, Insig-1, Insig-2, Site-1 protease, and Site-2 protease [6-10] – that constitute the “core” pathway for sterol-regulated activation of SREBPs from their membrane-bound precursor forms (pSREBPs) to their active, nuclear forms (nSREBPs). In the 24 years since the discovery of SREBPs, the function of every gene of the core SREBP pathway, with the exception of Site-2 protease, has been studied *in vivo* using knockout and/or transgenic mice.

The focus of this review will be the developmental and physiological functions of these 5 core SREBP pathway genes in mammals. In recent years, many functions have been assigned to SREBPs [11, 12] based on evidence using many experimental approaches. However, this review will be limited to evidence as revealed by the study of knockout and transgenic mice. Inasmuch as the best-understood function of these genes is the regulation of lipid synthesis, they have been studied most extensively in a subset of organs with either relatively high rates of lipid synthesis or high lipid contents, including the liver, digestive tract, skin, mammary gland, adipose tissue, and brain. However, notable phenotypes have been observed in other tissues less-classically associated with highly active lipid synthesis or storage, including pancreas, cartilage, immune system, kidney, ocular lens, and others. The role of these genes in the regulation of hepatic lipid synthesis has already been reviewed in great detail [1, 13, 14] and so here is not addressed.

An inclusive listing of published phenotypes from germline and tissue-specific knockout and transgenic mouse models for genes of the SREBP pathway is shown in Tables 1 and 2. Floxed alleles of *Scap*, *Srebf2*, *Insig1*, and *Mbtps1* (Site-1 protease) were generated and have been bred to a variety Cre drivers, which are listed in Table 1 and in the text for particular studies. Regarding the tissue distribution of various components of the SREBP pathway, Scap, SREBP-2, Site-1 protease, and Site-2 protease are widely expressed [15-18]. SREBP-1c expression is highest in liver and adipose tissue and generally exceeds SREBP-1a expression in mouse tissues except for the small intestine, heart, thymus, and spleen [19, 20]. Insig-1 is widely expressed, with peak levels in the liver and adipose tissue [9, 21]. Insig-2 has two isoforms arising from the use of alternative promoters: Insig-2a is liver-specific and Insig-2b is ubiquitous [22]. The following discussion will focus on the roles of SREBP pathway genes during embryonic development as well as their tissue-specific functions in the digestive tract, immune system, and nervous system.

2. The SREBP Pathway: Sterol-dependent regulation of SREBP proteolysis

SREBPs are transcription factors of the basic helix-loop-helix-leucine zipper (bHLH-Zip) family. They are unusual in this family as they are synthesized as integral membrane proteins of the endoplasmic reticulum (ER). SREBPs consist of cytosolic NH₂-terminal and COOH-terminal domains separated by two transmembrane-spanning sequences and a short loop that projects into the ER lumen. The NH₂-terminal domain contains the bHLH-Zip region that must be proteolytically freed from the membrane before it may enter the nucleus

to activate transcription. Under low-sterol conditions, pSREBPs are transported by another integral membrane protein, Scap, from ER to Golgi, where they are processed by two sequential proteolytic cleavages. The first cleavage occurs within the short luminal loop and is catalyzed by Site-1 protease. This cleavage is rapidly followed by a second cleavage within the first transmembrane-spanning segment, and is catalyzed by Site-2 protease. As a result, the NH₂-terminal domain containing the bHLH-Zip region is freed from the membrane and may enter the nucleus to activate transcription. Under sterol-replete conditions, proteolytic activation of SREBPs is prevented by Insig-1 and Insig-2, a family of integral membrane proteins that bind Scap in a sterol-dependent fashion and retain the Scap-SREBP complex in ER [9, 10].

3. Developmental roles of SREBP Pathway Genes

An essential role for SREBP-2 and Site-1 protease during mammalian development can be inferred given that mice with homozygous, germline disruption of these genes lead to embryonic lethality [1, 18, 23, 24]. SREBP-1 is incompletely required during embryogenesis; a small minority of SREBP-1 null animals survives to adulthood [25], likely owing to the increases in SREBP-2 expression which may compensate for the loss of SREBP-1 by activating the transcription of genes usually favored by SREBP-1 [2]. A complete lack of nuclear SREBPs, as is seen in Site-1 protease deficiency, is associated with complete, early embryonic lethality and abnormal formation of epiblasts that do not survive past implantation, at approximately E4 to E5 [24]. Likewise, in unpublished observations from our laboratory, interbreeding of mice with heterozygous null mutations in Scap does not produce live pups with homozygous Scap deficiency. Taken together, these findings suggest that there exists a requirement for nSREBPs, particularly nSREBP-2, during embryogenesis. Presumptively, in this context, nSREBPs are required to support cholesterol synthesis to provide building blocks to support rapid cellular growth in the embryo.

This presumption was recently challenged by studies of mice with a gene-trapped SREBP-2 null allele [18]. SREBP-2 null embryos display near-total embryonic lethality between E12.5 and E14.5, and were notable in the absence of digit formation on the limb buds. In wild-type embryos, SREBP-2 expression was detectable in limb buds in areas of the forming digits that were fated to undergo apoptosis during digit specification. Total cholesterol and triglyceride contents of the SREBP-2 null embryos were similar to those of controls, even though the expression of genes required for both cholesterol and FA synthesis were reduced, implying reduced embryonic lipid synthesis. These grossly normal lipid contents in the SREBP-2 knockout embryos raised the question as to whether the developmental abnormalities seen with SREBP-2 deficiency can be solely attributed to bulk availability of lipids, or whether there was crosstalk between SREBP-2 and developmentally-critical signaling pathways, such as the sonic hedgehog (Shh) pathway. In support of the latter hypothesis, the expression of several Shh targets genes (including *Bmp4*, *Gli1*, *Grem1* and *Ptch1*) known to be important for limb patterning were found to be reduced in the SREBP-2-deficient embryos, leading to the speculation that SREBP-2 may impact Shh signaling.

The mechanism(s) by which SREBP-2 deficiency might alter Shh signaling are unknown. Sterols impact the Shh signaling pathway at multiple levels, through Shh itself, its receptor

Patched, and its downstream transducer Smoothened [26]. One possibility is that SREBP-2 deficiency, by altering lipid synthesis, is altering post-translational modification of Shh. Shh undergoes both cholesterylation and palmitoylation, both of which required for its normal activity [27]. It is also possible that alterations in sterol metabolism attributable to SREBP-2 deficiency alter the activity of Patched, which contains a sterol-sensing domain (SSD), a structure that it shares with Scap and HMGR [28]. In Scap and HMGR, the SSD is required for sterol-mediated feedback inhibition of their activities, by altering their trafficking or ubiquitination, respectively, although in the case of Scap the SSD may not function by direct binding of cholesterol [29]. Lastly, Smoothened, the target of Patched, is a GPCR-like receptor whose activity is regulated by oxysterols, in turn whose generation is dependent upon SREBP-2 [30]. Further studies will be required to define crosstalk between the SREBP and Shh pathways. The study of Vergnes, et al. [18] is not the first to observe gene expression signatures suggesting crosstalk between the SREBP pathway and critical developmental signaling pathways; for example, reduced expression of Wnt-responsive genes was noted in embryonic lung tissues of mice with Scap deficiency in surfactant-producing alveolar type-II cells [31].

The above-mentioned findings from mouse models in which the SREBP pathway is inactivated indicate that nSREBPs are generally required for embryogenesis. Studies on mice with constitutively-activated SREBPs, owing to knockout of the inhibitors of SREBP proteolysis, Insig-1 and Insig-2, also shed light on the consequences of developmental perturbations of sterol synthesis [32]. *Insig-1^{-/-};Insig-2^{-/-}* (*Insig*-double knockout or DKO) mice died in the perinatal period and nearly 100% were noted to have severe craniofacial malformations with facial or palatal clefting. The *Insig*-DKO embryos, as expected, had a dramatically elevated rate of sterol synthesis and accumulated large quantities of cholesterol. Unexpectedly, they also accumulated large amounts of post-squalene intermediates of the cholesterol biosynthetic pathway, such as lanosterol, zymosterol, and desmosterol. As a group, sterol intermediates accumulated 40-fold higher in the *Insig*-DKO embryos compared to control littermates. Treatment of pregnant dams with lovastatin, an inhibitor of cholesterol synthesis, nearly normalized these levels of sterol intermediates and ameliorated the craniofacial / palatal clefting of the *Insig*-DKO embryos. These findings suggested a teratogenic role for the abnormal accumulation of sterol intermediates, a feature found in common with several human malformation syndromes caused by inherited defects of distal enzymes in the cholesterol biosynthetic pathway. The prototypical member of this class of disorders is Smith-Lemli-Opitz syndrome, which is associated with deficiency of sterol ⁷-reductase [26]. Similar to the aforementioned study on gene-trapped SREBP-2 null mice [18], it is tempting to speculate that alterations in Shh pathway signaling, as has been associated with craniofacial malformation syndromes, could explain the malformations in the *Insig* knockouts. However, no alterations in the expression of Shh targets were found in dissected palatal or maxillar tissues in *Insig*-DKO embryos.

4. Tissue-specific roles of SREBP Pathway genes

4.1. Epithelial homeostasis in the gastrointestinal tract

The gastrointestinal tract, the small intestine in particular, is central to whole-body sterol metabolism [33]. The epithelial cells of the small intestinal mucosa integrate several modes of influx and efflux of cholesterol. They absorb exogenous cholesterol from the gut lumen, avidly synthesize cholesterol, uptake plasma cholesterol from lipoproteins like LDL, secrete cholesterol into the plasma packaged as chylomicrons, and excrete cholesterol into the gut lumen through a process termed transintestinal cholesterol efflux [34]. Of the “influx” modes, quantitatively sterol absorption and sterol synthesis are the most important. For example, in the small intestine of an adult mouse fed a standard chow diet, approximately 0.5 to 1 mg of cholesterol a day is both synthesized *de novo* and absorbed from the diet [35]. Furthermore, the small intestine is quantitatively second to liver in terms of *de novo* sterol synthesis in mice and rats. The intestine may synthesize more cholesterol than the liver in other species, such as rabbit, guinea pig, and possibly man [36-38].

SREBP-2 is a key regulator of epithelial sterol homeostasis that balances the gut luminal cholesterol uptake with the mucosal sterol synthesis. Ezetimibe is a cholesterol-lowering drug that blocks intestinal cholesterol uptake by inhibiting the luminal cholesterol transporter NPC1L1. Blockade of cholesterol absorption with ezetimibe potently activates proteolysis of SREBP-2 in enterocytes [39], which elicits a compensatory increase in intestinal sterol synthesis [40, 41]. Mice with intestinal *Insig* deficiency also have marked elevations in nSREBP-2 and increased rates of cholesterol synthesis which did not further increase with ezetimibe administration [42]. The latter finding argues that the NPC1L1-mediated sterol absorption supplies the regulatory pool of cholesterol in enterocyte ER membranes that act on *Scap* and *Insig* [43].

Most intestinal sterol synthesis occurs in the epithelial cells of the lower villus and intestinal crypts [44, 45], which coincides with peak expression of SREBP-2 and HMGCR along the crypt-villus axis [39] and where it is thought to sustain rapid cellular division. NPC1L1 is present throughout the villus and crypt except the crypt base [46]. The inhibition of SREBP-2 proteolysis by absorbed cholesterol metabolically programs the epithelial cells in a gradient along the crypt-villus axis, as well as along the longitudinal axis of the intestine, to favor either sterol absorption or synthesis [39]. When cholesterol is being actively absorbed, nSREBP-2 is concentrated at the lower villus and crypt since the upper villus cells are being adequately sourced by NPC1L1. When exposed to ezetimibe, the upper villus cells can no longer absorb sufficient cholesterol and feedback inhibition on SREBP-2 is relieved, thereby increasing sterol synthesis higher up in the villus. When viewed along the longitudinal axis of the small intestine, NPC1L1's inhibition of SREBP-2 activation is likewise readily apparent. Thus, nSREBP-2 is virtually undetectable in the jejunum, where NPC1L1 and cholesterol absorption is the greatest, but it rises in the distal ileum where NPC1L1 expression drops. When NPC1L1 is blocked by ezetimibe, jejunal SREBP-2 is upregulated, thereby driving sterol synthesis to replace the reduced sterol absorption [39]. In rodent models of bile diversion in which cholesterol absorption is impeded, the intestinal cholesterol synthetic rate is increased by approximately 10-fold [45] likely as a result of

elevated nSREBP-2. Whether the same happens and is of significance in humans undergoing biliary-diverting surgical procedures remains to be elucidated.

The essential role of lipid synthesis in the small intestine was demonstrated in mice with an inducible deletion of Scap in intestinal epithelia using *Villin-Cre-ERT²* (*Vil-Scap^{-/-}* mice). As a result of Scap deficiency, all nSREBPs were abolished in the intestine of these *Vil-Scap^{-/-}* mice, leading to reduced intestinal cholesterol and FA synthesis. Lethal intestinal injury developed within a few days of Scap ablation [47]. Histology revealed severe damage to both the villus and crypts of the *Vil-Scap^{-/-}* mice. This timing suggests that the injury of Scap disruption requires only one cycle of epithelial renewal to manifest, which takes 3–4 days in the mouse [48]. Scap deficiency also blocked the growth of intestinal crypts cultured *in vitro* as enteroids or mini-guts. Enteroid growth was rescued by exogenous cholesterol and oleate, both of which require SREBPs for synthesis. Although exogenous lipids rescued the crypts *in vitro*, *Vil-Scap^{-/-}* mice could not be rescued by feeding of large amounts of fat and cholesterol. It is possible that the dietary lipids do not reach the crypt cells. The lethality of Scap deficiency in intestine contrasts sharply with the non-lethality of Scap deficiency in the liver [49, 50]. In contrast to intestinal crypt cells that divide frequently, hepatocytes replicate much more slowly [51]. This difference may suggest that SREBPs are essential in rapidly-dividing cells. This phenomenon has been noted in CD8⁺ T cells and is described below [52]. Whether this phenomenon can be generalized to other tissues with rapid cellular turnover (bone marrow, epidermis, etc.) remains to be seen.

4.2. Immune Function

SREBPs have been shown to play multiple roles in the innate and the adaptive immune systems. The following section elaborates on three knockout models affecting macrophages and one affecting T cells. Functional requirements of SREBP pathway genes in other components of the immune system remain unknown. The contribution of SREBP-1a, the predominant SREBP1 isoform in macrophages, in response to bacterial stimuli was examined in mice with hypomorphic SREBP-1a alleles, termed SREBP-1a deficient (*Srebp-1aDF*) mice [20]. These mice were created by the insertion of a β -geo cassette into intron 1a of *Srebf1*, which resulted in a 95% reduction in SREBP-1a mRNA and no effect on SREBP-1c mRNA [53]. Survival of *Srebp-1aDF* mice was improved, compared to controls, when sepsis was induced by LPS or cecal puncture. The improved survival was associated with reductions in levels of pro-inflammatory cytokines including IL-1 β . As expected, FA synthesis was reduced in macrophages of the *Srebp-1aDF* mice. Unexpectedly, expression of NLRP1a, a component of the inflammasome, was also reduced in SREBP-1a-deficient macrophages. The reduction in NLRP1a in turn reduced caspase-1 activity, thereby preventing cleavage of pro-IL-1 β and secretion of IL-1 β . Thus, SREBP-1a is required for pro-inflammatory responses of the innate immune system, and its blockage in macrophages may be potentially of benefit in settings of life-threatening inflammation such as sepsis. In support of this hypothesis, preventing the proteolytic activation SREBPs by Scap ablation in LPS-stimulated cultured macrophages *in vitro* was similarly found to reduce levels of IL-1 β and caspase-1 [54].

On the other hand, SREBP-1a may also be needed for macrophage survival in other circumstances. To combat bacterial pathogens, macrophages must resist microbe-derived pore-forming toxins that activate cell death pathways including apoptosis [55]. SREBP-1a-deficient macrophages were more sensitive to apoptotic cell death induced by bacterial (*S. aureus*) alpha toxin, owing to reduced expression of Api6, an anti-apoptotic factor [56].

In contrast, other findings emphasized the importance of SREBP-1 in controlling the synthesis of anti-inflammatory unsaturated fatty acids in macrophages. Using a lipidomics strategy, an accumulation of unsaturated FAs such as EPA and DHA was observed in macrophages 24 hours after exposure to KLA, a component of LPS and specific activator of TLR4 [57]. Expression of SREBP-1 target genes that control synthesis of unsaturated FAs, such as SCD-1 and SCD-2, peaked at the same time. Their expression was reduced and accordingly low levels of unsaturated FAs were found in KLA-treated macrophages from *Srebp-1*^{-/-} mice at this delayed time point. The SREBP-1-deficient macrophages exhibited a hyper-inflammatory state with increased expression of multiple cytokines (*Cxcl1*, *Cxcl9* and *Il1a*). A similar exaggerated inflammatory response was induced by LPS injection in *Srebp-1*^{-/-} mice, with increased levels of plasma cytokines such as IL-6 and IL-1α that were normalized by EPA supplementation. Together, these data suggest that SREBP-1, by controlling the synthesis of anti-inflammatory omega-3 fatty acids, is required for the termination of TLR-4-mediated inflammatory responses in macrophages.

The role of Scap/SREBPs in the innate immune system in the context of viral, rather than bacterial, stimuli was studied by selective deletion of Scap in macrophages using *Lysozyme 2-Cre* (*LysM-Scap*^{-/-} mice) [58]. *LysM-Scap*^{-/-} mice were resistant to respiratory infection with MHV-68 and had increased expression of interferon-stimulated genes (ISGs) in the lung. As expected, cholesterol and FA synthesis were reduced in Scap-deficient macrophages. In cultured cells in which cholesterol synthesis was inhibited owing to deficiency of Scap, SREBP-2, or mevalonate kinase (an enzyme of the sterol biosynthetic pathway), interferon signaling was potentiated and ISGs (*Ifnb1*, *Mx1*, *Mx2* and *Ccl2*) were dramatically upregulated. Conversely, IFNβ reduced cholesterol synthesis in wild-type macrophages. Furthermore, the stimulation of ISGs caused by SREBP-2 deficiency occurred only in cells in which the STING pathway was functional. STING is an ER adaptor protein that activates a number of transcription factors including IRF3, a key mediator of the type I IFN response [59]. Taken together, these findings suggested that sterol biosynthetic flux suppresses interferon signaling, and vice versa, forming an STING-dependent inhibitory circuit in macrophages.

Thus, in macrophages SREBP-1 stimulates context-dependent pro-inflammatory, anti-inflammatory, and anti-apoptotic responses to bacterial stimuli while SREBP-2, by driving sterol biosynthetic flux, suppresses interferon responses to viral stimuli. The link between Scap and IFNβ signaling was further explored in a study using *in vivo* siRNAs against Scap [60]. Scap-directed siRNAs reduced Scap levels in the liver and in leukocytes. In contrast to the *LysM-Scap*^{-/-} model, siRNA-treated Scap deficient mice were more susceptible to viral infection (using HSV-1) and had reduced plasma levels of IFNβ. Scap interference reduced the expression of ISGs (*Ifna4*, *Ifnb1* and *Cxcl10*) in HSV-1-exposed macrophages. *In vitro* protein-protein interactions between Scap and STING as well as Scap

and IRF3 were demonstrated. This data led to the proposal that Scap mediates STING signaling by physically recruiting IRF3 to STING. While these two studies [58, 60] propose differing functions for Scap (i.e. either promoting or inhibiting type-I IFN response), they were performed with different methodologies in different contexts, and so additional studies may be required to clarify the complex interactions between sterol biosynthesis, Scap, and SREBPs in macrophage IFN signaling.

In contrast to the above studies on Scap function in macrophages, in which macrophage immune responses were altered in the absence of changes in macrophage number [58], studies in T cells reveal a role for Scap in immune cell proliferation. In order to mount an adaptive immune response after mitogen stimulation, T cells must transition from a quiescent state to an activated, proliferative state. This switch requires T cells to undergo metabolic reprogramming in order to support the demands of cell division and growth [61].

In a study of SREBP function in the adaptive immune system, Scap was deleted selectively in T cells using *Cd4-Cre* (*Cd4-Scap*^{-/-} mice) [52]. When compared to wild type controls, T cells derived from *Cd4-Scap*^{-/-} mice failed to increase their levels of cholesterol and FAs after mitogen stimulation. Mitogen-stimulated Scap-deficient T cells failed to enter the S phase of the cell cycle and proliferate. This proliferation defect was prevented by the addition of exogenous cholesterol. Production of antigen-specific CD8⁺ T cells was impaired in *Cd4-Scap*^{-/-} mice infected with LCMV, indicating an inadequate T cell response to viral pathogens. This data suggests that Scap is required for effector responses of activated T cells by providing cholesterol to support cell proliferation, similar to its role in sustaining the intestinal epithelia [47].

4.3. SREBPs and the central nervous system

The lipid composition of the brain is quite different from that of other organs. In mice, cholesterol concentration of the brain is approximately 15 mg / g tissue, which is about 7 times higher than that of whole-body cholesterol content [62]. Nervous tissue is rich in cholesterol because of its abundance of plasma membranes, which contain the large majority of cellular cholesterol. The plasma membrane surface area of dendrites, axons and synapses is enormous. In addition, synaptic membranes and myelin, which provides electrical insulation for axons, are particularly cholesterol-rich [63]. Since circulating lipoproteins do not cross the blood-brain barrier (BBB) under normal circumstances, brain cholesterol is synthesized locally [62]. Because the major function of SREBPs is to drive cholesterol synthesis, several groups of investigators have examined roles of Scap and SREBP-2 in supporting the cholesterol-rich CNS.

First, a loss of function of Scap in the brain was accomplished using *Nestin-Cre* (*Nestin-Scap*^{-/-} mice) [64]. Homozygous *Nestin-Scap*^{-/-} mice died in the perinatal period. They exhibited microcephaly and reduced brain cholesterol levels. Histological analysis of the brain revealed gliosis, a nonspecific pattern of glial cell hyperplasia associated with tissue injury. Heterozygous *Nestin-Scap*^{+/-} mice survived postnatally and had grossly normal brain size. However, heterozygotes exhibited numerous abnormalities with reduced brain sterol synthesis, motor deficits, behavioral dysfunction, and impaired synaptic transmission.

To study Scap function selectively in astrocytes, a subtype of glial cells that are a major site of cholesterol synthesis in the CNS [63], *GFAP-Cre* was used (*GFAP-Scap*^{-/-} mice) [65, 66]. Astrocyte Scap deficiency led to reduced expression of cholesterol and FA biosynthetic genes and reduced secretion of cholesterol and phosphatidylcholine from cultured astrocytes. *GFAP-Scap*^{-/-} mice also exhibited numerous abnormalities with premature mortality, microcephaly, abnormal synapse structure, and motor deficits. Surprisingly, the motor defects were corrected by feeding a high-fat diet (HFD). Essential FAs and plant sterols accumulated in the brains of HFD-fed *GFAP-Scap*^{-/-} mice, suggesting that the correction of neurological deficits was attributable to transfer of dietary lipids through the BBB.

Similar findings were noted in a study of SREBP-2 in astrocytes, also using *GFAP-Cre* (*GFAP-Srebp-2*^{-/-} mice) [67]. *GFAP-Srebp-2*^{-/-} mice displayed microcephaly, a small reduction in cortical cholesterol content, and multiple neurological deficits. Unexpectedly, these mice also displayed increased energy expenditure, decreased adiposity, and a shift in metabolism toward carbohydrate oxidation. This data confirms that SREBP-2 is important for cholesterol synthesis in astrocytes, which in turn is required for normal brain development. The mechanisms by which astrocyte SREBP-2 contributes to systemic metabolic regulation are difficult to pinpoint given the numerous abnormalities in the brains of the *GFAP-Srebp-2*^{-/-} mice.

Taken together, these studies on Scap and SREBP-2 in the CNS suggest that reducing cholesterol levels or cholesterol synthesis in the brain alters brain structure, leading to alterations in brain function. In contrast, other investigators have used SREBP-2 to produce excess cholesterol in the brain, which may be detrimental in some circumstances. Cholesterol is thought to accumulate in diseased areas of the brain in patients with Alzheimer's disease (AD), a common form of dementia in which misfolded amyloid- β protein forms toxic aggregates in the brain [68, 69]. In mice with transgenic overexpression of nSREBP-2 in the liver (*Tg-PEPCK-nSREBP-2* mice) [2], brain cholesterol levels were elevated [70]. Since amyloid- β toxicity to neurons is thought to be potentiated by oxidative stress and depletion of reduced glutathione (GSH) [71], brain GSH levels were measured and found to be reduced in *Tg-PEPCK-nSREBP-2* mice. Accordingly, isolated neurons from the transgenic mice were more susceptible to oxidative injury caused by amyloid- β peptide than controls. Lastly, amyloid- β accumulation and neurodegeneration were accelerated in *Tg-PEPCK-nSREBP-2* mice that also bore transgenes driving mutant amyloid precursor protein and presenilin-1 in the brain, a transgenic mouse model of AD [72, 73]. Taken together, these studies suggest, perhaps without surprise, that too much cholesterol, as well as too little, has detrimental effects on the CNS.

5. Other tissues

SREBP pathway genes impact diverse physiological functions in an array of tissues, and the above discussion of the digestive, immune, and nervous systems explores only a minority of the phenotypic abnormalities of mutant and transgenic animals involving SREBP pathway genes (Table 1 and Table 2). In one example, the disruption of both *Insig-1* and *Insig-2* in the epidermis led to alopecia caused by the buildup of toxic cholesterol precursors. Simvastatin,

which blocks synthesis of these precursors, ameliorated their hair abnormalities [74]. In another example, the disruption of Site-1 protease in cartilage led to generalized chondrodysplasia in which type II collagen secretion from chondrocytes is impaired [75-77].

Of tissues for which SREBP activity might be predicted to be important owing to high rates of sterol or FA synthesis, four organs stand out for which SREBP function has yet to be completely elucidated in mammals *in vivo*: adipose tissue, adrenal glands, testes, and ovaries. Early studies on SREBP-1 named it adipocyte determination- and differentiation-dependent factor 1 (ADD1), and demonstrated that SREBP-1 was critical for adipogenesis by driving the production of endogenous lipid ligands for PPAR- γ [5, 78]. Given this role of SREBP-1 in adipogenesis, unexpectedly, transgenic overexpression of nSREBP-1c led to lipodystrophy [79]. In contrast, transgenic overexpression of nSREBP-1a lead to increased FA synthesis in adipose tissue with hypertrophy of white and brown adipocytes [80]. Adiposity in *Srebp-1^{-/-}* and *Srebp-1c^{-/-}* mice is normal [25, 81, 82], although in these models SREBP-2 remains intact and may compensate for the loss of SREBP-1. How a total loss of nuclear SREBPs, as achievable by Scap or Site-1 protease deficiency, affects adipose tissue function remains unknown.

Regarding steroidogenic organs, the contribution of SREBPs to the production of steroid hormones has not yet been explored *in vivo*. In MA-10 Leydig cells, Scap deficiency reduced cyclic AMP-induced steroidogenesis [83]. This effect was attributed to reductions in SREBP-2 activation and reduced cholesterol synthesis. Whether this function is relevant *in vivo* in the testis or can be generalized to other steroidogenic organs, such as the ovary or adrenal gland, has yet to be answered.

A summary of physiologic processes in individual tissues impacted by disruption or overexpression of SREBP pathway genes is shown in Figure 1. The majority of the phenotypic abnormalities in knockout and transgenic mice are the primary or secondary effects of excess or insufficient lipid synthesis, consistent with the major function of SREBPs in controlling lipid synthesis. Excess lipid accumulation and/or lipotoxicity is seen in liver, lung, intestine, pancreas, skin, and brain when nSREBPs are made over-abundant owing to transgenic expression of truncated SREBPs, transgenic expression of a sterol-resistant mutant Scap, or by deletion of Insigs (Tables 1 and 2). Conversely, lower *in vivo* cholesterol and/or FA synthesis is present or implied by reductions in lipid contents or in mRNAs of various lipid biosynthetic genes in liver, lung, intestine, pancreas, brain, peripheral nerves, mammary gland, macrophages, T cells, kidney, and cartilage when SREBP activity is blocked owing to transgenic overexpression of Insig-1 or knockout of SREBP-1, SREBP-2, Scap, or Site-1 protease.

Tables 1 and 2 include a few exceptions to the rule that the phenotypic abnormalities of the described knockout and transgenic mice are caused by altered lipid synthesis. In pancreas, SREBP-1c controls insulin secretion by regulating the expression of granuphilin [84], a component of the exocytosis machinery for insulin granules. In the immune system, SREBP-1a controls macrophage function by regulating the expression of Nlrp1a and Ap16 [20, 56]. Site-1 protease and Site-2 protease activate a number of membrane-bound transcription factors in addition to SREBPs, such as ATF6 and the CREB3 subfamily [85].

As such, causal interpretation of cartilage abnormalities in Site-1 protease-deficient animals is complicated. Although reduced chondrocyte lipid synthesis was noted in Site-1 protease knockouts, a mechanistic link between entrapment of type II procollagen and reduced lipid synthesis is not established [75]. Thus, it is possible that the cartilage abnormalities associated with Site-1 protease deficiency are related to defective proteolysis of other transcription factors or of other molecules aside from SREBPs.

6. Conclusions and Future Directions

Regarding the relationship between SREBP pathway genes and human disease, recent interest has focused on the use of pharmacologic inhibitors of Scap or SREBPs for the treatment of hyperlipidemia and nonalcoholic fatty liver disease [86]. In humans and rodents with hepatic steatosis in the setting of obesity and insulin resistance, hepatic SREBP-1c is upregulated [87-89]. Blocking SREBP-1c by antagonizing Scap improves hepatic steatosis and hypertriglyceridemia in rodents and monkeys [50, 90, 91], and so be might be a therapeutic strategy for these conditions in humans.

SREBP-1c antagonism in the liver could be accomplished either directly by the inhibition of SREBP-1c or indirectly by inhibition of SREBP-2 [30], Scap, Site-1 protease, or Site-2 protease. The studies reviewed herein provide important clues as to which molecule would be the ideal target for pharmacologic inhibition. First, targeting Scap is superior to targeting SREBP-1c inasmuch as the former is more potent in lowering hepatic TGs in obesity-induced fatty liver than the latter [50]. Second, targeting Scap is superior to targeting Site-1 protease, and by extension Site-2 protease, as the latter are likely to produce undesirable off-SREBP effects in many tissues because of the ubiquitous expression of both proteases and since the proteolysis of other transcription factors, like ATF6, will be impacted. Third, partial inhibition of Scap, as has been achieved in rodents and monkey with siRNA-based therapies, has been shown to yield therapeutic benefits [50, 90, 91]. Lastly, partial loss of Scap is seemingly well-tolerated in liver and in extrahepatic tissues with the possible exception of the CNS, since mice with Scap haploinsufficiency in the brain display neuronal dysfunction [64]. However, the safety profile of lipid-lowering agents, including statins, on CNS function has been an area of controversy [92], although recent meta-analyses have not born out this statin safety concern [93]. Altogether, Scap remains a promising therapeutic target. In closing, a huge breadth of physiology has been revealed by the *in vivo* study of SREBP pathway genes since its discovery 24 years ago, with many unanswered questions yet to fill many more years of study.

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Abbreviations

AAV	adeno-associated virus
ACC	acetyl-CoA carboxylase
AD	Alzheimer's disease
ADD1	adipocyte determination- and differentiation-dependent factor 1
APP	amyloid precursor protein
Api6	apoptosis inhibitor 6
ApoE	apolipoprotein E
ATF6	activating transcription factor 6
bHLH-Zip	basic-helix-loop-helix-leucine zipper
BBB	blood-brain barrier
BMDMs	bone-marrow derived macrophages
CE	cholesteryl ester
CNS	central nervous system
CREB3	cAMP responsive element-binding protein 3
DHA	docosahexaenoic acid
DEN	diethylnitrosamine
DIO	diet-induced obesity
DKO	double knockout
DSS	dextran sulfate sodium
ENU	<i>n</i> -ethyl- <i>n</i> -nitrosourea

EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
FA	fatty acid
FAO	fatty acid oxidation
GFAP	glial fibrillary acidic protein
GIRK1	G-protein coupled inward rectifying K ⁺ channel
GPCR	G-protein-coupled receptor
GSH	glutathione
HFD	high-fat diet
HMGR	HMG-CoA reductase
HSV-1	herpes simplex virus-1
IFNβ	interferon β
Ihh	indian hedgehog
IRF3	interferon regulatory factor 3
IRF7	interferon regulatory factor 7
IL-1α	interleukin-1 α
IL-1β	interleukin-1 β
ISG	interferon-stimulated gene
KLA	Kdo2-Lipid-A
LCMV	lymphocytic choriomeningitis virus
LDL	low-density lipoprotein
LDLR	low-Density lipoprotein receptor
lop13	lens opacity 13
LPS	lipopolysaccharide
LXR	liver X receptor
Mbtps1	membrane-bound transcription factor peptidase, site 1
MHV-68	murine gammaherpesvirus-68
NLRP1a	NLR family, pyrin domain containing 1a
NPC1L1	Niemann-Pick C1-Like 1

nSREBP	nuclear SREBP
PC	phosphatidylcholine
PEPCK	phosphoenolpyruvate carboxykinase
PL	phospholipid
PNS	peripheral nervous system
PPAR-γ	peroxisome proliferator activated receptor- γ
PS1	presenilin1
pSREBP	precursor SREBP
PTHr-P	parathyroid hormone related peptide-receptor
rtTA	reverse tetracycline-controlled transactivator
SCD	stearoyl-CoA desaturase
Shh	sonic hedgehog
siRNA	small interfering RNA
SPC	surfactant protein C, SRE, sterol regulatory element, SREBP, sterol regulatory element-binding protein
SSD	sterol-sensing domain
STING	stimulator of interferon genes
tetO	tetracycline operator
TG	triglyceride
TLR4	Toll-like receptor 4
UPR	unfolded protein response

Highlights

- SREBPs are membrane-bound transcription factors that control lipid biosynthesis.
- Physiological functions of SREBP pathway genes have been extensively studied in mice.
- Scap sustains cell division in intestine and in T cells by driving lipid synthesis.
- SREBP-1, SREBP-2, and Scap play varied roles in innate and adaptive immunity.
- SREBPs provide cholesterol synthesis essential for brain development and function.

Figure 1.
Physiological processes affected by genetic manipulation of SREBP pathway genes in mice.

Table 1

A. Phenotypic comparison of knockout & mutant mice in SREBP pathway genes I				
Tissue	Cre line	PN* Viability	Phenotypic summary [#]	Ref.
Mutation desc.				
SREBP-1 (<i>Srebf1</i>)				
Whole-body (Isoform <i>1a</i> & <i>1c</i>)	na	~30% survival	General: ~70% of null embryos expire at E11.	[25,81,94]
			Liver: Reduced FA synthesis. Increase in SREBP-2. Hepatic TGs reduced in obesity but insulin sensitivity and adiposity unchanged.	
			Heart: Decreased expression of GIRK1 (K ⁺ channel) in atria causing susceptibility to ventricular tachyarrhythmia.	[95]
			Pancreas: Enhanced insulin secretion with palmitate-induced lipotoxicity in cultured pancreatic islets owing to reduced expression of granulophilin, an inhibitor of insulin secretion. In other studies, reduced glucose-induced insulin secretion and reduced TG levels in cultured pancreatic islets.	[84, 96, 97]
			Immune system: KLA-stimulation of macrophages produces a delayed peak of unsaturated FA synthesis that is blocked and associated with a hyper-inflammatory state in SREBP-1 nulls. The hyper-inflammatory response is blocked by treatment with unsaturated FAs.	[57]
			Kidney: Amelioration of diabetic proteinuria and glomerular injury.	[98]
Whole-body (Isoform <i>1c</i>)	na	Yes	General: Selective deletion of isoform 1c only. Normal survival.	[50, 82, 99-101]
			Liver: Reduced FA synthesis. Increase in SREBP-2. Hepatic TGs reduced in obesity but insulin sensitivity and adiposity unchanged. Hepatic TGs reduced in chronic ethanol feeding. Insulin- & LXR-induced FA synthesis blocked. Reduced postprandial Cry1 expression.	
			Mammary epithelium: Minor reduction in milk fat content but normal growth of pups nursed by SREBP-1c null dams.	[102]
			Kidney: Protection from obesity-related renal lipid accumulation and glomerulosclerosis.	[103]
			PNS: Hypermyelination of small nerve fibers, impaired nerve function. Reduced nerve FA synthesis. Neuropathy prevented by PPAR-α antagonist.	[104, 105]
Whole-body (Isoform <i>1a</i> hypomorph)	na	Yes	Protection from sepsis induced by LPS injection, cecal puncture. Reduced macrophage Nlrp1a expression. Protection of macrophages from bacterial toxin-induced apoptosis in vitro. Reduced hepatic ACC-2 and fasting TGs.	[20, 53, 56]
SREBP-2 (<i>Srebf2</i>)				
Whole-body (null)	na	No	Null embryos expire at E11.5-E14.5.	[25]

A. Phenotypic comparison of knockout & mutant mice in SREBP pathway genes I				
Tissue	Cre line	PN* Viability	Phenotypic summary [#]	Ref.
Mutation desc.				
Whole-body (gene trap)	na	Varies	Complex gene trap allele either null or hypomorphic for SREBP-2. Hypomorphs survive and have reduced hepatic sterol and FA synthesis. Near-complete lethality in nulls by E14.5, abnormal limb development.	[18]
Whole-body (<i>lop13</i> mutant)	na	Yes	<i>lop13</i> mutant mice (R1038C substitution) with cataracts, reduced ocular lens cholesterol, poor wound healing.	[106]
Liver	<i>Albumin-Cre</i>	Yes	Reduced hepatic cholesterol and FA synthesis. Loss of oxysterol agonists derived from flux through sterol biosynthetic pathway reduces LXR activation to reduce SREBP-1c expression.	[30]
CNS (Astrocyte)	<i>GFAP-Cre</i>	Yes	Microcephaly, impaired brain development, behavioral and motor defects. Increased whole-body carbohydrate metabolism.	[67]
Scap (<i>Scap</i>)				
Liver	<i>Mx1-Cre</i>	na	IFN-induced disruption. Reduced hepatic nuclear SREBP-1 and SREBP-2, cholesterol and FA synthesis, plasma and liver lipids. Compensatory increase in FA synthesis in adipose via SREBP-1c.	[49, 107, 108]
	<i>Albumin-Cre</i>	Yes	Near-normalization of hepatic TG content, elimination of hepatic steatosis in the setting of obesity (<i>ob/ob</i> and DIO models), without improvement in obesity or insulin sensitivity. Resistance to DEN-induced liver cancer.	[50, 109]
	na	na	AAV8-Cre-mediation deletion. <i>Scap</i> and <i>HDAC3</i> double knockout in liver reduces FA/TG synthesis and reduces FAO, causing severe steatohepatitis.	[110]
Intestine	<i>Villin-Cre-ERT²</i>	na	Reduced intestinal sterol and FA synthesis leading to severe injury to intestinal epithelia, death. Heterozygous knockouts normal (unpublished obs.)	[47]
Whole-body	<i>Ubc-Cre-ERT²</i>	na	Phenocopy of <i>Scap^{fl/fl}</i> ; <i>Villin-Cre-ERT²</i> mice. Lethal small bowel enteropathy	[47]
Mammary epithelium	<i>B-lacto-globulin-Cre</i>	Yes	Reduced in milk fat content and mammary FA synthesis with poor growth of pups nursed by <i>Scap</i> knockout dams.	[102]
PNS (Schwann Cell)	<i>mP0TOTA-Cre</i>	Yes	Hypomyelination and age-reversible gait abnormalities.	[111]

B. Phenotypic comparison of knockout & mutant mice in SREBP pathway genes II.				
Tissue	Cre line	PN* Viability	Phenotypic summary [#]	Ref.
Mutation desc.				
Scap (<i>Scap</i>), cont'd				
CNS (Astrocyte)	<i>GFAP-Cre</i>	Yes	Microcephaly, progressive motor deficits, abnormal synapses, and early mortality in adult mice. Reduced brain cholesterol and oleate. HFD prevents mortality & neurological deficits.	[65, 66]
Pan-nervous system	<i>Nestin-Cre</i>	No	Complete perinatal lethality at P0, decreased brain cholesterol, microcephaly, and gliosis. Heterozygous	[64]

B. Phenotypic comparison of knockout & mutant mice in SREBP pathway genes II.				
Tissue	Cre line	PN* Viability	Phenotypic summary[#]	Ref.
Mutation desc.				
			knockouts with reduced cholesterol synthesis, abnormal cognitive/behavioral functioning.	
Lung (Alv. type II cell)	<i>SPC-rtTA; (tetO)₇-Cre</i>	na	Decreased PL synthesis in type II alveolar cells, increased PL synthesis in lipofibroblasts, overall normal pulmonary surfactant.	[31, 112]
Immune System (T cells)	<i>Cd4-Cre</i>	Yes	Reduced sterol and FA synthesis in activated CD8 ⁺ T cells. Impaired clonal expansion in mitogen-stimulated T cells. Poor CD8 ⁺ response to LCMV.	[52]
Immune System (Macrophages)	<i>LysM-Cre</i>	Yes	Reduced macrophage sterol and FA synthesis. Protection from MHV-68 infection. Enhanced type I interferon response in macrophages.	[58]
	na	na	Retroviral transduction of Cre-ER ^{T2} followed by tamoxifen in BMDMs in vitro reduced macrophage IL-1 β expression and caspase-1 secretion.	[54]
Insig-1 (<i>Insig1</i>)				
Whole-body (null)	na	Yes	Slightly reduced body weight and length, small increase in fat mass percentage, small increase in adipose SREBP-1c, FA synthesis genes.	[113]
Liver	<i>Mx1-Cre</i>	na	Normal parameters of hepatic lipid metabolism.	[114]
Lung (Alv. type II cell)	<i>SPC-rtTA; (tetO)₇-Cre</i>	na	Normal parameters of pulmonary lipid metabolism.	[115]
Insig-2 (<i>Insig2</i>)				
Whole-body (null)	na	Yes	Normal systemic, hepatic, and pulmonary lipid metabolism under basal conditions, defective hypoxia-induced HMGR degradation in the liver.	[32, 114-116]
Insig-1 (<i>flox</i> or null) and Insig-2 (null) Double Knockouts				
Whole-body (Insig-1 and -2 null)	na	No	Complete perinatal lethality. Nearly all with midline facial deformities (cleft palate to cleft face). Defects ameliorated by maternal lovastatin treatment, which normalized elevated levels of embryonic sterol intermediates.	[32]
Liver	<i>Mx1-Cre</i>	na	Fatty liver, marked hepatic cholesterol and TG accumulation, abolished feedback inhibition of dietary cholesterol on hepatic SREBPs and HMGR.	[114]
Skin	<i>Keratin-14-Cre</i>	Survival to ~P42	Defective hair growth and hyperkeratosis of the skin, growth restriction and death as early adults. Topical simvastatin normalized elevated skin sterol intermediates and ameliorated hair and growth defects.	[74]
Intestine	<i>Villin-Cre</i>	Yes	Markedly elevated intestinal sterol synthesis leading to elevated plasma, intestinal and hepatic cholesterol levels. Normal intestinal morphology.	[42]
Lung (Alv. type II cell)	<i>SPC-rtTA; (tetO)₇-Cre</i>	na	Lung lipotoxicity with increased SREBP-1, cholesterol, and TGs in type II	[31, 115]

B. Phenotypic comparison of knockout & mutant mice in SREBP pathway genes II.				
Tissue	Cre line	PN* Viability	Phenotypic summary [#]	Ref.
Mutation desc.				
			cells. Lung inflammation, remodeling. Pulmonary surfactant PC reduced.	
Site-1 Protease (<i>Mbtps1</i>)				
Whole-body (null)	na	No	Null embryos expire prior to implantation with a failure to form an epiblast layer, between E4 to E5.	[23, 24]
Liver	<i>Mx1-Cre</i>	na	Reduced hepatic nuclear SREBP-1 and SREBP-2, cholesterol and FA synthesis, LDLR clearance, and plasma cholesterol.	[23]
Cartilage	<i>Col2A1-Cre</i>	No	Complete perinatal lethality. Chondrodysplasia and a lack of endochondral bone formation. Cartilage lacks type II collagen, trapping of type IIB procollagen in chondrocyte ER. Heterozygous knockouts are normal.	[75, 77]
Cartilage	<i>Col2A1-CreER^{T2}</i>	na	Chondrodysplasia with disruption of chondrocyte differentiation in young mice, absent <i>lhh</i> and PTHR-P expression in growth plates.	[76]
Bone (Osteocyte)	<i>Dmp1-Cre</i>	Yes	Normal long bone density and morphology with increased in bone stiffness. Hypertrophy & increased strength of skeletal muscle (soleus).	[117]
Bone, tendon, skin	<i>Col1A1-Cre</i>	Survival to ~P10	Decreased vertebral number, vertebral fusion and kinky tail mimicking the human condition of caudal regression syndrome.	[16]
Whole-body (<i>woodrat</i> mutant)	na	~40% survival	<i>woodrat</i> mutation (Y496C substitution) by ENU mutagenesis. Partial survival of pups, and complete maternal-zygotic embryonic lethality before E11. Coat hypopigmentation, sensitivity to DSS-induced colitis, reduced markers of the ER stress. Resistance to LCV infection through dendritic cell mechanism.	[118-120]

* Postnatal viability not assessed (na) in models using postnatal or inducible Cre activation.

[#] Phenotypes described are of homozygous mutant mice unless otherwise specified.

Table 2
Phenotypic comparison of transgenic models involving SREBP pathway genes

Gene	Tissue	Transgene	Phenotypic summary	Ref.
SREBP-1	Adipose	<i>Tg-aP2-nSREBP-1a</i>	Adipocyte hypertrophy with increased FA synthesis in adipose. Increased release of FAs from adipocytes causes fatty liver.	[80]
		<i>Tg-aP2-nSREBP-1c</i>	Generalized lipodystrophy with type 2 diabetes, fatty liver. Leptin corrects metabolic derangements. Systemic inflammatory state is present with infiltrating macrophages in adipose.	[79, 121-123]
	Liver (& Kidney)	<i>Tg-PEPCK-nSREBP-1a</i>	Liver: Markedly fatty liver with increased hepatic synthesis of cholesterol and FAs. Mild peripheral insulin resistance. Impaired liver regeneration after partial hepatectomy associated with increased expression of cell cycle inhibitor p21.	[124-126]
			Kidney: Minor transgene expression in kidney associated with glomerulosclerosis, proteinuria, and increased kidney TG content.	[127]
		<i>Tg-PEPCK-nSREBP-1c</i>	Liver: Mildly fatty liver with increased hepatic FA synthesis. No increase in cholesterol synthesis. With high-fat/high-fructose diet, metabolic syndrome develops and is ameliorated by eplerenone.	[3, 128]
			Kidney: Minor transgene expression in kidney associated with glomerulopathy.	[98]
	Liver	<i>Tg-alb-nSREBP-1a</i>	Fatty liver, increased adiposity and hyperinsulinemia, insulin resistance. Metabolic abnormalities normalized with mutant nSREBP-1a transgene (S63A/S117A/T426V) lacking MAPK phosphorylation sites.	[129]
		<i>Tg-alb-nSREBP-1c</i>	Mildly fatty liver, increased expression of FA biosynthetic genes, accumulation of monounsaturated FA in liver. Increased visceral adiposity and hyperinsulinemia, insulin resistance.	[130]
	Pancreas (β Cell)	<i>Tg-RIP-nSREBP-1a</i>	Low levels of transgene SREBP-1a expression result in reduced β -cell mass, impaired insulin secretion, glucose intolerance.	[131]
		<i>Tg-RIP-nSREBP-1c</i>	Lipotoxicity in pancreatic islets with TG accumulation, reduced β -cell mass, impaired insulin secretion, glucose intolerance.	[132]
SREBP-2	Liver	<i>Tg-PEPCK-nSREBP-2</i>	Liver: Mildly fatty liver, increased synthesis of cholesterol > FA.	[2]
			Brain: Cholesterol accumulation in the brain, oxidative stress indicated by reduced mitochondrial glutathione.	[70]
		<i>Tg-PEPCK-nSREBP-2; Tg-APP; Tg-PS1</i>	Brain: Triple transgenic mice also bearing transgenes for mutant APP and PS1 (Alzheimer's disease model). Neuronal dysfunction with inflammation, oxidative stress, and Tau pathology observed.	[72, 73]
	Pancreas (β -cell)	<i>Tg-RIP-nSREBP-2</i>	Severe lipotoxicity in pancreatic islets with CE accumulation, reduced β -cell mass, impaired insulin secretion, frank diabetes.	[133]
	Intestine	<i>Tg-villin-nSREBP-2</i>	Increased jejunal and hepatic cholesterol and TG, increased plasma cholesterol, normal intestinal morphology.	[134]

Gene	Tissue	Transgene	Phenotypic summary	Ref.
Scap	Liver	<i>Tg-PEPCK-Scap(D443N)</i>	Sterol-resistant Scap D443N substitution. Fatty liver with increased hepatic synthesis of cholesterol and FAs.	[135]
Insig-1	Liver	<i>Tg-ApoE-Insig1</i>	Enhanced inhibition of SREBP proteolysis by dietary cholesterol leading to reduced hepatic FA and cholesterol synthesis, blunted insulin-stimulated lipogenesis.	[136]