Review

SREBP: a novel therapeutic target

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Sterol regulatory element-binding proteins (SREBPs) are major transcription factors regulating the biosynthesis of cholesterol, fatty acid, and triglyceride. They control the expression of crucial genes involved in lipogenesis and uptake. In this review, we summarize the processing of SREBPs and their regulation by insulin, cAMP, and vitamin A, and the relationship between miRNA and lipid metabolism. We also discuss the recent functional studies on SREBPs. These discoveries suggest that inhibition of SREBP can be a novel strategy to treat metabolic diseases, such as type II diabetes, insulin resistance, fatty liver, and atherosclerosis.

Keywords: SREBP; insulin; cAMP; vitamin A; miRNA; therapeutic target

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Introduction

In the cell membrane of most vertebrates, cholesterol is an essential structural component. Owing to its amphipathy, cholesterol plays an important role in maintaining the fluidity of the cell membrane. Besides, cholesterol is a precursor of steroid hormones and bile acids synthesis. In contrast, high-level cholesterol in blood can lead to atherosclerosis (AS) [1]. Triglyceride (TG), composed by fatty acids (FAs) and glycerol through esterification, is another important lipid. High-concentration TG and FAs in blood are a chief risk factor for type II diabetes and obesity [2].

In fact, with the industrialization of the society, human dietary structure has been changed greatly. High-cholesterol and high-fatty foods are overeaten. Studies from different areas indicate that many physiologic and pathophysiologic processes are tightly related to lipid metabolism [3].

In mammals, the biosynthesis of cholesterol, FAs, and TG is tightly regulated by a family of transcription factors, called sterol regulatory element-binding proteins (SREBPs) [4]. SREBPs can promote the expression of genes of lipid biosynthetic and lipid uptake [5,6]. For SREBP plays a vital role in synthesizing of lipids, inhibition of SREBPs may be a useful strategy to treat type II diabetes, insulin resistance, fatty liver, and AS.

The Activation Process of SREBPs

SREBPs comprise a subclass of transcription factors of basic helix-loop-helix–leucine zipper (bHLH-LZ) [7,8]. In humans there are two SREBP genes, SREBP-1 and SREBP-2. The SREBP-1 gene can produce two proteins, SREBP-1a and SREBP-1c, derived by different promoters. SREBP-1 is most abundant in the liver and adrenal gland, whereas SREBP-2 is ubiquitously expressed [9].

SREBP-1a, with 1147 amino acids, is 47% identical to SREBP-2 in humans [9]. The SREBPs transcription-activation domain is located at the extreme N-terminus. For SREBP-1c has a shorter N-terminal transcription-activation domain than SREBP-1a, it shows a weaker transcriptional activity [9]. The transcriptional activity of SREBP-2 corresponds to SREBP-1a, on account of the almost same length of the N-terminal domain.

In humans, the SREBP-1c primarily regulates FA metabolism, such as fatty acid synthase (FASN) gene [5,10,11]. SREBP-2 is mainly responsible for cholesterol-related genes, such as HMG-CoA reductase (HMGCR), a rate-limiting enzyme in cholesterol synthesis, and low-density lipoprotein receptor (LDLR) gene [12]. SREBP-1a targets both sides of genes [5,10,11]. However, there are overlapped functions between individual SREBPs [13].

In spite of their distinct roles in lipid metabolism, SREBPs are all synthesized as endoplasmic reticulum (ER) membrane proteins and are activated by proteolytic cleavage in the Golgi through the same processing pathway [9]. After the N-terminal transcription-activation domain is released from membrane by proteolysis, the mature SREBP enters the nucleus to enhance the transcription of its target genes (Fig. 1).

In the proteolytic processing of SREBPs, an ER membrane protein called SREBP cleavage-activating protein (SCAP), with eight transmembrane helices, functions as a cholesterol sensor and transporter. The SREBPs are synthesized as ER transmembrane proteins in their precursor form.
Immediately after synthesis, the pre-SREBPs bind to SCAP. The amino acid sequence, MELADL, located in the cytosolic Loop 6 of SCAP, is required for COPII-coated vesicles binding, which transports the SCAP–SREBP complex from the ER to the Golgi apparatus [14,15]. When the cellular cholesterol level is low, SCAP binds to pre-SREBP, escorts it into COPII vesicles, and enters into the Golgi, where the site 1 proteases (S1P) and site 2 proteases (S2P) cleave the pre-SREBPs (Fig. 1) [16,17]. Thus, the N-terminal of SREBPs (n-SREBPs) enters the nucleus and binds the sterol regulate element (SRE) to promote the expression of lipogenic genes. When cholesterol accumulates in ER membranes and exceeds a sharp threshold of 4%–5% of total lipids, SCAP binds cholesterol that causes it binding to Insig, an ER-resident protein [4]. The binding of Insig prevents the COPII from recognizing the MELADL sequence in SCAP-Loop 6 [18]. The transport of SCAP–SREBP complex to the Golgi is then blocked. As a result, synthesis of cholesterol and FA declines (Fig. 1) [18].

Insig is an ER membrane resident protein with six transmembrane helices. There are two Insig isoforms, Insig-1 and Insig-2 [19,20]. Insig-1 is composed of 277 amino acids [21], and Insig-2 contains 225 amino acids in humans [22,23]. In animal cells, Insigs have two important functions: (i) causing the sterol-regulated-ER retention of the SCAP–SREBP complex that has just been described; (ii) mediating the sterol-dependent degradation of HMGCR that will be discussed later.

Insig regulates HMGCR degradation through a sterol-responsive feedback inhibition system [24]. In sterol-replete conditions, the NH2-terminal region of HMGCR binds to Insigs, which recruits a ubiquitin E3 ligase gp78. gp78 interacts with Insig by its NH2-terminal domain [25,26]. gp78 conjugates ubiquitin to HMGCR on lysine 89 and lysine 248 [27,28]. gp78 can also interact with p97/VCP, a hexameric ATPase [4,28,29] which works by making HMGCR accessible to the proteasome [30]. After being ubiquitinated by gp78, HMGCR is extracted from the membrane and degraded [24,26]. When cellular cholesterol is low, Insig dissociates from HMGCR, thus stabilizing HMGCR by preventing ubiquitination.

The Effects of Insulin on SREBPs

Generally, the insulin-signaling pathway starts at receptor-mediated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and/or IRS-2 [31]. IRS then activates phosphatidylinositol 3'-kinase (PI3K) (Fig. 2). PI3K targets and converts the membrane phospholipid phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [32]. PIP3 can interact with the Akt, recruiting Akt to membrane where it can be activated [33]. Akt is an important serine/threonine kinase whose functions are related to cell proliferation and survival, cell size, angiogenesis, metabolism and migration [32,33].

It is well known that insulin can potently induce de novo lipogenesis and regulate the activity of lipogenic enzymes [34]. Accumulating evidences have shown that insulin regulates SREBPs at multiple levels, including transcription of SREBPs mRNA, the proteolytic processing of SREBPs, and the stability and abundance of n-SREBPs.

Regulation of SREBPs mRNA

As a target gene of itself, SREBP mRNA can be induced by n-SREBP. In addition, when exposed to insulin, Srebp-1c mRNA is robustly elevated in mouse and rat livers [35]. When fasting, following the suppression of insulin level, the transcription of the Srebp-1c in the livers of mice is reduced [36]. In freshly isolated rat hepatocytes, insulin can induce the Srebp-1c mRNA by as much as 40-fold within 6 h [31,37]. Similarly, the Srebp-1c mRNA level falls when rats are treated with streptozotocin, which abolishes insulin secretion [38]. The levels of mRNA for
SREBP-1c target genes parallel the changes in SREBP-1c expression [10]. These results indicate that insulin can strongly induce Srebp-1c transcription.

This enhanced transcription is partly through PI3K/Akt-mTORC1 (mammalian target of rapamycin complex 1). Studies have shown that insulin can activate mTORC1 through Akt-mediated phosphorylation of tuberous sclerosis complex 2 (TSC2), thereby that inhibits TSC1/2 function [39]. TSC1/2 can suppress Rheb, which phosphorylates and activates mTOR [40,41].

In the presence of wortmannin, a PI3K inhibitor, the increase of Srebp-1c mRNA induced by insulin is blocked [35]. The active form of Akt can enhance the expression of SREBP-1 and its target genes. Most importantly, this increase of SREBP-1 is blocked by low concentrations of rapamycin (an mTORC1 inhibitor), indicating that the mTORC1 is required [35]. Porstmann et al. [42] have also shown that the elevatory effect of Akt on Srebp-1c mRNA is abscissed by RNAi-mediated knockdown of mTOR and raptor, two components of mTORC1. Together, these studies indicate that insulin enhances the transcription of Srebp-1c mRNA mainly through mTORC1 (Fig. 2).

Regulation of the proteolytic processing
Besides regulation of SREBP1c mRNA, insulin has been found to influence the proteolytic processing of SREBP through two different ways: (i) decreasing Insig-2 expression; (ii) promoting mTORC1-induced p70 S6-kinase (S6K) function (Fig. 2) [35].

In vivo, the levels of Insigs are correlated with insulin. When fasting (low insulin level), the mice exhibit an elevated transcription and translation of Insig-2. Then, the SREBP-1 transport is blocked and n-SREBP-1c cannot be produced in the liver, since Insig-2 retains the SCAP/SREBP complex in ER [43]. As a target gene of SREBP1, Insig-1 mRNA and protein levels are also reduced. When refeeding (high insulin level), the Insig-2 transcription is repressed. Insig-2 is rapidly degraded by gp78-mediated ubiquitination [44], releasing the SCAP–SREBP complex to transport to the Golgi. At the same time, n-SREBP-1c activates the Insig-1 gene, and Insig-1 mRNA and protein levels are restored [43]. These results reveal that insulin can enhance the SREBP proteolytic processing through regulating Insig-2 expression (Fig. 2).

Recently, Owen et al. [35] generated a line of transgenic rats that produce epitope-tagged SREBP-1c driven by a human APOE promoter/enhancer expression cassette. This system rules out the insulin’s regulation at the transcription level. The amount of endogenous Srebp-1c mRNA can be increased by 14-fold in the transgenic rats in the absence of inhibitors. When adding rapamycin, this increase can be blocked by 85%. Interestingly, when inhibiting S6K, one of the major downstream targets of mTORC1, by LYS6K2, the increase is not blocked [35]. Similarly, at the protein level, insulin can increase transgene-encoded n-SREBP-1c by 12-fold within 6 h. When adding LYS6K2, the increase of n-SREBP-1c can be reduced by 74%. But rapamycin blocks this increase only by 64%. In consideration of no effect in Srebp-1c mRNA, the LYS6K2 inhibition is special for protein processing and not for mRNA induction [35]. Taken together, insulin enhances SREBP processing through PI3K/Akt-mTORC1 to activate S6K (Fig. 2), but it is unknown as to how S6K enhances this processing.

It has been reported that insulin treatment can cause the phosphorylation of the ER-bound pre-SREBP-1c, which increases the affinity of the SCAP–SREBP-1c complex to the Sec23/24 proteins of the COPII vesicles [34]. Thus, the SREBP processing is accelerated. It indicates that insulin increases the phosphorylation of full-length SREBP-1c, and the increased phosphorylation partly through serine phosphorylation. Moreover, in the insulin-treated hepatocytes, pre-SREBP-1c not only increases serine phosphorylation, but also enriches in phosphothreonine [34]. These results indicate that insulin enhances both serine and threonine phosphorylation on pre-SREBP-1c [34]. This effect of insulin has been proved tightly linked to PI3K and PKB/Akt pathway [34].

It is known that S6K belongs to a family of serine/threonine kinases. And as a major substrate of PI3K and PKB/Akt, the activated S6K can enhance SREBP processing [35]. S6K may be the candidate kinase for insulin-induced phosphorylation of pre-SREBP.

Regulation of n-SREBP
In addition to regulation of SREBPs mRNA and proteolytic processing, insulin can regulate the stability and abundance of n-SREBPs. Studies have identified that insulin pathway has a bifurcation on Akt. One branch is to regulate SREBPs through activating mTORC1. Another branch is to prevent n-SREBP degradation through GSK3-Fbw7 pathway. Akt can phosphorylate glycogen synthase kinase 3 (GSK3) at its Ser-9, which inhibits glycogen synthase [32]. When n-SREBP-1 binds to DNA, this change recruits GSK3 [45], which phosphorylates n-SREBP-1 at Ser-434 [46]. In turn, two other sites (Ser-430 and Thr-426) of n-SREBP-1 are phosphorylated by 85%. Interestingly, when inhibiting S6K, one of the major downstream targets of mTORC1, by LYS6K2, the increase is not blocked [35]. Similarly, at the protein level, insulin can increase transgene-encoded n-SREBP-1c by 12-fold within 6 h. When adding LYS6K2, the increase of n-SREBP-1c can be reduced by 74%. But rapamycin blocks this increase only by 64%. In consideration of no effect in Srebp-1c mRNA, the LYS6K2 inhibition is special for protein processing and not for mRNA induction [35]. Taken together, insulin enhances SREBP processing through PI3K/Akt-mTORC1 to activate S6K (Fig. 2), but it is unknown as to how S6K enhances this processing.

As a downstream branch of Akt, mTORC1 controls SREBP-1c by regulating its transcription and processing which has just been described. Recently, Peterson et al.
[49] have shown that mTORC1 elevates n-SREBP abundance through a mechanism that requires Lipin-1, a phosphatidic acid phosphatase. The nuclear accumulation of Lipin-1 can repress SREBP-mediated transcription. mTORC1 can directly phosphateylate Lipin-1 on many residues, which promotes Lipin-1 nuclear exclusion and its cytoplasmic accumulation. Thus, the repression of Lipin-1 on SREBP is released (Fig. 2) [49]. The mechanism by which mTORC1/Lipin-1 regulates n-SREBP levels remains unknown. They suggest lamin A might be involved in this process.

In conclusion, Akt activates SREBP through at least two mechanisms: (i) enhancing the stability of n-SREBPs via inhibition of GSK3; (ii) increasing SREBP through activating mTORC1. The effects of mTORC1 on SREBP can be divided into three levels: (i) transcription level, mTORC1 can elevate SREBP-1c mRNA; (ii) proteolytic processing level, mTORC1 can enhance proteolytic processing through activating S6K; (iii) n-SREBP level, mTORC1 can repress Lipin-1 to increase n-SREBP abundance.

**Cross-talk between liver X receptor and insulin pathways**

Liver X receptor (LXR) is another important sterol-regulated transcription factor. There are two subtypes of LXRα and LXRβ. LXR forms heterodimers with retinoid X receptor (RXR) and regulates SREBP pathway at both the transcription and the proteolytic processing level.

LXR can promote SREBP at the transcription level, mainly because there are two LXR-responsive elements (LXREs) within the SREBP-1c gene promoter [50]. Whereas, there is only a single LXRE in the promotor of other LXR target genes, such as ATP-binding cassette subfamily A (ABCA1), cholesteryl ester transfer protein, plasma (CETP), and cytochrome P450 family 7 subfamily a (CYP7A) [51–53], suggesting a strong response of the SREBP-1c to LXR and RXR agonists. Actually, LXR or RXR agonists can markedly increase transcription of SREBP-1c and induction of FA synthesis even under conditions of sterol overload [43]. In contrast, polyunsaturated fatty acids inhibit LXRα/RXRα heterodimer binding to the LXREs in SREBP-1c promoter, as a result, SREBP-1c mRNA and lipogenesis decrease (Fig. 2) [54].

The native activators of LXR are oxysterols, such as 24, 25-exopycholesterol, and 25-hydroxycholesterol (25-HC) [55]. Therefore, oxysterols, including 24-HC, 25-HC, and 27-HC, are well-known inhibitors of SREBP processing, but they may not be clinically used to treat hyperlipidemia, since they also activate LXRα [55]. Recently, Zhang et al. [56] reported that synthetic LXR agonists elicited antiatherogenic activity in the absence of hepatic LXRo. The reason may be that the LXR target gene SREBP1 is mainly expressed in the liver. Liver-specific deletion of LXRα can dramatically decrease the expression of SREBP1 in the liver, and then attenuate the FA synthesis. But in the presence of LXR in the liver, the clinical use of LXR agonists is limited.

Intriguingly, although it can strongly activate SREBP-1c expression and elevate pre-SREBP-1c, LXR is insufficient to increase n-SREBP-1c abundance and enhance target gene expression [57,58]. It is implied that the function of increasing SREBP-1c mRNA by LXR is blocked because of the limited proteolytic processing. Studies confirm this notion showing that LXR can regulate Insig-2 expression. In contrast to insulin, LXR can elevate Insig-2 mRNA and protein, subsequent retention of SREBP-1c in the ER. In the presence of insulin, which down-regulates Insig-2, LXR agonist strongly induces transport of SCAP–SREBP complex to Golgi, thereby enhancing lipogenesis (Fig. 2). The cross-talking between LXR and insulin on regulating the Insig-2 expression indicates that a protect mechanism prevents lipid overabundance.

Finally, it has been reported that insulin can significantly up-regulate LXR expression [59]. Deletion of LXR in mice markedly suppresses insulin-mediated induction of an entire class of enzymes involved in both FAs and cholesterol metabolism [60]. So, LXR cross-talks with insulin play an important role in regulating both cholesterol and FA metabolism.

In conclusion, insulin regulates SREBP at multiple levels. The proteins involve mTORC1, GSK3, Insig-2, and LXR. The interaction and cross-talking among these factors are complicated. Further studies are required to understand the mechanisms in more detail.

**The Effect of cAMP/PKA and Vitamin A on SREBP**

**The effect of cAMP/PKA on SREBP**

Cyclic adenosine 3′,5′-monophosphate (cAMP), rose by glucagon, adrenalin, and other reagents, links a number of extracellular signals to a variety of cellular functions. The cAMP-dependent kinase, protein kinase A (PKA), has been shown to be involved in lipid metabolism. Under physiological conditions, hepatic lipogenic enzymes, such as FASN, stearoyl-CoA desaturase (SCD), and glycerol-3-phosphate acyltransferase (GPAT), are negatively regulated by the elevated intracellular level of cAMP [61].

A recent study has shown that PKA suppresses SREBP-1c by modulating the activity of LXR [62]. PKA phosphorylation of LXR impairs DNA-binding activity by preventing LXR/RXR dimerization, and then attenuates the transcription of SREBP1. Lu et al. found that the Ser338 located at the NH2-terminus of SREBP-1a is also a PKA phosphorylation site [63]. PKA phosphorylation of this site attenuates DNA occupancy. Ser314 of SREBP-1c, the
counterpart of SREBP-1a Ser338, is also phosphorylated by PKA. These findings indicate that the cAMP-PKA pathway decreases transactivation of SREBP-1 through phosphorylating SREBP-1 [63]. As a result, the expression of SREBP-1-targeted genes is reduced. These results suggest that nutritional levels regulate SREBP-1 and lipogenesis partially through modulation of LXR and SREBP1 activity by cAMP-PKA pathway.

**The effect of vitamin A on SREBP**

As an essential and lipophilic micronutrient, vitamin A (VA, retinol) plays a crucial role in the general health of an individual, such as vision, growth, and immune responses. VA is metabolized to retinoic acid (RA) for regulating gene expression. RA exists in multiple isomeric forms, such as all-trans RA and 9-cis RA [64]. It regulates gene expression through the activation of two families of nuclear receptors, RA receptors activated by all-trans RA and RXRs activated only by 9-cis RA [65,66].

In primary hepatocytes, retinal and RA synergizes with insulin to induce Srebp-1c expression. This induction is followed by the elevation of its target gene, FASN [67]. Over-expression of a dominant-negative form of SREBP-1c diminishes the RA-dependent increase in promoter activity [68]. Evidences show that retinoids regulate hepatic Srebp-1c expression through activation of RXR [67]. Retinoic acid receptor elements in its promoter have been identified [67]. The RA induces Srebp-1c expression, indicating the roles of vitamin A in regulation of lipogenesis gene expression.

**The Regulation of miRNA in Lipid Metabolism**

In addition to the classic transcriptional regulation of lipid metabolism (e.g. by SREBP and LXR), micro-RNAs (miRNAs) have been identified to be potent regulators of lipid metabolism at the post-transcriptional level [12].

miRNA belongs to a large family of small non-coding RNA and is single stranded in the mature form. miRNA plays an important role in regulating gene expression at the post-transcriptional level in metazoan animals, plants, and protozoa. miRNAs have wide biological functions in development, differentiation, metabolism, growth, proliferation, and apoptosis [12].

Recently, several miRNAs have been implicated in regulating lipid metabolism. Those miRNAs include miR33, miR-122, miR-370, miR-335, miR-378/378, miR-27, and miR-125a-5p [69].

**miR-33**

In humans, miR-33a is an intronic miRNA located within intron 16 of SREBP-2 gene. Another form of miR-33 named miR-33b locates within SREBP-1 [69]. miR-33a is co-transcribed along with SREBP-2 in both hepatocytes and macrophages. When cellular cholesterol depleted, the expression of miR-33a and SREBP-2 is comparable in many tissues [69]. miR-33 has three major functions: (i) preventing the intracellular cholesterol export; (ii) reducing the high-density lipoprotein (HDL) formation; (iii) crippling FA β-oxidation (Fig. 3).

The target genes of miR-33a are involved in cholesterol trafficking, including ATP-binding cassette, sub-family A member 1 (ABCA1), ATP-binding cassette, sub-family G (WHITE), member 1 (ABCG1) and Niemann-Pick disease, type C1 (NPC1) [12]. The first identified target gene of miR-33 is ABCA1. ABCA1 is responsible for the transport of cholesterol out of cell. The 3’UTR of ABCA1 contains three highly conserved binding sites for miR-33a and/or miR-33b [70,71]. Indeed, miR-33a strongly represses the expression of ABCA1 in a variety of cell types [70,71]. In contrast, inhibition of endogenous miR-33 results in an increased expression of ABCA1, and a concomitant increase in cholesterol efflux to apoA1, which is the first step in the generation of nascent HDL particles [70,71]. Taken together, these results demonstrate that ABCA1 is under the post-transcriptional control of miR-33 [43]. In addition to ABCA1, ABCG1 and NPC1 are also targets of miR-33. ABCG1, which contains two miR-33 binding sites in the 3’UTR in the mouse genome, mobilizes cellular-free cholesterol to mature HDL particles for efflux [71]. In humans, there are two miR-33 binding sites in the 3’UTR of NPC1 [70]. Through NPC1, cholesterol transports from lysosomal compartments to other parts of the cell in need. These results indicate that miR-33 controls cholesterol transport through the coordinated regulation of ABCA1, NPC1, and ABCG1 (Fig. 3) [69].

In addition, several genes regulating the FA β-oxidation have miR-33 binding sites in their 3’UTR,
such as carnitine palmitoyltransferase 1a (Cpt1α), carnitine O-octanoyltransferase (Crot), and hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase, beta subunit (Hadhb) [72]. Each of these genes plays a distinct role in the FA oxidation pathway (Fig. 3). Overexpression of miR-33 leads to CPT1α expression decreases, and cellular FA β-oxidation reduces [69]. With regard to the fact of miR-33b differing from miR-33a by only two nucleotides, it is likely that miR-33b has similar capability in reducing β-oxidation. Indeed, miR-33a and miR-33b can similarly target the 3’UTR of ABCA1, Crot, Cpt1α and Hadhb [69].

In conclusion, miR-33 cooperates with SREBPs and plays an important role in maintaining the intracellular cholesterol homeostasis. Anti-miR33 elevates ABCA1 levels and increases HDL level that holds tremendous potential for the treatment and/or prevention of coronary artery disease. Moreover, inhibition of miR-33 increases FA oxidation and reduces the accumulation of fat in the liver [69]. miR-33 shows the potential for therapies to treat a number of metabolic disorders.

miR-122 and miR-370

miR-122 is another micro-RNA regulating cholesterol metabolism. miR-122 accounts for 70% in all miRNAs in the adult liver [73]. Several groups have reported that inhibition of miR-122 in vivo has pronounced effects on cholesterol and FA metabolism [73–76]. In both mice and non-human primates, inhibition of miR-122 in the liver can lead to sustainable decrease in plasma cholesterol level [73–76]. That mice treated with antisense oligo nucleotides (ASO) to miR-122 shows decrease in both the LDL and HDL lipoprotein fractions, and reflects 25%–35% reductions in total cholesterol [75,77].

Although the miR-122 has an obvious function in down-regulating lipid level, no direct target genes of miR-122 have been identified. The molecular mechanisms need to be further investigated. Nevertheless, several genes, including FASN, Acaca acetyl-Coenzyme A carboxylase alpha (ACCA), and Acacb acetyl-Coenzyme A carboxylase beta (ACCB), which are involved in FA synthesis and oxidation, are altered by anti-miR-122 [12]. Non-toxic in primate studies suggests that inhibition of miR-122 might be feasible and potential for the treatment of metabolic syndrome in humans [78].

Another microRNA, miR-370, is reported to have similar effects with miR-122 on lipid metabolism [79]. Whereas, unlike miR-122 that directly promotes changes in lipogenesis, miR-370 is found to up-regulate the expression of miR-122 [79]. In human hepatic cell line HepG2, transfection of miR-370 up-regulates the expression of miR-122, maintains the miR-122 level, and also enhances the expression of SREBP-1c [12]. Furthermore, miR-370 targets the 3’UTR of Cpt1α, and down-regulates the expression of the Cpt1α gene as well as the rate of β oxidation (Fig. 3) [79].

These findings suggest that controlling the expression of miRNAs is likely to be a useful way to modulate lipid metabolism [12]. microRNA therapeutics in the treatment of metabolic diseases will be a promising strategy in the future.

Inhibiting SREBP is a Novel Strategy to Treat Metabolic Diseases

Inhibition of SREBP pathway can reduce lipid biosynthesis, which will lower the risk of metabolic diseases. Therefore, inhibition of SREBP pathway might be a potential approach to treat these diseases [80]. The complicated regulation of SREBP indicates that different strategies can be developed. These methods include stimulating the interaction between SCAP and Insig, increasing Insigs, depleting SCAP, inhibiting the S1P or S2P, miRNAs, and accelerating the degradation of n-SREBPs [80]. Recently, several studies using different ways to inhibit SREBP have demonstrated that inhibition of SREBP can ameliorate metabolic diseases, such as type II diabetes, insulin resistance, fatty liver, and AS (Fig. 4).

Tang et al. [80] used a cell-based reporter assay to screen for compounds that can regulate SRE-containing promoter. They have found a small molecule, betulin, which is abundant in birch bark. Betulin specifically inhibits the maturation of SREBP by inducing the interaction of SCAP and Insig, which leads to the ER-retention of SCAP–SREBP complex. Betulin decreases the biosynthesis of cholesterol and FA. In vivo, betulin ameliorates diet-induced obesity, decreases the lipid contents in serum and tissues, and increases insulin sensitivity. Furthermore, betulin reduces the size and improves the stability of AS plaques. As an SREBP inhibitor, betulin shows a multitude of beneficial effects in vivo. The finding of betulin proves the concept that inhibition of SREBP pathway can be a useful strategy to treat metabolism diseases.

Another study used the strategy of deletion of Scap in the liver. Moon et al. [81] have shown that deletion of
SCAP can abolish hepatic steatosis in insulin-resistant ob/ob mice or in high-fat-diet-fed mice. SCAP deletion can reduce lipid biosynthesis and prevent fatty liver, even in the condition of persistent obesity, hyperinsulinemia, and hyperglycemia [81]. This study demonstrates that activation of SREBP is essential for development of diabetic hepatic steatosis and carbohydrate-induced hypertriglyceridemia, and once again demonstrates that inhibition of SREBP has therapeutic potential.

Recently, Liu et al. [44] have generated liver-specific gp78 knockout (L-gp78−/−) mice. In the L-gp78−/− mice, Insig-1 and Insig-2 increase, which causes the inhibition of SREBP. Although the HMGCR is also higher in L-gp78−/− mice than in WT mice, the inhibition on SREBP is dominant. And the net effect of gp78 deficiency is decreasing lipid biosynthesis [44]. Meanwhile, the expression of FGF21 in gp78-deficient liver increases that activates brown adipocytes and enhances energy expenditure [44]. The L-gp78−/− mice is protected from diet/age-induced obesity, and improves hyperlipidemia and insulin resistance [44]. Thus, inhibition of gp78 may also provide a therapeutic avenue to treat metabolic diseases through suppressing SREBP pathway.

SREBPs play a critical role in lipid metabolism. Is there any potential side effect of inhibiting SREBPs? Statins, the most widely prescribed drugs to treat hypercholesterolemia, are inhibitors of HMGCR resulting in block cholesterol biosynthesis. Meanwhile, inhibition of cholesterol synthesis in the liver stimulates SREBP, and SREBP up-regulates LDLR, which increases LDL uptake and decreases plasma cholesterol level. One potential problem of inhibition of SREBPs is a reduction of LDLR expression, which may cause a rise in plasma LDL levels. In fact, data from several studies show that although the LDLR mRNA level is reduced after inhibition of SREBPs, the level of LDLR protein does not decline and LDL in plasma decreases in mice and hamsters [44,80,81]. LDLR preservation is likely attributable to another SREBP target gene, proprotein convertase subtilisin/kexin type 9 (PCSK9).

PCSK9 belongs to the protease K subfamily, which are proteolytic enzymes that cleave their substrate, producing biologically active molecules [82,83]. The expression of PCSK9 in HepG2 cells is highly dependent on sterols [84]. The minimal promoter region of the PCSK9 gene contains an SRE, which makes the transcription of PCSK9 dependent on sterols [84]. PCSK9 impairs the LDLR pathway by promoting the internalization and degradation of LDLR in the liver and thereby controls the level of LDL cholesterol in plasma [84]. Inhibition of SREBPs reduces the expression of PCSK9 and inducible degrader of the LDLR. This can explain the preservation of LDLR protein although SREBP pathway is inhibited.

Concluding Remarks

Along with pampering high-calorie diet, hyperlipidemia has becoming a worldwide problem. Hyperlipidemia is closely related to metabolic diseases, such as AS and type II diabetes. In vivo, SREBPs are major transcription factors that control the biosynthesis of cholesterol, FA, and TG. So, inhibition of SREBP will be a promising way of treating metabolic diseases. Recently, several studies have proved this notion in different ways but further work is needed.

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