Regulation of the SREBP transcription factors by mTORC1

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Abstract
In recent years several reports have linked mTORC1 (mammalian target of rapamycin complex 1) to lipogenesis via the SREBPs (sterol-regulatory-element-binding proteins). SREBPs regulate the expression of genes encoding enzymes required for fatty acid and cholesterol biosynthesis. Lipid metabolism is perturbed in some diseases and SREBP target genes, such as FASN (fatty acid synthase), have been shown to be up-regulated in some cancers. We have previously shown that mTORC1 plays a role in SREBP activation and Akt/PKB (protein kinase B)-dependent de novo lipogenesis. Our findings suggest that mTORC1 plays a crucial role in the activation of SREBP and that the activation of lipid biosynthesis through the induction of SREBP could be part of a regulatory pathway that co-ordinates protein and lipid biosynthesis during cell growth.

In the present paper, we discuss the increasing amount of data supporting the potential mechanisms of mTORC1-dependent activation of SREBP as well as the implications of this signalling pathway in cancer.

SREBPs (sterol-regulatory-element-binding proteins)
The expression of genes encoding enzymes required for fatty acid and cholesterol biosynthesis is regulated by the SREBPs [1]. SREBPs are members of the bHLH-Zip (basic helix–loop–helix leucine zipper) family of transcription factors and exist in three isoforms: SREBP1a, SREBP1c and SREBP2. The two SREBP1 isoforms (SREBP1a and SREBP1c) arise as a result of alternative transcription start sites within the Srebf1 gene and differ only in their first exons [2]. SREBP1c is the predominant isoform in vivo with high levels of expression in the liver, whereas SREBP1a is expressed at low levels in all tissues and SREBP2 is expressed mainly in the liver and in adipocytes [1]. In addition, the three isoforms exhibit differential transcriptional potency, with SREBP1a being the most transcriptionally active due to the longer acidic transactivation segments in its N-terminus [2]. Studies in genetically manipulated mice have revealed that SREBP1 function differentially in vivo; SREBP1c mainly activates genes involved in fatty acid biosynthesis, whereas SREBP2 preferentially activates genes involved in the cholesterol pathway [3]. SREBP1a appears to activate genes involved in both biosynthetic pathways [3]. A large number of transcriptional targets, including genes not previously linked to cholesterol or fatty acid metabolism, have been identified for the three transcription factors through gene expression microarrays and chromatin-binding studies [3,4].

The activity of SREBPs is regulated by cellular sterol levels. SREBPs are synthesized as inactive precursors and inserted into the ER (endoplasmic reticulum) membrane, where the C-terminal regulatory region interacts with SCAP (SREBP cleavage-activating protein) [5]. Under low-sterol conditions, the SREBP–SCAP complex binds to COPII vesicles and translocates to the Golgi, where SREBP undergoes a two-step proteolytic cleavage process controlled by S1P (site-1 protease) and S2P (site-2 protease) [6]. Upon cleavage, the N-terminal form of SREBP or mSREBP (mature SREBP) translocates to the nucleus where it binds DNA as a homodimer [7]. SREBP’s bind SREs (sterol regulatory elements) and E-boxes within the promoters of their target genes, which include FASN (fatty acid synthase), ACLY (ATP-citrate lyase) and SCD1 (stearoyl-CoA desaturase 1), as well as SREBP itself [3]. Conditions of sterol saturation in the ER membrane induce a conformational change in SCAP, resulting in its interaction with the insulin-induced proteins (INSIG1 and INSIG2) [8,9]. This reduces the affinity of SCAP for COPII vesicles, thereby retaining the SREBP–SCAP complex in the ER and preventing SREBP-mediated transcription [8,9].

Akt/PKB (protein kinase B) and mTORC (mammalian target of rapamycin complex) 1 signalling
Akt is a serine/threonine kinase and plays a vital role in many cellular processes including metabolism, proliferation, cell

Key words: Akt, cancer, lipid biosynthesis, mammalian target of rapamycin complex 1 (mTORC1), phosphorylation, sterol-regulatory-element-binding protein (SREBP).
Abbreviations used: ACLY, ATP-citrate lyase; COPII, coatomer protein II; EGR, epidermal growth factor receptor; eIF4E, eukaryotic initiation factor 4E; eIF4E-BP, eIF4E-binding protein; ER, endoplasmic reticulum; FASN, fatty acid synthase; GBM, glioblastoma multiforme; GSK3β, glycogen synthase kinase 3β; LDLR, low-density-lipoprotein receptor; MEF, mouse embryonic fibroblast; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PISX, phosphoinositide 3-kinase; PRAS40, proline-rich Akt substrate of 40 kDa; rapam, regulatory associated protein of mTOR; SCD1, stearoyl-CoA desaturase 1; S6K, 4E-BP; SREBP, sterol-regulatory-element-binding protein; SREBF1, full-length precursor SREBP; mSREBP, mature SREBP; S6K, S6 kinase; S6K1, target of rapamycin signalling; TSC, tuberous sclerosis complex.

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survival and growth control [10]. Akt is activated downstream of the lipid kinase PI3K (phosphoinositide 3-kinase) in response to growth factors. Aberrant activation of the PI3K/Akt signalling pathway via the constitutive activation of oncogenic Ras or the loss of the PTEN (phosphatase and tensin homologue deleted on chromosome 10) tumour suppressor protein is found in many cancers [11].

PI3K/Akt activation stimulates cell growth, and mTOR (mammalian target of rapamycin) has emerged as a vital downstream target of Akt in growth control [12]. mTOR is a conserved serine/threonine kinase that functions in two distinct complexes that are functionally different: mTORC1 and mTORC2 [12].

The activity of mTORC1 is regulated by a number of upstream signalling inputs and can be blocked by the mTORC1-specific inhibitor, rapamycin. Negative regulators of mTORC1 activity include interaction with PRAS40 (proline-rich Akt substrate of 40 kDa) and phosphorylation by TSC (tuberous sclerosis complex) 2, as well as by AMPK (AMP-activated kinase)-mediated phosphorylation in response to a low cellular energy status (reviewed in [13]). Akt is able to activate mTORC1 by phosphorylating both PRAS40 and TSC2, relieving their inhibitory effects, and activation of mTORC1 by Akt requires amino acids [14,15]. It is therefore considered that mTORC1 is a sensor of cellular nutrient and energy levels.

Activation of mTORC1 by Akt results in the direct phosphorylation of downstream targets that include the ribosomal S6K (S6 kinase) 1 and S6K2 and the eIF4E (eukaryotic initiation factor 4E) and 4E-BP1 and 2 (eIF4E-binding proteins 1 and 2) [10]. Activation of these proteins leads to increased mRNA translation, cell proliferation and cell growth. S6K1 participates in a basal mTORC1-dependent feedback mechanism by phosphorylating and inhibiting the upstream IRS (insulin receptor substrate) [16].

**Regulation of SREBP1 by the Akt-mTORC1 pathway**

An overview of the possible ways in which mTORC1 is regulating SREBP activity is shown in Figure 1. A number of studies have linked SREBP1 with the PI3K/Akt pathway via insulin signalling [17–20]. In addition, it has been shown that mTORC1 is required for de novo lipogenesis in rat livers and cultured hepatocytes [21,22]. We have previously shown that Akt regulates the expression of genes involved in lipogenesis through SREBP1 activation [23], and that mTORC1 is required for Akt-dependent lipogenesis [24]. Furthermore, we demonstrated that nuclear accumulation of mSREBP1 as well as the expression of the SREBP1 target genes FASN and ACLY is blocked by rapamycin. Silencing of the mTORC1-specific component raptor (regulatory associated protein of mTOR) resulted in attenuated expression of FASN and ACLY in response to Akt activation as well as a reduction in Akt-dependent de novo lipogenesis [24]. However, silencing of rictor (rapamycin-insensitive companion of mTOR; the mTORC2-specific component) did not have this effect [24], indicating that regulation of SREBP1 is mediated by mTORC1 and not by mTORC2. In addition, using reporter assays, we have observed that rapamycin reduces the transcriptional activity of SREBP1a (C.A. Lewis, unpublished work). Rapamycin decreases the expression of SREBP1 mRNA in human BJAB B-lymphoma cells and murine CTLL-2 T-lymphocytes [25] and it was recently reported that the insulin-induced increase in SREBP1c mRNA levels in rat livers and cultured hepatocytes requires mTORC1 [22]. However, inhibition of mTORC1 by rapamycin did not affect the accumulation of mSREBP1 protein in HepG2 cells (where the predominant isoform is SREBP1c) [26], possibly indicating that mTORC1-dependent regulation of SREBP processing is isoform specific.

In another study, it was shown that LDLR (low-density lipoprotein receptor)-mediated cholesterol ester accumulation in HepG2 cells in the presence of inflammatory cytokines was inhibited by rapamycin [27]. Furthermore, it was shown that rapamycin decreased mRNA levels of both SREBP2...
and SCAP, as well as preventing SCAP/SREBP ER to Golgi translocation under these conditions [27]. Rapamycin has also been shown to down-regulate the expression of the LDLR, although in an SREBP2-independent manner [28].

The role of post-translational modifications in regulating SREBP activity

The stability of SREBPs represents a major mechanism of regulation of their transcriptional activity. It has been shown that Cdk1 (cyclin-dependent kinase-1) phosphorylates and stabilizes mSREBP during mitosis [29] and that the stability and transcriptional activity of mSREBP can be regulated by SUMOylation [30] and acetylation [31] respectively. Furthermore, phosphorylation of mSREBP by GSK3β (glycogen synthase kinase-3β) at three residues in the mature protein results in the binding of the SCFβw7 ubiquitin ligase and the subsequent degradation of the protein by the proteasome pathway [26,32]. Interestingly, SCFβw7 also regulates the degradation of mTOR [33]. In addition, it has been demonstrated that increased degradation of mSREBP by the proteasome pathway occurs upon binding of mSREBP to the promoters of their target genes [34] thereby limiting SREBP-transcriptional activity. The stability of the full-length precursor SREBP (full-length precursor SREBP) is also regulated. It has been shown that overexpression of the TRC8 (translocation in renal carcinoma on chromosome 8)/RNF139 (RING finger protein 139) ubiquitin ligase results in increased degradation of fSREBP after long-term sterol depletion [35].

It is unclear if mTORC1 plays a role in regulating the stability of SREBP. We have demonstrated that rapamycin does not affect the stability of mSREBP1α and that Akt-induced stabilization of mSREBP1 is likely to occur via the inhibition of GSK3β [24]. It has also been reported that Akt is able to directly phosphorylate exogenously expressed SREBP1c in primary rat hepatocytes, although the exact residues that are being phosphorylated have not been identified [36]. It has been suggested that this phosphorylation results in increased association of the SREBP–SCAP complex with the sec23/sec24 proteins in COP II vesicles in this system [36]. However, direct phosphorylation of SREBP by mTORC1 has not been demonstrated so far. Some known targets of mTORC1-mediated phosphorylation contain a conserved TOS (TOR signalling) motif that is bound by raptor [37,38]. We identified a sequence within SREBP1 that shows similarity to the TOS motif, although we have not been able to observe a direct interaction between mTORC1 and SREBP1 (C.A. Lewis and A. Schulze, unpublished work). Using MS, we have identified several novel sites of phosphorylation within SREBP1α in insulin-treated cells (C.A. Lewis and A. Schulze, unpublished work). Interestingly, one of these sites is followed by a proline, and mTORC1 is known to regulate proline-directed phosphorylation [39]. However, it is also possible that this site is being targeted not by mTORC1 itself, but by a downstream kinase.

Activation of SREBP by mTORC1: a role for S6K and 4E-BP?

As previously discussed, the main downstream targets of mTORC1 are the S6Ks and the 4E-BPs. A number of studies have investigated expression of SREBP target genes in response to S6K modulation. Expression of SCD1 has been shown to be regulated downstream of S6K1 in the breast cancer cell line MCF7, although it was not investigated whether this was dependent on SREBP activity [40]. This study showed that SCD1 expression was down-regulated in MCF7 cells following treatment with rapamycin and after gene knockdown using two different siRNAs against S6K1 [40]. In contrast, another study reported that the inhibition of SCD1 expression by rapamycin in MCF7 and MDA-MB-468 cell lines lies downstream of SREBP and eIF4E [41]. Furthermore, silencing of S6K1 did not affect SREBP1 or SCD1 expression, indicating that SCD1 expression may be regulated by the mTORC1–4E-BP1 axis [41].

It was recently reported by Dülvel et al. [42] that S6K1 is required for SREBP1 processing and subsequent transcription of its target genes in MEFs (mouse embryonic fibroblasts). This group used a model that exploits the differences between mTOR signalling in wild-type and Tsc1- and Tsc2-null MEFs. Cells lacking TSC1 or TSC2 exhibit growth factor-independent activation of mTORC1, thereby isolating mTORC1 signalling from that of Akt. They used several approaches to demonstrate the requirement for SREBP1 and SREBP2 in mTORC1-induced expression of genes involved in fatty acid and sterol biosynthesis. Furthermore, they showed that the activation of mSREBP by mTORC1 occurs via S6K1. Ablation of S6K1 by RNAi in TSC-deficient cells resulted in a decrease in nuclear accumulation of mSREBP [42]. However, we have recently observed that SREBP processing still occurs following S6K1 ablation in immortalized retinal pigment epithelial cells (RPE-hTERT) in response to acute Akt activation [43]. Although silencing of S6K2 in this system did reduce the accumulation of mSREBP1, combined silencing of both genes did not have an effect. In addition, genetic deletion of both S6K1 and S6K2 in MEFs did not prevent SREBP processing [43]. It has been suggested that constitutive activation of mTORC1 by genetic ablation of Tsc1 or Tsc2 may result in signalling adaptations as a result of extensive feedback mechanisms in the mTORC1 signalling pathway [44]. Indeed, primary hepatocytes from S6K1 and S6K2 double-knockout mice still retain phosphorylation of S6 on serine residues 235 and 236, and this is mediated by the MAPK (mitogen-activated protein kinase) pathway [45]. In addition, it may be possible that ablation of both S6Ks results in feedback mechanisms that allow SREBP processing even in the absence of S6K2.

A role for SREBP in tumorigenesis

FASN and SREBP1 are overexpressed in a number of cancers, including breast and prostate cancers [46–48] and the role for the lipogenic phenotype in cancer pathogenesis has been reviewed extensively [49]. Mutations in the EGFR (epidermal
growth factor receptor) that lead to increased EGFR signalling are common in GBM (glioblastoma multiforme). It has recently been reported that GBM patients treated with the EGFR-inhibitor lapatinib exhibited reduced EGFR-P13K-Akt signalling and that this in turn led to a reduction in SREBP1 expression [50]. However, the present study did not observe reduced nuclear accumulation of SREBP1 following rapamycin treatment both in patients and in vitro [50]. These results indicate that in this particular context the P13K/Akt/SREBP1 signalling axis does not proceed through mTORC1.

We have shown previously that the increase in cell size in response to Akt activation is dependent on SREBP1 and 2 [24]. Furthermore, we demonstrated that silencing SREBP in Drosophila melanogaster resulted in reduction in cell and organ size [24]. These results suggest that protein synthesis and lipogenesis are being regulated in concert by mTORC1.

Furthermore, we demonstrated that silencing SREBP response to Akt activation is dependent on SREBP1 and 2 [24]. Furthermore, we demonstrated that silencing SREBP downstream of mTORC1. The exact regulatory mechanism of SREBPs by mTORC1 is required to elucidate the mechanism by which SREBP is regulated by this important signalling axis.

Future perspectives

The exact regulatory mechanism of SREBPs by mTORC1 remains unknown, but a growing number of studies place SREBP downstream of mTORC1. The apparent conflicting results in the literature surrounding SREBP regulation by mTORC1 are most likely due to the different genetic backgrounds in which the experiments have been performed. Furthermore, the regulation of SREBP by Akt and mTORC1 is likely to be highly complex, and may depend on the exact nature of the signalling input. Continued experimentation is required to elucidate the mechanism by which SREBP is regulated by this important signalling axis.

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References


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