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REVIEW

microRNA-29 mediates a novel negative feedback loop to regulate SCAP/SREBP-1 and lipid metabolism

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The membrane-bound transcription factors, SREBPs (sterol regulatory element-binding proteins), play a central role in regulating lipid metabolism. The transcriptional activation of SREBPs requires the key protein SCAP (SREBP-cleavage activating protein) to translocate their precursors from the endoplasmic reticulum to the Golgi for subsequent proteolytic activation, a process tightly regulated by a cholesterol-mediated negative feedback loop. Our previous work showed that the SCAP/SREBP-1 pathway is significantly upregulated in human glioblastoma (GBM), the most deadly brain cancer, and that glucose-mediated *N*-glycosylation of SCAP is a prerequisite step for SCAP/SREBP trafficking. More recently, we demonstrated that microRNA-29 (miR-29) mediates a previously unrecognized negative feedback loop in SCAP/SREBP-1 signaling to control lipid metabolism. We found that SREBP-1, functioning as a transcription factor, promotes the expression of the miR-29 family members, miR-29a, -29b and -29c. In turn, the miR-29 isoforms reversely repress the expression of SCAP and SREBP-1. Moreover, treatment with miR-29 mimics effectively suppressed GBM tumor growth by inhibiting SCAP/SREBP-1 and *de novo* lipid synthesis. These findings, recently published in *Cell Reports*, strongly suggest that delivery of miR-29 *in vivo* may be a promising approach to treat cancer and metabolic diseases by suppressing SCAP/SREBP-1-regulated lipid metabolism.

Keywords: Lipid metabolism; SCAP; SREBP-1; miR-29; glioblastoma; cancer

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Lipids are essential components of the cells, particularly phospholipids and cholesterol, constituting the basic structure of cell membrane system [1, 2]. Furthermore, lipids also serve as important signaling molecules, regulating various cellular functions [3]. Dysregulation of lipid metabolism contributes to the pathogenesis of various metabolic syndromes, i.e., atherosclerosis, steatosis, obesity and diabetes [4]. Therefore, interfering with the dysregulated lipid metabolism in metabolic diseases has been a long-term focus of basic research and pharmacological development [4, 5]. Nevertheless, the still incomplete understanding of the molecular mechanisms underlying the alteration of lipid metabolism significantly hinders progress.

The family of basic helix-loop-helix transcription factors, SREBPs (sterol regulatory element-binding proteins), plays a central role in lipid metabolism by controlling the *de novo* synthesis of fatty acids, phospholipids, cholesterol, and also

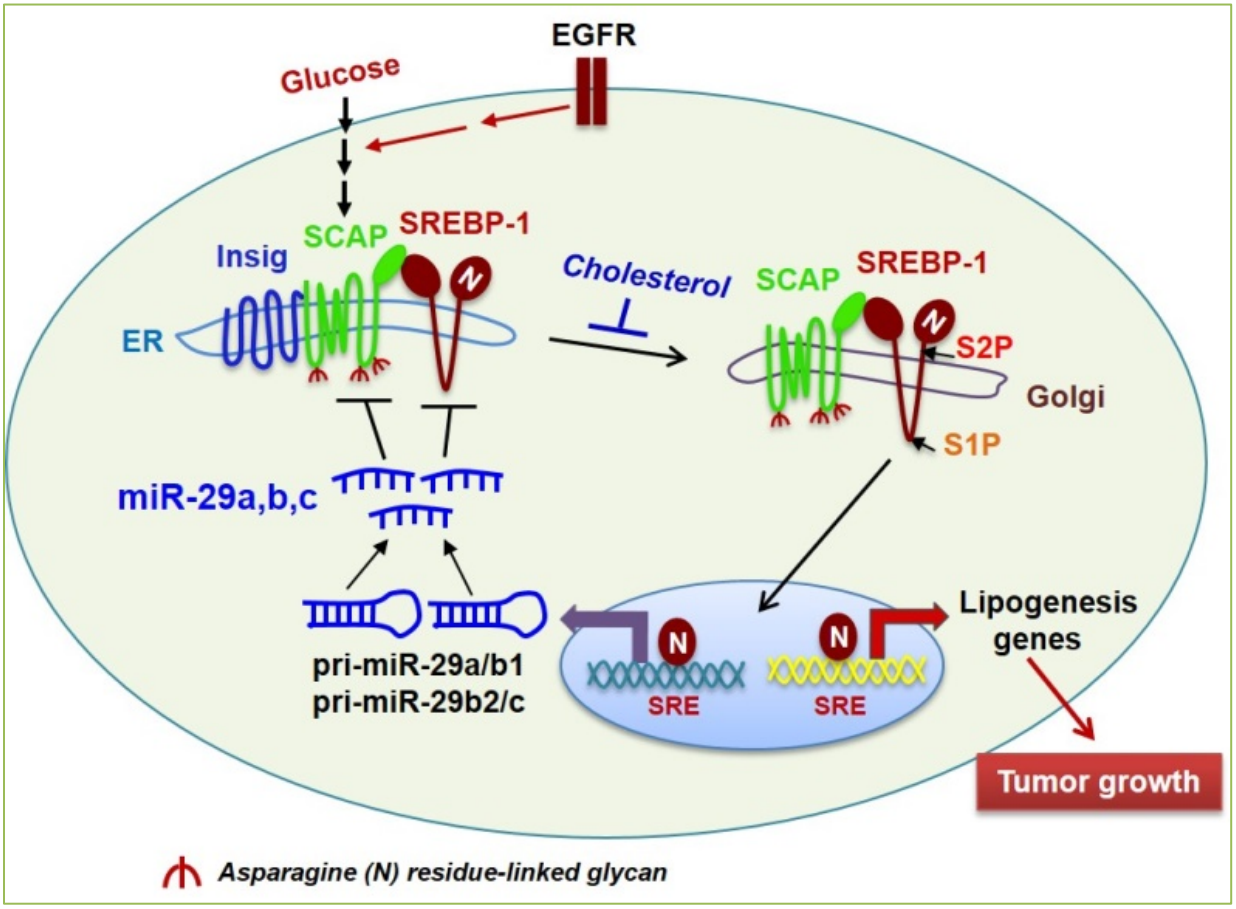

Figure 1. miR-29 mediates a novel negative feedback loop in SCAP/SREBP-1 signaling and regulates lipid metabolism. Our previous study showed that glucose-mediated SCAP *N*-glycosylation enables SCAP/SREBP-1 trafficking from the ER to the Golgi for subsequent proteolytic activation. Furthermore, EGFR signaling enhances glucose uptake, thereby increasing SCAP *N*-glycosylation and SREBP-1 activation to promote tumor growth [23-25]. High levels of cholesterol increase the association of Insig and SCAP, resulting in the retention of the complex in the ER [6]. Our newly discovered negative feedback loop shows that SREBP-1 transcriptionally activates the expression of pri-miR-29a/b1 and pri-miR-29b2/c, which generate the mature miR-29a, -29b and 29c. In turn, miR-29 reversely inhibits the expression of SCAP and SREBP-1 by binding to their 3'-UTRs, resulting in the downregulation of lipogenesis genes [55]. SRE, sterol regulatory element (SREBP-binding motif present in the promoters of SREBP target genes). S1P, site 1 protease. S2P, site 2 protease.

cholesterol uptake, which were initially discovered by Nobel Laureates Brown & Goldstein around 20 years ago [3-7]. SREBPs are comprised of three members, SREBP-1a, -1c and -2. SREBP-1a and -1c that are encoded by the same gene using different transcriptional start sites, resulting in a distinct exon 1 and around a 20 amino acid longer N-terminus in SREBP-1a than in -1c [8, 9]. SREBP-2 is encoded from a different gene and mainly controls cholesterol synthesis and uptake while SREBP-1c regulates fatty acid synthesis. In contrast, SREBP-1a is able to execute all three functions, i.e., fatty acid synthesis, cholesterol synthesis and cholesterol uptake [6, 10-14].

Brown & Goldstein put forth an elegant model of the regulation of SREBP activation through a cholesterol-mediated negative feedback loop (Fig. 1) [4, 6]. After translation, SREBPs immediately bind to the key protein, SCAP (SREBP-cleavage activating protein), to form a complex. SCAP further binds to Insig (insulin-induced gene protein), an endoplasmic reticulum (ER)-anchored protein, resulting in the formation of the Insig/SCAP/SREBP complex, which is retained in the ER by high levels of cholesterol [15-17]. When cholesterol levels decrease, SCAP dissociates from Insig, resulting in the degradation of Insig. SCAP then interacts with COPII proteins that translocate the SCAP/SREBP complex from the ER to the Golgi, where SREBPs are sequentially cleaved by site-1 and site-2 proteinases to release their transcriptionally active N-terminal fragments that enter into the nucleus to promote the transcription of lipogenic genes including *Insig-1* [4, 6, 10, 11, 17, 18]. Consequently, the levels of cholesterol and Insig are restored to bind again to the SCAP/SREBP complex, which

is then retained in the ER, resulting in the reduction of lipid synthesis and uptake (Fig. 1) [19-22].

To explore whether other factors are critical for SCAP/SREBP trafficking, we investigated the role of glucose-mediated *N*-glycosylation modification of the SCAP protein, and showed that it was a prerequisite step for SCAP/SREBP trafficking and activation upon cholesterol reduction [23-25]. We found that *N*-glycosylation stabilizes SCAP and reduces its association with Insig-1, allowing SCAP/SREBP movement from the ER to the Golgi (Fig. 1). Our study demonstrated that glucose is an essential activator of SCAP/SREBP trafficking, while cholesterol functions as a key inhibitor of this process [23-25].

Recent evidence shows that lipid metabolism is largely altered in cancer cells [26-33]. Our previous studies were the first to demonstrate that lipid metabolism is reprogrammed in glioblastoma (GBM) [29, 33-36], the most common primary brain tumor and one of the most lethal of all cancers [36-41]. Our data show that GBM tumors bearing amplification of the tyrosine kinase receptor, EGFR, or expressing the constitutively active EGFRvIII, which lacks a portion of the extracellular ligand binding domain due to the deletion of exons 2-7 of the *EGFR* gene [39, 42, 43], were greatly dependent on SREBP-1-mediated lipogenesis and cholesterol uptake for their rapid growth [34, 36, 44, 45]. We found that EGFR/EGFRvIII activates SREBP-1 via PI3K/Akt signaling to promote lipid synthesis [6, 10, 36], and that EGFR signaling enhances glucose uptake to promote SCAP *N*-glycosylation and SCAP/SREBP-1 trafficking [23-25].

SREBP proteins were recently reported to be upregulated in various cancers and now emerge as promising molecular targets for cancer treatment [33, 44, 46]. Nevertheless, the pharmacological development to directly target SCAP/SREBP has not been successful so far, and alternative means to block this pathway are needed. Thus, we turned our attention to microRNAs (miRNAs), small non-coding RNAs that greatly affect the expression and translation of a large number of genes [47,48]. miRNAs are involved in many biological processes, i.e., cell growth, development, differentiation, survival, etc. [47-49]. Moreover, miRNAs have been shown to be involved in tumorigenesis where they function as tumor suppressors or oncomiRs [50], and to regulate lipid metabolism [51-53].

We identified miRNA-29 as a critical mediator of a novel negative feedback loop in the regulation of SCAP/SREBP-1 signaling [54], providing a promising new approach to target GBM. The miRNA-29 family includes 3 members, miR-29a, -29b and -29c, which share the same seed sequence. miR-29b is encoded by pri-miR-29b1 and pri-miR-29b2, which are located on different chromosomes but generate the same mature miR-29b. Interestingly, pri-miR-29a and pri-miR-29b1 are both located on chromosome 7 and share the same promoter. Similarly, pri-miR-29b2 and pri-miR-29c are located on chromosome 1 and are co-transcribed by the same promoter [55,56].

In our study, we found that expression of all 3 mature miR-29s was positively correlated with SREBP-1 gene expression in samples from a large cohort of GBM patients with altered EGFR (amplification or mutation) [55]. Furthermore, activating EGFR/PI3K/Akt signaling via EGF stimulation significantly enhanced the expression of all 3 miR-29s in GBM cell lines. Interestingly, both SREBP-1a and -1c directly bind to the promoter region of miR-29a/b1 and miR-29b2/c, activating their expression and generating mature miRNA-29a, -29b and -29c. We also showed that the 3'-untranslated region (3'-UTR) of SREBP-1 has binding sites for miR-29, and demonstrated that miR-29a, -29b and -29c inhibited the mRNA and protein levels of SREBP-1 by directly binding to these complementary sites. Importantly, our intracranial GBM xenograft studies show that miR-29 treatment significantly suppressed tumor growth via inhibition of SCAP/SREBP-1 and lipid synthesis [54].

miR-29 has been shown to be transcriptionally inhibited by transcription factors such as c-Myc, TGF-β and *NF*-κB in cancer cells [57-59]. Our study was the first to show that miR-29 expression is controlled by SREBP-1, and that miR-29 is directly involved in the regulation of lipid metabolism. This newly discovered negative feedback loop regulation of SCAP/SREBP-1 by miR-29 further demonstrates that lipid homeostasis is elegantly regulated by multi-layer of mechanisms in addition to cholesterol and glucose regulation [23-25, 54]. Considering the simple synthesis and easy delivery of mature microRNAs, miR-29 treatment may be a feasible and promising approach to treat cancers and other metabolic diseases.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations

miR-29: microRNA-29; SREBPs: sterol regulatory element-binding proteins; SCAP: SREBP-cleavage activating protein; GBM: glioblastoma; Insig: insulin-induced gene; ER: endoplasmic reticulum; 3'-UTR: 3'-untranslated region.

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