Research progress in the targeted treatment of metabolic-related diseases with SQLE (#103618)

First submission

Guidance from your Editor

Please submit by **8 Aug 2024** for the benefit of the authors (and your token reward) .

Literature review article
Please read the 'Structure and Criteria' page to understand the reviewing criteria for this Literature review article.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the materials page.

2 Figure file(s)
1 Table file(s)
1 Other file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **STUDY DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Is the review of broad and cross-disciplinary interest and within the scope of the journal?
- Has field been reviewed recently. It there a good reason for this review (different viewpoint, audience etc.)?
- Introduction adequately introduces the subject and makes audience and motivation clear.

STUDY DESIGN

- Article content is within the Aims and Scope of the journal.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.
- Is the Survey Methodology consistent with a comprehensive, unbiased coverage of the subject? If not, what is missing?
- Are sources adequately cited? Quoted or paraphrased as appropriate?
- Is the review organized logically into coherent paragraphs/subsections?

VALIDITY OF THE FINDINGS

- **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
- Conclusions are well stated, linked to original research question & limited to supporting results.
- Is there a well developed and supported argument that meets the goals set out in the Introduction?
- Does the Conclusion identify unresolved questions / gaps / future directions?

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Research progress in the targeted treatment of metabolic-related diseases with SQLE

Mingzhu Chen [1], **Yongqi Yang** [2], **Shiting Chen** [1], **Zhigang He** [1], **Lian Du** [Corresp. 1]

[1] Chengdu University of Chinese Medicine, Chengdu, Sichuan Province, China

[2] Harbin Medical University, Department of Pharmacology, College of Pharmacy,, Harbin, Heilongjiang province, China

Corresponding Author: Lian Du
Email address: DULiandl@outlook.com

Metabolic-related diseases are a type of chronic disease caused by multiple factors, such as genetics and the environment. These diseases are difficult to cure and seriously affect human health. Squalene epoxidase (SQLE) is the second rate-limiting enzyme in cholesterol synthesis. It plays an important role in cholesterol synthesis and alters the gut microbiota and tumor immunity. Research has shown that SQLE is expressed in many tissues and organs and is involved in the occurrence and development of various metabolic-related diseases, such as cancer, nonalcoholic fatty liver disease, diabetes mellitus, and obesity. SQLE inhibitors, such as terbinafine, NB598, natural compounds, and their derivatives, can effectively ameliorate fungal infections, nonalcoholic fatty liver disease, and cancer. In this review, we provide an overview of the recent research progress on the role of SQLE in metabolic-related diseases. Further research on the regulation of SQLE expression is highly important for developing metabolic drugs with good pharmacological activity.

Research progress in the targeted treatment of metabolic-related diseases with SQLE

Mingzhu Chen1, Yongqi Yang2, Shiting Chen1, Zhigang He1, Lian Du1*

[1] School of Basic Medical Sciences, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, China.
[2] Department of Pharmacology, College of Pharmacy, Harbin Medical University, Harbin, Heilongjiang Province, China.

Corresponding Author:
Lian Du
No.1166 Liutai Avenue, Wenjiang District, Chengdu, China
Email address: DULiandl@outlook.com

Abstract

Metabolic-related diseases are a type of chronic disease caused by multiple factors, such as genetics and the environment. These diseases are difficult to cure and seriously affect human health. Squalene epoxidase (SQLE) is the second rate-limiting enzyme in cholesterol synthesis. It plays an important role in cholesterol synthesis and alters the gut microbiota and tumor immunity. Research has shown that SQLE is expressed in many tissues and organs and is involved in the occurrence and development of various metabolic-related diseases, such as cancer, nonalcoholic fatty liver disease, diabetes mellitus, and obesity. SQLE inhibitors, such as terbinafine, NB598, natural compounds, and their derivatives, can effectively ameliorate fungal infections, nonalcoholic fatty liver disease, and cancer. In this review, we provide an overview of the recent research progress on the role of SQLE in metabolic-related diseases. Further research on the regulation of SQLE expression is highly important for developing metabolic drugs with good pharmacological activity.

Keywords SQLE; metabolism; cancer; drug targets

Introduction

Rapid economic growth, coupled with changes in people's dietary habits and lifestyles, has led to a surge in mortality and morbidity associated with metabolism-related diseases, posing a substantial burden on public health systems and medical resources (Saklayen 2018). The currently available medications for the prevention and treatment of these diseases require long-term use, and there are issues of general efficacy, drug resistance, and adverse side effects. Therefore, the exploration for innovative approaches to prevent and control these conditions is important. Studies have identified squalene epoxidase (SQLE) as a key regulator of various physiological processes, including cholesterol biosynthesis, modulation of the gut microbiota, and regulation of tumor immunity. Consequently, the involvement of SQLE has been implicated in the development of various conditions, such as fungal infections, nonalcoholic fatty liver disease (NAFLD), cancer, and diabetes mellitus (DM). Understanding the precise role of SQLE in the pathogenesis of these diseases and its potential as a therapeutic target is essential for devising effective preventive and therapeutic strategies. By elucidating the regulatory mechanisms and molecular pathways mediated by SQLE in metabolism-related diseases, we can establish a solid theoretical foundation for developing targeted interventions. Here, we aim to provide a comprehensive overview of SQLE, beginning with its structural characteristics and its pivotal role in cholesterol synthesis. Subsequently, we will discuss the regulation, functional significance, and clinical implications of SQLE in the context of metabolism-related disorders. Finally, we will address the latest advancements in utilizing SQLE as a targeted therapeutic agent, thus paving the way for future research directions and clinical applications.

Intended audience and need for this review

SQLE is the second rate-limiting enzyme of cholesterol synthesis and plays an important role in cholesterol synthesis, alteration of intestinal flora, and tumor immunity. In addition, SQLE is expressed in many tissues and organs and is involved in the occurrence and development of a variety of metabolism-related diseases. However, current studies on its function, expression, role in metabolic diseases and development of clinical applications are not comprehensive. This article is thus concerned with the advancement of research into the role of SQLE in metabolic-related diseases over recent years. An in-depth study of SQLE expression regulation is of particular importance for the development of antimetabolic drugs with good pharmacological activity.

Survey methodology

The authors conducted an in-depth search on PubMed, Web of Science and Foreign Medical Literature Retrieval Service. The search was carried out by combining subject words and free words, and the following heading terms were used when performing the search: “SQLE”, “SQLE inhibitors”, “cancer”, “nonalcoholic fatty liver disease”, “diabetes mellitus”, “obesity”, “cholesterol synthesis”, “structure”, “activity regulation”,” function”, “transcriptional regulation”,” posttranscriptional regulation” and “metabolic disease”. This article is based on the published works of literature, which have been classified, organized, and searched by title, abstract, and full texts.

Structure, activity regulation, and function of SQLE

Structure of SQLE

SQLE was first discovered in rat liver microsomes in 1969 (Yamamoto & Bloch 1970). Like most cholesterol enzymes, SQLE is located in the endoplasmic reticulum or on lipid droplets, and the *SQLE* gene is located in the region of human chromosome 8q24.13 (Nagai et al. 1997).

As an essential lipid component of mammalian cell membranes, cholesterol is critical for cell survival and proliferation and coordinates multiple membrane receptor signaling pathways by maintaining the stability of lipid rafts (Dang & Cyster 2019). Almost all mammals can synthesize cholesterol from acetyl-CoA via 20 enzymatic reactions, including the mevalonate (MVA) pathway, SQLE biosynthesis, and subsequent reactions (Fig. 1). The two rate-limiting enzymes, HMGCR and SQLE, are important factors in the cholesterol synthesis pathway. SQLE is responsible for the first oxidative step in cholesterol synthesis; it oxidizes squalene to

epoxysqualene. When the activity of the enzyme lanosterol synthase, which converts epoxysqualene to lanosterol, is low, SQLE also converts epoxysqualene to deoxygenated squalene. The end product of this shunt pathway, 24(S), 25-epoxycholesterol, is a ligand for the hepatic X receptor, which increases ATP-binding cassette transporter protein A1 (ABCA1) levels to promote cholesterol efflux (Gill et al. 2011; Seiki & Frishman 2009). In conclusion, the catalytic reaction of SQLE is essential for cholesterol synthesis.

Figure 1 Cholesterol synthesis pathway centered on SQLE

Regulation and function of SQLE

SQLE is a direct target of sterol regulatory element-binding proteins (SREBPs), and SREBP2 is a transcription factor that regulates genes involved in cholesterol synthesis and homeostasis in a cholesterol-dependent manner. The SQLE proteins contain cholesterol-sensing structural domains that regulate the proteasomal degradation of SQLE. Thus, HMGCR-like SQLE activity is precisely regulated by intracellular cholesterol levels via feedback, which results in a second rate-limiting step in cholesterol synthesis (Ryder 1988; Ryder 1991). SQLE expression in cells is subject to complex regulation systems, including transcription, posttranscriptional regulation at the mRNA level, and posttranslational regulation.

Transcriptional regulation

The transcription factor SREBP2 directly regulates the mRNA levels of enzymes involved in cholesterol metabolism, such as SQLE, by binding to sterol regulatory element (SRE) sequences in the promoters of target genes (Brown et al. 2018). When the cholesterol level in the endoplasmic reticulum (ER) is less than 5% of the total intracellular lipid level, SREBPs are activated, and SCAP undergoes a conformational change to dissociate from the lnsig-1 protein. The SCAP-SREBP2 complex is then disassembled and detached from the ER membrane and transported to the Golgi complex via COPII vesicles. There, it is cleaved by site-1 protease (S1P) and site-2 protease (S2P) protein hydrolase cleavage sequentially and converted to activated nuclear SREBP2 (nSREBP2). Immediately thereafter, nSREBP2 enters the nucleus as a homodimer and binds to the sterol regulatory element (SRE) in the promoter of the target gene *SQLE* to increase SQLE mRNA levels (Griffiths & Wang 2021). In addition, oxygen sterol-binding protein-like 2 (OSBPL2) deficiency promotes nuclear entry of the SP1 transcription factor and SREBP2 in the SQLE promoter to upregulate SQLE expression and increase cholesterol and cholesteryl ester accumulation through inhibition of the AMP-activated protein kinase (AMPK) pathway (Zhang et al. 2019). In addition, the transcription factors NF-Y and SP1 act synergistically with nSREBP2 to upregulate *SREBP2* gene expression.

Posttranscriptional regulation

A large body of evidence suggests that the expression of long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs) is commonly dysregulated in human cancers. miR-133b (Qin et al. 2017; Wang et al. 2022), miR-205 (Kalogirou et al. 2021), miR-612 (Liu et al. 2020), miR-579-3p (Qian et al. 2023), and miR-1179 (Li et al. 2023a) can interact with SQLE mRNAs, act as negative regulators of gene expression at the posttranscriptional level, and play important roles in tumor cell differentiation, proliferation, and apoptosis through the miR/SQLE axis (Table 1). Qin et al. reported that in breast cancer, Lnc030 interacts with the K homology domain of the RNA-binding protein poly(rC)-binding protein 2 (PCBP2) structural domain 2, and the 3'UTR of SQLE mRNA binds to the K homology domain structural domain 3 of PCBP2. Lnc030 synergistically enhances the stability of SQLE mRNA with PCBP2. This leads to an increase in cholesterol synthesis, which, in turn, activates the PI3K/AKT signaling pathway and is involved in the regulation of the stemness characteristics of BCSCs (Qin et al. 2021). miR-133b and miR-205 were reported to reduce SQLE mRNA levels more rapidly by binding to the 3'UTR of SQLE mRNA (Kalogirou et al. 2021; Qin et al. 2017; Wang et al. 2022). circ_0000182 was shown to cause SQLE overexpression by sponging miR-579-3p. This miR then loses the ability to regulate SQLE and thus promotes cholesterol synthesis and proliferation in gastric adenocarcinoma cells (Qian et al. 2023).

Table 1. Relationships among miRNAs, SQLE and cancer

Posttranslational regulation

SQLE is also posttranslationally regulated. The regulation of SQLE protein stability and activity, mainly through the cholesterol membrane-associated ring-CH-type finger 6 (MARCH6)-proteasomal degradation axis, is dependent on specific structural domains in SQLE proteins (Sharpe et al. 2020). These domains include the cholesterol-dependent amphiphilic helical structure formed by the Gln62-Leu73 sequence (Chua et al. 2017), which can be embedded in the hydrophobic interior of membranes while interacting with the hydrophilic environment at the membrane surface. It regulates SQLE by interacting with cholesterol molecules and plays an important role in cholesterol-dependent degradation. The first 100 amino acids of SQLE (SQLE N-100) are attached to the ER membrane in the form of a re-entrant loop (Howe et al. 2015), which senses cholesterol in the cytoplasm. When intracellular cholesterol accumulates, the anchoring of the SQLE protein to the ER membrane tightens. This results in partial exposure of the amphipathic helical structure of the Gln62-Leu73 sequence to the cytoplasmic environment, preventing proteasomal degradation. The ubiquitination process also requires the E2 ubiquitin coupling enzyme J2 (UBE2J2). MARCH6 approaches the SQLE protein and recognizes serine residues near the cholesterol-dependent amphiphilic helix in the SQLE protein. UBE2J2 works in concert with MARCH6 to attach the ubiquitin molecule from the E1 enzyme to the SQLE protein to be ubiquitinated to label it as a protein to be degraded so that it becomes a ubiquitin-

protease substrate of the degradation system. The valosin-containing protein (VCP) is then recruited to extract the ubiquitinated SQLE substrate, and it dissociates from the ER. The VCP then cooperates with other proteins to mediate entry into the proteasomal degradation pathway. Excess cholesterol can stimulate SQLE degradation by inhibiting MARCH6 self-degradation (Sharpe et al. 2019).

The proteasomal degradation pathway of MARCH6-VCP can be regulated independently of cholesterol. The N-terminus of SQLE is partially degraded through a unique ubiquitination pathway, which leads to the conversion of full-length SQLE to a truncated SQLE. The enzyme activity of the truncated SQLE is associated with cholesterol resistance, implying that the function of SQLE is not completely lost under high cholesterol conditions. Thus, the function of this gene may complement cholesterol metabolism under cancer conditions (Coates et al. 2021). In addition to cholesterol, squalene directly binds to the SQLE N-100 structural domain to change its conformation. This results in the inability of MARCH6 to ubiquitinate SQLE or label it for degradation, thus increasing the stability of SQLE (Nathan 2020). Unsaturated fatty acids (USFAs) can also stabilize SQLE levels via the regulatory blockade of SQLE ubiquitination by MARCH6. Upregulation of the cancer-associated microprotein CASIMO1 increases SQLE levels by interacting with SQLE proteins (Polycarpou-Schwarz et al. 2018).

Overall, the main mechanism of SQLE regulation is the cholesterol-dependent feedback regulation of SQLE by the SREBP2 transcriptional and ubiquitin proteasomal degradation pathways. SREBP2 activation in tumor tissues leads to high SQLE expression. The activation of oncogenes, deletion of cancer suppressor genes, and deletion of miRNAs and cancer-associated proteins directly or indirectly upregulate SQLE expression. Different tumor cells or specific subpopulations have specific SQLE regulatory mechanisms.

Figure 2. The main mechanism of SQLE regulation

Since the epoxidized squalene derivative lanosterol is a component of fungal membranes, SQLE has long been investigated as an antifungal target with increasing relevance to human health and disease. Many studies have shown that SQLE can meet the high energy requirements for the rapid growth of diseased cells, and the dysregulation of SQLE has been found in a variety of metabolic diseases. This has become a hot topic in the field of targeted diagnosis and therapy.

The role of SQLE in metabolic diseases

The role of SQLE in the pathogenesis of nonalcoholic fatty liver disease

In recent years, nonalcoholic fatty liver disease (NAFLD) has replaced viral hepatitis as the most common chronic liver disease in China, and the number of NAFLD patients in China is expected to reach 314 million by 2030 (Nan et al. 2021). NAFLD is the hepatic manifestation of metabolic syndrome and includes simple steatosis to nonalcoholic steatohepatitis (NASH). Liu et al. reported that SQLE was closely associated with the development of NASH (Liu et al. 2021a), and SQLE expression was significantly upregulated in both NAFLD patients and mouse models. Specific overexpression of *SQLE* in mouse hepatocytes triggered spontaneous NAFLD by promoting cholesterol synthesis and accumulation and binding to carbonic anhydrase (CA3). This promotes the activation of sterol regulatory element-binding protein 1c (SREBP1C), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), stearoyl-CoA desaturase-1 (SCD1) and other adipogenic and triglyceride biosynthesis genes, thereby inducing hepatic de novo lipogenesis and activating the NF-κB inflammatory pathway. These processes promote the pathogenesis and progression of NASH.

The role of SQLE in cancer

There is growing evidence that cancer is also a metabolic disease involving tumor cell proliferation, energy metabolism, and dysregulation of immune surveillance. Reprogramming of cholesterol metabolism in tumor cells involves synthesis, uptake, esterification, efflux and transformation processes (Huang et al. 2020), which promote tumorigenesis. Studies have shown that SQLE is aberrantly expressed in a variety of malignant tumors and influences tumor cell proliferation, migration or invasion through pathways such as cholesterol synthesis, tumor immune infiltration and immunotherapy, and intestinal ecology. The level of SQLE expression may be correlated with aspects of cancerous tissue lesions, ethnicity, and the stage of the disease (D'Arcy et al. 2015; He et al. 2021; Jun et al. 2021; Li et al. 2022). The expression of SQLE is significantly upregulated in nasopharyngeal carcinomas (Li et al. 2023a), leukemias (Song et al. 2021), pancreatic cancer (Wang et al. 2022), and hepatocellular carcinoma (Sui et al. 2015).

p53 directly reduces SQLE expression in an SREBP2-independent manner, inhibiting cholesterol production in vivo and in vitro and leading to tumor growth inhibition (Sun et al. 2021). In prostate cancer cells, the involvement of SQLE in cholesterol synthesis consumes large amounts of NADPH and activates DNA methyltransferase 3A (DNMT3A). This triggers loss of function or reduced expression of the PTEN gene, which drives cholesteryl ester accumulation and subsequent SOAT1 activation via the PTEN/PI3K/AKT/mTOR pathway (Yue et al. 2014). These intertwined cascade reactions amplify the oncogenic effects of SQLE. Aberrant activation

of the ERK signaling pathway promotes cancer cell growth and proliferation, apoptosis, invasion and metastasis, and angiogenesis and is tightly associated with cancer development. The control of cholesterol biosynthesis associated with SQLE is markedly increased in patients with colorectal cancer. He et al. revealed that SQLE deficiency in colorectal cancer reduces intracellular cholesterol levels and decreases osteotriol (the active form of vitamin D3), leading to reduced levels of cytochrome P450 family 24 subfamily a member 1 (CYP24A1), the inhibition of ERK phosphorylation and CRC cell proliferation (He et al. 2021).

In addition to the cell-intrinsic effects of SQLE, it may also play a role in tumor growth through host-microbiota interactions. The intestinal flora is a diverse and surprisingly numerous microbial community present in the human gut that has been associated with inflammatory/immune diseases, metabolic disorders, and malignancies (Toya et al. 2021). In recent years, SQLE has been shown to be involved in tumor growth regulation by affecting the composition and function of gut microbes, thereby influencing metabolite production and modulating the immune response. Li (Li et al. 2022) et al. reported that SQLE-induced dysregulation of gut microbes promotes intestinal barrier dysfunction and proliferation of the colonic epithelium in germ-free mice and that the metabolism of secondary bile acids disrupts intestinal barrier function. Additionally, these authors reported that the downregulation of the tight junction proteins Jam-c and occludin causes a "leaky gut", which ultimately induces a proinflammatory response, and that the transplantation of feces from SQLE transgenic mice into germ-free mice impairs the intestinal function and proliferation of the colorectal cancer (CRC) epithelium.

SQLE plays an important role in tumor immunomodulation, and an algorithmic analysis of databases showed that SQLE mRNA was expressed at higher levels in head and neck squamous cell carcinomas (Liu et al. 2021b) (HNSCC) than in normal tissues. The expression of SQLE in glioblastoma (GBM) patients was significantly correlated with tumor-infiltrating lymphocytes, immune stimulants, immunosuppressants, and MHC molecules (Ye et al. 2023). WU (You et al. 2022) et al. experimentally demonstrated that SQLE expression was upregulated in pancreatic adenocarcinoma (PAAD) patients and was negatively correlated with prognosis. SQLE can affect the immune microenvironment and immunotherapy outcome of PAAD patients by regulating the infiltration of tumor immune cells and the expression of ICT, and this kind of metabolic intervention-based immunotherapy is beneficial for overcoming the bottleneck of cancer treatment.

Role of SQLE in DM

DM is a chronic metabolic disease involving elevated blood glucose levels and disturbances in the metabolism of glucose, proteins, and fats (Singh et al. 2021). Transcriptomic studies have revealed the upregulation of SQLE in peripheral monocytes from patients with atherosclerosis, and the cholesterol metabolism gene network represents a molecular link between obesity/inflammation and its most important complication, type 2 diabetes, and cardiovascular

disease. There is a link between cholesterol and type 2 diabetes that regulates this metabolic network, which includes SQLE (Ding et al. 2015).

The pathogenesis of diabetes is a complex process involving many genes. The overexpression of SQLE gene in diabetes increases the number of genes that promote **cholesterol synthesis,** increase cholesterol accumulation, and promote diabetes. The GE team (Ge et al. 2020) identified the differentially expressed genes (DEGs) involved in the pathogenesis of diabetes in mice and suggested that SQLE proteins, as one of the core differentially expressed proteins, are involved in the pathogenesis of diabetes. In addition, RNA-seq and qRT–PCR revealed that SQLE was downregulated in the liver, whereas SQLE protein expression was significantly upregulated according to Western blotting. This is possibly due to posttranscriptional modifications of the SQLE protein, leading to sustained expression of the protein (Ge et al. 2020). In addition, in patients with diabetes, who often have disorders of lipid metabolism, SQLE levels are increased, and impaired cholesterol efflux can lead to the development of DM and fatty liver, which can significantly impact atherosclerosis and dyslipidemia (Ahmadi et al. 2022).

Role of SQLE in obesity

Further studies revealed the potential role of SQLE in obesity. The Fob3b obesity quantitative trait locus (QTL) is one of the gene regions affecting body weight, adiposity, or other obesity-related phenotypes in mice, and SQLE has been suggested to be an important candidate gene. The differences in SQLE expression between high-fat diet-fed mice and transgenic mice carrying the Fob3b QTL region fed a low-fat diet suggest that SQLE may play an important role in regulating body weight and fat deposition. In addition, alterations in the Fob3b allele may increase cholesterol biosynthesis in mice with a high fat content, which in turn leads to increased cholesterol deposition in adipocytes, thereby exacerbating obesity (Stylianou et al. 2005) Therefore, therapeutic strategies targeting SQLE may help prevent and treat obesity and its associated diseases.

Obesity is a metabolic disorder associated with excessive fat accumulation [40] and is a major risk factor for type 2 diabetes and cardiovascular disease (La Sala & Pontiroli 2020). Alterations in the cholesterol gene network, which includes SQLE and is key in the cholesterol synthesis pathway, have been molecularly linked to obesity, inflammation, type 2 diabetes mellitus, and cardiovascular disease and may be a hallmark of obesity-associated diseases [41]. Further studies revealed the potential role of SQLE in obesity. The Fob3b obesity quantitative trait motif is one of the gene regions affecting obesity-associated phenotypes, and SQLE is considered an important candidate gene. Differences in SQLE expression between high-fat mice and synthetic mice carrying the Fob3b QTL region from low-fat mice suggest a possible role for SQLE in regulating body weight and fat deposition. Alterations in the Fob3b allele may increase cholesterol biosynthesis in high-fat-content mice, which may lead to elevated levels of

cholesterol deposition in adipocytes, thereby exacerbating obesity (Stylianou et al. 2005). Therefore, therapeutic strategies targeting SQLE may help prevent and treat obesity.

Clinical correlation between SQLE and metabolic diseases

SQLE and nonalcoholic steatohepatitis

NAFLD is not a simple benign disease, and according to the global data assessment in 2015, the number of deaths of NAFLD-HCC patients in China accounted for 10.5% of the total number of HCC deaths in the same period (Nan et al. 2021). Studies have shown that SQLE is significantly upregulated in NAFLD-HCC patients, and in mice with hepatocyte-specific overexpression of SQLE, NAFLD-HCC is driven by increases in cholesterol biosynthesis and the NADP/NADPH ratio (Liu et al. 2018). This induces oxidative stress, which activates the DNA methyltransferase 3A-mediated PTEN/PI3K/AKT/mTOR signaling pathway to promote NAFLD-HCC carcinogenesis. The SQLE inhibitor terbinafine inhibited NAFLD-HCC cell proliferation and tumor development in a mouse model. Du's team found (Sun et al. 2021) that *P53* regulates cholesterol synthesis by inhibiting the transcription of SQLE, thus exerting an inhibitory effect on NAFLD-HCC. The combination of the SQLE inhibitor terbinafine and the CA3 inhibitor acetazolamide in the treatment of NASH was superior to either drug alone. Additionally, SQLE and CA3 can be used as noninvasive markers for the diagnosis of NAFLD or NASH, which confirms that SQLE/CA3 is a new target for the diagnosis and treatment of NASH.

The above studies revealed the molecular mechanisms of NAFLD and NAFLD-HCC development, identified SQLE as a key factor in these mechanisms and a new target for drugs, and revealed that the antifungal drug terbinafine can inhibit tumor development.

SQLE and cancer

SQLE is involved in hormone signaling, and in prostate cancer, it is closely associated with high Gleason scores (Stopsack et al. 2017), correlates with metastasis, distinguishes tumors at high risk of metastasis, and is a strong predictor of fatal prostate cancer (Stopsack et al. 2016). In breast cancer, SQLE overexpression is usually associated with tumor aggressiveness, recurrence, and overall survival time, and breast cancers with amplification of 8q24.11-13 (a region that includes the SQLE gene) imply a poorer prognosis (Helms et al. 2008a). The mRNA expression of SQLE has been associated with a poorer prognosis of estrogen receptor-positive (ER+) phase I/II breast cancer (Helms et al. 2008b).

Poor drug response to letrozole and poor progression-free survival with adjuvant tamoxifen has been reported in SQLE-overexpressing breast cancer patients (Simigdala et al. 2016). In HCC, SQLE is an independent risk factor for overall survival, and high levels of SQLE expression significantly correlate with advanced tumor histological grade and elevated levels of alpha-fetoprotein. Thus, SQLE may serve as a novel prognostic biomarker (Liu et al. 2018; Shen et al. 2020). However, in colorectal cancer patients, the prognostic value of SQLE is related to tumor progression. Higher levels of SQLE in tumors are associated with poorer overall survival in patients with stages II and III disease, but lower levels of SQLE expression in tumors with stage T4 or IV disease predict a poorer prognosis (Kim et al. 2019). In pancreatic cancer, high expression of SQLE and other genes involved in cholesterol production is associated with resistance to radiotherapy (Souchek et al. 2014). For squamous cell carcinoma of the lung, SQLE is closely associated with poor differentiation, clinical stage, and lymphatic metastasis, which predict a poor prognosis; thus, it has become a novel molecular marker for lung cancer (Zhang et al. 2014). In uveal melanoma (Xu et al. 2019) and head and neck squamous cell carcinoma (Liu et al. 2021b), SQLE was associated with poor prognosis. Daunorubicin-resistant leukemia cells express higher levels of SQLE than daunorubicin-sensitive leukemia cells (Stäubert et al. 2016). In addition, cholesterol is a sex hormone precursor; therefore, the overexpression of SQLE is associated with adverse effects of hormone therapy. In conclusion, high levels of SQLE in most tumors predict poor prognosis, including tumor recurrence, tumor metastasis, and a short overall survival time. The role of SQLE in tumor development and progression has been demonstrated through basic research and clinical analyses, and SQLE may be a new target for cancer therapy.

SQLE and DM

Data from the International Diabetes Federation (IDF) indicate that diabetes is one of the fastest-growing global health emergencies of the 21st century, and 537 million people were living with diabetes in 2021. This number is projected to reach 643 million by 2030, and the prevalence of diabetes in 2045 is predicted to be 783 million (Einarson et al. 2018). DM is a syndrome of metabolic disorders, often characterized by disorders of lipid metabolism. The levels of SQLE are increased in patients with diabetes, and impairment of cholesterol efflux can lead to the development of DM and fatty liver, which can have a significant impact on atherosclerosis and dyslipidemia (Ahmadi et al. 2022). Weight loss alters monocyte cholesterol metabolism-related pathways and reduces SQLE levels. Thus, SQLE may serve as a potential therapeutic target for obesity-associated T2DM (Ding et al. 2022). Overexpression of SQLE gene in diabetes increases the expression of genes that promote cholesterol synthesis, increases cholesterol accumulation and promotes diabetes. DEGs related to the pathogenesis of diabetes were identified in diabetic mice, indicating that the liver of diabetic mice expresses 27 DEGs and that the SQLE is upregulated in diabetic mice. These findings further support the important role of SQLE in diabetes (Ge et al. 2018).

SQLE and obesity

Globally, obesity rates are increasing, and obesity is strongly associated with diseases such as hypertension, diabetes, cancer, and cardiovascular disease. This has led to increasing obesity-related healthcare costs. The cost of managing obesity-related morbidity and mortality accounts for between 3% and 21% of national health budgets (Ahmed & Konje 2023). Studies have shown significant upregulation of SQLE among obese patients and a decrease in SQLE after weight loss interventions [42]. Farfarb extract is considered to have potential for the treatment of obesity through the modulation of SQLE activity. Farfarb extract may affect fat metabolism by modulating the cholesterol biosynthesis pathway, thus exerting a therapeutic effect on obesity (Wang et al. 2017). This provides a promising approach for the development of new obesity treatments, but further studies are needed to confirm their safety and efficacy.

SQLE-targeted therapeutic strategies

SQLE has been reported as an oncogene in various cancers. Moreover, the dysregulation of SQLE has been associated with the inhibition of apoptosis and increased cell proliferation and invasiveness, and a high abundance of SQLE in tumors indicates a poorer prognosis. As a novel and attractive therapeutic target for anticancer treatment, SQLE has been increasingly used in preclinical studies to reveal its antitumor effects and related mechanisms. The first SQLE-targeted inhibitors disrupted the synthesis of ergosterol in antifungal bodies, thereby killing or inhibiting the fungus (Barrett-Bee & Dixon 1995). SQLE inhibitors can be classified as allylamines, natural compounds, and their derivatives according to their structure. Currently, research on the novel use of established SQLE inhibitors is increasing, and targeting SQLE is considered a new and promising therapy for metabolic diseases.

Allylamine

Since Georgopoulos et al. discovered that naftifine has high broad-spectrum antifungal activity in 1981, it has become the cornerstone for the commercialization of next-generation inhibitor drugs, such as butenafine and tolnaftate. The main SQLE inhibitor used in preclinical antitumor studies is terbinafine, which has been shown to inhibit cell proliferation, induce G0/G1 cell cycle arrest, apoptosis, and autophagy by inhibiting SQLE or SQLE-independent inhibition, and slow tumor growth in vivo in a dose-dependent manner.

The compound NB598, obtained by modification of the aromatic moiety of terbinafine, is another highly specific inhibitor of mammalian SQLE, with the best response in neuroblastoma and lung cancer and good drug sensitivity in small-cell lung cancer cell lines (Mahoney et al. 2019). Further modification of NB598 yielded silyl derivatives that also have the ability to inhibit the enzymatic activity of SQLE. However, preclinical studies revealed that

gastrointestinal toxicity and dermal toxicity were not tolerated by dogs and monkeys treated with a gavage of allylamine inhibitors (NB-598 and cmpd-4") for small cell lung cancer treatment (Nagaraja et al. 2020). Naftifine and terbinafine, which are used as antifungal agents, cause similar adverse effects, and this toxicity may limit the potential therapeutic benefit of metabolic disease treatment. This toxicity is attributed to the fact that the site of action of both terbinafine and NB-598 is Y195, and the tertiary amine group in the inhibitor structure forms a hydrogen bond with Y195. This prevents Y195 from interacting with glutamine (Q168) at position 168, inhibiting the conversion of SQLE to the active state. Thus, all the catalytic reactions of SQLE are inhibited, resulting in greater neurological and dermal toxicity (Nagaraja et al. 2020; Padyana et al. 2019). The IC50 values of allylamine inhibitors in mammalian cells are several orders of magnitude greater than those in fungi, and large doses are often required to achieve therapeutic efficacy, such as antitumor effects. Hence, there is a need for careful assessment of the tolerance of adverse effects (Ryder 1988).

Natural compounds and their derivatives

Many natural compounds and derivatives may be clinically safe SQLE inhibitors that can effectively and selectively inhibit SQLE enzyme activity. For example, Abe et al. (Abe et al. 2000) reported that green tea polyphenols, the main component of which is galloyl-containing epigallocatechin gallate (EGCG), noncompetitively inhibit SQLE by scavenging reactive oxygen species from the active site of the enzyme. The team synthesized galloyl groups, such as dodecyl ester and gallate dodecyl ester, as SQLE inhibitors, which are widely used in food additives for antioxidant purposes. The metabolites of EGCG are also inhibitory, and other plant extracts, such as beta-carotene, anthocyanins, tannins, fo-ti, and rhubarb, are also rich in galloyl groups. Grape skins and red wine are rich in the galloyl polyphenolic compound resveratrol, which reversibly and noncompetitively inhibits SQLE enzyme activity, with cholesterol-lowering and cardiovascular disease-preventive effects. Although EGCG still has few side effects when consumed at high doses, it has low bioavailability and a short half-life to reach effective therapeutic concentrations.

Ellagitannin analogs of pedunculin and eugenol also showed significant inhibitory efficacy. Gupta et al (Gupta & Porter 2001) reported that garlic and its derivative compounds were effective at inhibiting SQLE. Unlike tea polyphenols, which inhibit SQLE enzyme activity through a different mechanism, garlic extracts induce an irreversible inhibitory effect on SQLE activity. This is attributed to the high aryl cysteine content of garlic, which binds to the active region of the SQLE enzyme, rendering it inactive. Additionally, garlic allyl sulfide derivatives, though not highly specific to SQLE, interact with other proteins and inhibit SQLE enzymatic activity in Schwann cells, penetrating the blood–brain barrier. Consequently, this inhibition leads to decreased cholesterol synthesis and squalene accumulation, impacting myelin sheath formation and neurotransmission. Ultimately, this process results in segmental demyelination and peripheral nerve paralysis. The unique properties of natural SQLE inhibitors found in garlic

may serve as a foundation for developing clinically safe SQLE inhibitors. Moreover, different types of SQLE inhibitors offer diverse frameworks for creating novel compounds that mitigate side effects and enhance affinity. However, further investigation is required to determine their therapeutic potential in treating metabolic diseases.

Unlike the mechanism of inhibition by tea polyphenols, garlic allyl sulfide derivatives, which are not highly selective for SQLE, can also interact with other proteins and inhibit the enzymatic activity of SQLE in Schwann cells through the blood–brain barrier. This leads to the inhibition of cholesterol synthesis and squalene accumulation, which affects myelin sheath formation and severely impedes neurotransmission, ultimately leading to segmental demyelination and paralysis of peripheral nerves. The specific nature of these natural SQLE inhibitors may be a starting point for the development of clinically safe SQLE inhibitors, and the different types of SQLE inhibitors that provide different frameworks for the development of novel SQLE inhibitors to eliminate side effects and improve affinity and whether they contribute to the treatment of metabolic diseases need to be further verified.

Conclusions

In summary, an increasing number of preclinical studies have shown that SQLE is closely related to the occurrence and development of various metabolic diseases and has even become an independent prognostic factor for these diseases, providing new ideas for the targeted diagnosis and treatment of malignant tumors.

SQLE plays a role in cancer, NAFLD-HCC, DM and obesity and has potential for use in treating hyperlipidemia. Allicin has been reported to exert an antihyperlipidemic effect. Transcriptomic analysis revealed that allicin significantly regulated 148 genes, including SQLE (Zhang et al. 2023). Allicin regulates cholesterol synthesis by upregulating the SQLE gene, thus demonstrating its therapeutic effect on hyperlipidemia. Therefore, a comprehensive understanding of the role of SQLE in the regulation of dyslipidemia provides an important theoretical basis for further research and the development of therapeutic strategies for dyslipidemia. In addition to affecting metabolism-related diseases, SQLE has potential use in livestock regulatory networks, regulating muscle growth to improve beef yield by inhibiting the proliferation and promoting the differentiation and apoptosis of skeletal muscle-derived mesenchymal stem/stromal cells (Zhang et al. 2020) and ultimately affecting lactation yield and quality by promoting the proliferation, cell cycle, and apoptosis of mammary epithelial cells. Active substances in chlorophyll-like plants bind directly to SQLE proteins, causing hepatorenal toxicity and impairing the SREBP2/HMGCR/SQLE/LSS pathway, thereby disrupting cholesterol production (Li et al. 2023b). Targeting SQLE could allow for the future development of pharmacological agents that are similar to the structure of chlorophylls to be considered a therapeutic strategy for targeting SQLE. Compared to HMG-CoA reductase, SQLE is located downstream of the mevalonate synthesis pathway, and inhibition of SQLE does not modulate the effect of the nonsteroidal product on the normal physiology of the cell; therefore, SQLE is expected to be a highly selective drug target with few side effects. Therefore, existing drugs or molecular structures can be modified and combined with multiple effective inhibitors to further

improve targeting and reduce toxicity. However, clinically, strong evidence is needed to validate the role of SQLE in the prevention and treatment of metabolic diseases.

Acknowledgments

Thank you to every member of the lab.

References

Abe I, Seki T, Umehara K, Miyase T, Noguchi H, Sakakibara J, and Ono T. 2000. Green tea polyphenols: novel and potent inhibitors of squalene epoxidase. *Biochem Biophys Res Commun* 268:767-771. 10.1006/bbrc.2000.2217

Ahmadi A, Bagheri Ekta M, and Sahebkar A. 2022. Mechanisms of antidiabetic drugs and cholesterol efflux: A clinical perspective. *Drug Discovery Today* 27:1679-1688. 10.1016/j.drudis.2022.02.006

Ahmed B, and Konje JC. 2023. The epidemiology of obesity in reproduction. *Best Pract Res Clin Obstet Gynaecol* 89:102342. 10.1016/j.bpobgyn.2023.102342

Barrett-Bee K, and Dixon G. 1995. Ergosterol biosynthesis inhibition: a target for antifungal agents. *Acta Biochim Pol* 42:465-479.

Brown MS, Radhakrishnan A, and Goldstein JL. 2018. Retrospective on Cholesterol Homeostasis: The Central Role of Scap. *Annu Rev Biochem* 87:783-807. 10.1146/annurev-biochem-062917-011852

Chua NK, Howe V, Jatana N, Thukral L, and Brown AJ. 2017. A conserved degron containing an amphipathic helix regulates the cholesterol-mediated turnover of human squalene monooxygenase, a rate-limiting enzyme in cholesterol synthesis. *J Biol Chem* 292:19959-19973. 10.1074/jbc.M117.794230

Coates HW, Capell-Hattam IM, and Brown AJ. 2021. The mammalian cholesterol synthesis enzyme squalene monooxygenase is proteasomally truncated to a constitutively active form. *J Biol Chem* 296:100731. 10.1016/j.jbc.2021.100731

D'Arcy M, Fleming J, Robinson WR, Kirk EL, Perou CM, and Troester MA. 2015. Race-associated biological differences among Luminal A breast tumors. *Breast Cancer Res Treat* 152:437-448. 10.1007/s10549-015-3474-4

565 Dang EV, and Cyster JG. 2019. Loss of sterol metabolic homeostasis triggers inflammasomes -
566 how and why. *Curr Opin Immunol* 56:1-9. 10.1016/j.coi.2018.08.001
567 Ding J, Lohman K, Kritchevsky SB, Parks JS, Hoeschele INA, Nicklas BJ, Demons J, and Liu Y.
568 2022. 1428-P: Monocyte Cholesterol Metabolism during Weight Loss and Glycemic
569 Improvement. *Diabetes* 71. 10.2337/db22-1428-P
570 Ding J, Reynolds LM, Zeller T, Müller C, Lohman K, Nicklas BJ, Kritchevsky SB, Huang Z, de la
571 Fuente A, Soranzo N, Settlage RE, Chuang C-C, Howard T, Xu N, Goodarzi MO, Chen
572 YDI, Rotter JI, Siscovick DS, Parks JS, Murphy S, Jacobs DR, Post W, Tracy RP, Wild
573 PS, Blankenberg S, Hoeschele I, Herrington D, McCall CE, and Liu Y. 2015. Alterations
574 of a Cellular Cholesterol Metabolism Network Are a Molecular Feature of Obesity-
575 Related Type 2 Diabetes and Cardiovascular Disease. *Diabetes* 64:3464-3474.
576 10.2337/db14-1314
577 Einarson TR, Acs A, Ludwig C, and Panton UH. 2018. Prevalence of cardiovascular disease in
578 type 2 diabetes: a systematic literature review of scientific evidence from across the
579 world in 2007-2017. *Cardiovasc Diabetol* 17:83. 10.1186/s12933-018-0728-6
580 Ge Q, Feng F, Liu L, Chen L, Lv P, Ma S, Chen K, and Yao Q. 2020. RNA-Seq analysis of the
581 pathogenesis of STZ-induced male diabetic mouse liver. *Journal of Diabetes and its*
582 *Complications* 34. 10.1016/j.jdiacomp.2019.107444
583 Ge Q, Zhang S, Chen L, Tang M, Liu L, Kang M, Gao L, Ma S, Yang Y, Lv P, Kong M, Yao Q,
584 Feng F, and Chen K. 2018. Mulberry Leaf Regulates Differentially Expressed Genes in
585 Diabetic Mice Liver Based on RNA-Seq Analysis. *Frontiers in Physiology* 9.
586 10.3389/fphys.2018.01051
587 Gill S, Stevenson J, Kristiana I, and Brown AJ. 2011. Cholesterol-dependent degradation of
588 squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA
589 reductase. *Cell Metab* 13:260-273. 10.1016/j.cmet.2011.01.015
590 Griffiths WJ, and Wang Y. 2021. Sterols, Oxysterols, and Accessible Cholesterol: Signalling for
591 Homeostasis, in Immunity and During Development. *Front Physiol* 12:723224.
592 10.3389/fphys.2021.723224
593 Gupta N, and Porter TD. 2001. Garlic and garlic-derived compounds inhibit human squalene
594 monooxygenase. *J Nutr* 131:1662-1667. 10.1093/jn/131.6.1662
595 He L, Li H, Pan C, Hua Y, Peng J, Zhou Z, Zhao Y, and Lin M. 2021. Squalene epoxidase
596 promotes colorectal cancer cell proliferation through accumulating calcitriol and
597 activating CYP24A1-mediated MAPK signaling. *Cancer Commun (Lond)* 41:726-746.
598 10.1002/cac2.12187
599 Helms MW, Kemming D, Pospisil H, Vogt U, Buerger H, Korsching E, Liedtke C, Schlotter CM,
600 Wang A, Chan SY, and Brandt BH. 2008a. Squalene epoxidase, located on
601 chromosome 8q24.1, is upregulated in 8q+ breast cancer and indicates poor clinical
602 outcome in stage I and II disease. *Br J Cancer* 99:774-780. 10.1038/sj.bjc.6604556
603 Helms MW, Kemming D, Pospisil H, Vogt U, Buerger H, Korsching E, Liedtke C, Schlotter CM,
604 Wang A, Chan SY, and Brandt BH. 2008b. Squalene epoxidase, located on
605 chromosome 8q24.1, is upregulated in 8q+ breast cancer and indicates poor clinical
606 outcome in stage I and II disease. *British Journal of Cancer* 99:774-780.
607 10.1038/sj.bjc.6604556
608 Howe V, Chua NK, Stevenson J, and Brown AJ. 2015. The Regulatory Domain of Squalene
609 Monooxygenase Contains a Re-entrant Loop and Senses Cholesterol via a
610 Conformational Change. *J Biol Chem* 290:27533-27544. 10.1074/jbc.M115.675181
611 Huang B, Song BL, and Xu C. 2020. Cholesterol metabolism in cancer: mechanisms and
612 therapeutic opportunities. *Nat Metab* 2:132-141. 10.1038/s42255-020-0174-0
613 Jun SY, Brown AJ, Chua NK, Yoon JY, Lee JJ, Yang JO, Jang I, Jeon SJ, Choi TI, Kim CH, and
614 Kim NS. 2021. Reduction of Squalene Epoxidase by Cholesterol Accumulation

615 Accelerates Colorectal Cancer Progression and Metastasis. *Gastroenterology* 160:1194-
616 1207.e1128. 10.1053/j.gastro.2020.09.009
617 Kalogirou C, Linxweiler J, Schmucker P, Snaebjornsson MT, Schmitz W, Wach S, Krebs M,
618 Hartmann E, Puhr M, Müller A, Spahn M, Seitz AK, Frank T, Marouf H, Büchel G,
619 Eckstein M, Kübler H, Eilers M, Saar M, Junker K, Röhrig F, Kneitz B, Rosenfeldt MT,
620 and Schulze A. 2021. MiR-205-driven downregulation of cholesterol biosynthesis
621 through SQLE-inhibition identifies therapeutic vulnerability in aggressive prostate cancer.
622 *Nat Commun* 12:5066. 10.1038/s41467-021-25325-9
623 Kim JH, Kim CN, and Kang DW. 2019. Squalene Epoxidase Correlates E-Cadherin Expression
624 and Overall Survival in Colorectal Cancer Patients: The Impact on Prognosis and
625 Correlation to Clinicopathologic Features. *J Clin Med* 8. 10.3390/jcm8050632
626 La Sala L, and Pontiroli AE. 2020. Prevention of Diabetes and Cardiovascular Disease in
627 Obesity. *International Journal of Molecular Sciences* 21. 10.3390/ijms21218178
628 Li C, Wang Y, Liu D, Wong CC, Coker OO, Zhang X, Liu C, Zhou Y, Liu Y, Kang W, To KF,
629 Sung JJ, and Yu J. 2022. Squalene epoxidase drives cancer cell proliferation and
630 promotes gut dysbiosis to accelerate colorectal carcinogenesis. *Gut* 71:2253-2265.
631 10.1136/gutjnl-2021-325851
632 Li W, Jiang X, and Zhao L. 2023a. Hsa_circ_0028007 regulates the progression of
633 nasopharyngeal carcinoma through the miR-1179/SQLE axis. *Open Med (Wars)*
634 18:20230632. 10.1515/med-2023-0632
635 Li Z, Fan Q, Chen M, Dong Y, Li F, Wang M, Gu Y, Guo S, Ye X, Wu J, Dai S, Lin R, and Zhao
636 C. 2023b. The interaction between polyphyllin I and SQLE protein induces hepatotoxicity
637 through SREBP-2/HMGCR/SQLE/LSS pathway. *J Pharm Anal* 13:39-54.
638 10.1016/j.jpha.2022.11.005
639 Liu D, Wong CC, Fu L, Chen H, Zhao L, Li C, Zhou Y, Zhang Y, Xu W, Yang Y, Wu B, Cheng G,
640 Lai PB, Wong N, Sung JJY, and Yu J. 2018. Squalene epoxidase drives NAFLD-induced
641 hepatocellular carcinoma and is a pharmaceutical target. *Sci Transl Med* 10.
642 10.1126/scitranslmed.aap9840
643 Liu D, Wong CC, Zhou Y, Li C, Chen H, Ji F, Go MYY, Wang F, Su H, Wei H, Cai Z, Wong N,
644 Wong VWS, and Yu J. 2021a. Squalene Epoxidase Induces Nonalcoholic
645 Steatohepatitis Via Binding to Carbonic Anhydrase III and is a Therapeutic Target.
646 *Gastroenterology* 160:2467-2482.e2463. 10.1053/j.gastro.2021.02.051
647 Liu Y, Fang L, and Liu W. 2021b. High SQLE Expression and Gene Amplification Correlates
648 with Poor Prognosis in Head and Neck Squamous Cell Carcinoma. *Cancer Manag Res*
649 13:4709-4723. 10.2147/cmar.S305719
650 Liu Y, Lu LL, Wen D, Liu DL, Dong LL, Gao DM, Bian XY, Zhou J, Fan J, and Wu WZ. 2020.
651 MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid
652 reprogramming. *J Hematol Oncol* 13:12. 10.1186/s13045-019-0841-3
653 Mahoney CE, Pirman D, Chubukov V, Sleger T, Hayes S, Fan ZP, Allen EL, Chen Y, Huang L,
654 Liu M, Zhang Y, McDonald G, Narayanaswamy R, Choe S, Chen Y, Gross S, Cianchetta
655 G, Padyana AK, Murray S, Liu W, Marks KM, Murtie J, Dorsch M, Jin S, Nagaraja N,
656 Biller SA, Roddy T, Popovici-Muller J, and Smolen GA. 2019. A chemical biology screen
657 identifies a vulnerability of neuroendocrine cancer cells to SQLE inhibition. *Nat Commun*
658 10:96. 10.1038/s41467-018-07959-4
659 Nagai M, Sakakibara J, Wakui K, Fukushima Y, Igarashi S, Tsuji S, Arakawa M, and Ono T.
660 1997. Localization of the Squalene Epoxidase Gene (SQLE) to Human Chromosome
661 Region 8q24.1. *Genomics* 44:141-143. 10.1006/geno.1997.4825
662 Nagaraja R, Olaharski A, Narayanaswamy R, Mahoney C, Pirman D, Gross S, Roddy TP,
663 Popovici-Muller J, Smolen GA, and Silverman L. 2020. Preclinical toxicology profile of
664 squalene epoxidase inhibitors. *Toxicol Appl Pharmacol* 401:115103.
665 10.1016/j.taap.2020.115103

Nan Y, An J, Bao J, Chen H, Chen Y, Ding H, Dou X, Duan Z, Fan J, Gao Y, Han T, Han Y, Hu P, Huang Y, Huang Y, Jia J, Jiang J, Jiang Y, Li J, Li J, Li R, Li S, Li W, Li Y, Lin S, Liu J, Liu S, Lu L, Lu Q, Luo X, Ma X, Rao H, Ren H, Ren W, Shang J, Shi L, Su M, Wang B, Wang R, Wei L, Wen Z, Wu B, Wu J, Xin S, Xing H, Xu J, Yan M, Yang J, Yang J, Yang L, Yang Y, Yu Y, Zhang L, Zhang L, Zhang X, Zhang Y, Zhang Y, Zhao J, Zhao S, Zheng H, Zhou Y, Zhou Y, Zhuang H, Zuo W, Xu X, and Qiao L. 2021. The Chinese Society of Hepatology position statement on the redefinition of fatty liver disease. *J Hepatol* 75:454-461. 10.1016/j.jhep.2021.05.003

Nathan JA. 2020. Squalene and cholesterol in the balance at the ER membrane. *Proc Natl Acad Sci U S A* 117:8228-8230. 10.1073/pnas.2003388117

Padyana AK, Gross S, Jin L, Cianchetta G, Narayanaswamy R, Wang F, Wang R, Fang C, Lv X, Biller SA, Dang L, Mahoney CE, Nagaraja N, Pirman D, Sui Z, Popovici-Muller J, and Smolen GA. 2019. Structure and inhibition mechanism of the catalytic domain of human squalene epoxidase. *Nat Commun* 10:97. 10.1038/s41467-018-07928-x

Polycarpou-Schwarz M, Groß M, Mestdagh P, Schott J, Grund SE, Hildenbrand C, Rom J, Aulmann S, Sinn HP, Vandesompele J, and Diederichs S. 2018. The cancer-associated microprotein CASIMO1 controls cell proliferation and interacts with squalene epoxidase modulating lipid droplet formation. *Oncogene* 37:4750-4768. 10.1038/s41388-018-0281-5

Qian CJ, Zhou YX, Wu LK, Wang YC, Teng XS, and Yao J. 2023. Circ_0000182 promotes cholesterol synthesis and proliferation of stomach adenocarcinoma cells by targeting miR-579-3p/SQLE axis. *Discov Oncol* 14:22. 10.1007/s12672-023-00630-5

Qin Y, Hou Y, Liu S, Zhu P, Wan X, Zhao M, Peng M, Zeng H, Li Q, Jin T, Cui X, and Liu M. 2021. A Novel Long Non-Coding RNA lnc030 Maintains Breast Cancer Stem Cell Stemness by Stabilizing SQLE mRNA and Increasing Cholesterol Synthesis. *Adv Sci (Weinh)* 8:2002232. 10.1002/advs.202002232

Qin Y, Zhang Y, Tang Q, Jin L, and Chen Y. 2017. SQLE induces epithelial-to-mesenchymal transition by regulating of miR-133b in esophageal squamous cell carcinoma. *Acta Biochim Biophys Sin (Shanghai)* 49:138-148. 10.1093/abbs/gmw127

Ryder NS. 1988. Mechanism of action and biochemical selectivity of allylamine antimycotic agents. *Ann N Y Acad Sci* 544:208-220. 10.1111/j.1749-6632.1988.tb40405.x

Ryder NS. 1991. Squalene epoxidase as a target for the allylamines. *Biochem Soc Trans* 19:774-777. 10.1042/bst0190774

Saklayen MG. 2018. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep* 20:12. 10.1007/s11906-018-0812-z

Seiki S, and Frishman WH. 2009. Pharmacologic inhibition of squalene synthase and other downstream enzymes of the cholesterol synthesis pathway: a new therapeutic approach to treatment of hypercholesterolemia. *Cardiol Rev* 17:70-76. 10.1097/CRD.0b013e3181885905

Sharpe LJ, Coates HW, and Brown AJ. 2020. Post-translational control of the long and winding road to cholesterol. *J Biol Chem* 295:17549-17559. 10.1074/jbc.REV120.010723

Sharpe LJ, Howe V, Scott NA, Luu W, Phan L, Berk JM, Hochstrasser M, and Brown AJ. 2019. Cholesterol increases protein levels of the E3 ligase MARCH6 and thereby stimulates protein degradation. *J Biol Chem* 294:2436-2448. 10.1074/jbc.RA118.005069

Shen T, Lu Y, and Zhang Q. 2020. High Squalene Epoxidase in Tumors Predicts Worse Survival in Patients With Hepatocellular Carcinoma: Integrated Bioinformatic Analysis on NAFLD and HCC. *Cancer Control* 27:1073274820914663. 10.1177/1073274820914663

Simigdala N, Gao Q, Pancholi S, Roberg-Larsen H, Zvelebil M, Ribas R, Folkerd E, Thompson A, Bhamra A, Dowsett M, and Martin L-A. 2016. Cholesterol biosynthesis pathway as a novel mechanism of resistance to estrogen deprivation in estrogen receptor-positive breast cancer. *Breast Cancer Research* 18. 10.1186/s13058-016-0713-5

717 Singh A-K, Yadav D, Sharma N, and Jin J-O. 2021. Dipeptidyl Peptidase (DPP)-IV Inhibitors
718 with Antioxidant Potential Isolated from Natural Sources: A Novel Approach for the
719 Management of Diabetes. *Pharmaceuticals* 14. 10.3390/ph14060586
720 Song Y, Tian S, Zhang P, Zhang N, Shen Y, and Deng J. 2021. Construction and Validation of a
721 Novel Ferroptosis-Related Prognostic Model for Acute Myeloid Leukemia. *Front Genet*
722 12:708699. 10.3389/fgene.2021.708699
723 Souchek JJ, Baine MJ, Lin C, Rachagani S, Gupta S, Kaur S, Lester K, Zheng D, Chen S,
724 Smith L, Lazenby A, Johansson SL, Jain M, and Batra SK. 2014. Unbiased analysis of
725 pancreatic cancer radiation resistance reveals cholesterol biosynthesis as a novel target
726 for radiosensitisation. *Br J Cancer* 111:1139-1149. 10.1038/bjc.2014.385
727 Stäubert C, Krakowsky R, Bhuiyan H, Witek B, Lindahl A, Broom O, and Nordström A. 2016.
728 Increased lanosterol turnover: a metabolic burden for daunorubicin-resistant leukemia
729 cells. *Med Oncol* 33:6. 10.1007/s12032-015-0717-5
730 Stopsack KH, Gerke TA, Andrén O, Andersson SO, Giovannucci EL, Mucci LA, and Rider JR.
731 2017. Cholesterol uptake and regulation in high-grade and lethal prostate cancers.
732 *Carcinogenesis* 38:806-811. 10.1093/carcin/bgx058
733 Stopsack KH, Gerke TA, Sinnott JA, Penney KL, Tyekucheva S, Sesso HD, Andersson SO,
734 Andrén O, Cerhan JR, Giovannucci EL, Mucci LA, and Rider JR. 2016. Cholesterol
735 Metabolism and Prostate Cancer Lethality. *Cancer Res* 76:4785-4790. 10.1158/0008-
736 5472.Can-16-0903
737 Stylianou IM, Clinton M, Keightley PD, Pritchard C, Tymowska-Lalanne Z, Bünger L, and Horvat
738 S. 2005. Microarray gene expression analysis of theFob3bobesity QTL identifies
739 positional candidate geneSqleand perturbed cholesterol and glycolysis pathways.
740 *Physiological Genomics* 20:224-232. 10.1152/physiolgenomics.00183.2004
741 Sui Z, Zhou J, Cheng Z, and Lu P. 2015. Squalene epoxidase (SQLE) promotes the growth and
742 migration of the hepatocellular carcinoma cells. *Tumour Biol* 36:6173-6179.
743 10.1007/s13277-015-3301-x
744 Sun H, Li L, Li W, Yang F, Zhang Z, Liu Z, and Du W. 2021. p53 transcriptionally regulates
745 SQLE to repress cholesterol synthesis and tumor growth. *EMBO Rep* 22:e52537.
746 10.15252/embr.202152537
747 Toya T, Ozcan I, Corban MT, Sara JD, Marietta EV, Ahmad A, Horwath IE, Loeffler DL, Murray
748 JA, Lerman LO, and Lerman A. 2021. Compositional change of gut microbiome and
749 osteocalcin expressing endothelial progenitor cells in patients with coronary artery
750 disease. *PLoS One* 16:e0249187. 10.1371/journal.pone.0249187
751 Wang C-C, Yen J-H, Cheng Y-C, Lin C-Y, Hsieh C-T, Gau R-J, Chiou S-J, and Chang H-Y.
752 2017. Polygala tenuifolia extract inhibits lipid accumulation in 3T3-L1 adipocytes and
753 high-fat diet–induced obese mouse model and affects hepatic transcriptome and gut
754 microbiota profiles. *Food & Nutrition Research* 61. 10.1080/16546628.2017.1379861
755 Wang S, Dong L, Ma L, Yang S, Zheng Y, Zhang J, Wu C, Zhao Y, Hou Y, Li H, and Wang T.
756 2022. SQLE facilitates the pancreatic cancer progression via the lncRNA-TTN-AS1/miR-
757 133b/SQLE axis. *J Cell Mol Med* 26:3636-3647. 10.1111/jcmm.17347
758 Xu Y, Han W, Xu WH, Wang Y, Yang XL, Nie HL, Yao J, Shen GL, and Zhang XF. 2019.
759 Identification of differentially expressed genes and functional annotations associated
760 with metastases of the uveal melanoma. *J Cell Biochem* 120:19202-19214.
761 10.1002/jcb.29250
762 Yamamoto S, and Bloch K. 1970. Studies on Squalene Epoxidase of Rat Liver. *Journal of*
763 *Biological Chemistry* 245:1670-1674. 10.1016/s0021-9258(19)77144-0
764 Ye Z, Ai X, Yang K, Yang Z, Fei F, Liao X, Qiu Z, Gimple RC, Yuan H, Huang H, Gong Y, Xiao
765 C, Yue J, Huang L, Saulnier O, Wang W, Zhang P, Dai L, Wang X, Wang X, Ahn YH,
766 You C, Xu J, Wan X, Taylor MD, Zhao L, Rich JN, and Zhou S. 2023. Targeting

Microglial Metabolic Rewiring Synergizes with Immune-Checkpoint Blockade Therapy for Glioblastoma. *Cancer Discov* 13:974-1001. 10.1158/2159-8290.Cd-22-0455

You W, Ke J, Chen Y, Cai Z, Huang ZP, Hu P, and Wu X. 2022. SQLE, A Key Enzyme in Cholesterol Metabolism, Correlates With Tumor Immune Infiltration and Immunotherapy Outcome of Pancreatic Adenocarcinoma. *Front Immunol* 13:864244. 10.3389/fimmu.2022.864244

Yue S, Li J, Lee SY, Lee HJ, Shao T, Song B, Cheng L, Masterson TA, Liu X, Ratliff TL, and Cheng JX. 2014. Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. *Cell Metab* 19:393-406. 10.1016/j.cmet.2014.01.019

Zhang C, Zhang H, Zhang M, Lin C, Wang H, Yao J, Wei Q, Lu Y, Chen Z, Xing G, and Cao X. 2019. OSBPL2 deficiency upregulate SQLE expression increasing intracellular cholesterol and cholesteryl ester by AMPK/SP1 and SREBF2 signalling pathway. *Exp Cell Res* 383:111512. 10.1016/j.yexcr.2019.111512

Zhang HY, Li HM, Yu Z, Yu XY, and Guo K. 2014. Expression and significance of squalene epoxidase in squamous lung cancerous tissues and pericarcinoma tissues. *Thorac Cancer* 5:275-280. 10.1111/1759-7714.12087

Zhang M, Zou X, Du Y, Pan Z, He F, Sun Y, and Li M. 2023. Integrated Transcriptomics and Metabolomics Reveal the Mechanism of Alliin in Improving Hyperlipidemia. *Foods* 12. 10.3390/foods12183407

Zhang R, Deng Y, Lv Q, Xing Q, Pan Y, Liang J, Jiang M, Wei Y, Shi D, Xie B, and Yang S. 2020. SQLE Promotes Differentiation and Apoptosis of Bovine Skeletal Muscle-Derived Mesenchymal Stem Cells. *Cell Reprogram* 22:22-29. 10.1089/cell.2019.0077

Figure 1

Figure 1. Cholesterol synthesis pathway centered on SQLE

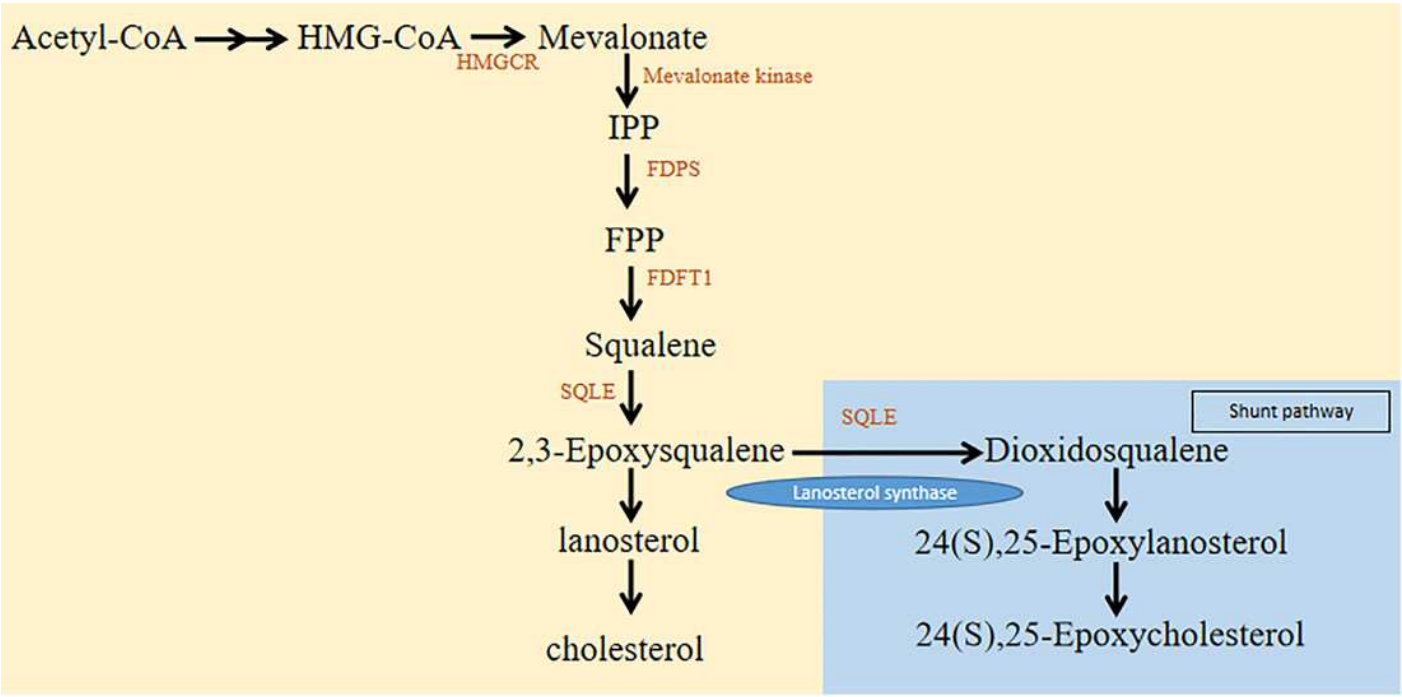

Table 1(on next page)

Table 1. Relationships among miRNAs, SQLE and cancer

Table 1. Relationships among miRNAs, SQLE and cancer

miRNA	cancer cells	miRNA expression level SQLE expression level	Function	Overexpressed	Ref
miRNA-205	Prostate cancer	Downregulated	Overexpressed	Promotion of cell proliferation and androgen receptor	[19]
miRNA-133b	Pancreatic cancer	Downregulated	Overexpressed	Promotion of cell proliferation, migration, and invasion	[20]
miRNA-133b	Esophageal squamous cell carcinoma	Downregulated	Overexpressed	Promotion of cell proliferation, migration, invasion	[21]
miRNA-579-3p	Gastric adenocarcinoma	Downregulated	Overexpressed	Promotion of cell proliferation	[22]
miRNA-584-5p	Head and neck squamous cell carcinomas	Downregulated	Overexpressed	Promotion of cell proliferation, migration, and invasion	[23]
miRNA-1179	Nasopharyngeal carcinoma	Downregulated	Overexpressed	Promotion of cell proliferation and inhibition of apoptosis	[24]
miRNA-363-3p	Pancreatic Cancer	Downregulated	Overexpressed	Promotion of cell proliferation, regulation of tumor immune cell infiltration and expression of immune checkpoints	[25]
miRNA-612	Hepatocellular carcinoma	Downregulated	Overexpressed	Promotion of cell invadopodia, EMT, migration, and invasion	[26]

Figure 2

Figure 2. The main mechanism of SQLE regulation