Point-by-point responses to the editor and reviewers

Responses to the Editor:

1. The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

   Response: All approved funding application forms have been uploaded.

2. The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

   Response: The original figure documents in PowerPoint format has been uploaded

Responses to Reviewer 1:

The review article entitled" Abnormal lipid synthesis as a therapeutic target of cancer stem cells" describes the lipid anabolism alterations that promote the survival of CSCs, including de novo lipogenesis, lipid desaturation, and cholesterol synthesis. Abstract covered main contents properly. The introduction is well organized with sufficient literature review. The authors clearly emphasize the molecular mechanism between lipid synthesis and stem cell survival, the signal transduction pathways involved. The findings summarized here are very important to the scientific community and may use in new drug development. However, there are some minor concerns to answer before considering further.

Minor concerns

1. The authors mentioned there are figures, but I do not see any figures submitted. It is better to include two to three figures in the article.

   Response: Thank you for your comments and advice. There are three figures mentioned in the article about "The origin of CSCs and the regulatory pathways involved." "The alteration of lipid metabolic pathways in tumors and CSCs." "The signaling pathways involved in lipid metabolism in CSCs.". All figures areat the end of the article.

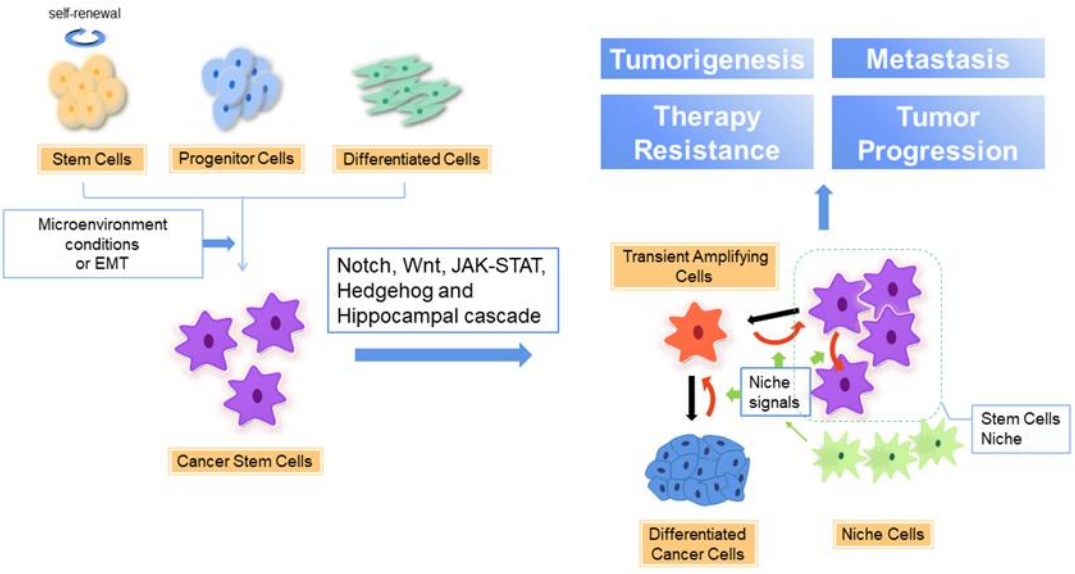


Figure 1 The origin of CSCs and the regulatory pathways involved. The origin of

CSCs and the regulatory pathways involved. There are two possible origins of CSCs, one is normal stem cells/progenitor cells, and the other is fully differentiated cells. CSCs are closely related to tumor microenvironmental factors. In the process of epithelial-mesenchymal transformation, cancer cells acquire stem cell-like characteristics. The differentiation direction of CSCs progeny is determined by niche signal, and the available niche space determines the number of progeny stem cells. When there is no space available in the niche, the stem cells divide into transient amplification (TA) cells, which divide and differentiate rapidly. At the same time, niche cells reprogram TA cells and differentiated cells into CSCs by niche signals[7]. CSCs are important subsets of tumor cells, which are regulated by a variety of signal pathways, including Notch, Wnt/β-catenin, Hippo and Hedgehog signaling, which are the main causes of cancer initiation, progression, metastasis, therapy resistance and relapse.

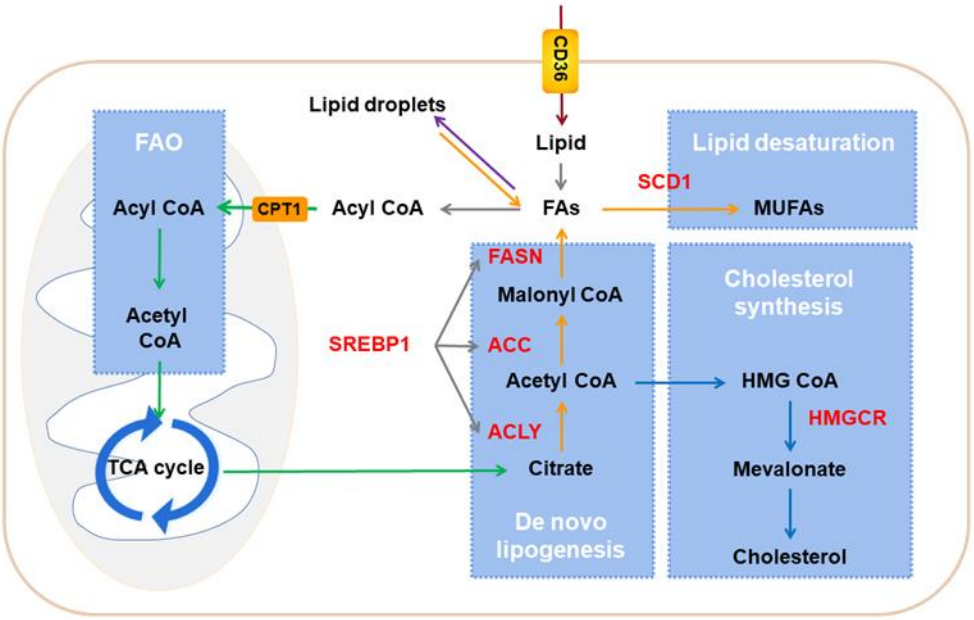


Figure 2 The alteration of lipid metabolic pathways in tumors and CSCs. CSCs enhance lipid metabolic activities, such as fatty acid synthesis, fatty acid oxidation and lipid storage to promote self-renewal and proliferation. Key enzymes that control lipid metabolism (red letters) are considered to be ideal therapeutic targets for CSCs. CPT1: carnitine palmitoyl-transferase 1; FAO: fatty acid oxidation; TCA cycle:

tricarboxylic acid cycle; CD36: cluster of differentiation 36; FA: fatty acid; FASN: fatty acid synthase; ACC: acetyl-CoA carboxylase; ACLY: ATP citrate lyase; SREBP1: sterol-regulatory element binding protein 1; SCD1: stearoyl-CoA desaturase 1; MUFA: monounsaturated FA; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase.

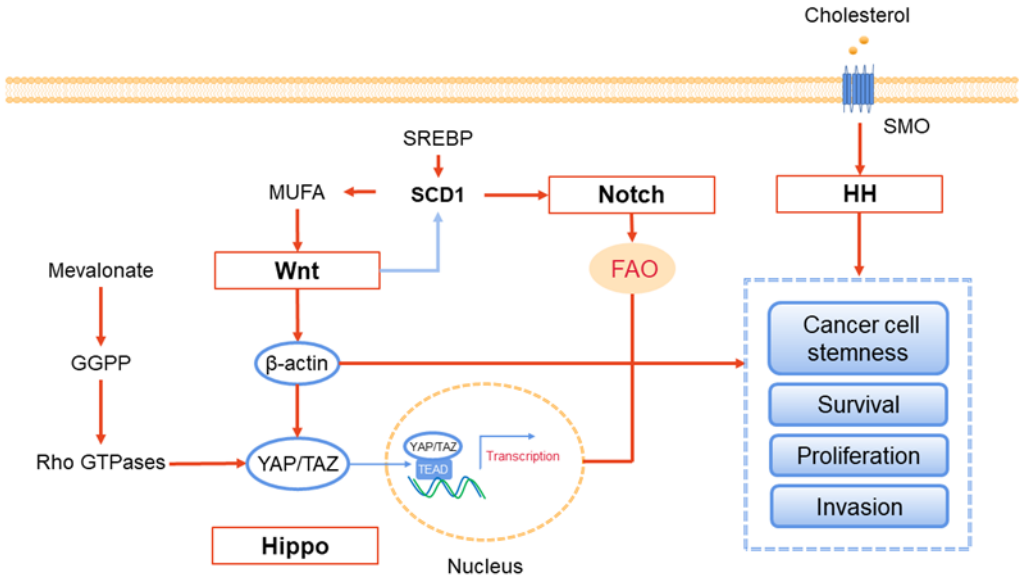


Figure 3 The signaling pathways involved in lipid metabolism in CSCs. There are four major signaling pathways, including Notch, Wnt, Hippo, and Hh signalling, involved in lipid metabolism to maintain cell stemness, sustain their survival, proliferation as well as invasion. GGPP: geranylgeranyl pyrophosphate; MUFA: monounsaturated fatty acids; YAP: yes-associated protein; TAZ: transcriptional co-activator with PDZ-binding motif; SREBP: sterol regulatory element-binding protein; SCD1: stearyl coenzyme A desaturase 1; TEAD: transcriptional enhanced associate domain; FAO: fatty acid oxidation; SMO: Smoothened; HH: Hedgehog.

2. Suggesting to add a table.

Response: Thank you for your suggestion. In the article, we summarized a table about inhibitors related to lipid synthesis enzymes of CSCS, which is also at the end of the article.

Table 1 Inhibitors related to lipid synthesis enzymes of CSCs

Metabolism type	Targeting enzyme	Drug	Cancer type	Metabolic processes or signaling pathways involved	Study type
Lipogenesis	FASN	Cerulenin	Glioma Stem Cells[68], Pancreatic CSCs[70]	FASyn	Preclinical Trial
	FASN	TVB-2640	NSCLC and breast cancer[154]	FASyn	Clinical Trial
	ACC	Soraphen A	Breast CSCs[65]	FASyn	Preclinical Trial
	ACC	ND-646	Non-small cell lung CSCs[156]	FASyn	Preclinical Trial
	ACC	Leptin	Breast CSCs[155]	TAK1-AMPK signaling	Preclinical Trial
Lipid desaturation	SCD1	CAY10566	Ovarian CSCs[51], Glioblastoma CSCs[167]	NF-κB pathway, ER Stress	Preclinical Trial
	SCD1	A939572	Liver cancer[160], etc.	MUFA synthesis	Preclinical Trial
	SCD1	MF-438	Colon CSCs[133], Lung CSCs[92]	Wnt, Notch, and YAP/TAZ signaling	Preclinical Trial
	SCD1	PluriSIn＃1	Colon CSCs[133], Liver	Wnt/β-catenin and Notch	Preclinical Trial

			CSCs[168]	signaling	
	Delta 6 desaturase	SC-26196	Ovarian CSCs[51]	Polyunsaturated fatty acids synthesis	Preclinical Trial
Cholesterol synthesis	SREBPs	25-HC or fatostatin	Colon CSCs[106]	FASyn and cholesterol synthesis	Preclinical Trial
		Pyrvinium pamoate	TNBC CSCs[161]	Cholesterol biosynthesis	Preclinical Trial
	HMGCR	Simvastatin	Breast CSCs[103]	Cholesterol biosynthesis	FDA-approved cardiovascular system drug

FASyn: fatty acids synthesis; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductas

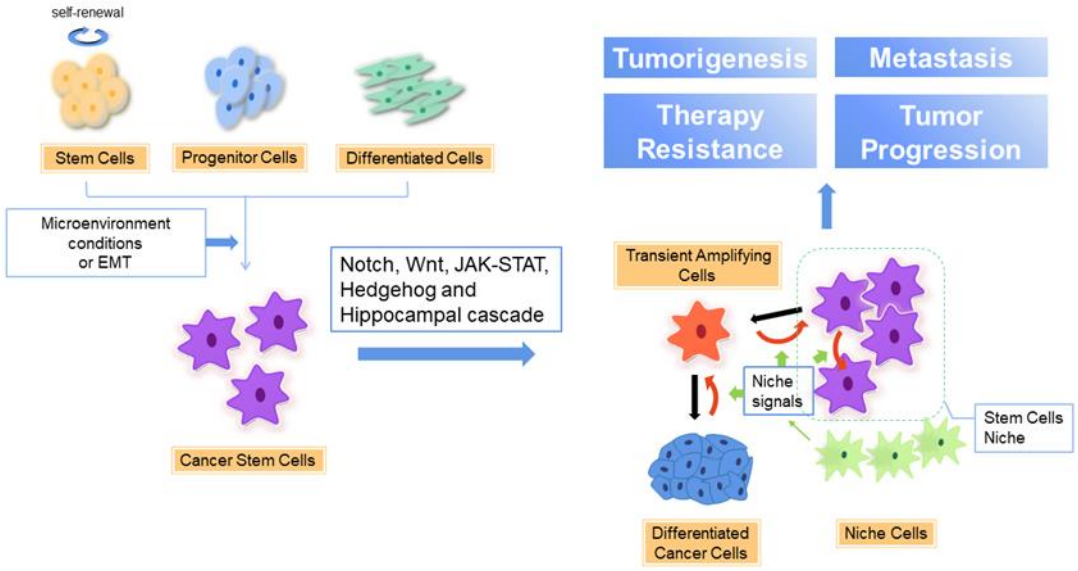
Responses to Reviewer 2:

This review manuscript addressed the important role of lipid metabolism in CSCs and its potential application in anti-CSC therapeutics. The recent relevant research advances were extensively summarized in the paper. The authors mainly discussed the lipid anabolism alterations in CSCs, the mechanism of lipid synthesis reprogramming in CSCs, and potential anti-CSC therapeutic strategies of targeting lipid synthesis. Though the review reflects the replica of the Huangcan Li review which was published in Theranostics in 2020 with subtitles and some contents (e.g. figure 1, the section of fatty acid synthase), the content widely varies with new information and recent references in the present review.

Major concerns:

1. Figure 1 did not correctly reflect CSC hierarchy. Suggest that change to the linear CSC hierarchy demonstrating the cellular plasticity among CSCs, progenitor cells and fully differentiated cancer cells (pls refer to the review 'Cancer stem cells revisited. Nat Med. Hans Clevers. 2017).

   Response: Thank you for your valuable comment. In this paper, we have added a large number of new literatures and information on the basis of previous researchers The possible origins of CSCs in Figure 1 have been revised. The transformation relationship between CSCs and fully differentiated cancer cells has been added.



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2. In introduction session, the author did not address microenvironmental conditions that may influence the functions of CSCs clearly. The content regarding to EMT seems irrelevant to the topic. Please rewrite this part, and discuss several factors involving in the TME that could fluence CSC functions with a few examples/

references.

Response: Thank you for your comment. In the introduction, we add new information on the importance of angiogenesis, hypoxic niche and extracellular matrix in maintaining the stemness of glioblastoma stem cells [1]. And myofibroblasts can secrete factors to establish the niche of colon cancer CSCs and restore the stemness of highly differentiated cancer cells[2]. We also modify the section about the link between EMT and CSCs. Many studies have shown that EMT promotes the transition from non-CSCs to CSCs [3-6].

References:

[1] Sattiraju A, et al. Glioblastoma Stem Cells and Their Microenvironment. Adv Exp Med Biol **1041**, 119-140 (2017).

[2] Vermeulen L, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol **12**, 468-476 (2010).

[3] Nieto MA, et al. EMT: 2016. Cell **166**, 21-45 (2016).

[4] Chaffer CL, et al. Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. Cell **154**, 61-74 (2013).

[5] Schmidt JM, et al. Stem-cell-like properties and epithelial plasticity arise as stable traits after transient Twist1 activation. Cell Rep 10, 131-139 (2015).

[6] Mani SA, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell **133**, 704-715 (2008).

3. Some findings discussed in the main text lack proper citation. Pls double check the text, and ensure accurate citation where needed. Just some examples: a. in 'Metabolic reprogramming in CSC' session, you need to cite the reference for each cancer type so the readers could easily appreciate for further reading if necessary. "The metabolic phenotype of CSCs may depend on the microenvironment to a great extent. Several studies conducted on a variety of cancer types, such as ovarian cancer (add citation), nasopharyngeal carcinoma (add citation), hepatocellular carcinoma (add citation), osteosarcoma (add citation), breast cancer (add citation), and glioblastoma (add citation), suggest that CSCs

showed a greater reliance on glycolysis for energy supply compared with other differentiated cancer cells in vitro and in vivo[28-32]" b. run-on sentences. Pls rewrite and make it concise. Also, pls add the citation. "The oxidative phosphorylation (OXPHOS) promotion system changes the carbon source from glucose to galactose in vitro, thus forcing pancreatic ductal adenocarcinoma (PDAC) cells to utilize OXPHOS, traditional CSC features are significantly increased, defined by the expression of multiple CSC biomarkers, enhanced activity of the NANOG promoter and self-renewal ability, and most importantly, significantly increased tumorigenicity in vivo."

Response: Thank you for your valuable advice.

a. We have checked all references and made changes according to your suggestions.

b. The sentence has been modified to "it is found that pancreatic CSCs (PaCSCs) are enriched in the oxidative phosphorylation (OXPHOS) promotion system using galactose instead of glucose as carbon source in vitro. And the CSC features are significantly increased, which are characterized by the expression of multiple CSC biomarkers, the overexpression of stem-related pathways, the enhancement of self-renewal ability and the significant improvement of tumorigenicity in vivo. Meanwhile, OXPHOS promoted the immune escape properties of PaCSCs" and added the citation.

4. Add a figure illustrating the signaling pathways involved in lipid metabolism in CSCs. It would help readers well appreciate the crosstalk among signaling pathways, lipid metabolism and CSCs.

Response: Thank you for your suggestion. We added a figure about " The signaling pathways involved in lipid metabolism in CSCs".

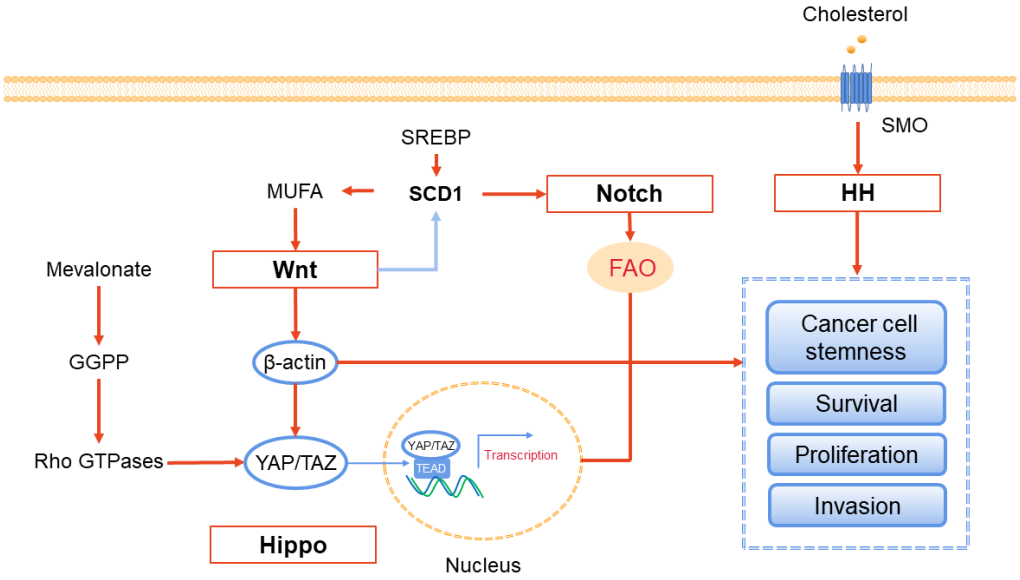

Figure 3 The signaling pathways involved in lipid metabolism in CSCs. There are four major signaling pathways, including Notch, Wnt, Hippo, and Hh signalling, involved in lipid metabolism to maintain cell stemness, sustain their survival, proliferation as well as invasion. GGPP: geranylgeranyl pyrophosphate; MUFA: monounsaturated fatty acids; YAP: yes-associated protein; TAZ: transcriptional co-activator with PDZ-binding motif; SREBP: sterol regulatory element-binding protein; SCD1: stearyl coenzyme A desaturase 1; TEAD: transcriptional enhanced associate domain; FAO: fatty acid oxidation; SMO: Smoothened; HH: Hedgehog.

5. In Table 1, pls add one column indicating the study type, either preclinical trial or clinical trial. It would be better to include the clinical trial number if possible.

   Response: Thank you for your suggestion. The study type has been indicated in Table 1 according to your suggestion.

Minor concerns:

1. Key words: change 'treatment' to 'anti-cancer therapeutic'

   Response: Thank you for your comment. The keyword "treatment" has been changed to "anti-cancer therapeutic".

2. The authors use unusually long, run-on sentences to describe much of their

reviewed material, which makes it challenging to read. The paper would be strengthened if professional editors were to improve the English usage and style.

Response: Thank you for your comment. The manuscript has been carefully checked and revised by the MedE Editing Service

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http://meditorexpert.com

EDITORIAL CERTIFICATE

(Ref. YTNVME-MS2021031410U)

We herein certify that the following document has been edited for English language by a native English speaking medical editor at MedE Medical Editing Group. The edited paper has reached grade A in language evaluation for SCI journals.

Manuscript title

Abnormal lipid synthesis as a therapeutic target of cancer stem cells

First author affiliations

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Responses to Reviewer 3:

Specific comments: 1) Page 1, Line 5: “Sun Yat-Sen University” should officially be Sun Yat-sen University [small letter in sen]. 2) P9, parag. #3: “Decreasing the level of SCD1 and MUFA synthesis promote apoptosis of leukemia and lymphoma cells [87].” [use promotes]. 3) P12, parag. #2: “The RasGTPase superfamily affects a variety of cellular processes in cancer progression and participate in EMT, tumor progression, metastasis, and chemotherapy resistance.” [use participates]. 4) P15, parag.#3: “HH signaling pathway, which is responsible for the signal transmission from [the] cell membrane to [the] nucleus, is a highly conservative pathway.” [add the before organelle termonolgy].

Response: Thank you for your valuable comment. The problems mentioned above have been modified according to your suggestions.