RESPONSE TO REVIEWERS:

Thank you for your constructive comments on our manuscript. The reviewer`s comments were very helpful for us to improve our manuscript. Our responses to the reviewer`s suggesting have been listed below.

Reviewes`s code: 03489187

Comment 1:

In the session "Oncogenes and tumor suppressor genes involved in glucose metabolic reprogramming during carcinogenesis", please prepare a new table summarizing oncogenes and cancer suppressor genes with their target genes.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have prepared a new table (Table 1) to summarize oncogenens and cancer suppressor genes with their targets as listed below:

Table 1 Oncogenes and tumor suppressor genes involved in glucose metabolic reprogramming during carcinogenesis

	Genes	Targets	Ref.
Oncogenes	HIF-1	HK1	[55]
		HK2	[55]
		GAPDH	[55]
		PKM	[55]
	Myc	LDHA	[58]
		GLUT1	[59]
		HK2	[60]
	CD147	MCT1	[62]
		GLUT1	[62]
	PIM1	GLUT1	[65]

		PKM2	[65]
Tumor suppressor	P53	GLUTs	[66]
genes	RRAD	GLUT1	[67,68]
		HK2	[67]

Ref: references, GLUT: glucose transporter, HK: hexokinase, PKM: pyruvate kinase isoform M, LDHA: lactate dehydrogenase A, MCT1: monocarboxylate transporter1, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, RRAD: ras-related associated with diabetes

We have renumbered the second table and listed all the abbreviations below the table as:

Ref: references, GLUT: glucose transporter, HK2: hexokinase2, PKM2: pyruvate kinase isoform M2, PFK: phosphate fructose kinase, LDHA: lactate dehydrogenase A, MCT1: monocarboxylate transporter1, AMPK: AMP-activated protein kinase

Comment 2:

Please prepare the legend for Figure 1. Put an ascending or downward arrow beside each modulated protein in the figure.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we revised Figure 1 and added the figure legend for Figure 1 as:

Reprogramming of glucose metabolism-related enzymes and transporting proteins in HCC. The expression of GLUT1, GLUT2, HK2, HKDC1, GAPDH, PKM2, LDHA and MCT4 are up-regulated in HCC glycolysis pathway. *GLUT* glucose transporter, *HK* hexokinase, *G6P* Glucose-6-phosphate, *GPI1* glucose-6-phosphate isomerase1, *F6P* fructose-6-phosphate, *PFK* phosphofructokinase, *FBP* fructose-1,6-bisphosphatase, *ALDA* aldolase A, *DHAP* dihydroxyacetone phosphate, *TIM* triosephosphate isomerase, *G3P*

glyceraldehyde-3-phosphate, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *PG* phosphoglycerate, *PGAM* phosphoglycerate mutase, *ENO* enolase, *PEP* phosphoenolpyruvate, *PKM2* pyruvate kinase isoform M2, *PFK* phosphate fructose kinase, *LDHA* lactate dehydrogenase A, *MCT4* monocarboxylate transporter4

Comment 3:

Abstract: First paragraph, lines 7-10. This sentence seems incomprehensible. Please rewrite clearly with terms genetic mutations, epigenetic modulations including non-coding RNAs, oncogenes, tumor suppressor genes, signaling pathways, and glycolytic enzyme protein etc.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised the sentences in Abstract section (lines 13-16) as:

The regulation of metabolic reprogramming in cancer is complex and may occur via genetic mutations and epigenetic modulations including oncogenes, tumor suppressor genes, signaling pathways, non-coding RNAs (ncRNAs), and glycolytic enzymes etc.

We have revised some of the Abstract (lines 2-7 and 11-13) as:

Despite the progress in diagnosis and treatment, the overall prognoses of HCC patients remain dismal due to the difficulties in early diagnosis and the high level of tumor invasion, metastasis and recurrence. It is urgent to explore the underlying mechanism of HCC carcinogenesis and progression to find out the specific biomarkers for HCC early diagnosis and the promising target for HCC chemotherapy.

Such metabolic reprogramming could be considered as a critical link between the different HCC genotypes and phenotypes.

Comment 4:

INTRODUCTION: Second paragraph, line 1. Is 1920 correct? Reference by Warburg [reference 4] was published in 1956.

Response to comment: We appreciate the reviewer`s suggestion.

We have carefully checked the literatures and corrected "1920s" as "1950s".

As early as the 1950s, Otto Heinrich Warburg first characterized cancer cell metabolism. Cancer cells principally use the glycolysis pathway to metabolize glucose and generate ATP whether there is sufficient oxygen present.

Comment 5:

INTRODUCTION: Second paragraph, lines 6-11. These sentences seem redundant. Please delete.

Response to comment: We appreciate the reviewer`s suggestion.

We have deleted the redundant sentences and revised the sentence (lines -7) as:

In the 1980s, the availability of ^{18}F-deoxyglucose positron emission tomography (FDG-PET) pushed the study of tumor metabolism to the climax.

Comment 6:

INTRODUCTION: Second paragraph, lines 17-20. Please list references.

Response to comment: We appreciate the reviewer`s suggestion.

According to the reviewer`s suggestion, we have added two related references in second paragraph, lines 17-20 as:

Moreover, recent studies of metabolomics offer new mechanistic insights into aerobic glycolysis and provide promising individualized therapeutic strategies by targeting the Warburg effect for treatment of HCC [13, 14].

13 **Geschwind JF**, Georgiades CS, Ko YH, Pedersen PL. Recently elucidated energy catabolism pathways provide opportunities for novel treatments in hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2004; **4**(3): 449-457. [PMID: 15161443 DOI: 10.1586/14737140.4.3.449]

14 **Ko YH**, Verhoeven HA, Lee MJ, Corbin DJ, Vogl TJ, Pedersen PL. A translational study "case report" on the small molecule "energy blocker" 3-bromopyruvate (3BP) as a potent anticancer agent: from bench side to bedside. *J BIOENERG BIOMEMBR* 2012; **44**(1): 163-170. [PMID: 22328020 DOI: 10.1007/s10863-012-9417-4]

Comment 7:

Reprogramming of gene metabolism-related enzymes and carrier protein in HCC: They used the term "carrier proteins" in the title and text. It might cause misunderstanding. I think "transporting proteins" is appropriate.

Response to comment: We appreciate the reviewer`s suggestion.

According to the reviewer`s suggestion, we have replaced all the inappropriate term "carrier proteins" with "transporting proteins" in the title and text.

Comment 8:

Reprogramming of gene metabolism-related enzymes and carrier protein in HCC: Second paragraph, lines 10-11. This sentence is unclear: "…prognostic significance of positive GLUT2 expression in HCC" ?

Response to comment: We appreciate the reviewer`s suggestion.

According to the reviewer`s suggestion, we have revised the sentence (Second paragraph, lines 10-11) as:

Another study demonstrated that positive GLUT2 predicts worse prognosis in HCC patients.

Comment 9:

Reprogramming of gene metabolism-related enzymes and carrier protein in HCC: Third paragraph, lines 10-11. This sentence seems incorrect and being

overstated. Reference 29 comprises clinical study and in vitro experiments. Repression of Wnt/beta catenin pathway was not proved directly in this article. "... inhibited HCC cellular proliferating and migration in vitro, probably by repression of ...".

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised the sentence (Third paragraph, lines 10-11) as:

The latest study showed that HKDC1, a newly discovered HK family member, was up-regulated in HCC with poorer prognosis and inhibited HCC cellular proliferating and migration in vitro, probably by repression of the Wnt/beta-catenin pathway.

Comment 10:

Oncogenes and tumor suppressor genes involve in glucose metabolic reprograming during carcinogensis: Please introduce oncogenes (definition, classification and activation) briefly in the beginning of the first paragraph.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have added some sentences to briefly introduce the oncogenes in the beginning of the first paragraph as:

Oncogenes are a number of important genes which are over-expressed or mutated in cancer cells that triggered the tumor initiation and maintained the tumor progression. Based on the biological functions, oncogenes are usually classified as growth factors, receptor tyrosine kinases, cytoplasmic tyrosine kinase, regulatory GTPase and transcription factors. The activation of oncogenes is complex and may be attributed to the genetic mutations and the tumor microenvironment. Hypoxic microenvironment is a crucial factor in the activation of some oncogenes.

Comment 11:

Oncogenes and tumor suppressor genes involve in glucose metabolic reprograming during carcinogensis: First paragraph, line 8. Please correct GAPD to GAPDH.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have corrected "GAPD" to "GAPDH":

Hamaguchi, et al. analyzed 22 glycolysis-related genes in HCC samples and identified 10 potential transcriptional targets of HIF-1α including HK1, HK2, GAPDH and PKM.

Comment 12:

First paragraph, lines 22-25. In Ref. 66, CD147 facilitate MCT1-mediated lactate export in HCC, which seems conflict to Ref.52 (MCT1 was reduced in HCC. Please comment about this discrepancy.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised these sentences as:

The latest study observed the reduced expression of MCT1 and MCT2 in HCC [50]. However, the data from another study showed that MCT1 was over-expression in HCC cells which facilitates the lactate exporting and promotes HCC glycolysis [51]. Therefore, further studies are still needed to illuminate the specific role of MCT1 and MCT2 in HCC glycolysis and progression.

Comment 13:

Signaling pathways involved in glucometabolic reprograming: First paragraph, line 4. The term "oxidative metabolism" is unclear. "oxidative phosphorylation" would be more appropriate.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised the sentence as:

The activation of AMPK by energetic stress promotes the switching from glycolysis to oxidative phosphorylation.

Comment 14:

Signaling pathways involved in glucometabolic reprograming: First paragraph, line 4. Please change the word "reverse" to more appropriate (clear meaning) one.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have corrected the word "reverse" with "inhibits".

This switching inhibits the "Warburg effect" in rapidly proliferating cells, including tumor cells to spare glucose and restore energy homeostasis.

Comment 15:

Signaling pathways involved in glucometabolic reprograming: In the first paragraph, authors described AMPK pathway. Are there any studies investigating AMPK signaling pathway in HCC cells?

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we carefully checked the literatures and found an article investigated the role of AMPK signaling pathway in Ciliary neurotrophic factor induced HCC glucose uptake. We have concluded the results from this study at the end of the first paragraph (lines 17-19) and listed the reference:

In HCC, AMPK signaling pathway was reported to participate in the ciliary neurotrophic factor (CNTF) induced GLUT4 translocation and glucose uptake [76].

76 Hu X, Zhao Y, He X, Li J, Wang T, Zhou W, Wan D, Wang H, Gu J. Ciliary neurotrophic factor receptor alpha subunit-modulated multiple downstream signaling pathways in hepatic cancer cell lines and their biological implications. HEPATOLOGY 2008; 47(4): 1298-1308. [PMID: 18307269 DOI: 10.1002/hep.22163]

Comment 16:

Signaling pathways involved in glucometabolic reprograming: First paragraph, lines 19-20. Is the statement "glycolytic carriers and enzymes" OK? "Glucose transporters and glycolytic enzymes" would be more appropriate.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised this sentence as:

Considering the effect of AMPK on the inhibition of glucose uptake in transformed cells, further investigations are greatly needed to clarify the role of AMPK on glucose transporters and glycolytic enzymes in cancer cells.

Comment 17:

Signaling pathways involved in glucometabolic reprograming: Second paragraph, line 7. The term "relies on" is unclear. Please change to other appropriate ward(s).

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised "relies on" to "mediated by":

Regulation of glucose metabolism by PI3K/Akt signaling is mediated by glycolytic enzymes.

Comment 18:

Advance in HCC therapy by targeting glucose metabolism. Lines 2 and 5. According to the title, the use of "HCC" (not "cancer") would be more clear.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we have revised "cancer" to "HCC":

The metabolic shift from oxidative phosphorylation to aerobic glycolysis in **HCC** not only provides abundant ATP for sustaining survival but also offers a favorable microenvironment for tumor progression. As one of the "hallmarks" of cancer, metabolic reprogramming relies on metabolic enzymes, thus providing many potential targets that could be exploited in **HCC** therapy.

Comment 19:

Advance in HCC therapy by targeting glucose metabolism. Flavonoids First paragraph, line 3. What is phloretin? Please describe briefly.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, sentence has been added in this paragraph to briefly describe the phloretin as:

Phloretin is a natural phenol which could be extracted from manchurian apricot and apple tree leaves.

Comment 20:

Conclusion (20) Please rewrite more concisely. For example, lines 1-6 are not necessary.

Response to comment: We appreciate the reviewer`s suggestion.

In accordance with the Reviewer's comment, we rewrote the colnlusion part as:

The reprogramming of glucose metabolism in cancer is a multi-factor and multi-step process, which can be regulated by oncogenes, oncogenic signaling pathways, and even noncoding RNAs. The developments in the study of cancer metabolism greatly enriched the understanding of carcinogenesis and afforded numerous potential targets to hit the Achilles` heel of cancer. The agents that target glycolytic enzymes directly and glycolysis-related pathways indirectly showed some promising effects in HCC prevention and therapy in the laboratory. However, the limitation of glycolysis targeted anti-cancer therapy should be noted. As multiple enzymes catalyze multiple steps in the process, there is a complex compensatory mechanism in cancer metabolism. Therefore, the inhibitors that specifically target a single modulator of glycolysis may not have a prominent or persistent effect on cancer metabolism in the human body. In the future, the effects of combination drug therapy should be evaluated. Moreover, noncoding-RNAs, which target multiple glycolysis-related enzymes and pathways, are also needed to be carefully considered in future studies.

Reviewes`s code: 00051373

Comment:

The current editorial is wonderful written and extensive explore the regulatory mechanism of glucose metabolism and the non-coding microRNA in the development of hepatocellular carcinoma. In my opinion, it should be accepting to publish without alter.

Response to comment: Thank you for your comments.

Reviewes`s code: 01453976

Comment:

This is an excellent manuscript reviewing the advances in the understanding of the reprogramming of glucose metabolism in hepatocellular carcinoma and

its associated applications in potential therapeutics. The manuscript was well written. The literature was cited appropriately. This current version of the manuscript is acceptable for publication.

Response to comment: Thank you for your comments.