RESPONSE TO REVIEWER COMMENTS:

REVIEWER COMMENTS ARE IN ITALICS

OUR RESPONSES FOLLOW AND ARE IN NORMAL FONT (IN SOME CASES, PHRASES THAT SHOULD BE EMPHASIZED ARE IN BOLD)

Reviewer #1

(Remarks to the Author)

1, COMMENT: *"However, is PKCδ as a pivotal gene that affects the progression of liver cancer, or provide a new biological marker for the diagnosis and a molecular target for treatment of liver cancer in the future?*"

OUR RESPONSE: We thank you for providing important insights. In this revision, we consider PKCδ to be a critical gene for liver cancer. The important point is that PKCδ secretion occurs in liver cancer cells. In fact, overexpression of PKCδ in cancer tissues has been reported, suggesting that a substantial amount of PKCδ may be present extracellularly. Extracellular PKCδ binds to GPC3 in the autocrine machinery and increases proliferation, which may contribute to tumor growth. The fact that the antibody administration caused shrinkage of tumor in xenograft mice supports our opinion. These findings suggest that PKCδ is important for liver cancer and provides the concept for a therapeutic target.

Additionally, we found that PKCδ in the blood was elevated in liver cancer. We compared the ability of PKCδ to discriminate liver cancer with AFP and PIVKA-II, which are known biomarkers, and found PKCδ to be superior although the number of N is currently small. This suggests that PKCδ may be a promising blood biomarker.

We have included a sentence in a revision as follows;

Page 13, line 11; "This high tendency in serum PKCδ levels was also noted in a limited number of AFP- and PIVKA-II-negative liver cancer patients."

Reviewer #2

(Remarks to the Author)

1, COMMENT: *"Most of the references were more than 10 years ago. The authors should try to*

summary the lasted developments. The more recent references should be added."

OUR RESPONSE: Thank you for making an important point. Most of the recent reports on PKC in liver diseases have been focused on intracellular activity. In this review, we describe that induction of activity at the plasma membrane occurs in chronic hepatitis and hepatic cirrhosis. Six papers from 2018-2020 were added for references.

We have enlarged some sentences as follows;
Page 10, line 2; "Indeed, liver damages have been reported to induce inflammation and PKCδ translocation to the plasma membrane[62, 63]. PKCδ activation has been observed in tissues of patients with non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), and mouse model of hepatic cirrhosis[64-67]."

2, COMMENT: *"In Table 1. The relation between subcellular localizations and functions of PKCδ in liver cancer, the Function and Mechanisms were too simple. Please use a sentence rather than a word.*"

OUR RESPONSE: We thank you for providing important insights. We have given the sentence in the Function and Mechanism section of table1. The table is shown below. The table is also attached at the last of the Revised Figures file.

Response	Localization	Function	Mechanisms
ANXA3 expression	cytosol/plasma membrane	interacts with PKCδ and inhibits apoptosis	p38MAPK activation
ROS	nucleus	activates PKCδ and induces apoptosis	activates caspase 3 and induces cleavage of PKCδ
Claudin-1	cytosol/plasma membrane	enhances the ability to cell migration/invasion	induces c-Abl-PKCδ signaling

mtROS	plasma membrane	induces gene expression for cell migration	triggers oxidation of HSP60 and then induces MAPK activation
HIF-2α expression	cytosol/plasma membrane	induces cell migration	phosphorylates PKCδ at Tyr311
HSP27 expression	cytosol/plasma membrane	inversely correlates with tumor malignancy	p38MAPK activation by PKCδ induces phosphorylation of HSP27
No response	extracellular space/cell surface	enhances cell proliferation	activates MAPK signaling

3, COMMENT: *"For STRUCTURAL FEATURES OF PKCδ, maybe the authors can add a protein structure figure to show its structure clearer."*

OUR RESPONSE: We thank you for pointing out. At present, the structure of PKCδ is not known precisely as a whole molecule, because the regulatory and kinase regions are known only separately. PKCδ is a unique protein with many domains, including lipid binding, intermolecular binding, and nuclear localization signal distributed throughout the protein. In this respect, we believe that the current style, which is closer to the amino acid structure, has the advantage of accurately describing the domain organization.

4, COMMENT: *"For different section level, please use different font size or add section level numbers, such as format like 2.3."*

OUR RESPONSE: Thank you for your advice. As you suggested, I have enlarged the font size of the phrase indicating the section level.

We have enlarged some phase as follows;

Page 3, line 14; “**INTRODUCTION**”

Page 4, line 21; “**STRUCTURAL FEATURES OF PKCδ**”

Page 6, line 3; “**FUNCTIONAL FEATURES OF INTRACELLULAR PKCδ IN LIVER CANCER**”

Page 9, line 14; “**SUBCELLULAR LOCALIZATIONS AND FUNCTIONS OF PKCδ**”

Page 15, line 6; “**CONCLUSION**”

5, COMMENT: “*In Figure 3, was it one cell? Was the line the cell membrane? It can be improved by adding more explanation words and colors.*”

OUR RESPONSE: Thank you for your suggestion. I apologize for the confusing figure. This picture illustrates a single cell, and the line represents the cell membrane. Therefore, I added the word "plasma membrane" to the part adjacent to the line.

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(Remarks to the Author)

1, COMMENT: *"Authors found that PKCδ in the blood was elevated in liver cancer, had compared the ability of PKCδ to discriminate liver cancer with AFP and PIVKA-II, which are known biomarkers, and found PKCδ to be superior although the number of N is currently small. How to suggesting or confirmed that PKCδ may be a promising blood biomarker. Is this PKCδ level in blood or in liver tissues? or in AFP-NEGATIVE or lower level HCC?*"

OUR RESPONSE: We thank you for providing important insights. First of all, we have confirmed the detection of PKCδ in blood not only by ELISA but also by immunoblotting. As for the availability of serum PKCδ, we have found that serum PKCδ is significantly higher in AFP-negative and/or PIVKA-negative liver cancer patients than in patients with chronic hepatitis or cirrhosis, or healthy individuals. These findings are reported in the cited reference Yamada et al, Cancer Res, 2021.

2, COMMENT: "*Many studies have shown that PKCδ promotes the survival of multiple types of cancers, including non-small cell lung cancer, breast cancer, pancreatic cancer, chronic lymphocytic leukemia, and liver cancer. How about the PKCδ expression as a promising circulating marker for HCC?"*

"

OUR RESPONSE: Thank you for making an important point. Almost all of the papers show intracellular expression of PKCδ, and we demonstrate that the extracellular secretion of PKCδ is specific to liver cancer. The extracellular secretion of PKCδ is a unique phenomenon that we have discovered for the first time, and we consider it to be of great significance in explaining the pathogenesis of liver cancer. We are now investigating the mechanism of PKCδ secretion, which is thought to be specific to liver cancer.