Dear editor in-chief and reviewers:

We gratefully appreciate for your valuable comments. Each suggested revision and comment, brought forward by the editors and reviewers is accurately incorporated and considered. Then we make a point-by-point response to all the issues that are raised in the peer-review report, and the revisions are indicated in the manuscript.

Reviewer 1:

Specific Comments to Authors:

In this review, the multiple roles of Smad4 have been summarized, including its identification, basic structure, expression, and regulation, and the indispensable role of in the TGF-β signaling pathway. The author further detail the function of Smad4 in regulating tumorigenesis, cell stemness as well as drug resistance, and the usage of a single blockade of Smad4 or in combination with multiple immunotherapy regimens to draw attention to antitumor potential as a target for immunotherapy.

Comment 1: The author have reviewed the opposite roles of Smad4 in the process of pancreatic cancer and hepatocellular carcinoma, but the specific mechanisms by which Smad4 affects tumor development are not completely clarified.

Reply 1: Thank you for your valuable comments. We have supplemented the specific mechanisms in which Smad4 is involved in tumorigenesis. The changes we made, which have been marked in red font in this word. "As a tumor suppressor gene, Smad4 exerts its inhibitory effect on tumor cells primarily via the canonical TGF-β signaling pathway[46]. The mechanism of this inhibition, which prevents carcinogenesis, involves the role of Smad4 in inhibiting the tumor-promoting activity of proinflammatory cytokines, inducing the cell cycle arrest and promoting the apoptosis through activating the TGF-β/BMP-

Smad4 axis. However, once the Smad4 gene is mutated, TGF-β cannot induce G1 or G2 cell cycle arrest and switch from tumor suppressor to tumor promoter, leading to tumor growth and metastasis[10, 47]"

In addition, we have added the simple description of three different mechanisms, which may be related to Smad4 deletion in the promotion of pancreatic cancer.

"Therefore, Smad4 dysfunction may be considered an advanced event in pancreatic cancer. Mechanically, the behavior that Smad4 deletion accelerates the progression of pancreatic cancer may be related to the increased expression of HNF4G, PAR-4 and PGK-1[51-53]."

Comment 2: Although Smad4 plays its tumor-suppressing role mainly through the TGF-β/Smad signaling pathway, Smad4 inactivation may also affect stem cells behaviors through the BMP/Smad pathway, thus accelerating tumor development, which should be further confirmed.

Reply 2: Thanks a lot for your kind comments. We have added the relationship between BMP signaling and stem cell fate decisions, and clarified that Smad4 may affect stem cell proliferation and differentiation through the BMP/Smad pathway. The all changes we have made in the manuscript are marked in red font below.

"BMP signaling regulated stem cell activation during hair regeneration[65]. In addition, BMP combines with leukemia inhibitory factor (LIF) to maintain the self-renewal of embryonic stem cells[66]. Subsequent studies documented that stem cell fate decisions induced by BMP may be related to Smad4. BMP/Smad axis regulated the proliferation and differentiation of alveolar stem cells. BMP suppressed the proliferation of alveolar type 2 epithelial cells (AT2s), while antagonists promoted the self-renewal of AT2s at the expense of differentiation[67]. Besides, BMP restricts the self-renewal of intestinal $Lgr5^+$

stem cells by Smad4-mediated transcriptional repression and thus prevents excessive proliferation of epithelial cells[68]. Therefore, the inactivation of Smad4 may counteract the inhibitory effect of BMP on stem cell proliferation, contributing in this manner to the occurrence of cancer cells."

Comment 3: There are few studies on the application of Smad4 in combination therapy, and the precise mechanism of combination therapy has not been revealed.

Reply 3: According to your professional suggestions, we searched the studies on the application of Smad4 in combination therapy on the PubMed, and found that only two literatures meet our requirements. Therefore, we quoted the two articles in our manuscript, and the changes we have made are marked in red font in this word. You can also find the revisions in our manuscript. We really appreciate all of your valuable comments and supports.

"Mariathasan and coworkers[102] showed that the combined application of TGF-β inhibitors together with anti-PD-L1 antibodies decreased the TGF-β level and promoted the infiltration of T-cells into tumor cells, thereby enhancing anti-tumor immune response and leading to tumor apoptosis. Kassardjian and Wang[103] found Smad4-positive tumors had better response to neoadjuvant therapy, and the lymph node metastasis rate of Smad4 positive tumors was significantly lower, suggesting Smad4 plays an important role in neoadjuvant therapy. In our previous research[104], we combined oncolytic virus therapy and targeted gene therapy to design a new oncolytic adenovirus CD55-Smad4, which can stably produce Smad4 protein in vitro and in vivo. CD55-Smad4 significantly inhibited proliferation, metastasis, and stemness of CRC cells. All these show that the combination therapy targeting Smad4 has great potential."