Answering Reviewers

Review 1

Thank you very much for your comments, we have revised our manuscript according to your questions and suggestions:

1) Abstract: Methods is too long, while Results should be written in detail.

The Methods have been shortened and the Results have been written in detail, with word count more than 120.

2) Introduction: The last paragraph is a conclusion of this study, which should not be written in Introduction.

We have deleted the last paragraph of Introduction and added one sentence to lead to our study.

3) Immunohistochemistry: How many experienced pathologists diagnosed the staining of PAR2? In addition, the antibody used in this study should be demonstrated.

These details have been added in the Materials and Methods part.

4) Results: The authors stated that PAR2 could predict the patient prognosis. Then, it should be analyzed whether PAR2 expression level by IHC is an independent factor or not by Cox-hazard model.

Multivariate Cox regression analysis revealed that PAR2 was an independent prognostic marker for the OS time of HCC patients (hazard ratio, 1.814; P = 0.041) (Table 3).

5) In IHC study, which part of the cancer cells was stained, nucleus, cytoplasm, or membrane? In addition, the patient distribution of IHC score should be shown (score 0, __ patients; score 1, __ patients, …).

The cytoplasm membrane was stained in IHC study; this information was added to the first part of Results. The patient distribution of IHC score was shown in Table 1.

6) In wound healing assay, rate of decrease of the wound breadth should be shown.

In wound healing assay, the area of occupation in different groups have been

shown in Figure 4.

Review 2

Thank you very much for your kind suggestions, especially the part concerning the mechanisms that how did PAR2 induce EMT in HCC cells. We have revised our manuscript according to your comments:

Major Comments:

The manuscript implies that PAR2 promotes EMT in HCC cells partly by activating the ERK signaling pathway. It is unclear if this ERK activation is constitutive or occurs in response to growth factors present in the serum. The authors could examine if growth factors such as HGF promotes PAR2-mediated EMT. In this context, the authors could quote a recent page published in WJG on HGF-induced EMT in HCC cells (World J Gastroenterol. 2017 Sep 28;23(36):6639-6649).

This is a very attractive point about the upstream of PAR2. As this study mainly focused on the downstream signaling of PAR2, we discussed this possibility in the Discussion part and quoted the recent published paper (World J Gastroenterol. 2017 Sep 28;23(36):6639-6649). In our following study, we will focus on this upstream aspect of PAR2.

It has been reported in several previous studies that EMT is modulated by the induction of different transcription factors (SNAI1, SNAI2, ZEB1, ZEB2, TWIST1). Particularly, ERK has been implicated in inducing EGR-1 (EMBO J 2006; 25: 3534-3545). It would be informative if the authors could examine EGR-1 expression is modulated by PAR-2.

We examined EGR-1 and Snail expression in different HCC cell clones and found that EGR-1 is activated by ERK, then activated EGR-1 promoted Snail expression (Figure 7).

In the wound healing assay, the increased wound closure caused by overexpressed PAR2 (Fig. 4C) could result from increased cell proliferation. To avoid this complication, it would be necessary to inhibit cell growth (for example using hydroxylurea; Ref: J Hepatol 2011; 55: 1300-1308) and

evaluate cell migration alone. However, the increased matrigel invasion caused by PAR2 is supportive of increased EMT.

After the wounds were created, cells were cultured in FBS-free medium, which was described in the Materials and methods part. So cell proliferation will be inhibited and will not influence wound closure. What's more, as is shown in Figure 3, cell proliferation ability was almost the same in the first 2 days in different cell clones, so the wound closure will not be affected by cell proliferation.

Minor comments:

First paragraph of the discussion is redundant as most of the points are already described in the introduction. Instead, it would be nice if the authors could suggest some future line of research based on their findings.

We have discussed some future line of research based on our findings in the fifth paragraph of Discussion part.

The author could indicate the rationale behind the use of HepG2 and SMMC-7721 cell lines in this study.

HepG2 and SMMC-7721 are the most common HCC cell lines and PAR2 played important role in these cell lines instead of others, so we chose these cell lines in our study.

Review 3

Major points

1) Basically, PAR2 is activated (without its own ligand) via its N-terminal cleavage by several proteases. I agree with PAR2's role in in vivo model (such as xenograft model) since many proteases would exist in transplanted tumors. However, can HCC cell lines produce active form of protease? The authors had better refer the others' paper reporting the protease production by HCC or other cancer cell lines.

This is a very interesting direction for the following study of PAR2, we have discussed it in the fifth paragraph of Discussion part. PAR2 is activated by several proteases especially serine proteases, many study reported that serine proteases played very important role in HCC cells, so if serine proteases are responsible for PAR2 activation is worth researching in the following study.

2) From this study results, I strongly convince that PAR2 promote HCC cells' EMT. However, what subcellular molecules are responsible for EMT? How about SNAIL or SLUG?

It has been reported that ERK was implicated in inducing EGR-1 (EMBO J 2006; 25: 3534-3545), then promoted Snail expression. We examined EGR-1 and Snail expression in different HCC cell clones and found that EGR-1 is activated by ERK, then activated EGR-1 promoted Snail expression. So Snail is responsible for PAR2 induced EMT (Figure 7).

Minor points

1) In the abstract part, the sentence "PAR2 could promote proliferation and metastasis of~" is not understandable. The authors performed overexpression or knockdown of PAR2 by lentivirus-mediated RNA interference. Therefore, the authors had better describe the method of PAR2 gene manipulation in the abstract.

We have revised the Abstract according to your suggestion and the editor'advise.

2) What cell expresses PAR2 in HCC tissue? The authors had better explain PAR2-expressing cell and the intracellular localization of PAR2.

It is reported that HCC cells and hepatic stellate cells (HSCs) (Mol Cancer 2016;15:54.) could express PAR2 in HCC tissue. We have described the intracellular localization of PAR2 in the first part of Results.