March 13, 2014

Dear Editor,

Thank you and the reviewers for the encouraging comments on our manuscript and the constructive criticism.

Please find enclosed the edited manuscript in Word format (file name: manuscript_revised.doc).

Title: Distinct antifibrogenic effects of erlotinib, sunitinib and sorafenib on rat pancreatic stellate cells
Author: Anne Elsner, Falko Lange, Brit Fitzner, Martin Heuschkel, Bernd Joachim Krause, Robert Jaster

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 8215

To meet the reviewers concerns, we have performed additional experiments and included new data into the manuscript (new figures 4 and 5).

The novel results show that sorafenib and sunitinib strongly inhibit collagen synthesis in pancreatic stellate cells (both drugs) and reduce transforming growth factor beta 1 levels in culture supernatants (sorafenib only). Together, they provide further evidence for antifibrogenic effects of tyrosine kinase inhibitors and support the conclusions that were drawn in the manuscript. The manuscript has been revised thoroughly according to the suggestions of the reviewers. Furthermore, the format has been updated. The changes in the manuscript are highlighted in yellow.

Below, we provide a point-to-point response to all comments of the referees.

We hope that the manuscript, in its revised form, is now acceptable for publication in WJG as a brief or original article.

Sincerely yours,

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Point-to-Point Response:

Reviewer 1:

The author's conclusion that the tested SMI display antifibrogenic effects in vitro, which should be further evaluated in preclinical studies seems to be good and promising, but as author's statement, in vivo efficacy should be added in the current results of in vitro study.

We thank the referee for the advice. The study was designed as an *in vitro* investigation with the aim to gain mechanistic insights into the action of the investigated kinase inhibitors at the molecular level. Animal experiments were beyond the scope of the current study but will be the next logical step in follow-up investigations. We have however performed a variety of additional *in vitro* experiments to address the questions raised by the referee (see below).

The final surrogates are prerequsitie including
1) real changes of collagen and SMA

We have now analyzed type I collagen and α-SMA on the protein level as well. The immunoblot data (new Fig. 4) indicate a strong inhibition of collagen synthesis in pancreatic stellate cells by both sunitinib and sorafenib, thereby confirming the results on the mRNA level. With respect to α-SMA, all three drugs showed little effect. For erlotinib and sunitinib, this result is in agreement with the mRNA expression data. For sorafenib, the data (which also include the results of confocal imaging; Fig. 6), are more heterogeneous. This is discussed in detail the revised manuscript.

2) real measurements of fibrosis markers in addition to current SMA
In addition to α-SMA, we have now measured protein levels of type I collagen as one key marker in pancreatic fibrosis (see point 1).

3) the changes of TGF-beta before and after kinase inhibitor
To address the question raised by the reviewer, TGF-β1 levels in tissue culture supernatants were measured by ELISA. Of the three drugs, only sorafenib significantly reduced the concentration of TGF-β1 in the supernatants (new Fig. 5). Since the drug did not inhibit mRNA expression of TGF-β1 but displayed growth-inhibitory effects, we consider the reduction of TGF-β1 protein levels as an indirect effect of sorafenib that is linked to its antiproliferative action.

4) presentation of confocal imaging in addition to the changes of molecules.
The effects of sorafenib on α-SMA were also studied by confocal microscopy (Fig. 4). Together with the mRNA and immunoblot data, they are evaluated the sections Results and Discussion. We did not include more confocal microscopy data since they did not provide additional mechanistic insights into the action of the investigated drugs.

Together, the new data provide further support for the hypothesis that sorafenib is the most efficient antifibrotic drug of the three investigated kinase inhibitors. We would like to thank the referee for the suggestions, which were helpful to sharpen our conclusions.

Reviewer 2:

There is still no specific antifibrotic therapy available for clinical application. This study explored the antifibrogenic effects of three clinically available small molecule kinase inhibitors (SMI), erlotinib, sunitinib and sorafenib on PSC and analyzed the basis of their action. It is found that these three SMI showed distinct antifibrogenic effects on PSC. It is showed that sorafenib and sunitib, but not erlotinib, efficiently blocked activation of the AKT pathway; and erlotinib and sunitinib, but not sorafenib, significantly reduced the expression of transforming growth factor-β1. It is helpful for us to evaluated the antifibrogenic effects of the tested SMI in preclinical studies. This is a well conducted and well written study. The experiments are described in detail, the results are shown nicely and the figures are impressive.

We thank the referee for these encouraging comments.