

June 17, 2013



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3510-revised version.doc).

**Title:** Smoothelin, a new marker to determine the origin of liver fibrogenic cells.

**Author:** Sébastien Lepreux, Christelle Guyot, Fabrice Billet, Chantal Combe, Charles Balabaud, Paulette Bioulac-Sage, Alexis Desmoulière

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 3510

**The manuscript has been improved according to your suggestions:**

**Commentaire [1]:** *It's too short. Please add it to 80 words. Thank you!*

This part (Methods of the abstract) has been modified according to this commentaire.

**Commentaire [2]:** *It's too short. Please add it to 120 words. Thank you!*

*Authors should present exact P value where necessary and must provide relevant data to illustrate how it is obtained, e.g.,  $6.92 \pm 3.86$  vs  $3.61 \pm 1.67$ ,  $P = 0.002$ . Otherwise, it is not accepted.*

This part (Results of the abstract) has been modified according to this commentaire.

The instructions for the redaction of the abstract have been respected: AIM (no more than 20 words); METHODS (no less than 80 words); RESULTS (no less than 120 words); CONCLUSION (no more than 26 words).

**Commentaire [3]:** *Please reformat all the reference numbers like this one ([1] → ctrl+shift+=). Please check throughout. Normal line space is required. Thank you!*

All the reference numbers have been reformatted according to this commentaire.

**Commentaire [4]:** *Information is lost during format conversion, please add it.*

This has been done.

**Commentaire [5]:** Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

PMID (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>)

DOI (<http://www.crossref.org/SimpleTextQuery/>) (Please begin with DOI: 10.\*\*)

For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.

When provided, PMID and DOI information has been added.

**Commentaires [6] [7] [8]:** Please use uniform legends for the same subjects. For example: Figure 1 Total title. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...etc.

The legends of the figures have been reformatted.

A summary (core tip) of less than 100 words to outline the most innovative and important arguments and core contents have been included.

**In addition, the manuscript has been improved according to the suggestions of reviewers:**

**Reviewer number 00503628**

*"Colocalization by confocal microscopy... may be convincing."*

As suggested by the reviewer, we have performed confocal microscopy to confirm the expression of both the proteins, smoothelin and  $\alpha$ -smooth muscle actin, in the same cells. Confocal microscopy analysis brings conclusive evidence that myofibroblasts express smoothelin.

In addition, a quantitative evaluation of the cells expressing  $\alpha$ -smooth muscle actin alone or  $\alpha$ -smooth muscle actin and smoothelin has been included.

This information has been added together with a figure (Figure 3) illustrating the co-expression of  $\alpha$ -smooth muscle actin and smoothelin in the same cells.

*"At the very least, demonstrating that the antigen is in fact smootheling may be a significant improvement."*

We underline that the antibodies used in this work have been extensively used and that their specificity has been clearly documented.

**Reviewer number 00504232**

*"Nonetheless, it could be more convincing if integrated by immunohisyochemistry experiments showing a co-localization of the markers alpha-SMA and smoothelin in the same cell. The immunohistochemistry experiments should be integrated by immunofluorescence analysis of alpha-SMA and smoothelin, to highlight their co-localization in the cytoskeleton of the same cell."*

As previously indicated, double immunofluorescence has been performed to highlight the co-localization of  $\alpha$ -smooth muscle actin and smoothelin in the same cells.

*"The immunohistochemical detection of the fibronectin ED-A isoform, also involved in myofibroblast differentiation, could also be helpful to support the data."*

It is well known that the ED-A isoform of fibronectin is expressed by myofibroblasts. However,  $\alpha$ -smooth muscle actin remained the "gold standard" marker to characterize fully differentiated myofibroblasts.

**Reviewer number 00503442**

We thank the reviewer for all these suggestions. We agree that the number of cases that we have investigated is low. However, this brief article 1) underlines that myofibroblasts can derive from smooth muscle cells particularly in advanced stages of fibrosis/cirrhosis and 2) demonstrates that smoothelin is an interesting biomarker to determine the state of fibrosis of a pathological liver.

We thank the reviewer for his/her positive evaluation of our work (classification Grade B).

I hope that this new version of our manuscript will be accepted for publication in the *World Journal of Gastroenterology*.

I am looking forward to hearing from you.

Many thanks.

Sincerely yours,

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