Author's response to reviews

Title: The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion

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Version: 4 Date: 9 June 2010

Author's response to reviews: see over
June 8, 2010

Dear Editor,

We are submitting our revised manuscript addressing the reviewer critiques.

In addition, I have a very special request and was wondering if you might be able to help. My NIH R01 competing renewal is undergoing review this current cycle and the final day to submit supplementary information for that process is June 15th. This manuscript is a key component of that review. If accepted, a rapid communication of this acceptance would help me out immensely in the grant review process, and if I could submit this information to NIH by June 15th that would make a significant difference in my favor. I’m hoping that if at all possible you might maximally expedite this re-review to determine if our manuscript is now suitable for publication.

Thank you for your understanding of this unique request and for your assistance.

Kind Regards,

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**Referee 1**

**Reviewer's report**

**Title:** The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion

**Version:** 3 **Date:** 3 April 2010

**Reviewer:** Jan Gettemans

**Reviewer's report:**
OK now

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that i have no competing interests

Referee 2

Reviewer's report
Title: The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion

Version: 3 Date: 8 April 2010
Reviewer: Eduard Ryschich

Reviewer's report:
Additional experiments and analyses have sufficiently improved the manuscript quality, although not all comments were thoroughly addressed. On my opinion, the manuscript has achieved the level of potential acceptance.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'

Referee 3

Reviewer's report
Title: The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion

Version: 3 Date: 7 April 2010
Reviewer: Steven Akiyama

Reviewer's report
The manuscript by Kamarajan, Garcia-Pardo, D'Silva, and Kapila “The CS1 segment of fibronectin is involved in human OSCC pathogenesis by mediating OSCC cell spreading, migration, and invasion” is a revised version of a previously submitted manuscript. It describes the localization of the fibronectin alternatively spliced CS1 segment in human oral squamous cell carcinoma (OSCC) and characterization of possible functions of the CS1 segment. Immunohistochemical analyses were performed with normal human and oral squamous cell carcinoma tissue. OSCC cells were also assayed in cell spreading, migration, and invasion. This revised manuscript is of only marginal improvement because the authors appear to have been less than enthusiastic about following the recommendation of the reviewers. Thus, this reviewer recommends that publication be declined pending major compulsory revisions as recommended in the original reviews. Specific examples of excellent recommendations that would have made this paper much, much stronger had they been followed include:

1. The authors still need control panels in Figs 1B, plus data in Table 1 to show that total fibronectin is not changed in the tissue samples or to show that the changes in the amount of CS-1 in the tumors is not due to there simply being differing amounts of fibronectin present in these samples. The revised figures show only blots of total fibronectin expressed by the expressed by OSCC cells and normal keratinocytes, not the tissues.

To address this concern, we re-examined CS1 expression along with fibronectin expression in serial sections of normal and OSCC tissues. There were significant differences in CS1 but not in total fibronectin expression between normal and OSCC tissues. This new data is included in Figure 1C.

2. The analysis of tumor cell adhesion to substrates prepared from immobilized CS-1 and control peptides really needs to be done. The authors’ claim that this work is “beyond the scope of this current study” is, in the opinion of this reviewer, without merit. Two papers from 20 years ago describe how to do this (Humphries et al, (1987) J Biol Chem 262:6886 and Humphries et al, (1986) J Cell Biol.103:2637). If the authors were able to procure true ‘scrambled” peptide for a new set of experiments, they should be able to get the peptides for
the analysis of tumor cell adhesion to immobilized peptide substrates as described in the two Humphries papers.

In response to this query, we further confirmed the ability of CS1 peptides to mediate spreading using immobilized peptides. OSCC cells were plated onto surfaces coated with immobilized peptides that were coupled with BSA. Under these conditions, significant cell spreading was again observed in CS1-BSA coated wells compared to those coated with Scr-BSA, whereas wells coated with CS1 blocking peptide-BSA (VLA4 inhibitor) inhibited OSCC spreading. This additional data is included in Figure 2C.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.