Reviewer's report

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

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Reviewer: Eduard Ryschich

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This manuscript evaluates the expression of CS1 segment of fibronectin in OSCC tissue and studied its relationship to the migratory activity of tumor cells as well as to FAK function in vitro. The subject of the study is up-to-date and can be of interest for researchers in the field of oral oncology. However, mechanisms of CS1 action were evaluated superficially and there are also a number of other concerns.

Major points:

1. The authors analysed the CS1 expression in tissue microarrays and tried to use the intensity of immunohistochemical staining to compare CS1 expression between different groups. This kind of analysis is questionable, since the intensity of the staining is a very variable and subjective parameter. Furthermore, a small size of the tissue sample in microarray does not represent a total tumor, whose antigen distribution can be very heterogeneous. To make solid conclusions regarding any differences in CS1 expression, the authors should use quantitative methods such as ELISA in combination with the microdissection of stroma or tumor cells.

2. The authors studied CS1 expression in tumor tissue. However, they did not include any information about distribution of CS1 between intracellular compartment and extracellular matrix. Since VLA-4 is a cell surface receptor, expression of CS1 in extracellular matrix may have a higher relevance for the initiation of tumor cell spreading and migration. As depicted by some images in the manuscript, there is an intracellular and intranuclear staining of CS1 in some cells. The relevance of such differential expression of SC1 should be also shown and discussed.

3. The authors showed CS1 expression in four cell lines, but they analysed spreading and invasion only in one of them. Migration activity of different cell lines as well as the response to stimuli can be very heterogeneous. The authors should show whether CS1 has the same effects in different cell lines. To make any conclusions about the role of CS1 in OSCC, the authors have to compare CS1-mediated effects on tumor cell migration of different cell lines.

4. The main mechanism of CS1 action is the interaction with its counter-receptor VLA4. The authors used the inhibition of the binding between VLA4 and CS1, but they do not show any data of VLA4 expression in examined cell lines and tissues. Although the authors cited two publications (25, 32; page 12) as a
reference of VLA4 expression, these papers do not actually provide information about expression of VLA4 in OSCC cell lines. A detailed characterisation of VLA4 expression in used cell lines, analysis of human samples and the correlation between VLA4 expression and functional parameters of migration have to be included.

5. As previously described by the authors, the blockade of alpha 4 integrin increases tumor cell migration (Ref. 32). This discrepancy to the results of the present study should be discussed. The authors should also discuss how SC1 interacts with other fibronectin variants which could be expressed in OSCC tissue.

6. Adhesive interactions between VLA-4 and fibronectin represent the first step of initiation of tumor cell spreading and migration. Thus, the analysis of tumor cell adhesion to fibronectin-coated surface (CS1 and other variants) with or without blockade of VLA-4 should be performed to evaluate adhesive properties of CS1.

Minor points:

1. The section „Immunohistochemistry“ (page 6 in bold) should be changed to „Tissue microarrays“.
2. The description of some methods (immunohistochemistry/microarrays, immunoblot) should be shortened.
3. Catalog numbers should be removed. Names of antibody clones should be added.
4. Specifications of Calcein AM and fluorimeter are missing.
5. In vitro study: CS1 content in supernatant (measured by ELISA) will better reflect the CS1 release than measuring of CS1 content in the cell lysate.
6. A repeated presentation of the same data (Tab. 1 vs. Fig. 1C) is confusing.

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

**Quality of written English:** Needs some language corrections before being published

**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'