Reviewer's report

Title: Collagen reorganization at the tumor-stromal interface facilitates local invasion

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Reviewer: Thomas Dittmar

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Comments Revised Manuscript „Collagen reorganization at the tumor-stromal interface facilitates local invasion“ Provenzano et al.

The authors have revised the manuscript accordingly to the comments given by the reviewers, thereby improving the quality of manuscript. However, to me, there are still some points that should be revised prior to publication (see below):

1) Is the question posed by the authors new and well defined?
   Yes!

2) Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
   Yes

3) Are the data sound and well controlled?
   The reviewer is thankful to the authors for giving a short introduction how tumor cells were visualized by their FAD autofluorescence. This is a critical point that has to be added to the figure legends. Additionally, single tumor cells should be marked by an arrow or arrowhead.

For TACS-2 the tumor mass/ collagen lattice border is clearly visible, but this is not the case for TACS-3 due to tumor invasion and tumor-mediated collagen reorganization. For instance, 6C is higher magnification of a region shown in 6A and single tumor cells are marked by a “*”. In their covering letter the authors stated out that tumor cells were visualized by their FAD autofluorescence. Thereby, dark round structures are the nucleus surrounded by a “ring” of FAD signal from the cytoplasm. To me the FAD autofluorescence of single tumor cells is difficult to see in e.g. Fig. 6C. What is the FAD autofluorescence and what are the collagen fibers? In Fig. 6D a tumor cell in contact with collagen fibers is clearly visible (by the way, I am still searching the region that has been magnified in 6D).

It is not clear to me why the authors do not use different colors to distinguish between tumor tissue (and single tumor cells) and the collagen structure. As stated out in their covering letter the emission signals (of collagen and FAD) can be differentiated after simultaneous excitation. For instance, collagen fibers could appear in green (e.g. see reference 13) and tumor cells in red or vice versa, or solely collagen fibers appear in green and the tumor cells/ tissue in grayscale.

“The beta-1 integrin experiment”: As stated out above it is pretty difficult to distinguish between the FAD autofluorescence of tumor cells and collagen fibers at higher magnifications. Staining with a beta-1 integrin antibody would help to discriminate tumor cells from collagen fibers. A FITC-conjugated anti-mouse/ rat CD29 antibody is available form BioLegend (#102205) and it is not necessary to fix the samples. Viable tumor cells (and tissues) can be stained with fluorochrome labeled antibodies, incorporated in the collagen lattice and analyzed subsequently. Alternatively, liquid collagen and the antibody solution can be mixed first and than combined with the tumor cells/ explants (for instance see Maaser et al. (1999), 10(10): 3067-79 [However, Maaser et al. solely used tumor cells for their research but not tumor explants. Nonetheless, it is feasible to stain viable cells with fluorochrome labeled antibodies within a collagen lattice and to analyze them]).

However, this experiment was just a suggestion not to prove tumor cell-collagen fiber interactions but rather to discriminate between tumor cells and collagen fibers. As mentioned above, it is pretty difficult to distinguish between tumor cells and collagen fibers in a grayscale image. Why not stain the collagen fibers and the tumor cells with different colors?
4) Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes!

5) Are the discussion and conclusions well balanced and adequately supported by the data?
Yes

6) Do the title and abstract convey what has been found?
Yes!

7) Is the writing acceptable?
Yes!

What next?: Accept after minor essential revisions

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No

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