Reviewer's report

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

Version: 2 Date: 27 August 2006

Reviewer: Claudia Binder

Reviewer's report:

General

Interactions between tumor cells and components of the stromal compartment are difficult to investigate in in vitro models as they are strongly influenced by experimental conditions. The authors, who focus on collagen reorganization in the tumor microenvironment, provide interesting new data using two promising methods for in vivo imaging. However, there are several points which need to be addressed:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The experimental conditions have to be clarified. The authors make a strong point that the investigations have been performed in live tissues. So, supposedly, the experiments have been done with live animals which had to be prepared for the procedure in some way (anaesthesia? skin incision to expose the tumor? etc.). However, there is no description of these procedures in the text, except the citation nr 26, which may contain these details, but is not accessible for me. In case, all of the described experiments have been performed with tissue biopsies as described in “tumor explants and collagen gel culture” (p. 4), then they represent “ex vivo” investigations and should be referred to as such.

2. The presented data clearly demonstrate that MPLSM and SHG are suitable to detect collagen reorganization in the tumor microenvironment. However, as the authors come to rather far-reaching conclusions regarding the diagnostic utility of these methods (e.g. abstract), it would be necessary to present the respective evidence in the form of larger series of carcinomas investigated in comparison with benign glands in all states of development (virgin, pregnant, involution). The number of presented cases (for the malignant ones only given in the legends and difficult to find, for the normal glands not indicated) does not seem sufficient for these conclusions. Especially, as both the PyMT and the Wnt 1 mouse model are not necessarily transferable to human breast cancers, usually not virally induced and without deregulated canonical Wnt signaling. Thus, the investigated numbers should be clearly indicated in “Methods” and the conclusions restricted to the proven findings.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. From most of the figure legends it is not clear which kind of mouse has been investigated, this should be changed.
2. Spelling errors: "samples" instead of sample (p 6, 5th line from below), to visualize of collagen.."of" should be taken out(figure legend 3)
3. The “PyVT” mouse should be the PyMT mouse, if it is the same mouse as in the cited reference
4. The histological findings should be specified according to the usual classification of breast malignancies: Mostly, the investigated samples are referred to as “tumors” (e.g. in “Tumor Explants…”, p. 4; “pre-palpable tumors…”, p. 10 line 1; etc.) without stating whether these are adenomas, carcinomas in situ or frankly invasive carcinomas.
5. P10, line 8: “high localization…”, probably meaning “high density”?
6. The chapter “Results” contains a lot of interpretations, speculations and citations of data of other authors (e.g. end of first paragraph p. 7, end of first paragraph p. 9, second paragraph p. 10, end of first paragraph p. 11), which better belong into the discussion. The “unpublished observations” on tumor formation in “appropriately crossed” col 1a-1 mice (p. 9) should either be omitted, if these are not own observations, or further specified. In this context, it is surprising that a recent paper, which shows diminished hyperplasia and tumorigenesis in PyMT mice crossed with collagen knock outs (J Clin Invest 2005, 115:1163) and fits very well with the presented data, is not cited in the discussion.
7. In the conclusion, the statements on tumor cell contractility, GTPases, Rho and ROCK should be omitted, as they are speculative and not underlined by the presented experiments.

Discretionary Revisions (which the author can choose to ignore)
As the tumor cells in some of the pictures are difficult to recognize, it would be nice to see the different imaging results (H&E, MPLSC, SHG) of the same tumor region placed next to each other, like in the supplementary Fig 3.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests