Author's response to reviews

Title: Remodeling of extracellular matrix by normal and tumor-associated fibroblasts promotes cervical cancer progression

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Author's response to reviews: see over
Dear Professor Solera,

We were pleased to read the positive comments of the Reviewers and we are grateful for their useful critics. Please find attached our revised manuscript entitled “Remodeling of extracellular matrix by normal and tumor-associated fibroblasts promotes cervical cancer progression” for consideration of publication.

As you can see, we performed several improvements in the text and provided exhaustive answers to all questions have been raised.

Responses to authors:

**Reviewer 1 – Martin Götte:**

“The methods are largely described in sufficient detail, however, insufficient information is provided on the TMA patient samples: More information (age, stage of cervical carcinoma) should be provided at least in a Supplementary table?”

According to this comment, we retrieved clinical and pathological data from our archive records. These data are included on Table 1, and on page 6 line 120-123. In addition, we refer to a previous publication (ref.17) including the same samples.

“What was the medical indication for taking the normal control samples? Please provide more information, also on the primary tissues used to generate fibroblast cultures.”

Normal tissues for primary cell culture were taken from hysterectomy specimens containing clearly separable, cancer-free region. Correction: on page 6 line 130-134.

“Some language corrections are required”

The manuscript has been edited by a native American scientist. The corrected parts are marked by red colour. We are grateful for the constructive remarks and suggestions of the reviewer.
Reviewer 2 – Ines Lohse:

Major Compulsory Revisions

“The manuscript would benefit from a spellcheck and major editing by a native English speaker. Missing punctuation and grammatical discrepancies make it hard to follow the story”

The manuscript has been edited by a native American scientist. The corrected parts are marked by red colour.

“Growth signals generated by the tumorous extracellular matrix...” Please change this sentence. The extracellular matrix doesn’t generate growth signals but rather contains signalling molecules deposited by CAFs and tumor cells. This sentence is rather confusing.”

According to this comment we corrected the sentence as: Growth signals generated by the stromal cells of the tumor are mediated to the cancer cells by integrins (on page 5 line 98-99).

“Please describe CSCC7 cells better, what markers do they express? How was purity and stability of the line evaluated?”

According to this comment the following sentence was added to page 7-8, line 161-163: „HPV16 positive CSCC7 cells exhibit a clear epithelial morphology. When growing in monoculture, they form nests. They are positive for pan-cytokeratin but negative for vimentin.

In contrast, fibroblasts are vimentin positive, pan-cytokeratin negative cells with spindle-like morphology and elongated, oval nuclei.”

“How was purity of the NF and TF line evaluated? How many passages were used for this experiment? Please comment on culture conditions (Oxygen concentration). What is the rationale for the medium change at passage 3? Were the NF established from areas adjacent to the tumor or cancer-free areas?”

Normal tissues for primary cell culture were taken from macroscopically cancer-free regions of hysterectomy specimens (on page 6 line 130-134).

We added the following text to page 8 line 162-167: „An average of <3% epithelial cell contamination was found in established fibroblast cultures. CSCC7 cells were routinely cultured..."
in RPMI-1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/mL penicillin, and 100 µg/mL streptomycin. All cell lines were cultured in a humidified 95% air/5% CO\textsubscript{2} incubator at 37°C. Cell lines between 8\textsuperscript{th} and 12\textsuperscript{th} passage have been used. Within this period both fibroblast and CSCC7 cells proved to be stable. The reason of changing culture medium was that Amino Grow Plus medium is designed to establish primary culture, and it is an expensive material. As after growing out from the small tissue pieces, fibroblasts did not need to be grown in this medium, we switched to regular culture medium.

„Figures 3, 4, 5 and 6 are described twice. It makes the second part feel very repetitive, especially since they message is similar. While I appreciate that the authors intent to discuss the TF/NF and tumor cells separately, I would make more sense for the reader to merge these sections.”

To avoid repetitions we removed the sentence on page 15 line 316-317 from the fibroblast section. Authors feel that discussing the characteristics of fibroblasts and tumor cells in a common section makes the story difficult to understand. That is why we prefer to keep the two sections separated. We do hope that the reviewer understands our point.

„How were tumor cells identified in this experiment? It can’t be excluded that these cells are fibroblasts or immune cells.”

As immune cells do not survive cultivation, their presence can be excluded. Benign cells, like fibroblasts do not grow on the top of each other, this is a characteristic feature of malignant cells. Morphology of cells spreading on the fibroblast lawn clearly correspond to the cancer cells (large nuclei, with variable size and irregular feature, prominent nucleoli, decreased cytoplasm/nucleus ratio fulfill the criteria of malignancy). Vimentin and pan-cytokeratin staining served to differentiate between cell types, and corroborated our morphological observations.
Minor Essential Revisions

„Please comment on whether Ethics approval was obtained for patient samples used on the TMA.”

Yes, the Ethical Board of the university approved the sample collection. In accordance, the following sentence was added to page 6 line 123-126: „Tissue blocks were collected from the 1st Department of Obstetrics and Gynecology, Semmelweis University with permission, approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (TUKEB permit number: 95/1999).”

“Was the counterstain really H&E? Generally only Eosin is used.”

Thank you, for this remark, we used hematoxylin counterstain to detect nuclei. The sentence is corrected (on page 6 line 129).

„The table should be moved to supplementary as there is no benefit from it being part of the main manuscript.”

We included the table describing the antibodies in the main text as BMC Cancer publish no supplementary material.

„Which 2 types of fibroblasts were used?”

On page 10 line 212: medium conditioned by normal fibroblasts (NF) and tumorous fibroblasts (TF).
„Fig. 3: please add error bars to the growth curves.”
Done.

„Fig. 5/6: some of the plots are very blurry and hard to read. Please improve picture quality.”
As the zymography bands were generated by digestion, the method necessarily provides blurry signals. We only could improve the plots by manipulating the image, which should be avoided in our view.

„Page 19, line 436: Change “indicate” to suggest.”
Done on page 20 line 448.

„Please remove the location (A,B, ect) from the figure title.”
The mentioned parts of figure titles are removed (on page 27-30 lines 704, 745, 758).

**Discretionary Revisions**

„Page 21, line 469: Is that data part of another manuscript? It would be interesting to include it here.”
The changes of syndecan-1 will be more extensively analyzed and published in a separate paper.

We do hope, that the manuscript in present form meets the requirement of BMC Cancer.

Hoping your favourable decision
Sincerely Yours

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