Author's response to reviews

Title: Simultaneous expression of flotillin-1, flotillin-2, stomatin and caveolin-1 in non-small cell lung cancer and soft tissue sarcomas.

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Author's response to reviews: see over
Dear Editor,

We are grateful to you and the reviewer for the opportunity to improve our manuscript. We revised article’s text and tables. Please, find below point-by-point response to the comments and description of the changes. We hope that you will find our responses substantiated and complete.

Response to the reviewer’s comments:

1. Non-small cell lung cancer origins from epithelial tissue, while soft tissue sarcomas come from mesenchymal tissue. The molecular mechanism of carcinogenesis is different in epithelial tissue and mesenchymal tissue. It is rare that they were involved in the same study.

We agree completely with the reviewer’s statement, and believe that the inclusion of these two very diverse (at the molecular level) types of cancer represents a strength, not a weakness, of our work, suggesting that we have described a relatively general cancer phenomenon as opposed to one limited to only epithelial or mesenchymal tumors. Furthermore, the main aim of our project was the investigation of the role of microdomain-forming proteins (MFP) in cancer, and the two groups of tumors served as models for this analysis. Our study represents the first combined analysis of MFP and the first stomatin expression assessment in carcinogenesis processes as well as there is a lack of data about flotillins expression changes in tumors of any origins. As described, we uncovered correlations common to both groups of tumors that could point on more general mechanisms.

Another argument for choosing these tumors was previous works devoted to caveolin-1 investigations, where similar down-regulation of caveolin-1 was found in soft tissue sarcomas and NSCLC, because caveolin-1 may function as a tumor suppressor gene as well as an oncogene depending on the histogenesis of the tumor.

2. There is comparation in expression of microdomain-forming proteins mRNA in STS between soft tissue sarcomas and relative normal tissue in Table 3. However, there is no the similar data for NSCLC sample. The parameters in different type of tumors should be equal.

This information was embedded in the main text of the article and we had omitted detailed comparison because it is not carrying anything new. Nevertheless, we have included the requested information as an additional table (Table 1).

According to point 1 and 2, I suggest authors to split the manuscript into two parts, NSCLC and soft tissue tumor, respectively.

The part “Results” had presented in accordance with this suggestion. As highlighted above, the main focus of the study were the proteins involved rather than the specific tumors utilized. This is why we have left the “Discussion” “as-is”, in a more generalized form, to allow to us better prioritize the obtained results and highlight the main idea.

3. This study used western blot to investigate the protein expression in tumor tissue. Western blot just test the total target molecule expression in whole sample including tumor cells and mesenchymal cells in tumor tissue. Western blot is difficult to locate the target molecule as well. If is available, it is better to use immunohistochemistry to make the microdomain-forming proteins location clear.
We agree that immunohistochemistry (ICH) can also be used to interrogate protein expression in tumor tissue, but ICH has its own limitations. IHC does not allow comparing tumor and normal tissues precisely because normal tissue also can be immunoreactive. In this situation we should consider only tumor tissue, without taking into account normal tissue, or provide a subjective comparison, evaluating staining intensity by eye (using automated methods for this analysis would not have been possible due to the limited amounts of tumor and normal material available). Using Western blot we were able to compare how the expression levels of the protein have changed in the tumor tissue compared to the normal one without subjectivity, and in our opinion, it is more fruitful to compare tumor and normal samples than tumor samples between each other. As noted by the reviewer, a tumor sample includes a mesenchymal component, but this is also present in a normal tissue specimen, hence when conducting a Western blot this mesenchymal “background” will be subtracted, while if ICH were used and a not-quite-representative field of view was chosen, this background would be different. We histologically verified all of our samples before investigation and include in the study only specimens with minimal mesenchymal cell impurity; this is why we believe that the mesenchymal cells do not introduce significant error in the result.

Another reason for choosing Western blot analysis over ICH was the possibility of distinguishing different protein isoforms, which is especially important in the case of stomatin. Stomatin is a poorly studied protein and until 2012 it was considered that it has only one isoform, but now four isoforms have been described. Commercially available antibodies against stomatin react with all four isoforms. Using Western blot we were able to differentiate between the different isoforms.

Response to the Editor’s comment:

"The authors have addressed some of the reviewers’ comments. The manuscript will be strengthened if a more detailed analysis of the correlation between caveolin-1, stomatin, flotillin-1 and flotillin-2 expression and survival is performed and presented. To provide more useful information, it should be done within each stage group, and presented in a graph form as well. A expanded discussion on the results of this analysis should be included."

Before article writing we conducted exhaustive statistical analysis and include in the text the more meaningful results. We also performed detailed correlation analysis which was suggested by the editor. One of the limitations of Spearman’s rank correlation test is a size of sampling. When we split our sampling by histology (for example, taking into account only adenocarcinomas) and analyzed correlations within subgroups divided in according clinicopathological characteristics we did not received statistical significance, likely not because of lack of correlation (r can be equal to 0.6) but because of small sample size (~10 samples). Introducing statistically significant results into the manuscript only for bigger groups can cause seeming differences between samples which they really do not have. By this reason we could not analyze the whole NSCLC sampling within each stage group, for instance, in group pT4 there are 9 samples (for detail, see Table 6) and that is why we combined similar smaller groups. Nevertheless, we included correlation results for adenocarcinomas and SCC in Table 2. We also inserted an additional table as suggested by the reviewer and revised the text regarding survival analysis.