September 14, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4975.doc).

Title: "Assessment of tumor antigen and MHC molecule expression in glioblastoma multiforme cells for immunotherapeutic interventions"

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Name of Journal: *World Journal of Immunology*

ESPS Manuscript NO: 4975

The manuscript has been improved according to the suggestions of reviewers:

1 Revision has been made according to the suggestions of the reviewer

(1) the reviewer #1 asked for in situ immunohistochemistry of the tumors of origin for the markers analyzed in our study. We are very sorry, but this form of analysis cannot be performed on original tumor material because we do not have sufficient material here for.

(2) similarly, he or she asked for a phenotypic characterization of freshly isolated cells by FACS. Again, we are very sorry, but this would have had to be done immediately at the time point of model generation. We missed this for the models described in this manuscript. However, we are very happy with this comment and will follow this in future studies!

(3) next, he or she suggests to perform IF in vitro on cell cultures at several time point. First, we want to state that we analyzed the markers included into our study on cells in different passages – ranging from 10 to 35 – using FACS staining. The data presented in the manuscript are truly representative since we did not observe strong variations in marker expression over time. There has been some fluctuation; but this was most likely due to splitting-effects (i.e. when we analyzed the cells on the day immediately after splitting). Thus, the data presented are from cultures that have been at least 3 days old (after last splitting). Secondly, we beg for pardon that in our lab FACS is the standard method; we have only marginal experience with IF and strongly prefer FACS especially for analysis of extracellular markers. In fact, almost all of the markers analyzed in this manuscript are membrane-proteins.

(4) moreover, he or she suggests to discuss about the apparently discrepancy with respect to the MHC class II results. What could be the bases of the enormous differences of MHC class II expression in the different cell lines? We added a comment on this to the discussion part. But frankly, nobody really knows the biological background for this phenomenon. Recently, we were involved in a similar analysis in colorectal cancer. The basic findings are similar there. Thus, we initiated a study to unravel the possible importance of this observation.

(5) in addition, we were asked to discuss possible further mechanisms of immune evasion other than low expression of MHC in cancer cells. Kindly, he or she provided a number of references here for. We were happy to follow this suggestion and included a more detailed discussion of this very interesting topic into the discussion part of the manuscript.

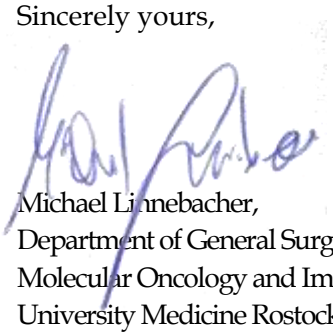
(6) finally, he or she suggests to deliver the information on the number of passages, our cell lines underwent before the evaluations. This information has been incorporated into the body of the manuscript. We want to thank the reviewer for this very helpful hint, since our main line of argumentation builds on the use of low-passage cell lines instead of "old" ones.

(7) reviewer #2 suggests to analyze further markers, i.e. molecules involved in the tuning and efficacy of the immune response. We totally agree that this kind of analysis would be of great interest. However, we chose to omit these further analyses for the present manuscript and would like to support our view with the following arguments: one can always find more interesting markers and the focus of the present study was to characterize a panel of novel GBM cell lines in order to prove that they may be of help for future tumor-immunological research. The markers selected for the present study cover tumor antigens and basic molecules of importance for in vitro tumor immunology. We are aware of the fact that this information does not cover the whole picture of GBM's interaction with the human immune system and we hope to be able to efficiently follow up this line of research further in the next years. Moreover, the molecules suggested by him or her are typically up-regulated on tumor cells when the tumor cells directly interact with T cells: PD-L1 and Fas on tumor cells is higher expressed upon interaction with PD-1 and FasL expressing T cells. We have experience with in vitro co-cultures of tumor cells and immune cell subsets and will readily pick up this valuable hint of the reviewer in future studies. In fact, we started analyzing the molecules suggested by him or her and we hope to be able to publish these results soon in one of our next publications.
Lastly, we want to hint on the fact that all of our tumor models – and currently, more than 50 cell lines (GBM, colorectal cancer and some other entities) have been generated from our group – will be available upon request for the research community. Thus, many more analyses can, and we are confident that they also will, be performed by us and hopefully many other groups worldwide.

2 The manuscript has been reviewed again by a native speaker of American English, i.e. the first author of the manuscript! We would like to stress this fact, since no further mistakes have been found and we find the judgment of reviewer #2 regarding the quality of written English quite offending.

Thank you again for publishing our manuscript in the *World Journal of Immunology*.

Sincerely yours,



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