Author's response to reviews

Title: Viral-mediated oncolysis is the most critical factor in the late-phase of the tumor regression process upon vaccinia virus infection

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Author's response to reviews: see over
Dear Prof. Ramzi Mohammad, Dr. Asfar Azmi, and Dr. K. M. Rahman,

Thank you for reviewing our manuscript and for considering the publication of our manuscript. We included all the reviewer comments into the revised form of the manuscript and listed, further, a point-by-point response to the minor concerns below:

Referee 1:

-point 1-

In our study, we are mainly focusing on microscopic techniques to analyse the tumor microenvironment upon viral infection. The used microscopic and imaging techniques offers the great opportunity to us not only to characterize the qualitative and quantitative expression level of different markers in whole tumor cross sections, but also give us insights into localization and microenvironmental interactions of labelled cells and structures. However, if we are preparing whole tumor lysates for western blot analysis, the information about the marker localization becomes totally lost.

The suggested western blot analysis of necrotic marker expression in control and infected tumors 42 days post infection appears us impracticable, because of an appropriate lack of necrotic marker for western blot analysis of whole tumor lysates. A typical necrotic marker like HMGB1 is under normal, healthy conditions intracellularly localized. Due to necrotic cell lysis proteins like HMGB1 are released from dying cells – so that finally the “extracellular localization” of a specific protein acts as a marker for necrosis. In cell culture, such experiments are easy to carry out, because cell lysates can be clearly separated from supernatants. But in cell lysates from whole organs there is no possibility to separate the “intracellular” from the “extracellular” milieu.

In Fig. 1 of the manuscript, we showed that viral infected tissue (GFP) in whole tumor cross sections was not labelled for actin and cell nuclei, which indicate tissue destruction. Further, in brightfield pictures the infected areas appear white, which is a typical morphological feature of necrotic tissue with extensive calcification. Recently, we have analyzed the cell death characteristics of GLV-1h68 infected cancer cells, and could show that this VACV strain induces necrosis instead of apoptosis (Gentschev et. al., 2009).

Since the presented images in Fig. 1 of the manuscript clearly show the virus-induced tissue destruction and our previous studies indicate that GLV-1h68 induces necrotic instead of apoptotic cell death in cancer cells, we think that western blot analysis of necrotic marker in infected and uninfected tumors does not necessarily support the main statements of the manuscript.

-point 2-

The discussion was truncated. Please see the revised manuscript.
The statistical analysis of the microarray data was elaborated. Please see in the Methods section of the revised manuscript.

Minor typos were corrected.

Referee 1:

The discussion was truncated. Please see the revised manuscript.

My colleagues and I would like to thank you in advance for considering the publication of our manuscript.

Yours sincerely,

S. Weibel