Reviewer's report

Title: TLR9 expression in glioma tissues correlated to glioma progression and the prognosis of GBM patients

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Chao et al. address interesting question regarding the relation between TLR9 expression, glioma progression and prognosis. Previous studies in mouse tumor-models have demonstrated that administration of TLR9 agonists can have positive effects on induce anti-tumor responses. Some controversies exist in literature as to whether TLR9 activation is beneficial in all circumstances and to which cells in glioma tissues express TLR9.

Major points:

A significant part of the data (tables and figures) presented by Chao et al. are not novel but rather confirm data present in literature:

1) TLR9 is expressed on glioma tissue samples and glioma cell lines on RNA level (Meng et al., Andalussi et al.) and on protein level: Meng et al.)
2) TLR9 is expressed on glioma tissue samples and glioma cell lines on protein level (Meng et al.)
3) Increased in-vitro invasiveness of glioma cell line after CpG stimulation (in fact, Merell et al. demonstrate that this is induced by secretion of matrix-metalloproteinase-13).

Furthermore, TLR9 mRNA expression is rather difficult to analyze as it is encoded by as single exon gene. Without DNase treatment on the RNA samples, inclusion RT+ and RT- samples, and semi-quantitative PCR it is difficult to draw unequivocal conclusions regarding the presence or absence of physiologically significant amounts of TLR9 mRNA. Also regarding the TLR9 staining it remains unclear how-specific this staining really is as a true positive control and a negative control (isotype matched primary control Ab) are missing. Finally, also a number of the functional experiments are poorly controlled.

The claim of Chao et al. that high TLR9 expression (in 128 grade IV gliomas) correlates with poor prognosis is in itself interesting. Others did not find such a correlation (Meng et al.). Unfortunately, patient selection, patient-characterization, parameter-definition and method to address this point are completely insufficient. This opinion will be highlighted by giving some examples:

1) Chao et al. analyse not all 128 patients in their study but select 69 patients that are ‘recurrent or dead before July 2009’. This selection criterion may.will
introduce a strong selection-bias.

2) Table 3 in fact suggests that selection-bias may have occurred because clinical parameters that have been previously shown to strongly influence prognosis in GBM patients are not correlated to PFS in this study (KPS, Extent of resection; table 3).

3) One of the clinical parameters the authors use is DFS: disease free survival'. On page 12 the authors conclude that „...extent of resection were not associated with DFS in GBM“'. This is curious, if you have complete resection of the GBM you have a DFS > 0 days. If you have a partial resection one must conclude that the DFS is 0 days).

Taken all this into account the current manuscript of Chao et al. does not provide sufficient new insights and experimentally supported conclusions with respect to our current understanding and the significance on TLR9 expression on gliomas.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interest.