Reviewer's report

Title: Evaluation of Immunological Escape Mechanisms in a Mouse Model of Colorectal Liver Metastases

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The manuscript by Grimm et al. introduces a model for colorectal cancer liver metastases based on the intraportal injection of CT26 murine colon carcinoma cells. Such a model is of interest for many different questions in colorectal cancer, despite the fact it circumvents the classical metastasis procedure and thus might neglect processes that occur through selection during metastasis in human tumors. The authors set out to investigate some of the mechanisms responsible for metastasis formation and outgrowth and concentrated on the analysis of immune escape mechanisms. This work led to several conclusions, which are however inadequately supported by the data provided within the manuscript.

Major points

1- The authors show that over time (up to 20 days) the tumor volume increases 5 times between day 10 and day 20. Therefore it is not surprising that also the number of TILs of different nature increases. For a better representation of this infiltration the authors stain the tissues with antibodies characteristic for certain subpopulation, CD4, CD8, CD25 and FoxP3 and suggest an increase of all four populations. Unfortunately, they only show CD4 and Foxp3 in Fig2c. While an increase in FoxP3 between day 10 and day 20 is visible, no difference can be detected between CD4 day 10 and CD4 on day 20. According to the picture given in the manuscript it remains unclear how counting of the cells could be done properly and it would be of interest to see, how the TIL proportion varies in primary (human) tumor samples of different size.

2- The same figure identifies the FoxP3 population as CD4 helper T cells, staining with CD8 and merging the pictures would have been an appropriate control, which however is missing.

3- The potentially increased infiltration of T cells is attributed to an increased level of cytokines namely IL-10, TGFβ and TNF. While this seems to be true for IL-10, the increase in TGFβ and even more in TNF is extremely weak. This is confirmed via immunohistochemical analysis of positive cells. Counting of cytokine positive cells is extremely critical because cytokines are released and cannot be attributed to individual cells on tissue any more. Such an analysis requires proper co-staining of tumor cells and/or TILs and intracellular cytokines as well as highly preserved tissue architecture. None of these conditions were used, therefore it is unclear how reliable these data are.
4- Similar problems occur with the observation of FAS and FASL deregulation. The counterattack theory is discussed in a highly controversial manner, which is in part due to the fact that it is hard to prove in vivo. The observation by Grimm et al. that FASL increases, and it increased in tumor cells, while FAS receptor declines indeed supports this view. But, due to the fact that on the same tissue (Fig 4c) only FASL is shown but not FAS, is again a lack of support for the conclusions the authors draw in the results and discussion section. Why did they not stain FAS? The PCR data suggest a decline and the authors show tumor tissue with adjacent normal liver? This is similar important for Fig 4d, here the authors show a co-staining of CD8 plus cells, assumed to express FAS. This is very hard to see, the better magnification given in the lower panel would also have been beneficial for the upper panel.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests