Reviewer's report

Title: Growth and metastasis of B16-F10 melanoma cells is not critically dependent on host CD73 expression in mice

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Reviewer: John Stagg

Reviewer's report:

In this study, the authors report that CD73 gene-deficiency is not associated with decreased B16-F10 tumor growth or lung metastasis, in contrast to what has been reported in previous studies. In this study, the authors used a distinct CD73 gene-targeted mouse (i.e. a floxed CD73 mouse), different from the other studies, and MRI monitoring to show that neither complete loss of host CD73 expression nor endothelial-specific loss of CD73 affected B16-F10 tumor growth.

Major Compulsory Revisions:

Intriguingly, the authors report that upon intradermal tumor cell injection, peritumoral edema was significantly reduced in CD73-/- mice. The cause of this phenotype was not investigated. Does this reflect decreased inflammation? The authors performed bone marrow chimeras in which WT mice were transplanted with WT or CD73-/- bone marrow. These BM chimeras did not show decreased peritumoral edema, suggesting that CD73 on non-hematopoietic cells mediated the observed decreased peritumoral edema. However, no experimental data is shown to support this. To confirm a role for non-hematopoietic CD73, the authors must perform the appropriate chimeras by transplanting CD73-/- mice with WT or CD73-/- bone marrow. The authors further proposed that endothelial-CD73 is involved in the decreased peritumoral edema observed in CD73-/- mice. Surprisingly, however, they did not perform the experiment in endothelial-specific CD73-deficient mice.

The FACS data of tumor-infiltrating immune cells show minimal T cell infiltration in both WT and CD73-/- mice. FITC levels make the analysis difficult to interpret, i.e. there is no clear CD4 or CD8 T cell population visible. Analysis at earlier time-points, optimization of T cell staining, and absolute CD45 counts is recommended.

The authors report that complete loss of host CD73 has no effect on B16-F10 lung mets after i.v. injection, in contrast to previous studies. In their experiments, only one dose of B16-F10 cells was tested. The authors must test several doses in order to conclude. While the reasons for such discrepancy are unclear, the study would benefit from a detailed phenotype analysis of MHC molecules and NK cell ligands on the B16-F10 cells used. The difference in tumor growth in WT versus control LoxP mice is particularly worrisome. As pointed out by the authors, it suggests genetic differences favouring B16 tumor growth in the CD73 LoxP mice. This could be the result of impaired NK cell activity. This could
explain the discrepancy between this study and previous published study.

Minor Essential Revisions:

1. “Targeted deletion of CD73 on endothelium was confirmed by histochemistry and qRT-PCR analysis.” – Please show data.

2. In the result section, the authors write: “Tumor growth and edema formation was significantly reduced when compared with the respective experiments in global (Fig. 2C-D) and endothelium specific CD73 mutants”. This sentence is not clear.

3. The authors write: “it should emphasized that clinical studies testing CD73 expression on tumor cells as a prognostic marker have yielded contradictory results”. Reference to primary research articles (instead of reviews) is recommended.

4. Peritumoral edema was found to be significantly attenuated in CD73-deficient mice. Could this explain the previously reported reduced tumor growth of primary B16F10 tumors in CD73-deficient mice?

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have non-financial competing interests in relation to this paper; my laboratory studies the role of CD73 in cancer