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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Burghoff et al present an interesting paper showing that CD73 expression on host cells, particularly on endothelial and hematopoietic cells, does not modulate tumor growth and metastatic spread of B16-F10 melanoma cells.

The membrane protein CD73 catalyzes dephosphorylation of extracellular AMP to adenosine. As stated by the authors (Introduction section) “Recent studies have investigated the role of CD73 expressed on host cells in different tumor cell models (i.e. colon adenocarcinoma, melanoma, lymphoma) using CD73-/- mice, pharmacologic inhibition of CD73 or anti-CD73 antibodies, which all suggested a stimulating effect of host CD73 on local tumor growth and metastatic spread. Although, not all studies showed a clear promoting role of CD73 on tumor progression, especially when using less immunogenic tumor cell lines. Moreover systemic application of inhibitors or antibodies targets CD73 on tumor cells as well as host immune cells and vascular endothelium to the same extent.” Thus the authors concluded that more studies were required to further specify the role of host CD73 on local tumor growth and metastatic spread. Nevertheless this referee has a number of concerns that should be considered by the authors.

1. Results: B16-F10 cells showed very little CD73 and negligible AP activity. Neither complete loss of host CD73 nor specific knockout of CD73 on endothelial cells or hematopoietic cells affected tumor growth after subcutaneous or intradermal tumor cell application. Also lung metastasis after intravenous B16-F10 injection was not altered in CD73-/- mice. However, the major problem with B16-F10 cells is that most of its adhesion and growth factor profiles are unlike those of its human melanoma counterparts. Even the enzymes used for invasion into tissues, the ability of cells to overpower the immune system, the antiapoptotic mechanisms, and many other cancer cell hallmarks do not reflect the human disease [Journal of Investigative Dermatology (2010) 130, 911–912]. In fact in different human melanoma cells, upregulated expression of ecto-5#-nucleotidase is associated with a highly invasive phenotype. Therefore my first question is why the authors selected a murine model that appears far from the main molecular characteristics of human melanomas.

2. A2A adenosine receptor protects tumors from antitumor T cells [Proc Natl Acad Sci U S A. 2006 Aug 29;103(35):13132-7]. Indeed, the inhibition of antitumor T cells via their A2AR in the adenosine-rich tumor microenvironment may explain the paradoxical coexistence of tumors and antitumor immune cells in some cancer patients (the "Hellstrom paradox"). ATP, ADP and AMP are present
in the human blood stream. Therefore it appears that A2A/A2B receptors (if overexpressed) in tumor cells and not in host cells are the critical factors….A2A/A2B receptor antagonists were effective in reducing the metastasis of tumors expressing CD73 endogenously (4T1.2 breast tumors) and when CD73 was ectopically expressed (B16-F10 melanoma) [Proc Natl Acad Sci U S A. 2013 Sep 3;110(36):14711-6].

3. Other potential mechanisms should be taken into account. For instance, as recently proposed, antagonism of adenosine A2A receptor expressed by lung adenocarcinoma tumor cells and cancer associated fibroblasts inhibits their growth [Cancer Biol Ther. 2013 Sep 1;14(9):860-8]. Not only could there be prevention of negative signaling in T cells within the tumor microenvironment and inhibition of angiogenesis, but also an inhibitory effect on tumor-promoting, immunosuppressive cancer-associated fibroblasts and a direct inhibitory effect on the tumor cells themselves. Besides, and closely related, extracellular ATP (which highly increases in fast-growing tumors or hyperinflamed tissues) exerts opposite effects on activated and regulatory CD4+ T cells via purinergic P2 receptor activation [J Immunol. 2012 Aug 1;189(3):1303-10].


**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:**

This referee has no competing interests in relation to the paper