Reviewer's report

Title: PAF-R dependent pathways control tumor growth and tumor response to chemotherapy

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Reviewer: Raymond Konger

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Oliveira et al. present a number of somewhat loosely interrelated, but interesting findings: First, they show that the PAF-R antagonist WEB2170 inhibits the growth of Ehrlich Ascitic Tumor (EAT) cells in vivo. In addition, WEB2170 was shown to block PGE2, VEGF, and NO production in the ascites fluid in mice inoculated ip with EAT cells. They also show that EAT cell growth in vivo was also stimulated by the addition of apoptotic, but not viable thymocytes; importantly, WEB2170 was shown to block the ability of apoptotic thymocytes to stimulate EAT cell growth in vivo. Finally, they show that both WEB2170 and the chemotherapeutic agent DTIC inhibit growth of B16F10 melanoma cells in vivo, but that only the combination of WEB2170 and DTIC resulted in prolonged survival. The ability of WEB2170, DTIC and WEB2170+DTIC to alter B16F10 apoptosis, MVD, and the number of COX-2 or Galectin-3 positive cells by immunohistochemistry was shown. However, the conclusions reached by the authors is in some cases premature, particularly in regards to the data with B16F10 cells. The authors need to provide additional evidence that B16F10 cells express the PAF-R in vivo or alter their conclusions. In addition, the manuscript is in general poorly written and requires significant editorial revisions.

Major compulsory revisions:

1. The authors report that B16F10 tumors were positive for the PAF-R by IHC, but do not show the data. Previous reports have shown that B16F10 cells lack PAF-R expression in vitro (Bussolati et al, Am J. Pathol, 157(5); 1713-1725, 2000 (See fig 4))(Im et al. Cancer Res., 56: 2662–2665, 1996) (Biancone et al, Clin Cancer Res, 9:4214-4220, 2003). The authors need to show their IHC data and also provide additional evidence to support their contention that the B16 tumor cells expressed the PAF-R and that this expression was necessary for their observed effects. This is important, as the authors speculate that the ability of WEB2170 to inhibit the growth of B16F10 cell growth in vivo was due to the presence of PAF-like molecules on apoptotic tumor cells.

2. The authors argue that the ability of WEB2170 to inhibit B16F10 tumor growth was restricted to the initial phase of tumor growth as there was no survival advantage to WEB2170 treatment. However, they only measured tumor volumes up to day 12, in which WEB2170 treatment very effectively inhibited tumor growth. Did the authors record tumor volumes at later time points? To make this argument, they need to extend these studies out further to determine whether
WEB +/- DTIC inhibited growth throughout the course of treatment. A more likely explanation for their data is that the change in survival could be mediated by reductions in metastasis by WEB2170 +/- DTIC. Given that B16F10 cells metastasize readily, it is unclear why the authors failed to examine this aspect. It should be noted that the ability of PAF-R antagonists to inhibit B16F10 melanoma metastasis has been previously reported (Im et al., ibid). The authors should at least acknowledge this possibility in their discussion.

3. The reliance on immunohistochemical analysis for Fig 5 and to assess PAF-R expression (not shown) is somewhat troubling as IHC is prone to artifacts. Moreover, the changes noted are minor. The absence of images of the staining patterns is also a concern as the reader has no way to judge the quality of the immunolabeling. The authors should ideally show images of their staining patterns or provide an alternative methodology to support their findings (e.g. RT-PCR or immunoblot data or PGE2 quantitation).

Minor Essential Revisions:

1. Numerous typographical and grammatical errors are noted. The manuscript requires significant editorial revision to improve readability of the manuscript.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

**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 interests