Reviewer's report

Title: Efficacy of Wnt-1 monoclonal antibody in sarcoma cells

Version: 1 Date: 4 November 2004

Reviewer: Jeffrey A Toretsky

Reviewer's report:

General
In the manuscript by Mikami et al, authors investigate the role of Wnt-1 signaling pathway inhibition in sarcoma cells by a monoclonal antibody and siRNA. Their results suggest that inhibition Wnt-1 signaling in sarcoma cells induces apoptosis. However, work presented in this manuscript is merely replication of the author's previous publication with a new cell line. The results are extremely over interpreted and data lack several key controls.

-------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The authors discuss sarcoma as one disease identity (when in fact A204 is a rhabdomyosarcoma cell line) and then report conclusions as if they would apply to all sarcomas. Sarcoma is a general classification for tumors originating from mesenchymal tissue. Each type of sarcoma has different pathologic mechanism and responds to different treatment modalities. Results from a single cell line cannot be generalized to the whole family of sarcomas.
2. Patient samples were described as lung metastasis of sarcoma. What was the primary tumor pathology for these lesions? The whole tumor tissue was homogenized and used in cell culture experiments. The results given in Figure 1 and Figure 4 are therefore difficult to interpret since there is no way of knowing if the effect is on tumor or stroma. Furthermore, western blots shown in Figure 1 do not prove that these cells have an active (autocrine or paracrine) Wnt signaling. The presence or absence of beta-catenin and cyclin-D1 could result from many cell signaling processes and are not specific to Wnt-1. In order to be convincing, Wnt-1 treatment with and without antibody are necessary to support the cause/effect relationship with these primary tumor cells.
3. The Wnt-1 antibody is problematic due to a lack of binding and specificity validation. Even though the monoclonal Wnt-1 antibody has been published earlier, neither this manuscript nor the original one addresses the issue of cross reactivity. This antibody was raised against a 12 aa long peptide. Wnt-3, Wnt-5a, Wnt-8b, and Wnt-16, all have similar domains with 8-9 identical aa. This clearly taints the conclusions of the entire paper. Specifically, the western blot in Figure1 is not convincing. There are multiple bands and high background. In addition, peptide competition of antibody effects would have helped to support Wnt-1 specificity.
4. Figure-3b shows results of an siRNA experiment. However, the figure lacks a control, Wnt-1 western blot, to show that siRNA works in this cell line. Earlier published work on other cell lines does not guarantee the outcome in this particular cell type. The effect on beta-catenin is very limited compared to Figure-3a.
5. Apoptosis is described based upon only one Annexin V binding study and the results lack information about PI uptake as a control for non-apoptotic cell death.
6. The abstract is poorly written.

-------------------------------------------------------------------

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Origin of A-204 cell line, an embryonic rhabdomyosarcoma, should be given.
2. To eliminate the possibility of simple general toxicity, the Wnt-1 monoclonal antibody should be tested on a cell line that does not express any Wnt-1.
3. The sequence of the siRNA and protein effects should be shown.
4. Whenever a cell fractionation is done (Figure-1 and Figure-3), it would be best to show the whole cell lysate as a control.

Discretionary Revisions (which the author can choose to ignore)

What next?: Reject because too small an advance to publish in any journal
Level of interest: Too insignificant to warrant publication in any journal
Quality of written English: Needs some language corrections before being published
Statistical review: No
Declaration of competing interests:
I declare that I have no competing interests