Reviewer's report

Title: Intra-tumoral delivery of IL-18 naked DNA induces T-cell activation and Th1 response in a mouse hepatic cancer model

Version: 2 Date: 16 January 2007
Reviewer: Richard Heller

Reviewer's report:

General

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

1. The authors discuss delivery of a plasmid encoding for IL-18, but give very little detail as to how it is actually delivered. They cite several references that use electroporation to deliver. Does this imply that the authors also used electroporation? How did they access the liver? Was the injection done through the skin without an incision? All of these questions need to be addressed before publication.

2. In the background section the authors discuss actions of IL-18 and its similarities to IL-12. One of the references cited to document these statements is (5). This reference is not related to IL-18 or cytokine delivery or tumor therapy. This needs to be corrected.

3. In the methods section the authors discuss inducing tumor formation in mice. They mention the mice were treated after tumor formation, but give no information as to the size of the tumors at time of treatment or how they were treated (procedure).

4. In the results and discussion section, under Levels of transgene expression in treated tumor site, middle of the paragraph, it is not clear what the authors mean when they report IL-18 protein levels as 500-900 µg of liver tissue.

5. In the results and discussion section, under In vivo kinetic study of immune cell population in spleen, the end of the section it is not clear how the authors can describe their expression as "high level". Expression is at best 2-2.5 fold higher than background. It also does not seem reproducible since in Figure 2 (which these results refer to) 25 µg dose up to 7 days has lower positive cells than the control and 10 µg is higher. While 50 µg does show some induction of cells it is not clear what is happening when the 10 and 25 µg doses are evaluated. The authors do not discuss these results adequately.

6. In the results and discussion section, under Regression of established CT26 hepatic tumor after direct intratumoral mIL-18 gene transfer. The authors only follow the tumors for 14 days which not long enough to make a true evaluation of the effect of therapy. It is also not clear there is regression of the tumor. It would appear that there is a slowing of growth but without information regarding the starting size it is hard to determine if there is truly regression of the tumors. The authors also do not indicate how the measurements were made.

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. Under Flow Cytometric Assessment of Intracellular Cytokine Production, first sentence "with" should be changed to "was".

2. Under In vivo kinetic study of immune cell population in spleen the first sentence, change "day 2, 5, 7 days after..." to "2, 5 and 7 days after..."

3. The results presented in Figures 1, 2 and 4 there is no indication as to the number of mice or replicate experiments. It is important to include this information. It is also not clear how many replicates were done for the results reported in figure 5 as well.
Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

**Quality of written English:** Needs some language corrections before being published

**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.