Reviewer's report

Title: Poly I:C enhances cycloheximide-induced apoptosis of tumor cells through TLR3 pathway

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Reviewer: Kiyoshi Takeda

Reviewer's report:

General
In this manuscript, the authors analyze the expression and apoptotic activity of TLR3 in tumor cells. TLR3 is expressed in a variety of human and mouse tumor cell lines. Treatment with the TLR3 ligand poly I:C increases sensitivity to cycloheximide (CHX)-induced apoptosis in HeLa and MCA38 cells. Neutralizing Ab against TLR3 blocks TLR3-mediated induction of apoptosis.

A major concern is that this study is repetitive to previous studies (Ref 12, 13).

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

1. Unlike the previous studies (Ref 12, 13), TLR3 stimulation itself does not induce apoptosis. The authors should make this point clear.

2. It is very ambiguous why the authors use CHX, but not other apoptosis-inducing agents, to induce apoptosis. The authors should state the reason why CHX is used.

3. It has been shown in other studies (Ref 12, 13) that TLR3 directly triggers apoptosis, and that TLR3-dependent induction of type I IFNs is responsible for apoptosis induction. CHX is predicted to inhibit de novo protein synthesis of type I IFNs, resulting in decreased sensitivity to apoptosis. But this is not the case. As the authors describe in the Discussion, they should show that CHX unexpectedly increases protein expression of IFN-alpha/beta by ELISA.

4. I wonder how apoptotic cells are counted in Fig. 3 B, C. Are these Annexin V-positive cells? If so, data in Fig. 3B should fit with those in Fig. 3A.

5. The authors analyze surface expression of TLR3 in Fig.1. However, TLR3 is known to be expressed in the endosomal compartment. Therefore, intracellular staining of TLR3 should be performed.

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

6. In page 3, lane 2, 'dsRNA virus' should be 'viral dsRNA'.

7. On page 8, the authors describe that RIG-I also initiates cellular responses.
However, a recent study (Nature 441, 101, 2006) showed that another RNA helicase MDA-5 recognizes poly I:C.

Discretionary Revisions (which the author can choose to ignore)

**What next?:** Reject because too small an advance to publish

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Acceptable

**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.