Answering the Reviewers and the Editor

November 12, 2015

Dear Editor,

Please find the enclosed the edited manuscript in Word format (file name: 22310-miniReview.doxc).

Title: Naked DNA in cells: an inducer of MHC molecules to evoke autoimmune responses?

Author: Yuqian Luo, Aya Yoshihara, Kenzaburo Oda, Yuko Ishido, Naoki Hiroi, Koichi Suzuki

Name of journal: *World Journal of Translational Medicine*

ESPS Manuscript NO: 22310

Manuscript Type: Minireviews

The manuscript has been substantially revised according to the suggestions of the Reviewers and the Editor. 1) **Table 1** and **Figure 1** are provided in the revised manuscript to significantly improve the quality of this manuscript. 2) The revised parts are indicated in red letters in the manuscript. 3) 12 new references are added to the Reference list accordingly, whose PMID/DOI are provided whenever available. 4) A signed Conflict-of-interest Statement is enclosed (file name: 22310-Copyright Assignment.pdf). 5) An Audio Core Tip is provided (file name: 22310-Audio Core Tip.mp3). 6) The point-to-point response to the Reviewers' comments and the detailed descriptions of the revisions we made can be found at below. 7) The revised manuscript has also been proof read by an English editing service. We believe all the revisions have significantly strengthened the manuscript and make it now suitable for publication in *WJTM*.

We thank you for publishing our work in *WJTM*. If there is any point we can further clarify, please address all correspondence to me at the address below.

Sincerely,

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Comments of the Reviewers

This is a well-written review. I only have two suggestions for improvement: 1) It would be good to provide a figure describing the proposed activation/signaling pathways. 2) Part are the article and the abstract discusses data showing that dsDNA can induce an mature APC phenotype even in non-APCs. Is there any evidence that these cells can actually function as APCs and activate T cells?

Response of the Authors:

We thank the appreciative comments and constructive suggestions of the Reviewers, and have improved the manuscript accordingly as followings:

Figure 1 that summarizes the proposed cytosolic dsDNA signal pathways is provide, and cited in the main text accordingly (Page 13-15). Figure title and legend are presented on Page 27.

To our knowledge, there is so far no direct evidence explicitly showing that non-professional APCs alone can activate T cells upon stimulation by cytosolic dsDNA. This lack of evidence might be due to the self-MHC restriction principle that requires a stringent experiment condition using MHC-100% matched non-APCs and T cells. Nevertheless, a substantial amount of evidence supports that cytosolic dsDNA like has such potentials to enable non-APCs to activate T cells. Moreover, aberrant expression of MHC molecules in epithetical cells found in various autoimmune disorders, although a definitive mechanism is yet elucidated. We have revised the manuscript to clarify this point accordingly as followings:

"endocrine epithelial cells in autoimmune target tissues (**Table 1**) such as pancreatic beta cells of insulin-dependent diabetes[1]" (Page 6)

"Transgenic mouse strains harboring MHC linked to insulin promoter overexpress MHC molecules in pancreatic beta cells and spontaneously develop insulin-dependent diabetes[6-8]." (Page 6)

"In particular, direct evidence has shown that MHC-expressing thyroid epithelial cells are potentially competent APCs to present antigens to activate T cells. MHC class II-positive human thyroid follicular cells were able to present a influenza-specific peptide to a human T-cell clone, a reaction which was abrogated by anti-MHC class II antibodies[3]. Lectin-induced MHC class II-positive human thyroid cells in monolayer culture were able to induce a proliferative reaction in autologous T cells, a phenomenon not found with MHC class II-negative cells[18]. Wistar rats are susceptible to the induction of experimental autoimmune thyroiditis. A cloned Wistar thyroid epithelial cell line (WRT) was shown to be directly recognized by Wistar rat lymphoid T cells that were both MHC class I- and class II-restricted[19]. When CBA mouse lymphoblasts generated on co-culture with monolayer syngeneic thyroid epithelial cells were injected either intravenously or into the thyroid lobes of intact CBA recipients, thyroiditis appeared within three weeks[20]. All these evidence suggest that the potential ability of thyroid epithelial cells as APCs to directly interact with T cells in a MHC-restricted

manner likely precipitates autoimmune response in the thyroid, although whether exposure to cytosolic dsDNA would substantialize such potential in non-immunes cells needs to be further tested by experiments." (Page 8-9)

We provide **Table 1** that lists aberrant MHC expression in non-professional APCs reported in autoimmune disorders in the revised manuscript (Page 26), and is cited in the main text accordingly (Page 6)