

Comment 1

1. The authors are unclear how the cell cycle was studied (Materials and Methods paragraph 2.5.3).

Response: Cell cycle distribution was evaluated using an Epics XL-MCL flow cytometry (BECKMAN coulter, USA). Results were analyzed using ModFit LT V3.1 (BECKMAN coulter). These statement was illustrated in the revised Materials and Methods paragraph “Cell cycle and apoptosis assay”.

2. In Figure 2C the western blot plot of Tg737 after anti-miR-548a-5p blocking shows several bands of different size for Tg737. What are these extra bands and why they don't appear in the other Tg737 blots? The authors need also to state the molecular weight of the bands for Tg737 and b-actin.

Response: There were some non-specific bands in Figure 2C. It may be accused of the polyclonal anti-Tg737 antibody. The polyclonal antibody may result in some non-specific binding, applying to western blot. However, the target bands were clear and satisfying. In addition, the molecular weight of Tg737 is 94kd and that of β -actin is 43kd.

3. In Figure 2 the authors show that overexpression of miR-548a-5p reduced by 2-fold the expression Tg737, but when miR-548a-5p was blocked there was 4x more Tg737.

What do the authors think about this? Is there anything else involved in regulation of Tg737 mediated by blocking of miR-548a-5p?

Response: We found that overexpression of miR-548a-5p reduced the expression Tg737, and when miR-548a-5p was blocked there was increased expression of Tg737. However, the trend was not completely matched between miR-548a-5p overexpression and miR-548a-5p blocking. The incompletely matched trend mentioned above may be blamed to the varied transfection results between miR-548a-5p overexpression and miR-548a-5p blocking. Nevertheless, the data could illuminate the interaction between miR-548a-5p and Tg737. We dare not say that there was any other mechanism involved in regulation of Tg737 mediated by blocking of miR-548a-5p.

comment2

Good work

Response: No response.

comment3

Authors

- (1) The importance of the research and the significance of the research contents; The authors of this article have been evaluated the interrelation between Tg737 and microRNA-548a-5p (miR-548a-5p), and how this relation correlates with hepatocellular

carcinoma cells (HCC) proliferation and apoptosis. The authors suggest "...
miR-548a-5p negatively regulates tumor inhibitor gene Tg737 and promotes
tumorigenesis progress in vitro and in vivo, indicating its potential as a novel therapeutic
target for HCC. .." The importance and significant of the research contents is high,
because this article present the new therapeutic strategies which may be useful to limit
HCC growing and metastasis (inhibition of miR-548a-5p).

Response: No response.

(2) The novelty and innovation of the research; Zhao Ge et al. present the evidence that "...
miR-548a-5p negatively regulates tumor inhibitor gene Tg737 and promotes tumorigenesis
progress in vitro and in vivo..." . The novelty of the research represents the idea that
inhibition of miR-548a-5p may limit HCC growth and miR-548a-5p expression may be the potential
predictor of tumor that respond to Tg 737 –targeting therapies.

Response: No response.

(3) Presentation and readability of the manuscript; The original article is well organized and
classically presented scientific article, well readable manuscript.

Response: No response.

(4) Ethics of the research. All animal procedures were performed in accordance with a
protocol approved by the Fourth Military Medical University Animal Care and Usage

Response: No response.

Committee SPECIFIC COMMENTS Title: accurately reflects the major topic and contents of
the study (may not a very good idea to abbreviation in the title). Abstract: it gives a clear

delineation of the research background, objectives, methods, results, conclusions and key words. Abstract contain the main point presented in this original article. As summarized, the article highline that novel therapeutic strategies would be the forefront of HCC treatment in the near future. Introduction: present relevant information about primary hepatocellular carcinoma, Tg737 gene as a tumor suppressor gene in multiple cancers, microRNAs (miRNAs) as a group of important endogenous modulators of gene function at the posttranscriptional level and they interrelation, which influence the HCC growing and metastasis. Materials and Methods This part of the article is well organized; contain information which help the riders to understand the methodology of the study, which evaluate interrelation between Tg737 and microRNA-548a-5p in in vitro and in vivo. Contain section with statistical analysis. Results This part of article help the riders to illuminate the role of Tg737 in HCC cell proliferation, to show how miR-548a-5p acted on Tg737, to detect the impacts of miR-548a-5p on HCC cell proliferation and to illustrate that Tg737 is a functional target of miR-548a-5p. All of this information are supported by good quality figures and graphs. Discussion. Zhao Ge et al present the idea that miR-548a-5p negatively regulates tumor inhibitor gene Tg737 and promotes tumorigenesis progress. The author suggests that the inhibition of miR-548a-5p may be new therapeutic strategies to limit tumorigenesis progress. Conclusions The authors had presented valuable conclusion that provide novel evidence that miR-548a-5p negatively regulates tumor inhibitor gene Tg737 and promotes tumorigenesis progress in vitro and in vivo. References: references are appropriate, relevant, and updated. Tables and figures: figures and graphs are relevant and useful.

RESPONSE: It was not a good idea to abbreviation in the title. It has been revised in

newly submitted manuscript.

comment4

1.What about the originality of the HCC cell lines, eg, HCC cell lines HepG2 and MHCC97-H?

RESPONSE: HCC cell lines such as HepG2 and MHCC97H were used as cancer model for scientific research universally. We use these cell line for research previously. miR-548a-5p negatively regulates tumor inhibitor gene Tg737 and promotes tumorigenesis progress in vitro and in vivo, indicating its potential as a novel therapeutic target for HCC. HepG2 and MHCC97H played as two regular hepatocellular carcinoma cell lines was involved in the research.

2.Polish the MS. such as, line 175, "200 cells" should be written as two hundred cells."2×10⁶

HepG2 cells", line 181, "Kit-8(CCK-8)" should has blank interval.

RESPONSE: We have got these details revised in the revised manuscript.

3.The name of cell line should be consistent, eg, MHCC97-H, and MHCC97H (line 192).

RESPONSE: We have polished the manuscript and authorized biomedical editing companies to ensure the language quality of the revised manuscript.

4. Which strain of the nude mice? BALB/c?

RESPONSE: BALB/c nude mice was used in the research. We have illustrated it in the revised manuscript.