

## ANSWERING REVIEWERS

January 24, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 7023\_revised 013114.doc).

**Title:** Novel method for extracting exosomes of hepatocellular carcinoma cells

**Authors:** Lin Zhu, Xiuhua Qu, Yulin Sun, Yangming Qian and Xiaohang Zhao

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 7023

We greatly appreciate the comments of the Reviewers, and the manuscript has been improved according to their suggestions.

### **1 The format has been updated**

(1) We have revised the "Columns" of the manuscript according to the "ESPS-Columns scope note" and corrected the address of the corresponding author.

(2) We added the "COMMENTS" section according to the format for brief articles.

### **2 Revisions have been made according to the suggestions of the reviewers**

#### **Comments from Reviewer 1:**

The authors show a new method of exosomes extraction and analysis that may be of general interest.

**Response:** We appreciate the comments from the reviewer.

#### **Comments from Reviewer 2:**

The article entitled "A novel method for extracting exosomes of hepatocellular carcinoma cells" by Lin Zhu et al. describes a new extraction method for exosomes from hepatocellular carcinoma cells and shows that protein TGM2 can be secreted through an exosome-mediated non-classical secretion pathway. The methodology and the data reported are of great interest. There are several grammatical mistakes in English.

**Response:** We thank the reviewer for noting the grammatical mistakes, and we have polished the language.

#### **Comments from Reviewer 3:**

**Comment:** The most important thing is about a novelty. However I read the manuscript carefully, I think this manuscript has no novelty. Although authors have explained that the use of ExoQuick is novel methods, ExoQuick is commercial products and it is used in the world already. This manuscript only showed ExoQuick use to extract exosomes from hepatocellular carcinoma cell lines.

**Response:** We appreciate the comments from the reviewer. Although ExoQuick is a commercial product, the study of exosomes derived from tumor cells using the product is minimal. A search on Pubmed yields only such three papers, including those on lung cancer cells and serum from melanoma patients. We added the study of exosomes secreted from hepatocellular cancer cells using ExoQuick, and there is no paper that compares the traditional method and ExoQuick. Furthermore, one of the hepatoma-associated proteins, tissue transglutaminase 2 (TGM2), was first found in the exosome of the human liver cancer cell line Huh-7. This protein can be secreted through an exosome-mediated non-classical secretion pathway and is a potential tumor marker.

**Comment:** There is no data compared to these methods however authors have described the comparison exosomal proteins from traditional and ExoQuick,. Fig 2 and 3 are not reflected the comparison between traditional methods and ExoQuick methods. It should be need to compare between exosome extracted from traditional methods and ExoQuick, not whole cell lysates.

**Response:** We thank the reviewer's comment. Compared with previous methods, our results mainly focus on the comparison of protein quantity and exosome morphology, showing that the traditional method for extracting exosomal proteins is less productive The concentration of exosomal proteins extracted by the ExoQuick method was approximately 780  $\mu\text{g}/10^8$  Huh-7 cells, and therefore, it was 19 times higher than that of exosomes extracted in the traditional manner.

**Comment:** Citation number is incorrect and there are typographical errors. Please check and correct adequately.

**Response:** We appreciate the reviewer noting this, and we have corrected the references.

**Comment:** What did you discuss on paragraph 1 in discussion? I think this is the only explanation about exosomes.

**Response:** We thank the reviewer for this comment, and we modified paragraph 1 of the discussion.

### **3 References and typesetting were corrected**

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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