ANSWERING REVIEWERS

December 3, 2014

Dear Editor and Reviewers,
We greatly appreciate your efforts in our manuscript. Your comments are very helpful to improve the quality of our paper. We have revised the manuscript according to your comments and the revised manuscripts have been submitted. The following is the point-by-point explanation. Thank you for your attention.

Please find enclosed the edited manuscript in Word format (file name: 15030-revised.docx).

Title: Multiphoton microscopy for tumor regression grading after neoadjuvant treatment for colorectal carcinomas

Author: Lianhuang Li, Zhifen Chen, Xingfu Wang, Shuangmu Zhuo, Hongsheng Li, Weizhong Jiang, Guoxian Guan and Jianxin Chen

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15030

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated.

2. Revision has been made according to the suggestions of the reviewer

(1) Advantages and disadvantages of multiphoton microscopy in comparison with other techniques should be systematically addressed.

Answer:

In the revised manuscript, we have supplemented some explanations:

Please see Page 9, 1st paragraph, Line 5: "…… However, evaluating TRG using current approaches such as CT, magnetic resonance imaging, and positron emission computed tomography is challenging, as these medical imaging technologies lack sufficient resolution[23].

MPM relies on nonlinear optical processes to achieve high resolution imaging of biologic tissues, and can detect cellular and subcellular tissue microstructures. Compared with its single-photon counterpart, TPEF offers an inherent optical sectioning property and deep penetration, and the nonlinear scattering from non-centrosymmetric structures provides complementary information to visualize endogenous structures in intact tissues. Residual tumor cells are detected by the TPEF signal, and the SHG signal is used to detect fibrotic tissue. The SHG:TPEF ratio can be used to distinguish fibrosis from submucosal and serosal tissue, as well as to quantify the fibrotic change, which has been proposed as a diagnostic

indicator for gastrointestinal diseases[24,25]."

(2) Minimal tumour residues in a broad fiber matrix is hard detectable using conventional microscopy. Is there any evidence that multiphoton microscopy could overcome the problem?

Answer:

According to the nonlinear spectral analysis, residual tumor cells can generate TPEF signal (color-coded red), while the fibrotic tissues can emit SHG signal (color-coded green). Thus, these provide a possibility of identifying minimal tumor in a fiber matrix by MPM. Our results demonstrated that MPM has the ability to distinguish the residual tumor cells from the fibrotic tissues (Figure 3).

(3) Scarring includes accumulation of different collagen types. Collagen composition could be of interest to further characterize tumour regression. Is there any data available that multiphoton microscopy is able to categorize scarred tissues?

Answer:

Scarring is an accumulation of different collagen types, and is in particular characterized by an excessive deposition of collagen type I. According to previous publications, collagen type I, II and III generate SHG signals in tissues, while collagen type IV does not; furthermore, collagen I and III can be differentiated by SHG microscopy because they have different morphology, and collagen I and II can be identified through the far-field polarization SHG (P-SHG) measurements. Therefore, MPM should be able to categorize scarred tissues although we have not done these researches.

[References:

1. Strupler M, et al. Optics Express, 2007, 15: 4054-4065;

2. Egeblad M, et tal. Current Opinion in Cell Biology, 2010, 22: 697-706;

3. Su PJ, et al. Biophysical Journal, 2011, 100: 2053–2062;

4. Suzuki M, et al. Proc. of SPIE, 2012, 8226: 82263F-1-9;

5. Theodossiou TA, et al. Biophysical Journal, 2006, 91: 4665–4677;

6. Lo´pez-Novoa JM, et al. EMBO Mol. Med., 2009, 1: 303-314.]

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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