

November 22, 2015

Ferruccio Bonino, MD, PhD

Editor-in-Chief, *World Journal of Gastroenterology*

Dear Dr. Bonino,

Please find enclosed our manuscript titled “**Sphingosine Kinase 1 is upregulated with LPAR2 in Human Colorectal Cancer**” for publication in the *World Journal of Gastroenterology*. < ESPS Manuscript NO: 22930>

As requested by the Editor, the manuscript has been revised. With respect to the comment from reviewers, please see our ‘Response to the Reviewer’s comment’ below.

We believe the manuscript has substantially improved, and look forward to hearing from you at your earliest convenience.

Sincerely yours,

Dai SHIDA, MD, PhD

Colorectal Surgery Division, National Cancer Center Hospital

Email: dshida-tyk@umin.ac.jp

Response to the Reviewer's comment.

Reviewer1 (**Reviewed by 03062291**)

1. This is quite an interesting study showing positive correlation between SphK1 and LPAR2 and the development of colorectal. However, the results are solely relied on RT PCR data without further validation. What would be highly advisable is to screen the replicas of the same tissue samples on a protein level as most of the known functions for the above two genes are performed by encoded proteins.

The point which reviewer mentioned may be the limitation of this study. However, in our previous paper we have already confirmed mRNA expression of LPA receptors measured by real-time RT-PCR is in consistency with that measured by Northern blot analysis [19]. In addition, in this study, we examined real-time RT-PCR of LPA receptors and SphK1 several times for each sample, and the results were highly reproducible (data not shown). Thus, the data of mRNA expression obtained from RT-PCR method in this study seemed very reliable. Moreover, several groups including us have already confirmed correlation between mRNA expression and protein expression using Western blot analysis and immunohistochemical staining of LPA receptors as well as SphK1[13, 19, 28]. From these reasons, we consider our mRNA results are reliable and have significant meanings in reality, although further investigation will be needed.

We added this paragraph in the Discussion section (page13 line17 – page14 line7).

Another minor point: it is suggested to incorporate reference numbers within the text.

We have confirmed the reference numbers within the text. One reference (Chomczynski P *et al.*) was deleted. Several references (#4, 8, 18) may bother the reviewer because the official name of LPA receptors had changed. LPAR2 was originally named as EDG4, and LPAR1 was previously named as LPA₁.

Reviewer2 (**Reviewed by 02533652**)

1. This study by Dr. Shida is one of the interesting manuscripts in the field of molecular mechanisms of colorectal cancer implicating the role of phosolipid modulating enzymes in the development of CRC. My only issue with the manuscript is that there is only one kind of experimentation done to assess the expression of the molecules... and the conclusion are heavily based on the expression of SphK1 and

LPA₂ RNA levels using RT-PCR. This has many times proven deleterious when the same were replicated with another technique to ascertain the results. I would recommend that another technique to ascertain the results should be incorporated in the manuscript for more assurance. Furthermore, the authors are advised to properly cite the references within the main body of the paper. The authors should also expand the abbreviation when use first, then only can authors abbreviate the long terms when used second time. I would

The point which reviewer mentioned may be the limitation of this study. However, in our previous paper we have already confirmed mRNA expression of LPA receptors measured by real-time RT-PCR is in consistency with that measured by Northern blot analysis [19]. In addition, in this study, we examined real-time RT-PCR of LPA receptors and SphK1 several times for each sample, and the results were highly reproducible (data not shown). Thus, the data of mRNA expression obtained from RT-PCR method in this study seemed very reliable. Moreover, several groups including us have already confirmed correlation between mRNA expression and protein expression using Western blot analysis and immunohistochemical staining of LPA receptors as well as SphK1[13, 19, 28]. From these reasons, we consider our mRNA results are reliable and have significant meanings in reality, although further investigation will be needed.

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The authors should also expand the abbreviation when use first, then only can authors abbreviate the long terms when used second time. I would

Thank you. We checked is throughout our paper.