Reviewer's report

Title: Increased Diacylglycerol Kinase zeta Expression in Human Metastatic Colon Cancer Cells Contributes to Enhanced Invasion by Augmenting Rho GTPase Activity

Version: 1 Date: 5 July 2013

Reviewer: Helan Xiao

Reviewer's report:

-Major Compulsory Revisions

1. The physiological meaning of the work is unclear. The methods are unsuitable and the number of samples are very limited. The underlying mechanism is not properly studied and discussed. The conclusion is not sound.

SW480 and SW620 are the ATCC cell immortalized cell lines from the same patient. They are not representative cells lines for the control and metastatic colon cancer cells.

To draw the solid conclusion, the author should use at least normal (non-cancer colon cells) and metastatic colon cancer cells from at least 3 different patients, and at least 3 different cell lines from ATCC.


3. The author should pick 3 clones of the shRNA DGKzeta knockdown stable cell lines for experiments.

4. In Material and Methods in Invasion Assay part, the author said that "count 5 fields of view". How many fields and views in total? How many cells in total views? How many cells in these 5 counted fields? Please clarify the quantification.

5. In Fig 1, the author should add a western to test phosphorylatedDGKzeta in Fig1A and add quantification of relative phosphorylated DGKzeta level.

The author only tested SW480 and SW620 cell lines. They should test at least 3 control and 3 patients cells, at least 3 control and 3 metastatic cancer ATCC cell lines.

6. In Fig 2E, the relative RhoA activity is counted by GST-RBD/tubuli. It should be GST-RBD/RhoA,, so there is no big difference between SW480 and SW620.

The figure legends for Fig 2D and 2E are missing.
7. in Fig 3A, a control (tubulin) western blot should be added.

8. in Fig 4A, it should test phosphorylated DGKzeta. the author should also quantify relative phosphorylated DGKzeta level.

9. in Fig 5, the author should show the data of SW480, 3 vector stable cell lines, and 3 shRNA DGKzeta stable cell lines.

in Fig 5A, add control tubulin western blot.

10. in Fig 6B, the author should show the invasion assay data of 3 vector stable cell lines, and 3 shRNA DGKzeta stable cell lines.

-Minor Essential Revisions

1. there should be a clarification between cell invasion and cell migration. The author did the double chambers transwell cell invasion assay. So the results support the cell invasive property, not migration. The running title shoule be "DGKzeta controls cancer cell invasion".

2. in Abstract line 12, it should be shRNA silenced SW620 cell, not siRNA.

3. in Material and Methods in Antibodies part, add the catalog number of the antibodies. Make a table of all the antibodies and list the source, catalog number, final concentration, experimental condition (temperature and time).

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.

Helan Xiao 7/4/2013