PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Immunohistochemical analysis of TRIM59: A novel multiple cancer biomarker
AUTHORS	Xuan, Jim W ; Khatamianfar, Vida; Valiyeva, Fatma; Rennie, Paul; Lu, Weiyang; Yang, Burton B.; Bauman, Glenn; Moussa, Madeleine

VERSION 1 - REVIEW

REVIEWER	Shigetsugu Hatakeyama, M.D., Ph.D., Professor, Hokkaido University Graduate School of Medicine I have no conflict of interest.
REVIEW RETURNED	26-May-2012

GENERAL COMMENTS	Previously, the authors have reported that TRIM59 gene was upregulated in SV40 Tag oncogene-directed transgenic mouse prostate cancer models and that two phosphorylated forms of TRIM59 were observed by using purified TRIM59 proteins from mouse prostate cancer models at different stages. Furthermore, TRIM59 knockdown caused S-phase cell-cycle arrest and cell growth retardation. In this paper, the authors demonstrate that TRIM59 is highly expressed in several epithelial cancer tissues isolated from human samples and that TRIM59 may be used as a novel tumor marker for detecting early tumorigenesis. This manuscript contains some important issues, such as identification of high expression of TRIM59 in several cancer tissues including prostate, mammary gland, and so on. This paper might be of broad interest in the field of clinical oncology. Specific points 1. The specificity of antibodies is very important for immunohistochenical analysis. The authors should confirm the specificity of TRIM59 antibodies to analyze human tissues. The authors showed that human cell lines such as PC3, LNCap and HEK293 express TRIM59 in a supplemental figure. The authors should perform immunocytochemical analysis and immunoblot analysis using TRIM59 knock-down cells (293, PC3 or LNCap) to check your antibodies. Minor points 1. Since this manuscript has several mistakes such as typographical errors, authors should carefully check: Page 16, line 12, Page 18, line 14; Page 20, line 15 and others. 2. The authors should cite more publications about TRIM family-

	related carcinogenesis.

REVIEWER	Sha Tao Biostatistician Center for Cancer Genomics Wake Forest University USA no competing interest
REVIEW RETURNED	27-Jun-2012

THE STUDY	The manuscript needs to more proofreading in order to be more readable. A quick look I see a few problems: 1. page 12, line 50, "was significantly"; 2. Page 18, line41, "may also possibly"; 3. page 16, line 26, "in m this"
REPORTING & ETHICS	1. The supplementary figure 1 is partially identical to a 2011 paper on Mol Cancer Ther title "Characterization of the Oncogenic Activity of the Novel TRIM59 Gene in Mouse Cancer Models". The author needs to point to the reference as the copyright of the figure must have been transferred to the journal. 2. Page 18, line 20. Please add the source for the 30% claim.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Shigetsugu Hatakeyama, M.D., Ph.D., Professor,
Hokkaido University Graduate School of Medicine

I have no conflict of interest.

Previously, the authors have reported that TRIM59 gene was upregulated in SV40 Tag oncogene-directed transgenic mouse prostate cancer models and that two phosphorylated forms of TRIM59 were observed by using purified TRIM59 proteins from mouse prostate cancer models at different stages. Furthermore, TRIM59 knockdown caused S-phase cell-cycle arrest and cell growth retardation. In this paper, the authors demonstrate that TRIM59 is highly expressed in several epithelial cancer tissues isolated from human samples and that TRIM59 may be used as a novel tumor marker for detecting early tumorigenesis.
This manuscript contains some important issues, such as identification of high expression of TRIM59 in several cancer tissues including prostate, mammary gland, and so on. This paper might be of broad interest in the field of clinical oncology.

Specific points

1. The specificity of antibodies is very important for immunohistochenical analysis. The authors should confirm the specificity of TRIM59 antibodies to analyze human tissues. The authors showed that human cell lines such as PC3, LNCap and HEK293 express TRIM59 in a supplemental figure. The authors should perform immunocytochemical analysis and immunoblot analysis using TRIM59 knock-down cells (293, PC3 or LNCap) to check your antibodies.

Response : We performed this experiment and reported in the previous publication (Valiyeva et al 2011 Mol, Can Thr,) at p.1239 that “Probably due to this rapid effect, results of our Western blotting semiquantitative test on levels of TRIM59 and p-TRIM59 proteins in shRNA knockdown cells and in

PSP94-TRIM59 mice were marginal …….".

Minor points
1. Since this manuscript has several mistakes such as typographical errors, authors should carefully check: Page 16, line 12, Page 18, line 14; Page 20, line 15 and others.

Response : Page 16, line 12, 37 tumor types analyses.`` Changed to "37 tumor type analyses." n "Page 18, line 14; Maybe" Chaged to "may be"
Page 20, line 15 and others. "in eight types of tumor" changed to "in eight types of tumors" (we searched other places, and no one found).

2. The authors should cite more publications about TRIM family-related carcinogenesis

Response: We have quoted one new and the last review paper "Coleman,M.L., Marshall,C.J. and Olson,M.F. RAS and RHO GTPases in G1-phase cell-cycle regulation, Nature Review Molecular Cell Biology 2004; 5: 355-366."

Reviewer: Sha Tao
Biostatistician
Center for Cancer Genomics
Wake Forest University
USA

no competing interest

The manuscript needs to more proofreading in order to be more readable. A quick look I see a few problems: 1. page 12, line 50, "was significantly"; 2. Page 18, line41, "may also possibly"; 3. page 16, line 26, "in m this"

Response: We have corrected all these mistakes.

1. The supplementary figure 1 is partially identical to a 2011 paper on Mol Cancer Ther title "Characterization of the Oncogenic Activity of the Novel TRIM59 Gene in Mouse Cancer Models". The author needs to point to the reference as the copyright of the figure must have been transferred to the journal.

Response: We have addressed this issue in M&M that `` Supplement Fig. 1 provided details, which combined Supplement materials of (17) , and more informations.``

2. Page 18, line 20. Please add the source for the 30% claim.

Response: we have quoted one more and the last review paper "Coleman,M.L., Marshall,C.J. and Olson,M.F. RAS and RHO GTPases in G1-phase cell-cycle regulation, Nature Review Molecular Cell Biology 2004; 5: 355-366."

VERSION 2 – REVIEW

REVIEWER	Shugetsugu Hatakeyama, Professor Hokkaido University Graduate School of medicine, Japan
REVIEW RETURNED	07-Jul-2012

THE STUDY	1. Since this manuscript has several mistakes such as typographical errors, authors should more carefully check. Page 5, line 5: PI33K? (PI3K) Page 6, Table 1: is total patient number correct, 291 or 287? Page 7, line 8: form? (from) Page 14, table 2: in 35 TMA? (in 34 TMA) Page 16, line 11: in m this report? Page 16, line 13: The sentence “although TRIM59 is a normal gene involved in CDC (cell cycle division) regulation from G1 to S-phase involved in DNA S-phase and cell growth”. The authors should add the word “and” before “involved”? 2. The authors should cite more publications about TRIM family-related carcinogenesis. The paper “Coleman,M.L., Marshall,C.J. and Olson,M.F. RAS and RHO GTPases in G1-phase cell-cycle regulation, Nature Review Molecular Cell Biology 2004; 5: 355-366” is inappropriate. The authors should check more carefully.
RESULTS & CONCLUSIONS	The specificity of antibodies is very important for immunohistochemical analysis. The immmunoblot or immunocytochemical analysis about TRIM59-knockdown has not been shown in previous publication (Valiyeva et al 2011 Mol. Cancer Thr.). Actually, several supplemental data in this manuscript is used from the same figures in the authors’ previous publication (Valiyeva et al 2011 Mol. Cancer Thr.). However, the raw data is not shown in this paper: Probably due to this rapid effect, results of our Western blotting semiquantitative test on levels of TRIM59 and p-TRIM59 proteins in shRNA knockdown cells and in PSP94-TRIM59 mice were marginal (data not shown) (p.1239). The authors should perform immunocytochemical analysis or immunoblot analysis using TRIM59 knock-down cells (HeLa, 293, PC3 or LNCap) to confirm the reliability of your antibodies.
GENERAL COMMENTS	If a revised form is well addressed, editor may decide without my opinion.

VERSION 2 – AUTHOR RESPONSE

Reviewer: Shugetsugu Hatakeyama, Professor
Hokkaido University Graduate School of medicine, Japan

1. Since this manuscript has several mistakes such as typographical errors, authors should more carefully check.
Page 5, line 5: PI33K? (PI3K)
Response: yes, corrected

Page 6, Table 1: is total patient number correct, 291 or 287?
Response: 291 is correct.

Page 7, line 8: form? (from)
Response: yes, corrected

Page 14, table 2: in 35 TMA? (in 34 TMA)
Response: You are right. The TMA was 35 tumor TMA, here just list 34. However, we still call it 35 tumor TMA.

Page 16, line 11: in m this report?

Response: Already corrected in the first version.

Page 16, line 13: The sentence “although TRIM59 is a normal gene involved in CDC (cell cycle division) regulation from G1 to S-phase involved in DNA S-phase and cell growth”. The authors should add the word “and” before “involved”?
Response. Add “and”.

2. The authors should cite more publications about TRIM family-related carcinogenesis. The paper “Coleman,M.L., Marshall,C.J. and Olson,M.F. RAS and RHO GTPases in G1-phase cell-cycle regulation, Nature Review Molecular Cell Biology 2004; 5: 355-366” is inappropriate. The authors should check more carefully.

Response: This reference will meet reviewer’s question about “30% claim”. I cite another more clear ones on human cancer tumorigenesis with Ras mutations.

The specificity of antibodies is very important for immunohistochemical analysis. The immmunoblot or immunocytochemical analysis about TRIM59-knockdown has not been shown in previous publication (Valiyeva et al 2011 Mol. Cancer Thr.). Actually, several supplemental data in this manuscript is used from the same figures in the authors’ previous publication (Valiyeva et al 2011 Mol. Cancer Thr.). However, the raw data is not shown in this paper: Probably due to this rapid effect, results of our Western blotting semiquantitative test on levels of TRIM59 and p-TRIM59 proteins in shRNA knockdown cells and in PSP94-TRIM59 mice were marginal (data not shown) (p.1239).
The authors should perform immunocytochemical analysis or immunoblot analysis using TRIM59 knock-down cells (HeLa, 293, PC3 or LNCap) to confirm the reliability of your antibodies.
Response: I agree it will be perfect to demonstrate the reliability of the Ab used, if either by Western or immunocytochemistry (ICC) showed the protein level decreased or significantly decreased after KO of TRIM59 in cell lines.
As requested, I attach a figure in below (not shown in here but in an uploaded file) of such experiment we performed. The second row is tests of total cell lysates of 3 shRNA stable transfectant cell clones, which only one showed lower level of TRIM59 as compared with controls of shRNA negative plasmid transfectants (right lane). The third row shows IP purified TRIM59 proteins from Row 2. The bottom row is tests of phosphorylation of Row 3. As we have indicated the results was “marginal”, not very significant and was not repeating well.

We only performed Western blots experiments. Western blot is semi-quantitative, and may be better than ICC test in this regard.
As we have explained in the publication that our shRNA KO experiments showed a “hit-and-run” effect, a rapid change in effectors in Ras signal pathway, by both quantitative realtime RT-PCR (n=4) and by GeneChip results in transients or stable cell KO transfection. Since this is rapid and transient, so we confirmed Western results that no significant knockdown of TRIM59 protein could be observed. We have multiple lines of evidences to support this working conclusion (1) TRIM59 protein phosphorylation (2) p-TRIM59 correlation in GEM-CaP models (3) Transgenic mouse TRIM59 up regulation GEM-CaP models ……
Again, our strong points on antibody characterization are as we have repeatedly addressed, that the antibody raised against N-terminal 163aa sequence of TRIM59 protein (403aa) can recognize the same band as that of the antibody of the C-terminal 136aa, in Western