

Format for ANSWERING REVIEWERS



December 16, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: *WJH_granulysin_review(6207 revise).doc*).

Title: Granulysin and its clinical significance as a biomarker of immune response and NK-cell related neoplasm

Author: Masayuki Nagasawa, Kazuyuki Ogawa, Kinya Nagata, Norio Shimizu

Name of Journal: *World Journal of Hematology*

ESPS Manuscript NO: 6207

The manuscript has been improved according to the suggestions of reviewers:
(Correction or answer is written in red color.)

1. The title of the paper would better to show the target status of a biomarker. For example, biomarker for immune response and NK-cell related neoplasms.

Title was changed according to the suggestion.

2. Introduction, line 6: “only after 3 to 5 days” is not clear in Figure 1. Very weak signal on day 8?

“only after 3 to 5 days” was changed to “later”.

3. I. Structure and function, page 1, line 15: “figure 2” should be “figure 2a”. Please use the illustration with higher resolution.

“Figure 2” was changed to “figure 2a”.

4. I. Structure and function, page 1-2: Figure 3 should be quoted in this paragraph.

According to the comment, figure 3 was quoted in this paragraph.

5. II. Expression and cytotoxic activity, page 2: Please explain about the mechanisms of release of 9 kDa molecule in this section (Ca-dependent release).

Sentence below was added in this section.

9 kDa granulysin is released when co-cultured with target K562 cell and its release is prohibited by depletion of calcium, indicating Ca-dependent and trigger-dependent excretion of 9 kDa granulysin.

6. IV. Granulysin as a biomarker, 1: cell mediated immunity: Figures 4a and 4b were expected to be the data from reference No. 35. Is there any problem about the copyright of the publisher?

There is no problem about the copyright.

7. IV. Granulysin as a biomarker, 4: tumor immunity: Serum granulysin increases with aging (Figure 4a). This means that immune deterioration with aging is not related with the granulysin level. However, in patients with tumors, granulysin as well as general immune capacity might significantly reduced. Isn't it contradictory?

It is well known that NK activity increases with ageing until the age of 40 and decreases thereafter. Discrepancy between granulysin level and NK activity after the age of 40-50 is not well explained. One possibility is that the ratio of conversion from 15kDa to 9kDa is changed after the age of 40. We have no data concerning this issue, which should be investigated.

8. IV. Granulysin as a biomarker, 6: NK-cell related tumor or neoplasm: In Figure 6c, what were the bands around 50 kDa? How to read the levels of TNF- α and IFN- γ ? Were the data driven from other experiments?

50kDa band is a non specific band. TNF- α and IFN- γ were assayed by ELISA in the culture supernatant of cell lines that were used for western blot analysis.

9. Figure legends should be prepared for Figure 2b, 4a, and 6a.

Legends of figure 2b, 4a, and 6a were added to each figure.

Other revision

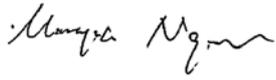
New information and reference were added as "Supplementation".

Core tip is added as follows.

Granulysin is a cytotoxic granular protein expressed in cytotoxic T-cells, NK-cells, and $\gamma\delta$ T-cells and has anti-microbial activity against microorganisms such as bacteria, fungi, mycobacteria, and parasites, as well as tumoricidal activity against some tumors. It is secreted constitutively and trigger-dependent manner. Clinically, serum granulysin is a unique biomarker for immune response and NK-cell related neoplasm.

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

Sincerely yours,



Masayuki Nagasawa, M.D., Ph.D.

Tokyo Bay Urayasu/Ichikawa Medical Center

Chair, Department of Hematology, Oncology and Immunology

3-4-32, Todaijima, Urayasu-city, Chiba 279-0001 Japan

Tel +81-047-351-3101 Fax +81-047-352-6237

E-mail: mnagasawa.ped@tmd.ac.jp

masayukin@jadecom.jp