Reviewer's report

Title: The immunomodulator PSK induces in vitro cytotoxic activity in tumor cell lines

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Reviewer: Kenichi Matsunaga

Reviewer's report:

-General
1) The article investigated the in vitro cytotoxic activity of the immunomodulator PSK against cancer cell lines.

The protein-bound polysaccharide PSK has been used clinically as an anti-cancer drug for more than 30 years in Asia, mainly in Japan. Efficacy and mode of action of PSK have been investigated and evaluated clinically as well as fundamentally for a long time by many researchers in the fields of oncology, immunology, etc. The drug has characteristically multiple modes of action; however, the in vitro anti-cancer activity has been insufficiently studied when compared to immunomodulating activity. Further, correlation of in vitro anti-cancer activity and in vivo anti-cancer activity remains unelucidated.

In the article, authors have analyzed the mechanism of in vitro anti-cancer effect of PSK at molecular level, and found that cytotoxic action of the drug on cancer cells was different according to histology of cancer cell lines, and that arrest of cell cycle, expression of caspase 3 and induction of apoptosis appear responsible mechanisms in PSK-effective cell lines. These findings are novel, and give some impact to researchers in related fields.

2) The assignments of the study described in the article are well defined and the methods used appear to be appropriate. Although some revisions are necessary as described below, the discussion and conclusions are well balanced and supported by the data.

-Minor Essential Revisions
1) In Abstract Section (page 2 line 2), it is required to correct CM.101 to CM-101.
2) It is required to correct the numerical value of the vertical axis (absorbance) of Figures 1, 2 and 3 from comma (,) to period (.). (For example, 0,2 â## 0.2)

-Discretionary Revisions
1) The title (page 1) appears inadequate, since it does not fully reflect author's findings. It is recommended to revise the title (for example, The immunomodulator PSK induces in vitro cytotoxic activity in tumor cell line through arrest of cell cycle and induction of apoptosis).
2) In Materials and Methods Section (page 5), physicochemical natures (for example, composition, components, etc.) of neuraminidase-treated PSK and
original PSK were not described. It is necessary to comment their differences for better understanding of possible active components or structures.

3) Also, in Discussion Section, interpretation of the results of experiments using PSK variants was not described. It is necessary to discuss or comment the results (for example, interpretations of possible component or structure responsible for the expression of cytotoxic activity, etc.).

4) Concerning the data of Table 1, Figures 1 to 3, it is required to do statistical analysis.

5) In Discussion Section (page 13 line 20 to 21), authors described a possible involvement of cytotoxic components of PSK in in vivo anti-metastatic capacity. Please explain the ground thought in fuller detail that anti-metastasis activity is due to cytotoxic components of PSK.


7) In Materials and Methods Section (page 4 lines 18), it is recommended to insert (PSK) after protein-bound polysaccharide K.

8) The names of the same cell lines are sometimes written different in the text; for example, ANDO-2 (page 5 lines 8, Legend of Figure 1), Ando 2 (page 5 lines 12 and page 5 lines 15), Ando-2 (page 9 lines 4) and ANDO2 (Table 1); Jurkat T cell leukemia (page 5 lines 9 and page 9 lines 4) and JURKAT leukemia (page 5 lines 21, Legend of Figure 1, Table 1 and Figure 2); HeLa (page 5 lines 8 and Figure 2), Hela (page 9 lines 13) and HELA (Table 1). In order to avoid confusing, authors are recommended to unite the name.

9) Neither the cell line IMIM PC-1 (page 5 lines 13) nor U937 (page 5 lines 21) in Materials and Methods Section appears to be used in the experiments. It is recommended to delete them.

10) Sentence of the page 5 lines 22 ended with RPMI. It is recommended to change full name of RPMI (for example, RPMI1640 with 10% heat-inactivated fetal bovine serum).

11) It is recommended to add 7-AAD, BrdU, Concan, LAK, NK and TMB in Abbreviation List (page 14).

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.