Author's response to reviews

Title: Essential oil of Pinus koraiensis leaves inhibits cell proliferation and migration via inhibition of p21-activated kinase 1 pathway in HCT116 colorectal cancer cells.

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Version: 3
Date: 29 June 2014

Author's response to reviews: see over
Dear Dr. Tom Rowles, Executive Chief Editor:

We thank reviewers and an editor for valuable comments to our manuscript entitled with “Essential oil of pinus koraiensis leaves inhibits cell proliferation and migration via inhibition of p21-activated kinase 1 pathway in HCT116 colorectal cancer cells” (Manuscript ID: 1655723695125979) Based on the reviewer’s comments, we revised them point by point as follows:

The followings are our point-by-point responses to reviewer’s comments

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Reviewer’s Report:

Major Compulsory Revisions

1. Experimentally, the effect of EOPK was shown on colorectal cancer cells, however there is not a control cell line included. It would be useful to show the cytotoxic effect of EOPK on non-tumor cells such as normal colon epithelial cells or maybe fibroblasts.

(Response) Thank you for your comments. Based on your comments, we evaluated the cytotoxicity of EOPK in NIH-3T3 cells as normal cell control in this revised MS. We found no significant cytoxicity of EOPK in NIH-3T3 cells as shown in our revised MS (Figure 1D).

2. Discussion part needs to be improved. Obtained data must be discussed more thoroughly. For example, is there a synergy between PAK-1 siRNA and EOPK and what might be the underlying mechanisms? A figure might be added to Show the interactions of key factors in signaling pathways.

(Response) We appreciate the suggestion. Discussion part was improved.
Minor Essential Revisions:

1. Manuscript needs to be carefully revised and corrected in terms of language and grammar. (Response) According to your advice, we used a professional language editing service (Editage by CACUS, English Editing certification number OJUTS_1).

2. Abstract needs to be written in more coherent manner to reflect the findings of the paper more precisely. (Response) Thanks. Based on your comment, we improved Abstract more clearly.

3. In methods, Cell growth assay part needs to written using passive sentences. (Response) Thanks. Corrected

Discretionary Revisions

1. Authors may show the degree of apoptosis using simple giemsa stainig or appropriate kits in cancer cells after treatment with EOPK. (Response) EOPK showed growth inhibitory effects in a dose dependent fashion. When HCT116 cells were further tested for EOPK-induced apoptosis, apoptotic cells were observed after 24h at 200 µg/ml EOPK. We confirmed 200µg/ml of EOPK induced sub G1 by FACS. FACS data were used instead of TUNEL staining or Giemsa staining.

2. The authors may demonstrate the cytotoxic effect of EOPK on cancer cells in short term culture (2 days) in addition to long term (5 day) culture. (Response) Thanks. The cytotoxic effect of EOPK on cancer cells in short term was performed by MTT assay.
Reviewer’s Report : 2

Comments:

1. In background section, authors should add some details about the study on genotoxicity and biological activity of EOPK with applied doses and cell lines for the comparison purposes (On the page 6, last paragraph).

(Response) We improved background section.

2. According to in my opinion all data should be able to repeated. On light of this point authors should be explain content of EOPK and collected time of *Pinus koraiensis* leaves on the material and methods section (page 7-preparation of EOPK). Because season always effect to content of the essential oils. Changing in the content also effects biological activity of essential oils.

(Response) Thank you for your comment. We agree with your opinion. The material and methods for EOPK were explained clearly in MS.

3. The cells used in each experiment should be specified in the material and methods section (siRNA transfection, wound healing, cell growth assay). Why did the authors choose the HCT116 cells specifically for cell cycle assay and proliferation assay?

(Response) Corrected. We chose HCT-116 cells, because when various colon cancer cells were tested for cytotoxic effect of EOPK, we found that EOPK significantly inhibited cell viability in HCT-116 cells(data not shown). Furthermore, as shown in Figure.1A, protein level of PAK1 was expressed strongly in HCT-116cells.

4. It can be used a positive control which drugs is used to treatment of colorectal cancer for all experiment to improvement of discussion. Or author should report the literature reference to which is referred the capacity of the drug in inhibiting cell proliferation and apoptosis on
colorectal cancer.

(Response) Thanks. We improved the discussion part including capacity of drugs in inhibiting cell proliferation and apoptosis on colorectal cancer.

5. Additionally, a successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. As the main aim of chemotherapy is the destruction of tumour cells without any undue influence on proper cells. It should be applied all of concentrations of EOPK on healthy cells.

(Response) we evaluated the cytotoxicity of EOPK in NIH-3T3 cells as normal cell control in this revised MS. EOPK had no significant effect on the viability of the normal fibroblast cells as shown in our revised MS (Figure 1D).

6. Recent results which is published papers in should be mentioned and commented in discussion.

(Response) Thanks. Added.

We would like to take this opportunity to express our sincere thanks to the reviewers and editor who identified areas of our manuscript that needed corrections or modification for improving the quality of our manuscript. We hope our revised manuscripts would be accepted for publication in your journal soon.

Sincerely yours,

Hyo-Jeong Lee, Ph.D.