

## Format for ANSWERING REVIEWERS



May 13, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17717-review.doc).

**Title:** Dexamethasone inhibits hypoxia-induced epithelial-mesenchymal transition in colon cancer

**Author:** Jung Ho Kim, You Jin Hwang, Sang Hoon Han, Young Eun Lee, Saerom Kim, Yoon Jae Kim, Jae Hee Cho, Kwang An Kwon, Ju Hyun Kim and Se-Hee Kim

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 17717

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

### EDITOR'S SUGGESTIONS

1) English language editing service

⇒ An English language editing service has reviewed the revised manuscript. We have attached the file containing the English language certificate.

2) The revisions

⇒ We have modified the manuscript based on this review and added the requested data and discussion. These are denoted by green-highlighted text.

3) A decomposable figure is required. It means that the fonts and lines can be edited or moved. The example is attached.

⇒ We have attached a ppt file.

### REVIEW

1) Reviewer 1

The authors address a clinically very interesting issue, considering the fact that most patients undergoing chemotherapeutic treatment for metastatic colorectal cancer also receive anti-emetic treatment with synthetic corticosteroids. There are emerging clinical data pointing in the direction of an unfavourable effect of dexamethason perioperatively on the survival of colorectal cancer patients. The underlying molecular mechanisms are unclear. Kim et al. aim to investigate the effects of dexamethason on hypoxia-dependent EMT in colorectal cancer cell lines. Their introductory remark about increasing incidence of colorectal cancer leads too far away from the topic. The statement that dexamethason is proven to have cytostatic effect in hematologic malignancies lacks references. Apart from that, the introduction is presenting the clinical problem in an effective manner. The method section is clear and concise. The results section guides the reader nicely through the experiments and presents the results in a clear manner. The figures add to the understanding. Would the authors in the discussion please add comments on the following issues: ? Why are two different cell lines used? ? Did the authors study any other markers of hypoxia (CA-IX)? ? What is the effect of dexamethasone on colorectal cancer cells under normoxic conditions? All in all, the paper is easily

read, addresses an interesting question and adds to the evidence in the field.

Their introductory remark about increasing incidence of colorectal cancer leads too far away from the topic.

- ⇒ We agree with reviewer's comment and have modified the manuscript. We removed the following sentence in the introduction (~~Page 4, lines 2-4~~): "The increasing incidence of colorectal cancer in economically developing countries is thought to reflect a combination of factors including smoking, obesity, physical inactivity, and changes in dietary patterns"

The statement that dexamethasone is proven to have cytostatic effect in hematologic malignancies lacks references.

- ⇒ Several references were updated or added.

Would the authors in the discussion please add following comments on the following issues?:

- ⇒ Thank you very much for your comments. We have modified the manuscript based on the review and added the requested data and discussion.

Why are two different cell lines used?

- ⇒ Colon cancer can be classified into two major groups: MSI and MSS. Because HCT116 and HT29 belong to the MSI (microsatellite instability) and MSS (microsatellite stable, chromosome instability) classifications, respectively, we used these cells as representatives of the two subtypes (Giacomini CP et al., 2005, Cancer Research).

Did the authors study any other markers of hypoxia?

- ⇒ Since dexamethasone showed an inhibitory effect on HIF-1 $\alpha$  and VEGF expression in hypoxia, we hypothesized that dexamethasone could control the HIF-1 $\alpha$  pathway in hypoxia. Therefore, we did not test any other markers of hypoxia.

What is the effect of dexamethasone on colorectal cancer cells under normoxic conditions?

- ⇒ It has reported that dexamethasone controls apoptosis under normoxia (Bansal N et al., 1991, FASEB J). But the effect of dexamethasone on EMT (Epithelial-Mesenchymal Transition) of colon cancer cells under normoxic condition remains elusive. Transforming-growth factor- $\beta$  (TGF- $\beta$ ) functions as a major factor of EMT by stimulating proliferation of mesenchymal origin cell types. However, this current study aims to investigate the effects of DEX on hypoxia-dependent EMT in colorectal cancer cell lines.

## 2) Reviewer 2

The author proposed to elucidate the effects of dexamethasone on hypoxia-induced EMT in colon cancer. Though the real-time PCR, Western blot, Transwell and Wound healing tests, the authors verify that dexamethasone inhibit HIF-1 $\alpha$  protein level and its downstream gene, VEGF mRNA level in hypoxic condition. In addition, dexamethasone down-regulated not only hypoxia-induced Snail and Slug mRNA levels, but also hypoxia-induced integrin  $\alpha$ V $\beta$ 6 protein level. Furthermore, reduced E-cadherin in hypoxic condition was found to be recoverable by treating with dexamethasone in colon cancer cell lines; dexamethasone blocked the migration and invasion of colon cancer cells in hypoxia. Thus, in all it suggested the potential role of dex in cancer therapy and the mechanism involved in and it is possible to accept this manuscript with minor improvements. Major issues 1. In the paper, they only told that DEX may have some role in EMT of cancer, but they can not tell us if the role is direct, for example, if there is no HIF-1 $\alpha$  signal pathway, what would happen? So, the author may perform some gene over-expression experiments which would testify if the role is only one or direct? 2. If the author can clarify the mechanism of the migration and invasion by DEX in colon cancer. Or try to discuss some of them. Minor concerns: 1. "These data suggest that Snail plays a major role as an inducer of tumor invasion andmetastasis[19]." For this sentence, there should be a space between "andmetastasis" and this should be take care in the whole text. 2. Both the cell lines of HCT116 and HT29 have got the same results with DEX, but if there is any other colon cancer cell line with the different characteristic or if it would relate with the colon cancer stem cell?

#### Major issues

1. In the paper, they only told that DEX may have some role in EMT of cancer, but they can not tell us if the role is direct, for example, if there is no HIF-1 $\alpha$  signal pathway, what would happen?

- ⇒ The hypoxic tumor microenvironment facilitates cancer progression. The key discovery of this study is the inhibitory effect of dexamethasone on hypoxia-induced EMT in colon cancer cells. Thus, it is necessary to determine whether dexamethasone directly contributes to HIF-1 $\alpha$  stability.
- ⇒ Dexamethasone is known to control the apoptosis of cancer cells under hypoxic conditions; however, its role on EMT in colon cancer cells remains unclear.

2. If the author can clarify the mechanism of the migration and invasion by DEX in colon cancer. Or try to discuss some of them.

- ⇒ Thank you very much for your comments. As described in the Discussion, a limitation of this study is the lack of a detailed molecular mechanism underlying the regulation of EMT by DEX in colorectal cancer cells.

#### Minor concerns

1. "These data suggest that Snail plays a major role as an inducer of tumor invasion and metastasis[19]." For this sentence, there should be a space between "and metastasis" and this should be taken care in the whole text.

- ⇒ We have modified this sentence to "These data suggest that Snail induces tumor invasion and metastasis."

2. Both the cell lines of HCT116 and HT29 have got the same results with DEX but if there is any other colon cancer cell line with the different characteristic or if it would relate with the colon cancer stem cell?

- ⇒ Nevertheless, HCT116 and HT29 cells were differently categorized as MSI (microsatellite instability) and MSS (microsatellite stable, chromosome instability) colon cancer subtypes, and yet they show similar effects in the function of dexamethasone on hypoxia-induced EMT. However, as aforementioned in the discussion, these effects could be cell type- or organ-specific. In addition, we preliminarily found that dexamethasone controls characteristics of colon cancer stem cell under hypoxia.

3 References and typesetting were corrected

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

Sincerely yours,

**Ju Hyun Kim and Se-Hee Kim**

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