Author's response to reviews

Title: Induction of G1 and G2/M cell cycle arrests by the dietary compound 3,3’-diindolylmethane in HT-29 human colon cancer cells

Authors:

Hyun Ju Choi (runchoi@hallym.ac.kr)
Do Young Lim (ldydo0@hallym.ac.kr)
Jung Han Yoon Park (jyoon@hallym.ac.kr)

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Dear Dr. Le Good,

Thank you for reviewing the manuscript named above. We would like to thank the reviewers for their helpful comments and suggestions. We have conducted additional studies, and have attempted to revise the manuscripts in accordance with the reviewer’s comments. Our answers to each one of the specific points raised are outlined below. All these changes have been highlighted in blue throughout the text.

Reviewer: Dr. Thomas Szekeres
We have conducted additional experiments and added the results showing that DIM induces both G1 and G2/M arrest in HT-29 human colon cancer cells in a dose-dependent manner (Fig. 1A). The old Fig. 1A has been replaced by the new Figure 1A. The legend for Figure 1 has been accordingly revised.

Reviewer: Dr. Farrukh Afaq
1. We have added dose-dependent data for G1 and G2/M phase arrest (Figure 1A) (See the comment for Reviewer Thomas Szekeres).

2. In our previous study, we showed that DIM did not influence the viability of the IEC-6 normal small intestinal epithelial cells [1]. We have added the following sentence to the Results section (page 7, line 19)

We have also reported that these concentrations of DIM do not affect the viability of IEC-6 cells, normal small intestinal epithelial cells [1].

3. Because HT-29 cells were determined to harbor a mutant p53 gene [2], we did not evaluate the status of p53 after DIM treatment. As the reviewer correctly pointed out, p21\textsuperscript{CIP1/WAF1} expression is generally controlled at the transcriptional level by both p53-dependent and -independent mechanisms [3]. In the present study, we observed that DIM induced p21\textsuperscript{CIP1/WAF1} mRNA levels in HT-29 cells, which harbor a mutant p53 gene. In addition, in our previous study, we demonstrated that p53 levels were not affected by DIM treatment in HCT116 human colon cancer cells, which contain the
wild-type p53 gene [1]. These results indicate that DIM increases p21\textsuperscript{CIP1/WAF1} levels via p53-independent mechanisms. The following paragraph was added to the Discussion section (page 11, line 12).

p21\textsuperscript{CIP1/WAF1} expression is usually controlled at the transcriptional level by both p53-dependent and -independent mechanisms [3]. In the present study, we noted that DIM induced p21\textsuperscript{CIP1/WAF1} mRNA levels in HT-29 cells (Fig. 3), which harbor a mutant p53 gene [2]. In addition, in our previous study, we demonstrated that p53 levels were not affected by DIM treatment in HCT116 human colon cancer cells, which harbor the wild-type p53 gene [1]. These results show that DIM increases p21\textsuperscript{CIP1/WAF1} levels via p53-independent mechanisms.

4. We have conducted additional experiments and added results demonstrating that DIM had no effect on the levels of p16 protein (Figure 3A) or mRNA (Figure 3B). The legend for Figure 3 has been revised accordingly. We have added the following paragraphs to the Introduction (page 4, lines 7) and Results sections (page 8, lines 15).

Introduction Section-- Several mammalian CDK inhibitors (CDKIs) have been identified. One group is the INK4 (inhibitors of CDK4) family, which has four members--p16\textsuperscript{INK4a}, p15\textsuperscript{INK4b}, p18\textsuperscript{INK4c} and p19\textsuperscript{INK4d}--all of which share the ability to control the G1/S transition [4, 5]. The second group of CDKIs includes p21\textsuperscript{CIP1/WAF1} and p27\textsuperscript{KIPI}. These proteins conduct crucial functions in cell cycle regulation via the coordination of internal and external signals that inhibit cell cycle progression at critical checkpoints [5].

Results Section-- We also examined the effect of DIM on the levels of p16\textsuperscript{INK/CDKN2} (also known as p16\textsuperscript{INK4c}) protein and mRNA. The levels of p16\textsuperscript{INK/CDKN2} protein or mRNA were not affected by DIM treatment (Fig. 3A and 3B).

5. The manuscript has been rechecked and grammatical errors have been corrected by a professional editing service.

We appreciate the thoughtful reviews of the reviewers, and hope that the manuscript will now prove adequate for publication in the BMC gastroenterology. We look forward to hearing from you regarding its status.

Sincerely,


