Author's response to reviews

Title: Griseofulvin stabilizes microtubule dynamics, activates p53 and inhibits the proliferation of MCF-7 cells synergistically with vinblastine

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Author's response to reviews: see over
Dear Dr. Juin,

Thank you for finding our manuscript MS: 1704876321323988 entitled “Griseofulvin stabilizes microtubule dynamics, activates p53 and inhibits the proliferation of MCF-7 cells synergistically with vinblastine” suitable for publication in BMC Cancer after minor revisions. The manuscript has been revised as suggested by the reviewers.

We are going through a period of serious fund crunch. We would highly appreciate if you could waive the article processing charge.

Thank you.

Warm Regards,

Dr. Dulal Panda
Professor
Indian Institute of Technology Bombay
India

Responses to reviewer’s comments:

Reviewer: Sheau-Yann Shieh
Query 1) In Fig. 1B, a G2/M arrest was implicated, but in fact, judging from the evidence provided later, an M phase arrest is likely. To distinguish the two, the authors should also perform antibody staining using anti-phosphohistone H3 before FACS analysis to directly assess the contribution of an M phase arrest.
Response: As suggested, we have performed antibody staining using anti-phosphohistone H3 and the result indicates that griseofulvin induces M phase arrest (Supplementary Figure 1). We have explained the result in the revised manuscript (Page 11 of the revised manuscript).

Query 2) For Fig. 1C, a kinetochore marker should be co-stained together with BubR1, as the spots appear rather large for kinetochore staining.
Response: As suggested, the cells have been co-stained with a mitotic check point protein Mad2 and a kinetochoric protein Hec1 (Figure 1C of the revised manuscript). The co-immunostaining of BubR1 and Hec1 has not been done as the primary antibodies for
both of these proteins available with us are from the same source (mouse).

Figure 1C of the previous manuscript showing BubR1 has now been presented as supplementary Figure 2 of the revised manuscript.

Query 3) In some of the immunofluorescence studies (Fig. 1D and Fig. 2), especially under high concentration of GF, nuclear fragmentation and blebbing are evident. Were these distinguished from the multinucleated cells and how? On a related note, for a multipolar spindle, multiple spindle poles (centrosomes) can be observed, and this evidence can be obtained by staining with anti-gamma-tubulin in the immunofluorescence studies. Furthermore, multinucleation may increase the >4N population upon FACS analysis, it is not obvious whether this is the case from the data presented. The author should either provide additional evidence or rephrase the statement.

Response: Thanks for the suggestion. We have confirmed the multipolar mitosis using anti-γ tubulin antibody (Supplementary Figure 4 of revised manuscript). We analyzed the cells in flow cytometer after GF treatment and did not find any significant increase in the cell population with more than 4N DNA content, indicating that that multiple nuclei in the cells are indeed due to the improper chromosome segregation and not due to the multiplication of DNA. We have rephrased the word “multinucleated” with “fragmented nuclei” in the revised manuscript.

Reviewer: claude prigent

Figure #1, GF does not activate the checkpoint protein BubR1! It inhibits microtubules dynamics, inhibiting spindle assembly and consequently inhibiting the extinction of the checkpoint. The checkpoint is not satisfied! This must be rewritten.

Response: Griseofulvin suppresses microtubule dynamics and perturbs microtubule-kinetochore attachment and inter-kinetochoric tension. This results in inhibition of extinction of the checkpoint proteins from kinetochores, which activates the spindle assembly checkpoint. We have modified the text as suggested.

Query 2: Figure #3 the images are not of very good quality!

Response: We are uploading high resolution tiff files of the Figure 3.

A: the effect of GF on interphase microtubules is not visible

Response: Griseofulvin exerted only a minimal depolymerization effect on the interphase cells (Page 13 and reference 2 of the manuscript). It has a prominent effect on the spindle microtubules.

B: I don’t see multipolar spindles, because I don’t see any pole.

Response: Merging of DNA (DAPI fluorescence) with the spindles has masked the poles. Hence, in the revised manuscript we have given only the spindle images (Figure 3B). In addition one more figure (supplementary Figure 4 of the revised manuscript), showing cells stained with anti-γ tubulin antibody has been incorporated to show the presence of multipolar spindles in GF treated cells.

Fig 3S F (GF + Vinblastine) show what multipolar spindles should look like.
Response: Merging of DNA (DAPI fluorescence) with the spindles masked the poles. We have provided only the spindle images in the revised manuscript. Multipolar spindles are highlighted by arrows (Supplementary Figure 3F).

Fig 3S A spindle without astral microtubules, Fig 3S B spindle with astral microtubules … why?
Response: The images were acquired using a wide-field fluorescence microscope; it was difficult to get both the astral microtubules and the spindle microtubules in one focal plane. To maintain similarities, we have removed the spindles with astral microtubules and provided a new image.

Figure 3C needs quantification (histogram with error bars)
Response: As suggested, a histogram with error bars has been provided in the revised manuscript (Figure 3D).