Peer Review File

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Reviewer A

Uremic retention solutes accumulated in uremic patient are regarded as toxins when they exert toxic effects on various systems. In the manuscript "The effect of p-Cresol, a uremic toxin, on cancer cells", authors explored that p-cresol had the similar effects on kidney cancer cells and liver cancer cells.

Couple questions are required to be answered before it will be accepted.

(1) How about the distributions of p-Cresol in human body? Please state in the introduction.
Reply: P-cresol, an end product of aromatic amino acids, is produced in the gut, metabolized in the liver and excreted in the urine and feces. The concentrations of total p-cresol in the blood, urine, liver and kidneys of uremic patients underwent hemodialysis are approximately 16 to 30-fold higher than those of non-hemodialysis patients.
Changes in the text: We have stated in the introduction.

(2) In the research, why to use 70 μM of p-Cresol, not 80 μM of p-Cresol? And how to determine the concentrations of p-Cresol? Please state in the methods.
Reply: In the study, different concentrations (0, 10, 20, 40, 70 μM) of p-cresol for 48 hours were selected to treat these cells according to the previous reference (0-100 μM; 0-48 h). If necessary, higher concentrations of p-Cresol (80-150 μM) will be used to treat these cells.
Changes in the text: We have stated in the methods.

(3) It was better to integrate figure 1A and 1B into an intact figure 1. The same to figure 2 and 3.
Reply: Thank you for your suggestion. We have integrated figure 1A and 1B into an intact figure 1, and the same to figure 2 and 3.
Changes in the text: We have integrated figure 1A and 1B into an intact figure 1, and the same to figure 2 and 3.

(4) Please perform statistical analysis in the figure 2.
Reply: Thank you for your remainder. We have added the data in the figure 2.
Changes in the text: We have added the data in the figure 2.

(5) The figure 3C was not clearly. So, it was necessary to replace it with a new.
Reply: Thank you for your reminder. We have replaced it with a new one.
Changes in the text: We have replaced it with a new one.

(6) Since the viability and proliferation of 786-O cells and HepG2 cells were unaffected by p-cresol treatment in the study, it was advised to add a positive control in the research.
Reply: Thank you for your suggestion. Previous studies have shown that high dose of p-cresol is cytotoxic to a variety of cells. In the present study, 0 to 70 μM p-cresol for 48 h

showed no effects on the viability and proliferation of 786-O cells and HepG2 cells; thus 40 μM p-cresol for 48 h was selected to investigate its effects on cell migration and invasion.
Changes in the text: We have explained it in the discussion.

(7) The viability and proliferation of 786-O cells and HepG2 cells were unaffected by p-cresol treatment in the paper. Whether there were effects of p-cresol on the primary cultured cancer cells? It was better to test the effects of p-cresol on cancer by animal model or primary cultured cancer cells.
Reply: Thank you for your suggestion. Biological function of p-cresol in animals needs further investigation.
Changes in the text: We have mentioned this limitation in the discussion.

(8) Since p-cresol is undetected in the serum of uremic patients, what is the point of this research? Or what the p-cresol was mainly applied in? Whether the p-cresol could be intake into human body? Please state in the discussion.
Reply: Thank you for your reminder. Endogenous p-cresol, originated from the bacterial fermentation of dietary tyrosine in the colon, is absorbed by the intestinal wall, then conjugated and metabolized in the liver, and eventually excreted in the kidney. P-cresol presented in serum of uremic patients as the form of p-cresyl sulphate or p-cresyl glucuronide. Though in the blood of HD patients, p-cresol represents only 2% of total p-cresol, it is the major component of total p-cresol in the liver since the liver is the main site of p-cresol metabolism. Thus, the tumor-promoting effect of p-cresol on HepG2 cells identified in this study is worthy to be given attention to.
Changes in the text: We have modified the words and stated in the discussion.

Reviewer B

Please revise the grammars of the article and ethical consideration of the journal.
Reply: We have revised as requested