Dear professor Zhang,

We would like to thank the editor and the reviewers for their conscientious reviews, and insightful comments and suggestions to improve the manuscript. In the response below, we have addressed all the concerns raised by the editor and the reviewers in the revised manuscript. We hope the editor and the reviewers will find that our revised manuscript has improved and is suitable for publication. All changes have been marked in blue.

I hope my paper could achieve the academic standards of your magazine and be published finally.Thank you very much.

Yours Sincerely,

Wenqi Cui and Lixuan Sang

Response to Reviewers Comments

Manuscript NO: 51370

Title: Caffeine and Its Main Targets of Colorectal Cancer

Authors: Wenqi Cui, Shitong Wang, Dan Pan, Bing Chang and Lixuan Sang

Reviewers' comments:

Reviewer #1: This manuscript is a good description of the state of art about the Caffeine and Its Main Targets of Colorectal Cancer.

Response: Thank you for your review and evaluation. I am very glad that this review can be approved by you.

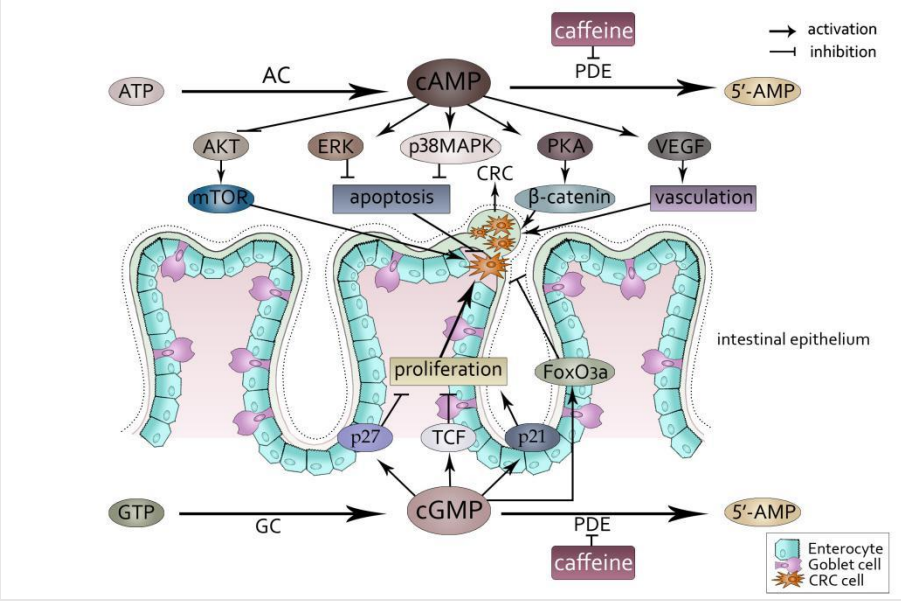
Reviewer #2: Caffeine and Its Main Targets of Colorectal Cancer In this review article, Cui et al. review the molecular mechanisms implicated in the influences of caffeine on colorectal cancer and intestinal homeostasis, including the modulation of gut microbiota. This review has encompassed the various aspects of the interactions between caffeine and the colon and presents nicely with delicate figures. There are, however, some issues to be clarified:

Major comments

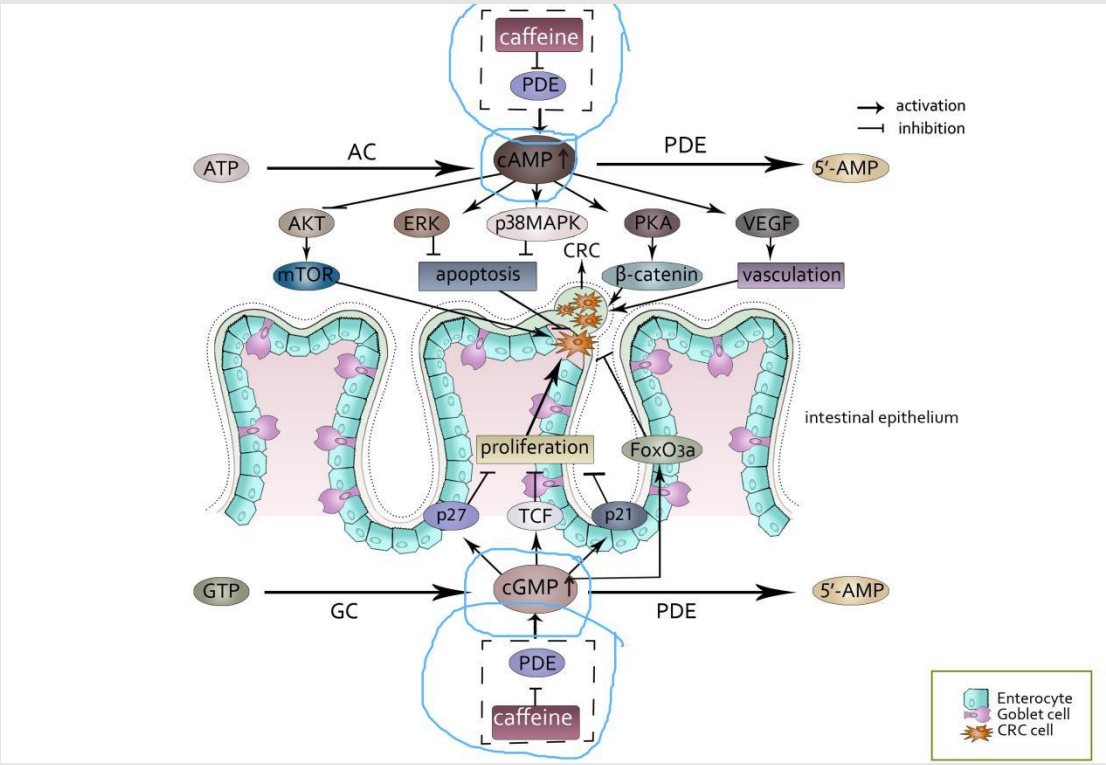
1. Fig.3 shows that caffeine only blocks the action of PDE and reduces the synthesis of 5'-AMP without influencing the main pathways (cAMP→AKt, ERK, p38MAPK…….) that promotes the carcinogenesis of colorectal cancer cells.

Response: Yes, thank you for pointing out the problem, it is an excellent advice. Our previous figure did not express the process in detail. For caffeine is competitive inhibitor of PDE, it can block the action of PDE without influencing the pathways. However the blockage of PDE can cause the accumulation of cAMP, and the accumulated cAMP can influence the pathways and targets(AKT,ERK,P38MAPK, PKA,VEGF).We now add two "↑"to emphasize that it is the accumulation of cAMP which cause the following changes then promote/inhibit CRC. Moreover, we express the process which involves accumulation of cAMP/cGMP caused by inhibition of PDE by caffeine clearer.The Fig.3 now modified as following and part that revised has been circled in blue:

Original drawing:



After revised:

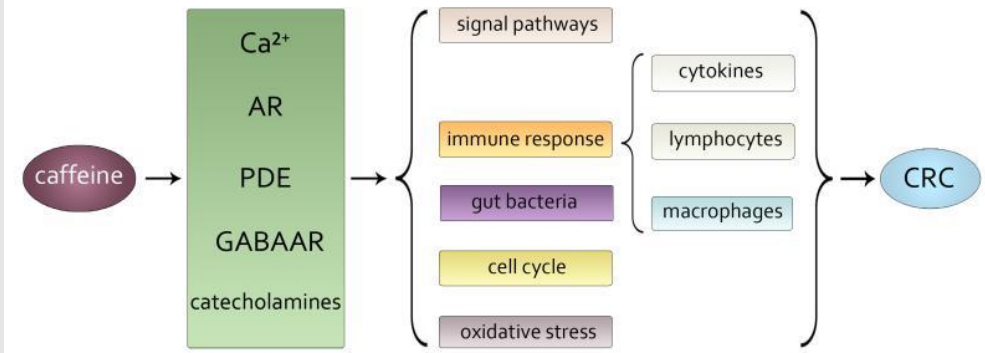


2.Likewise, the presentation of Fig.4 may mislead the readers to feel that caffeine is "promoting" the progression of colorectal cancer cells via the substances shown in this figure.

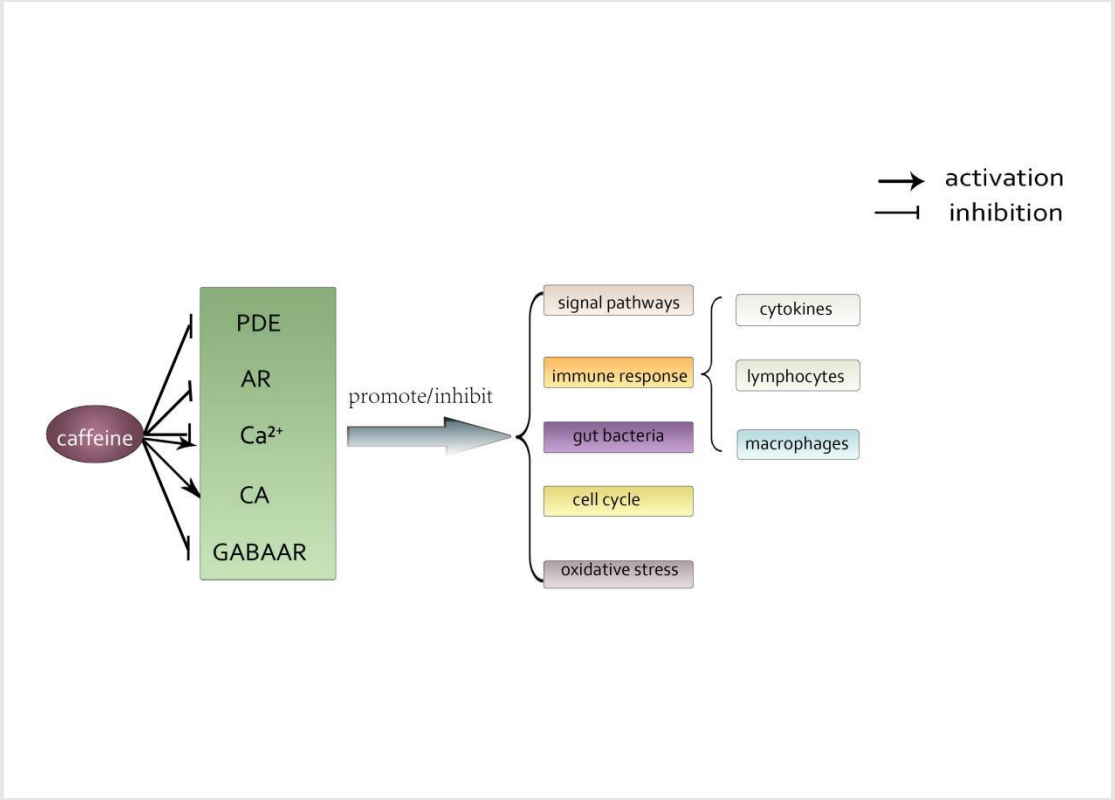
Response: Thanks for your reminding, our previous writing does cause ambiguity. Now we revise the photo annotate according to the comments of reviewers as following:The main acting sites and physiological processes modulated by caffeine on colorectal cancer involved in this article.

The Fig.4"→CRC"is deleted and now modified as following:

Original drawing:



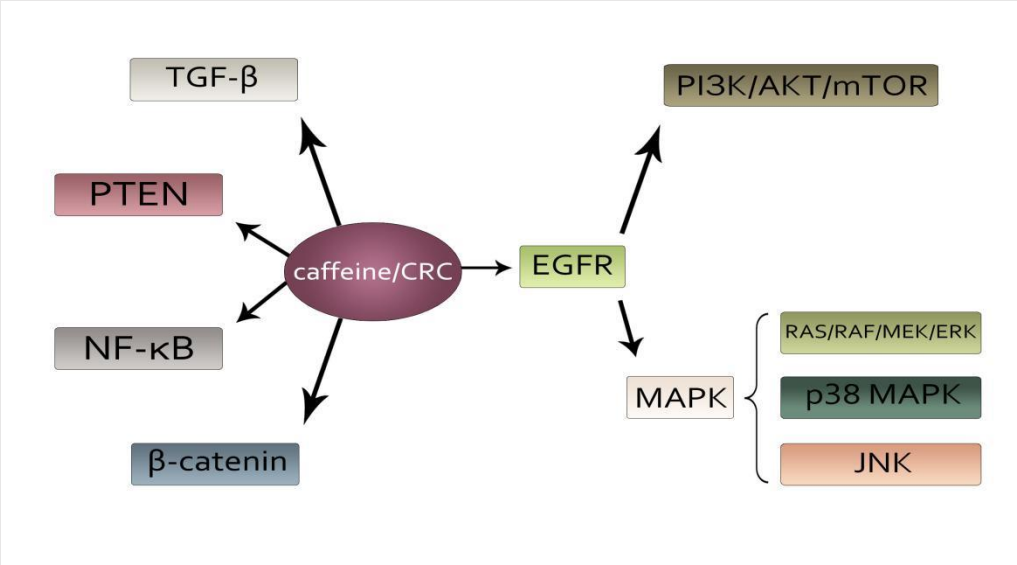
After revised:



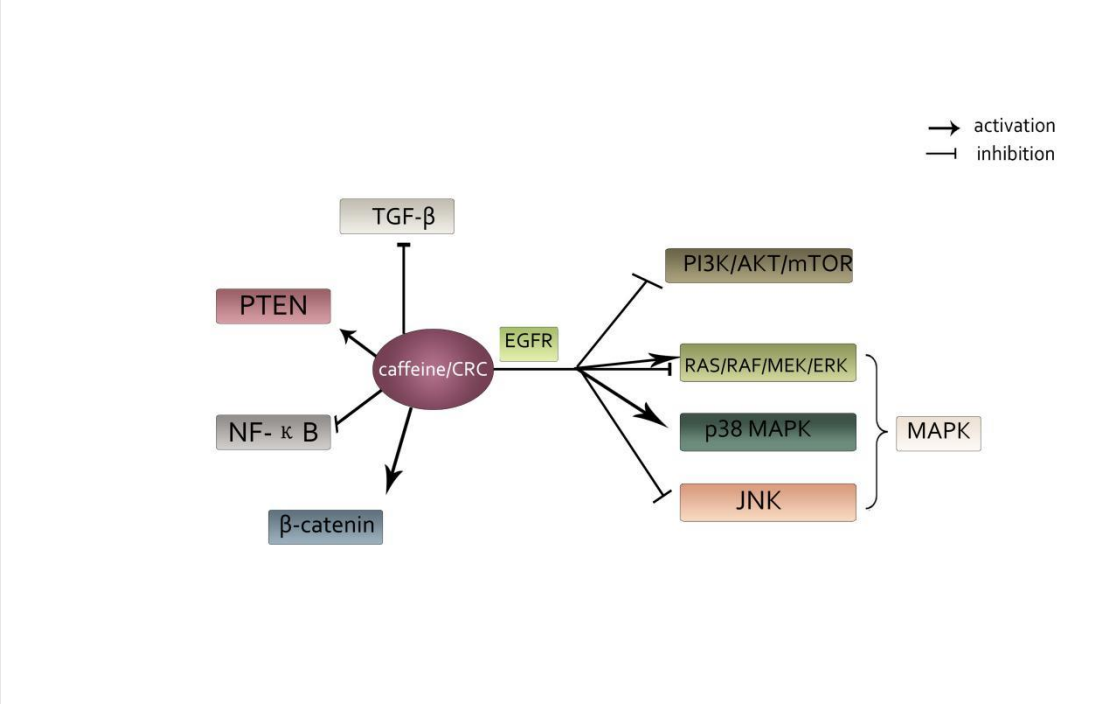
3. Please adjust the Fig.5, because it may also mislead the readers to feel that caffeine positively regulates TGF-β, EGFR……then promotes the growth of colorectal cancer cells.

Response:Yes, it is misleading for readers. Now we revised the figure as following:

Original drawing:



After revised:



Minor comments

1. Please delete "……..according to this article" at the end of figure legend 5, because this is a review article based upon the previous literature, but not an original article with novel findings.

Response:Yes, thank you for your suggestion and i am very sorry. This is a mistake in language and has been corrected as following:

The main signaling pathways associated with colorectal cancer that can be influenced by caffeine involved in this article.

Reviewer #3: This is an excellent review that summarizes the potential effects of caffeine and the involved mechanisms on coco-rectal cancer. However, the authors have overlooked an important aspect of caffeine: Due to its ability to increase intracellular cAMP, caffeine enhances the downstream effects of beta-adrenergic receptors that are coupled to the stimulatory G-protein Gs which increases cAMP by activating adenylyl cyclase.

There are numerous reports in the literature that beta-adrenergic receptor signaling in response to their physiological agonists epinephrine and norepinephrine or in response to psychological stress that increases the systemic levels of these stress neurotransmitters stimulate the growth and angiogenesis of coal-rectal cancer. These aspects and the stimulatory effects of caffeine on these pathways need to be added to the review.

Response:Yes,thank you very much for reminding us that important aspect to enrich our review. We add a new paragraph named "Stimulation of Adrenergic Signaling" in "caffeine "section as following:

Stress, as one of the environmental factors, is reported to enhance CRC cell growth in both vivo and vitro, and is linked to the occurrence and progression of CRC[61]. Catecholamines, including norepinephrine (NE) and epinephrine, are the primary neurotransmitters involved in stress response and originate from the sympathetic nerves of the autonomic system[62]. When people suffering from acute or chronic stress, both epinephrine and norepinephrine are elevated[61]. Caffeine ingestion is widely associated with

stimulation of the sympathetic nervous system and with subsequent elevations in the plasma concentrations of the catecholamines epinephrine and norepinephrine[63,64]. Catecholamines can stimulate beta-adrenergic receptors by beta-adrenoceptor-adenylylcyclase-protein kinase A cascade. Beta-adrenergic receptors belong to the family of G-protein coupled receptors and stimulation of the cascade can cause an accumulation of the second messenger cAMP then resulted in modulation of varied pathways[65]. They can influence a lot in CRC because beta-2 adrenergic receptors have a high expression level in the neoplastic cells from colorectal adenocarcinoma. Moreover it has significant association with tumor grading[66]. Meanwhile,it also have effect on tumor growth including promoting tumorigenesis, tumor cell proliferation, antiapoptotic mechanisms, and promoting metastasis by stimulating the expression of angiogenic growth factors such as VEGF and IL-6 and inducing epithelial to mesenchymal transformation (EMT), motility and invasion[67]. In addition, it can also cause the modulation of immune system. For example,it can induce a Th1/Th2 imbalance in the mouse immune system, which is considered critical during colon cancer progression[68].Moreover,use of beta-adrenergic receptors blocker has been proved to be associated with longer survival in patients with stage IV CRC[69].