李文森客座教授
系列演講

最高學歷 美國匹茲堡大學生理學博士

學術專長 血管增生粥狀動脈硬化、抗癌藥物研發、癌症生物學

論文撰寫系列

1. 如何有效率的撰寫科學論文	08/18 (四)
2. 撰寫科學論文常見的問題	08/25 (四)
3. 如何回覆論文審查者的意見	09/15 (四)

教學增能系列

1. 如何增進學生的學習興趣與效能	09/22 (四)
2. 考試命題的技巧	10/13 (四)
3. 英文授課技巧	10/20 (四)

研究計畫系列

1. 如何成功的申請研究計畫補助	11/17 (四)
2. 研究主題選擇及研究計畫的撰寫	11/24 (四)

現任

慈濟大學醫學系生理學科客座教授
臺北醫學大學名譽教授

經歷

臺北醫學大學教務長
臺北醫學大學醫學院醫學科學研究所所長
臺北醫學大學醫學系系主任
臺北醫學大學醫學院生理學科主任
臺大醫學院生理學研究所兼任教授
高雄醫學大學生理研究所兼任教授
嘉義大學兼任教授
高雄醫學大學醫學院客座教授
日本大學客座教授

主辦單位：醫學院醫學系生理學科
協辦單位：教師發展暨教學資源中心

如何回覆論文審查者的意見

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The process of manuscript review

The editorial decision

Outright acceptance (3-5%)

Outright rejection (60-80%)

Revision

- **Major revision**
- **Minor revision**

de novo submission

Reviewers' comments might be valuable, but not always correct.

There is no need to accept everything the reviewers' suggest

The reviewers can be wrong,

but the editor is never wrong!

How to response to reviewers' comments

Agree with reviewers' comments

Reviewer's comment

In Materials and Methods, there is no data regarding progesterone concentrations range, vehicle used, or vehicle maximum concentration established in cell cultures.

Authors' response

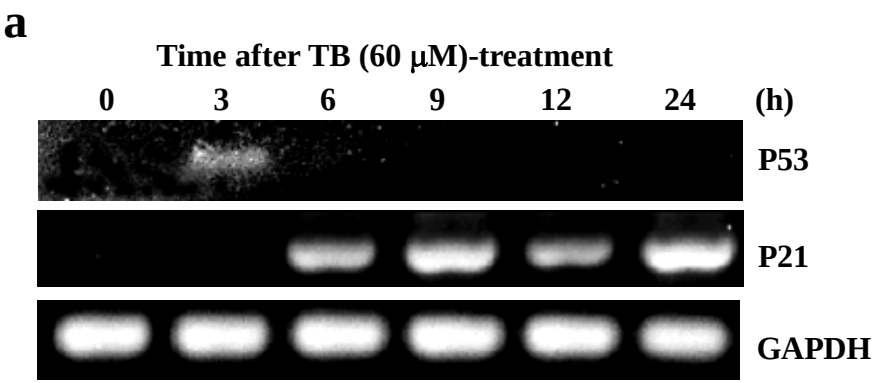
"As requested, we have added the information in the revised manuscript."

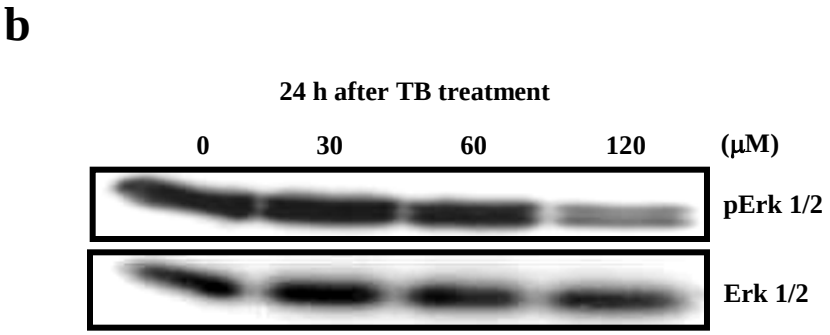
Reviewer's comment

Please, provide for Fig. 2c another data presentation with OD on the axe Y, as it has been done at the Fig. 5a.

Authors' response

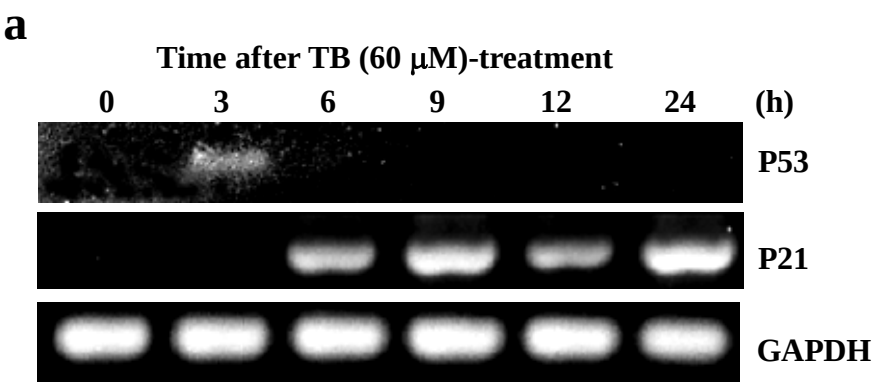
"As requested, we have changed the data presentation with OD on the axe Y of Fig. 2c. "

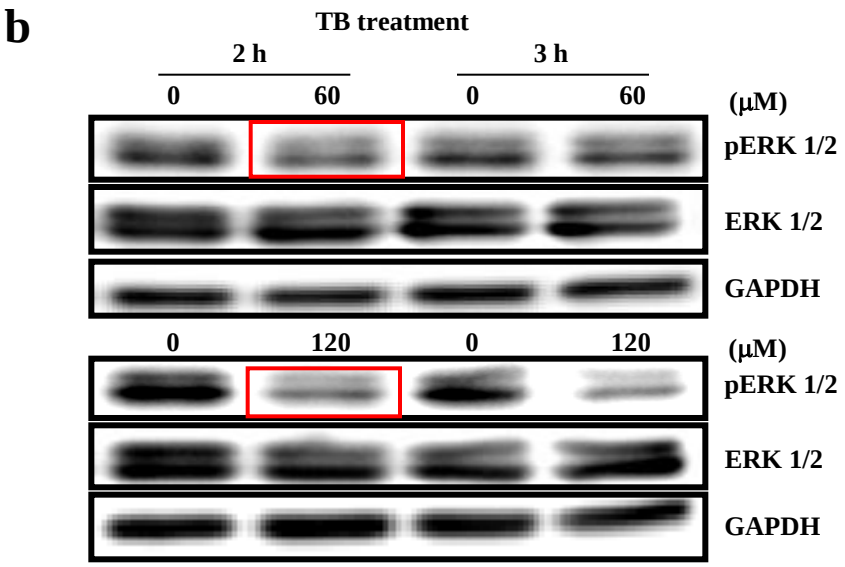




Reviewer's comment

Figure 2a. Erk1/2 phosphorylation is decreased at 24 h of exposure to 120 μM terbinafine. This decrease is difficult to resolve with the observed and robust induction of p53 mRNA at 3 h and 60 μM terbinafine exposure. Neither the timing nor the dose used support the conclusion that Erk inactivation is upstream of the p53 induction.





Authors' response

"We agree with reviewer's comment that a decreased Erk1/2 phosphorylation observed at 24 h of exposure to 120 μM terbinafine could not resolve with the observed and robust induction of p53 mRNA at 3 h and 60 μM terbinafine exposure. An additional experiment was performed to address this issue. As shown in the revised Figure 2a, the Erk1/2 phosphorylation is decreased at 2 h of exposure to 60 and 120 μM terbinafine. At 3 h terbinafine exposure, the Erk1/2 phosphorylation is still lower than the vehicle-treated cells. Figure 2a has been replaced with this new figure and added the information in the revised MS (lines 18-20, page 22). "

Reviewer's comment

To derive a rationale of the present finding in relation to cancer development and prevention, it will be desirable that some preliminary data is given on some cell lines for whether the compound is able to inhibit cell proliferation in tumor cells also, otherwise the article seems distantly related to the main thrust of the journal. Therefore, it is suggested that the data on decline in cancer cell proliferation should be included in the article, which it appears, authors have been able to generate some, as it has been mentioned in the conclusion part of the discussion. or else, it should be described sufficiently with the relevant literatures in the text.

Authors' response

We have added a new paragraph "The dependence of angiogenesis in the growth and metastasis of solid tumors is well recognized [21,22]. Lately, application of anti-angiogenic therapy has been suggested to be a potential therapeutic strategy against cancer development and metastasis. In this study, we evaluated the anti-angiogenic activity of DPTH-N10 and delineated the molecular mechanisms underlying. Noteworthy, our preliminary data showed that DPTH-N10 also concentration-dependently inhibited the proliferation of colon cancer cell line, COLO-205 (Lee et. al., unpublished data). "

Reviewer's comment

The demonstrated inhibition of HUVEC proliferation does not necessarily imply inhibition of tumour angiogenesis in vivo. Since the authors have claimed that terbinafine is an antiangiogenic agent it is necessary to include further experiments to demonstrate the antiangiogenic effect of terbinafine in vivo. For example, a possible in vivo effect of terbinafine on tumour-induced angiogenesis may be studied in a xenografted tumour model in the nude mouse. The tumour xenograft model has been successfully employed by this group (Lee et al. Int J Cancer 2003) and an antiangiogenic effect of terbinafine in vivo would greatly enhance the scientific value of the manuscript.

Authors' response

We appreciate the reviewer's comment on this issue. However, we do feel that the tumour xenograft model in the nude mouse might not be an ideal model for studying the anti-angiogenesis of terbinafine in vivo. In the revised MS, we have added the results from two additional experiments (capillary-like tube formation assay and chick embryo chorioallantoic membrane, CAM, assay) to demonstrate the antiangiogenic effect of terbinafine (the results shown in Figures 6 and 7 of the revised MS).

Disagree with reviewers' comments

Reviewer's comment

In Results section 3.1, which technique was used for cell number assays? This is not mentioned neither on Results nor in Figure 1c legend. If these findings were obtained by MTT assay, a technique that examined cell viability, decreased cell number (Page 3, line7) should be replaced by decreased cell viability.

Authors' response

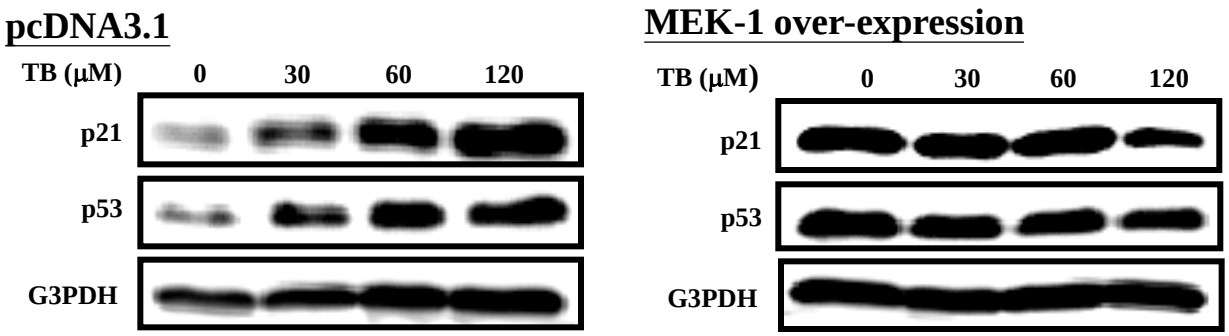
"The cell number was examined by MTT assay. We have added this information in the revised MS. The MTT assay has been used to examine both cell viability and cell number. Since treatment of the cells with progesterone at a concentration of 500 nM for 24 h did not cause cell death as examined by MTT assay, the results of the MTT assay after treatment of the cells with progesterone for 3 days reflect the cell number. Therefore, we would keep [decreased cell number]"

Reviewer's comment

The doses of ICI 182,780 appear to be massive. In as much as ICI 182,780 has a sub nM potency for estrogen receptors, why were such high doses of ICI 182,780 need to antagonize the estradiol effects? This suggests that ICI 182,780 is not functioning in this model through an ER mechanism. To clarify this issue, kinetic information on ICI 182,780 following the initial and final doses of the compound is needed. Alternatively, studies in estrogen receptor knockout mice would be instructive

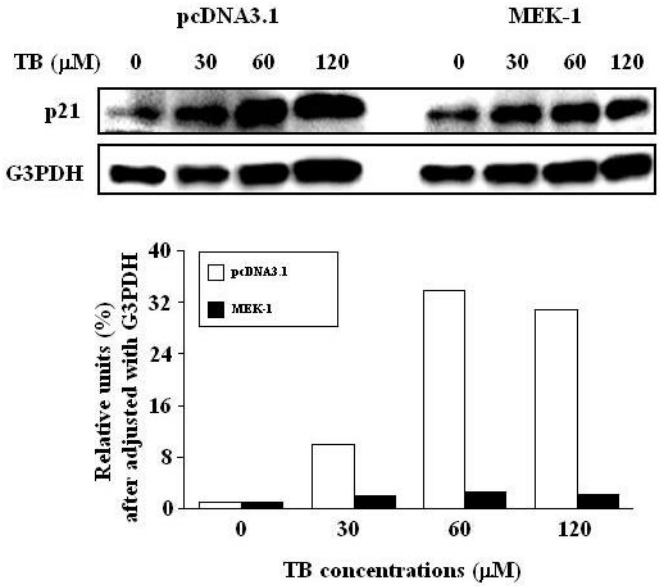
Authors' response

We appreciate the reviewer's comment on the issue of ICI 182,780 doses used in this study. As mentioned by the reviewer, ICI 182,780 has a sub nM potency for ERs at the in vitro study. However, our study was done in an *in vivo* setting and ICI 182,780 at a dose of 2 mg/kg/day, which was used in this study and has been used in many other labs[1] (Bakir et al., 2000, Circulation, 101:2341-23444), would give the blood levels of ICI 182,780 at a range of nM. Although we did not study the kinetic study of ICI 182,780, a clinical study showed that an 18 mg/day injection maintains blood levels of 25 ng/ml one week after beginning treatment (Bakir et al., 2000, Circulation, 101:2341-23444). We have added a reference (reference 7) in the revised MS (page 5, line 11).



Reviewer's comment

Figure 2c. It cannot be concluded from this figure that MEK over-expression abolishes the ability of terbinafine to induce p21 or p53 protein expression, but rather MEK over-expression itself induces p21 and p53 expression in the absence of terbinafine exposure. Thus, basal (untreated) levels are induced and terbinafine treatment does not induce them further.



Authors' response

A higher intensity of the p21 and p53 signals observed at the time 0 of the MEK-transfected cells as compared with vector-transfected cells was due to a longer exposure time of the film (different exposure time would cause a different intensity of the signal and these two members were not exposed at the same time). Importantly, terbinafine concentration-dependently increased the signals of p21 and p53 in the vector-transfected cells. In contrast, the signals of p21 and p53 were not changed significantly in the MEK-transfected cells. Accordingly, we conclude that MEK over-expression abolishes the ability of terbinafine to induce p21 or p53 protein expression. For your reference, the figure shown above is the membrane to detect the expression levels of p21 protein isolated from the vector-transfected and MEK-transfected cells and was exposed together.

Reviewer's comment

The authors used G3PDH as a loading control for phospho-ERk(pERK)/phospho-RAF (pRAF) proteins in all their experiments. While G3PDH is widely used, it however, is not an ideal control when quantifying for the levels of phospho-proteins. I strongly suggest that the authors include total ERK/RAF protein levels as internal controls in addition to G3PDH for quantifying pERK and pRAF in all their experiments.

Authors' response

We agree with the reviewer's comment that total protein levels might be a better internal control for quantifying the levels of phospho-proteins. In our study, however, the changes of phospho-ERK/phospho-RAF level were not observed until 40 min. In this case, the levels of total ERK/RAF might be changed. Therefore, we choice to use G3PDH as an internal control for protein loading, and our data showed that the intensities of G3PDH protein in each time point were almost identical, but the levels of phospho-ERK/phospho-RAF were increased at 40 min after magnolol treatment. In revised Figure 3a, 5b and 6, we have added the total ERK as a loading control and they showed an increase of pERK after 40-50 min after magnolol treatment.

Reviewer's comment

First, the authors do not provide any data or information that clearly shows or states that progesterone is an important physiologic regulator of endothelial cell function and, more specifically, proliferation. Thus, while these in-vitro studies are intriguing, their physiologic relevance is not known.

Authors' response

In the Introduction section of our manuscript, we did state that the effects of progesterone on endothelial cells have been documented. Vázquez et al. used the progesterone receptor knockout mice animal model to demonstrate that physiologic levels of progesterone could inhibit the proliferation of vascular endothelial cells and the rate of re-endothelialization, and thus impair vascular repair processes through progesterone receptor dependent pathways (9).

Reviewer's comment

Why did the authors show the transfection efficincy by cotransfection with the GFP-vector. This presumed that pcNA-Thy-1 vector and GFP-vector transfection efficiency are similar. Thy-1 can be easily detected by staining with antibodies. The tranfection efficiency should be shown by detection of Thy-1-positive cells upon transfection by Western Blot or FACS analysis.

Authors' response

To verify the transfection efficiency, HUVEC was transfected either with Thy-1-pCMS-EGFP (enhanced green fluorescent protein)-C1 or with pCMS-EGFP-C1, and then monitored using an inverted fluorescent microscope. We found that transfection efficiency of pCMS-EGFP-C1 and Thy-1-pCMS-EGFP-C1 is 55% and 40 %, respectively. The data shown in this manuscript were obtained from the cell transfected with Thy-1-pCMS-EGFP-C1 (we have added this information in the revised manuscript page 7, lines 1-3). The reason why we did not detect the Thy-positive cell was to avoid the staining of endogenic thy-1 immunoreactivity.

Reviewer's comment

Most of results were shown without basal status and therefore the readers did not know whether control cells were really activated. It is possible that folic acid only affects basal level.

Authors' response

There might be some misunderstanding. In fact, we did include the control group (Co) in each experimental design and the data from the control group (Co) have been presented in each Figure. We did define "Co" in the end of each figure legend.

Review's comment

Authors only provided some phenomenon about β-Sitosterol treated RASMCs. ... So, even if the authors perform additional experiments and clearly demonstrate that β-Sitosterol induces cell cycle arrest in smooth muscle cells, such data would only corroborate findings already obtained with other type of cells. Authors should examine more experiments to discover the molecular mechanisms, and then increase the scientific merit of this manuscript.

Authors' response

We appreciate the reviewer's comment. We would certainly continue some further studies to delineate the signaling pathway involved in the β-sitosterol-induced increase of p21 in the future. However, it seems that the reviewer has some misunderstood our data and we would also like to make it clear for the reviewer. First, Fig. 5A shows that the levels of p21 protein in RASMC were increased (not decreased as reviewer's comment) by β-sitosterol treatment. The increased p21 caused an increase in the formation of CDK2-p21 complex (Fig. 5B), subsequently decreasing the CDK2 activity (Fig. 5C). Although the anti-atherosclerosis activity of β-sitosterol has been suggested by previous in vivo studies, the cellular and molecular mechanisms underlying have not been studied. Yet, the references cited by the reviewer regarding the anti-proliferation and apoptotic activity induced plant sterols in various cancer cells did not provide complete evidence to prove that p21 is the major factor responsible for β-sitosterol-induced anti-proliferation activity and how p21 induction caused cell cycle arrest. To our knowledge, our present study demonstrates for the first time the cellular and molecular mechanisms underlying β-sitosterol-induced anti-proliferation activity. As Reviewer 3's comment, this paper is very much mechanistic focus. So, we are not just only provided some phenomenon about β-Sitosterol-treated RASMCs as commended by the Reviewer 1.

Authors' response

By the way, we are sorry for not being able to understand what the reviewer's following comment "β-Sitosterol induced cell proliferation and DNA fragmentation assay, as well as β-Sitosterol induced gene expression by RT-PCR and Western blot." So, we can not have any response on this comment.

Need further explanation

Reviewer's comment

Thymidine incorporation: the authors start at cell densities of 80% confluency, starve the cells and then apply their drug for 24h. Are the cells still in a log growth phase at the time of thymidine incorporation or did the cells already reach a growth plateau, where they reduce their proliferative and metabolic activity?

Authors' response

Although we placed the COLO-205 at a density of $1X10^4$ cells/cm^3 and rendered them for quiescent when the cells had grown to 70-80% confluence, the COLO-205 cells did not grow a single layer (they would pile up when the cells grow) and without contact inhibition. So the cells did not reach a growth plateau.

Reviewer's comment

Are there data indicating that the lethal activity of TB on fungi could be mediated by a modulation of signaling molecules of the Ras family in such organisms?

Authors' response

"To our knowledge, there are not data indicating that the lethal activity of TB on fungi could be mediated by a modulation of signaling molecules of the Ras family in such organisms."

Reviewer's comment

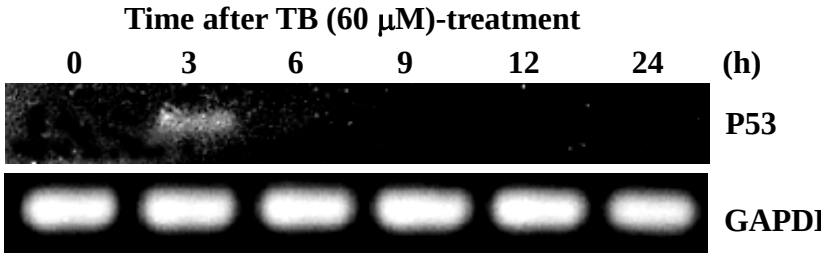


Fig.1a: I do not know why there was no any P53 at 6, 9, 12 and 24 h after TB-treatment.

Authors' response

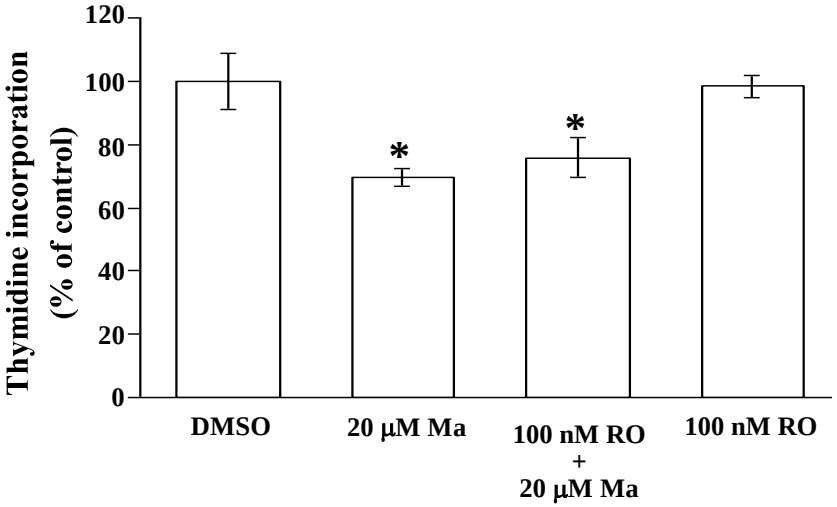
While we do not have the exact answer for why there was no any p53 at 6, 9, 12 and 24 h after TB-treatment, one possible explanation is the film exposure time is not long enough to detect the very weak expression levels and/or a short half-life of p53. When we studied the steroid effect on endothelial cell proliferation, we also found that the increased levels of p53 mRNA and protein lasted for only 2 h. For such a narrow window time in the increase of p53 deserves further investigation.

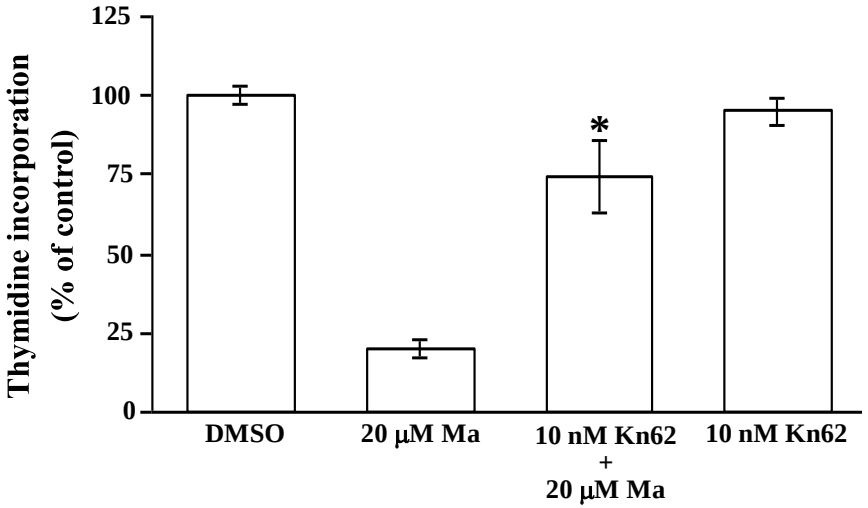
Reviewer's comment

The authors still need to provide information regarding the cytotoxicity of the inhibitors used in the study. Although they now provide the IC50's for each inhibitor, no mention is made regarding the cytotoxicity observed with each of the agents. This could be a confounding factor

Authors' response

The concentrations of these inhibitors used in this study did not cause any cytotoxicity as evidenced by no significant change in thymidine incorporation when the cells treated with inhibitors alone. We have added this information in the revised MS (page 7, lines 17-19).





Reviewer's comment

Figures 2-8, demonstrate the effect of Ma on thymidine incorporation. If you just consider the first two bars, indicating DMSO and Ma treatment in Figures 4b, 6b, 7a, 8b, the incorporation ranges from ~24 to 70 % (data normalized to DMSO), even though Ma was used at 20 μM concentration in these experiments. Assuming the assay conditions were all the same for thymidine studies, why is there a significant variation across different experiments?

Authors' response

It has been recognized that the cell density would affect the cytotoxic activity of certain anticancer agents (Kobayashi et al., Cancer Chemother Pharmacol 1992). Although we placed the COLO-205 at a density of $1X10^4$ cells/cm^3 and rendered them for quiescent when the cells had grown to 70-80% confluence in each experiment for studies of thymidine incorporation, the COLO-205 cells did not grow a single layer; they would pile up when the cells grow, which makes difficult in determining the cell confluence. Although the cell density among different experiments (different 24-well plates) might be different, the cell density of each well in a 24-well plate should be almost the same (evidenced by small standard error) and each figure shown in this paper was performed in cells grown in the same 24-well plate.

Reviewer's comment

Nevertheless, it would have been helpful, both from a technical and biological point of view, if similar experiments were performed on an additional Magnolol-sensitive cell line.

Authors' response

We would definitely be more careful in concerning the issue raised by the reviewer in our further studies.

Reviewer's comment

Did the authors evaluate the effect of ICI + SAH on the various outcome measures described in figures 2-5? It would have been interesting to see what effect, if any, ICI had on these measures as compared to E2 in the presence or absence of SAH.

Authors' response

The experiments of Figures 2-5 were conducted to explain the possible mechanisms underlying E2-induced protection of basilar arteries from vasospasm. Since the diameters of basilar arteries were not affected by ICI 182,780 treatment alone as shown Figure 1b, we did not examine the effect of ICI+SAH on the various outcome measured in Figures 2-5. We appreciate the reviewer's comment and would perform this study in our future studies.

Reviewer's comment

It is a concern that in the current concentration, 50 mg/kg, there was no significant toxicity observed, but the inhibitory effect at this dose was only 50%. A higher dosing may be tested to achieve a stronger efficacy and evaluate the toxicity.

Authors' response

We do appreciate the reviewer's valuable comment on the dose of TB used in the in vivo study. Due to time limitation for manuscript revision, we are not able to conduct a detailed in vivo study of dose effects of TB on the tumor growth and the toxicity. However, this important issue and some other potentially important issues will certainly be addressed in our further study. In this manuscript, we showed the result of our pilot in vivo study to demonstrate the anti-tumor activity of TB and the potential application of TB in oral cancer therapy.

Reviewer's comment

The title of the results section (line 93) is 'Effect of Kras activation on the P4-induced up-regulation of p53 in HUVEC'. Have the authors investigated if Kras is activated by P4 in HUVEC cells? The figure shows increased association with p-Src but not increased activation of Kras.

Authors' response

When Kras is activated, it will translocate from the cytosol to the cell membrane and further phosphorylate the direct effector protein, Raf-1(site Ser 338). Although we did not measure the Kras activity, we provided two pieces of evidence in this study to prove that Kras was activated in progesterone-treated HUVEC. (1) Treatment of HUVEC with progesterone for 5 min did not significantly affect the total protein level of Kras (Fig. 3A), but did increase the membrane translocation of Kras (Fig. 3B); (2) Progesterone treatment induced the recruitment and phosphorylation (site Ser 338) of Raf-1, a direct effector protein of Kras (Fig. 3C).

Reviewer's comment

In the central nervous system, NOS2 could play central roles in inflammation mediated by microglia and macrophages. Thus, the readers could be interested in NOS activation after ferrous citrate in other inflammatory cells. The authors could add their opinion about whether the similar pathways are involved in NOS2 induction in microglia and macrophages after ischemia and ferrous citrate treatment.

Authors' response

The reviewer did bring an interesting issue on the signaling pathways of ischemia or ferrous citrate-induced NOS2 induction in microglia or macrophages. Unfortunately, the evidence for ischemia- or FC-induced NOS2 induction in microglia or macrophages has not found. However, we have added the following information in the revised manuscript. "Since NOS2 could play central roles in inflammation mediated by microglia and macrophages in the central nervous system, it will be interested to know whether the similar pathways are involved in NOS2 induction in other inflammatory cells after ischemia or FC treatment. Although increases of interferon-γ-inducible macrophage nitric oxide generation through the NFκB-dependent pathway [50] and involvement of ROS in activation of NFκB in neutrophils [51] have been demonstrated, the direct evidence for molecular signaling pathways involved in ischemia- or FC-induced NOS2 induction in microglia or macrophages has not been found. Whether this signaling pathway involved in the FC-induced NOS2 induction is unique to cerebral endothelial cells still needs further investigation."

De Novo submission

De Novo: from the beginning

The reviewers of your manuscript have recommended major revisions with priority rankings that do not reach the point of acceptance for publication in Stroke at this time. After you have read the reviews, if you feel you can respond satisfactorily to the concerns of the reviewers with a revision of the paper, we would be prepared to consider such a revision as a new "De Novo" manuscript if received within 30 days. (Stroke)

The editors and reviewers have completed their examination of your manuscript, #ME-10-0148 Version 1: Progesterone receptor activation of extra-nuclear signaling pathways in regulating p53 expression in vascular endothelial cells.

While our reviewers agree that your subject is potentially important and interesting, they believe that the present study offers only preliminary new information. We regret therefore that we cannot accept your paper for publication in Molecular Endocrinology. However, the subject of your study is of interest to our readers and therefore we would encourage you to submit a revised manuscript to Molecular Endocrinology. While this revised manuscript would be considered as a new manuscript submission, you should indicate in your cover letter that this manuscript has been previously submitted and reviewed. Importantly, you should also highlight how the revised manuscript addresses all of the significant concerns of the reviewers. (Molecular Endocrinology)

You do not have to make every suggested change, but you do need to address all of the comments.

Be polite.

Avoid a defensive or confrontational tone in your response.

李文森客座教授
系列演講回饋

問卷連結
https://reurl.cc/yMMqRO

提供諮詢項目

(1) 生物醫學論文撰寫及投稿
(2) 生物醫學論文審查意見回覆
(3) 研究計畫撰寫
(4) 研究主題及方向
(5) 生物醫學論文及研究計畫修改
(6) 生物醫學實驗設計與生物統計
(7) 口頭演講及教學增能