Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The manuscript titled Effect of conditioned medium from neural stem cells on glioma progression and its protein expression profile analysis, by Zhang and colleagues, is an interesting study on the effect of CM derived from NSC cultures grown under normoxic and hypoxic conditions on the proliferation and invasiveness of glioma cells, and the identification of the protein NCAN as a mediator of these effects. The relevance of this work could be demonstrated should the authors be able to address some important doubts that arise while looking at the data presented: RESULTS SECTION Optimization of CM derived from normoxic and OGD preconditioning of SVZ NSCs / Figure 1: Besides an increased expression of HIF-1 in OGD-NSC cultures, no real comparison has been established between NSC cultures grown under normoxic vs those grown under hypoxic conditions: are there more neurospheres generated in hypoxia, are they bigger in size? When the cultures are grown as a monolayer in order to collect the CM, what is the timing to collect the CM? Is there any differentiation occurring in these cultures, are the proportions of undifferentiated cells in both cultures comparable? Are there more cells (therefore, more cells secreting factors into the CM) in the OGD-NSC-derived monolayers by the time the CM is collected?

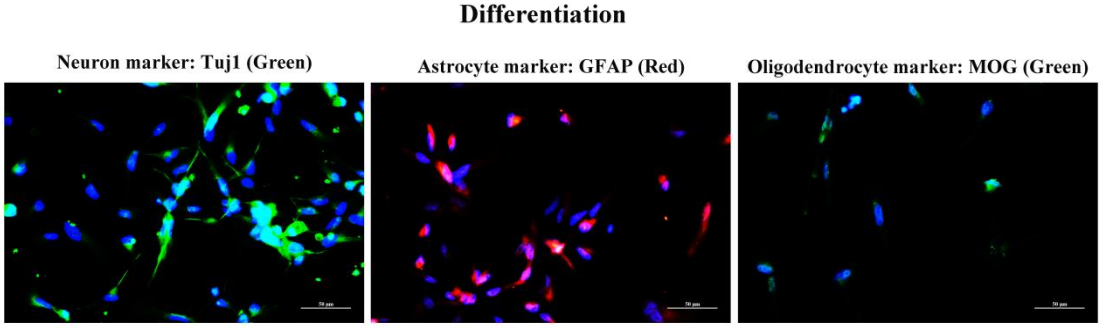
Response: Thanks for your comments. NSCs were suspension culture and formed neurosphere morphology, rather than monolayer culture. Under hypoxic condition, NSCs can generate more neurospheres, but not bigger in size. We used the same number of NSCs to culture for three days, collected the CM and quantified their protein concentration by BCA assay method, then used the consistent protein concentration of CM in each group for experiments.

In Figure 1, it is difficult to understand how, given that differentiation of the NSC cultures was

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

carried out by simply removing the growth factors bFGF and EGF and adding 2% FBS to the cultures, the images in Fig. 1C indicate a proportion of basically 100% of the cells expressing each of the differentiation markers studied (i.e. Tuj1, GFAP, MOG), in each of the images. Would this mean that each of the differentiating cells express all three markers at the same time? No-primary-antibody-controls should be provided to demonstrate that the staining shown in Fig. 1C is not unspecific and that the isolated cells are indeed multipotential; some double staining of differentiating NSC cultures with any combination of these three markers would also be highly desirable.

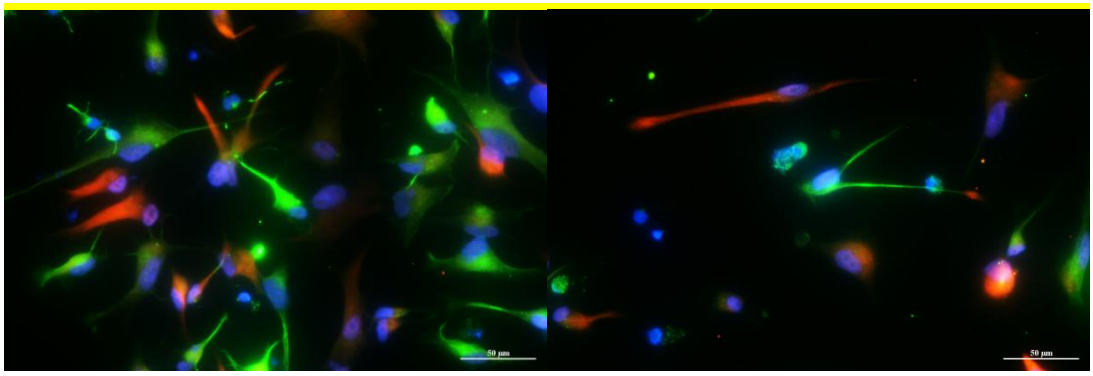
Response: Thank you for your comments. After removing the growth factors, the neural differentiation media was mainly used DMEM/F12 supplemented with 2% FBS to induce NSC differentiation. Fig. 1C showed that the expressions of differentiation markers were not 100%, the fluorescence background of original images maybe disturb the effects, and we have refined the figures.



And we also have done the double immunofluorescence of Tuj1 (neuron) and GFAP (astrocyte), as shown below:

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com



Neuron (Green) + Astrocyte (Red) + Oligodendrocyte (unlabeling)

NSC-derived CM promotes glioma proliferation and invasion / Figure 2: The authors talk about invasion/migration ability of the glioma cells. These are not interchangeable concepts. Since the authors have indicated in Materials and Methods that the transwell chambers were coated with Matrigel, they have conducted an Invasion assay and not a Migration assay, which is conducted through uncoated wells. Importantly, the authors show that NSC-CM has an effect on the proliferation of glioma cells. They record these differences within 1-4 hours of culture (they do not indicate what timing is represented in Figure 2A). It is therefore logical to think that an effect on the proliferation of these cultures would also have an effect on the numbers of cells that cross through the Matrigel coat in 24 hours. The invasion experiment must be re-evaluated taking into account the increased proliferation in the cultures exposed to NSC-CM. Otherwise, an increased number of cells crossing the membrane might be associated to an increased number of cells in the culture.

Response: Thanks for your suggestions. The glioma cells were co-cultured with the same protein concentration of CM (quantified by BCA method) for 24 hours, and the proliferation and invasion of glioma cells were examined. We mainly tested the invasion ability (pre-coated pore polycarbonate membrane with Matrigel) of glioma cells, and then quantified the number of migrated tumor cells through the pore of polycarbonate membrane to the other side. Furthermore, we have refined the related content in the revised manuscript.

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

NCAN protein accelerated the proliferation and migration of glioma cells: The authors demonstrate that treatment of glioma cells with NCAN leads to increased expression of RhoA and ROCK proteins in these cells. However, although they show that NCAN is expressed by both NSCs and glioma cells, they (surprisingly, in my opinion) do not present any data on whether culture of glioma cells in NSC-CM or in the presence of soluble NCAN has an effect on NCAN expression levels in the glioma cells themselves. Given that the authors have carried out a Western Blot to demonstrate NCAN expression in these cells, it would have been highly desirable that they also presented whether there is an effect of NSC-CM or soluble NCAN on NCAN expression in glioma cells. As I mentioned, it is strange not to have analyzed this possibility; it should, at least, be discussed why this experiment was not carried out. It is especially puzzling when the authors state in the last line of the first paragraph in the Discussion section that "…NCAN was expressed in glioma cells and played an important role in….", that could be interpreted as an indication of glioma-expressed NCAN playing a role in the reported results. The same objections raised above to the indistinctive use of migration/invasion and, especially, regarding the effect of NCAN on the proliferation of glioma cells and a subsequent possible effect on the number of cells that invade through Matrigel, apply to this section and to Figure 4.

Response: Thank you very much for your comments, the purpose of this study was to evaluate the effects of conditioned medium (CM) derived from SVZ NSCs on the cancer-related behaviors of glioma cells. And interestingly, the CM from SVZ NSCs promoted the proliferation and invasion of glioma cells, especially in the CM deprived from hypoxic NSCs (OGD-NSC-CM). Furthermore, we identified the secreted protein neurocan (NCAN) in OGD-NSC-CM by mass spectrometry analysis, and preliminary evidenced that NCAN played regulatory roles in mediating the progression of glioma cells. But we have not explored the potential mechanism of the CM or protein NCAN in regulating gliomas. In briefly, our findings provided important insights of CM derived from SVZ NSCs in the regulation of glioma, and next we will in-depth study the potential mechanisms.

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Other comments are: - Some details on the Materials and Methods section are missing, such as: specify when the NSCs are cultured as spheres or monolayers; for how long were the NSCs cultured as monolayer before staining the cultures for stem cell markers?; specify what "complete medium" means; what medium are the cells plated on for the invasion assays? - There are no scale bars In Figure 1 - The title of the legend for Figure 2 is not wholly descriptive. Rather, use something in the lines of "Effect of hNSC-CM on…" - Bibliographical references should be added all throughout the last paragraph in the section "Identification of proteins in the CM derived from SVZ NSCs by mass spectrometry", from "Furthermore, the upregulated proteins…" through to "…variety of matrix or transmembrane molecules" - Figure 4D: Indicate what -1 and -2 mean. Are these independent samples? - There are some mistakes in the English language. - There is no indication as to what MPPC stands for

Response: Thanks for your comments, and sorry about some inconvenience and inattention, we have added the scale bar in the revised Figures, updated and refined the related content of the M&M, Result and Discussion sections in the revised manuscript.

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: 1 Title. The title reflects the main subject/hypothesis of the manuscript. 2 Abstract. The abstract summarizes and reflects the work described in the manuscript. 3 Keywords. The key words reflect the focus of the manuscript. 4 Background. The manuscript adequately describes the background, present status, and significance of the study but some questions arise: - Please reduce the number of abbreviations (at least in headings). This will lead to a better understanding of the article. - You provide a definition for the terms SVZ and NSCs, but not for the term SVZ NSC.

Response: Thanks for your comments. We have added the abbreviative definition of SVZ NSCs.

5 Methods. Authors describe methods in adequate detail but some questions arise: - In the section "Statistical analysis" please more details on the methods applied to your data. - U87 and U251 glioma cells: Please describe in more detail these cell lines.

Response: Thanks for your suggestions. The methods of t-tests or ANOVA analysis were used to evaluate the statistical difference between two groups or more groups. U87 and U251 glioma cells were commonly used and commercialized glioma cell lines, obtained from ATCC.

6 Results. The study of effect of conditioned medium from neural stem cells on glioma progression and its protein expression profile analysis. The research objectives are achieved by the experiments used in this study but some questions arise: - Emerging data have confirmed that glioma spread to the SVZ is closely associated with decreased survival of patients and increased tumor recurrence. We believe that in the following text it would be more correct to give examples of studies in which only neural stem cells were studied and to estimate the prospects for their use.

Response: Thanks for your suggestions, we have added examples (such as patients with GBMs

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

contacting SVZ region have lower survival rates than those with GBMs contacting subgranulazr zone (SGZ), corpus callosum (CC), or cortex) in the revised manuscript.

7 Discussion. The manuscript interprets the findings adequately and appropriately. 8 Illustrations and tables. The figures and diagrams are sufficient, good quality and appropriately illustrative of the paper contents. The tables are absent. 9 Biostatistics. The manuscript meets the requirements of biostatistics. 10 Units. The manuscript meets the requirements of use of SI units. 11 References. The number of references is 33. It's OK for the basic research. 12 Quality of manuscript organization and presentation. The manuscript well organized and presented. The style and language are OK. 13 Research methods and reporting. Authors prepared their manuscript according to manuscript type and the appropriate category: a Basic Researh. 14 Ethics statements. The manuscript has serious problems in ethics: - SVZ NSCs obtained from the human fetuses of 10-14 weeks gestation were kept in our laboratory, as described before [19, 20] Please clarify this point. In case if your article is based on work with the cells that have not been approved by the ethics committee, it will not be allowed to publication.

Response: Thank you for pointing out of this. We have added the content about ethics statements in the M&M section of revised manuscript. SVZ NSCs obtained from the human fetal brain tissue were kept in our laboratory (approved by the Institutional Review Board of Zhongda hospital Southeast University), as our previously described and published [1-3].

[1] Zhang G, Zhu Z, Wang H, Yu Y, Chen W, Waqas A, Wang Y, Chen L*. Exosomes derived from human neural stem cells stimulated by interferon gamma improve therapeutic ability in ischemic stroke model. Journal of advanced research, 2020, 24: 435-445.

[2] Zhang G, Chen L*, Guo X, Wang H, Chen W, Wu G, Gu B, Miao W, Kong J, Jin X, Yi G, You Y, Su X*, Gu N. Comparative Analysis of microRNA Expression Profiles of Exosomes Derived from Normal and Hypoxic Preconditioning Human Neural Stem Cells by Next Generation Sequencing[J]. Journal of biomedical nanotechnology, 2018, 14(6): 1075-1089.

[3] 张桂龙, 陈陆馗*, 李炳乾, 蔡云朗.人源神经干细胞来源外泌体的提取鉴定及内吞作用[J]. 东南大学学报: 医学版, 2017, 36(6): 984-989.

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: Study topic may be relevant to those in the field. Study is well designed. However, few issues need to be addressed for improving the manuscript.

1. Manuscript pages need to be numbered for clarity.

Response: Thank you for your suggestions, we have added page number in the revised manuscript.

2. Neural Stem cells (NSC) are used in the study so if Human ethics committee and Stem Cell Research Committee approval were sought needs to be mentioned in the methods section. Researchers say NSC were isolated from Subventricular Zone of Brain of Human foetuses and preserved from previous study. Authors can provide details if they had sought approval to store / preserve NSC for future studies.

Response: Thank you for pointing out of this. We have added the content about ethics statements in the M&M section of revised manuscript. SVZ NSCs obtained from the human fetal brain tissue were kept in our laboratory (approved by the Institutional Review Board of Zhongda hospital Southeast University), as our previously described and published [1-3].

[1] Zhang G, Zhu Z, Wang H, Yu Y, Chen W, Waqas A, Wang Y, Chen L*. Exosomes derived from human neural stem cells stimulated by interferon gamma improve therapeutic ability in ischemic stroke model. Journal of advanced research, 2020, 24: 435-445.

[2] Zhang G, Chen L*, Guo X, Wang H, Chen W, Wu G, Gu B, Miao W, Kong J, Jin X, Yi G, You Y, Su X*, Gu N. Comparative Analysis of microRNA Expression Profiles of Exosomes Derived from Normal and Hypoxic Preconditioning Human Neural Stem Cells by Next Generation Sequencing[J]. Journal of biomedical nanotechnology, 2018, 14(6): 1075-1089.

[3] 张桂龙, 陈陆馗*, 李炳乾, 蔡云朗.人源神经干细胞来源外泌体的提取鉴定及内吞作用[J]. 东南大学学报: 医学版, 2017, 36(6): 984-989.

3. Methods section, neural differentiation media used is not clear. It needs to be mentioned if any

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

neural differentiation factors were added to culture and at what concentrations.

Response: Thank you for pointing out this, we have added the detail contents (DMEM/F12 supplemented with 2% FBS and 1% Penicillin-Streptomycin, removed growth factors) in the M&M section in the revised manuscript.

4. Concentration of conditioned media used in experiments, is not mentioned. It seems single concentration of CM was used, how was single concentration decided. If any experiment was done using varying concentrations of CM for finalizing particular concentration, it may be mentioned.

Response: Sorry about this inconvenience and inattention. We have added the related content of the concentrations of CM (quantified by BCA method) in the revised manuscript.

5. Authors may mention, how reproducibility of CM quality was ensured.

Response: Thanks for your comments. We used the same number of NSCs to culture for three days, then collected the CM and used the consistent protein concentration of CM in each group.

6. Scale bar to be provided in Figure 1 A, Figure 2B and 2D.

Response: We are very sorry for this negligence. We have updated the scale bar in the revised Figures.

7. Figure 1D, house-keeping marker protein data need to be provided as loading control.

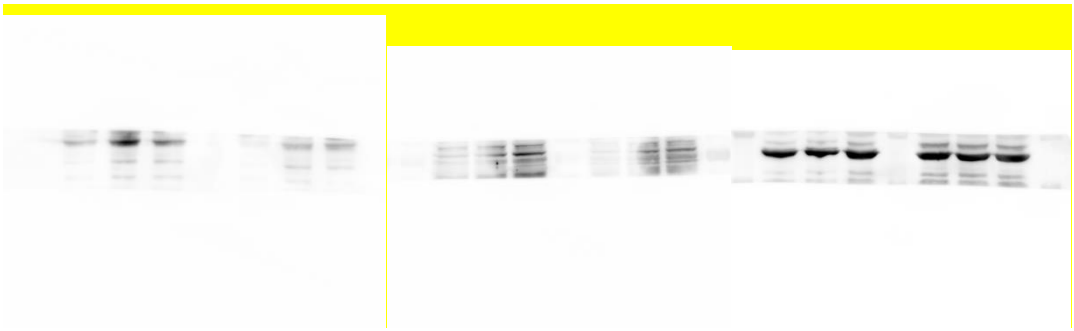
Response: Sorry about this inconvenience, we have added the loading control (GAPDH) in the revised Figure.

8. Figure 4E , original images or developed sheets may be shown for clarity.

Response: Thanks for your advice, the original images were shown in below:

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com



RhoA(21KDa) ROCK(158KDa) ACTIN(42KDa)

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The repetition number of each experiments for statistical analysis must be presented in this paper. The cytokine array results appear to be needed to confirm the detailed mechanism of conditioned medium. If this experiment had not been performed, the description for the reason would be recommended.

Response: Thank you very much for your suggestions. We have performed at least three repetitions in every experiment, and added the related contents in the M&M section. The cytokine array normal used the known cellular factors, such as inflammatory factors or chemokines，but mass spectrometry (MS) can find more known factors and potential unknown factors, the MS method is more superior than the cytokine array.

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: World Journal of Stem Cells

Manuscript NO: 57259

Title: Effect of conditioned medium from neural stem cells on glioma progression and its protein expression profile analysis

Reviewer's code: 02524648
Position: Editorial Board
Academic degree: PhD
Professional title: Postdoctoral Fellow, Senior Researcher
Reviewer's Country/Territory: Spain
Author's Country/Territory: China

Manuscript submission date: 2020-06-01
Reviewer chosen by: Le Zhang
Reviewer accepted review: 2020-09-07 09:05
Reviewer performed review: 2020-09-09 17:49
Review time: 2 Days and 8 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Baishideng Publishing Group

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

Please find below my comments to the revised version of Manuscript 57259, by Zhang and co-workers. Although the authors have made some changes to the text and figures, following reviewers' recommendations, they have not attended to a good number of the comments made in the earlier revision: - Some text has been added to the Materials and Methods and Results sections; however, this is still incomplete and does not clarify all the questions posed when trying to understand the experiments that have been carried out. - The questions by Reviewer 1 regarding an appropriate comparison of NSCs grown in normoxic and hypoxic conditions, beyond that of increased expression of HIF-1 has not been addressed. The authors have indicated that more neurospheres are generated under hypoxic conditions, what should be discussed in the text. Would this also be accompanied, for example, by differences in cell phenotypes in these neurospheres, greater proportions of stem cell-like cells per sphere? They seem to indicate that neurospheres are dissociated (not detached) into single cells and the same number of cells plated per well in order to collect the CM; but they do not explicitly say whether the dissociated cells are plated all under the same conditions or whether the cells from neurospheres grown in normoxic conditions are grown in normoxic conditions for three days and those from neurospheres grown under hypoxic conditions have been grown further in hypoxic conditions; in this case, it is to be expected that there has been greater proliferation in cultures in hypoxic conditions, factor that could itself have an effect on the CM collected; also, they would have needed to verify what happens, in terms of differentiation, to the two different types of dissociated cell cultures that could also have an effect in the CM composition. No appropriate comparison has therefore been established between the two CM-cell culture sources. - Regarding the immunostainings presented in Fig 1C, some of the staining patterns seem strange, most especially that of GFAP, often staining areas which do not seem to closely correlate to the presence of cells; it would still be advisable to present no-primary antibody-controls, tyo ascertain there is

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

no unspecific staining. Also, and although these data have not been introduced into the Ms, it is an error in the Tuj1/GFAP-double labelling figure to indicate that unlabelled cells correspond to oligodendrocytes. No oligodendrocyte marker has been used and therefore unlabelled cells cannot be identified as oligodendrocytes. - No normalization of the invasion results to the reported increased in proliferation, advised by Reviewer 1, has been conducted. This is an important point, since an increase in the number of cells crossing through the transmembrane on its own cannot be reliably interpreted if the increased proliferation rates are not taken into account. - No response has been provided either to the question by Reviewer 3 (point 4) as to how were the CM protein concentrations selected for the experiments (different concentrations are not indicated either in the text/figures, if a range of these concentrations were tested). They have not replied to the same reviewer's question (point 5) on the reproducibility of CM preparations. In addition, the authors have added a loading control Western blot to Figure 1D (in response to reviewer 3´s point 7) where they indicate that the protein shown is GAPDH; should this not be -actin, like in the rest of the blots? (This part of the work has not been discussed in the Materials and Methods section) - The English language should be edited, since there are errors throughout the text that may lead to misinterpretations and ambiguity.

Response: Thanks for your comments. The NSCs formed neurosphere morphology under the condition of suspension culture, rather than monolayer culture. Maybe you did not culture stem cells especially NSCs, these NSCs were cultured under NSC complete medium that inhibited the differentiation and maintained cell self-renew. Thus, under hypoxic condition, except generated more neurospheres, there was no difference between them. Furthermore, it was the most basic that the conditions of cell culturing and cell dissociating were constant. The number or

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-399-1568
E-mail: bpgoffice@wjgnet.com
https://www.wjgnet.com

concentration of CM collected from normoxic NSCs or hypoxic NSCs was definitely different each time, thus, we quantified the protein concentration of CM by BCA assay and used the consistent protein concentration for experiments. In addition, the composition of CM was also obviously different, so we used the MS proteomics technology to identify the differential expression proteins between normoxic CM and hypoxic CM. The positive expression of GFAP was mainly located in cytoplasm, so we can see that the staining of red color was around the nucleus. In the figures of Tuj1/GFAP-double labelling staining, we have further confirmed the characteristics of NSCs, and these figures were not shown in the manuscript. About Transwell assay, the cultivation time of cells was less than 16 h under the serum-free medium, the number of cells was about 5 × 10^4 U87 cells or 2 × 10^4 U251 cells each well, thus the increased cells were very few and the effect was negligible. We have added the concentration of CM in the M&M section if you took a careful look at the revised manuscript. And Reviewer 3 has accepted our responses. Finally, both GAPDH and ACTIN were the loading control that have been commonly used in Western blotting, so this question did not need to further explain.

RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: World Journal of Stem Cells

Manuscript NO: 57259

Title: Effect of conditioned medium from neural stem cells on glioma progression and its protein expression profile analysis

Reviewer's code: 00532996

Position: Editorial Board

Academic degree: BSc, MSc, PhD

Professional title: Assistant Professor, Research Scientist, Senior Scientist

Reviewer's Country/Territory: India

Author's Country/Territory: China

Manuscript submission date: 2020-06-01

Reviewer chosen by: Le Zhang

Reviewer accepted review: 2020-09-07 03:09

Reviewer performed review: 2020-09-11 18:09

Review time: 4 Days and 14 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

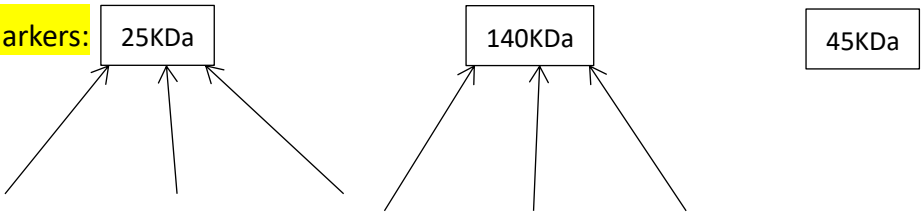
SPECIFIC COMMENTS TO AUTHORS

1. Most comments have been answered satisfactorily except one as shown below. 2. Authors should provide whole un-cropped images of the original western blots from which figures have been derived shown as supplemental

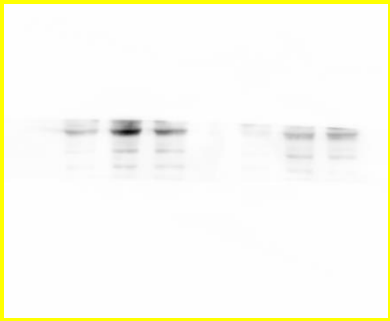
figures. They must also mark position of band of interest on unstained lanes image. 3. Figure 4E: With reference to images shown in original compared to revised, following observations are noted, a. Its observed that intensity of bands is different for RHOA and ROCK proteins in original versus revised manuscript. b. In revised image of ROCK, there is one extra lane noted and unlabeled band in U251MG panel. c. Band shape format seen in actin middle lane of U87MG doesn't match with that seen respective lanes in RHOA and ROCK. d. Authors should provide justification or they should mention if result was reproducible and confirmed by repeating experiment at least two times. They can provide data of two experiments. Alternatively, they can conduct fresh experiment and provide clear acceptable results.

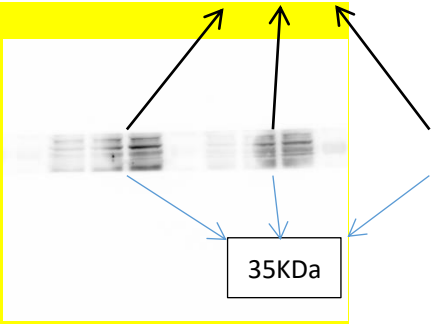
Response: Thanks for your comments, the original images were the same with the revised figures, the image gray or intensity was adjusted in the revised version. In these figures, U87 MG was shown in the left and U251 MG was shown in the right, both sides and the middle bands were the marker positions of prestained protein ladder. In the ACTIN image, you can see the markers (bands of both sides and the middle), the upper band was 45KDa and the lower band was 35KDa. In the ROCK image, the marker band was 140KDa. In the RhoA image, the marker band was not clear enough (25KDa). In addition, this experiment was repeated at least two times (as below), slight non-specific bands also existed, due to the reasons of primary antibodies or blocking reagent, but it did not affect the interpretation of results at all. We have replaced these figures by another clear version in the revised manuscript. Thank you again for your suggestions.

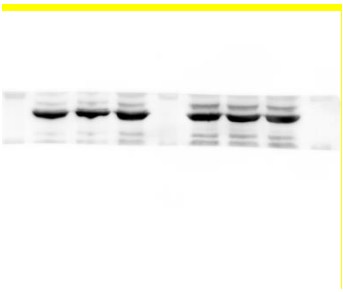
Original images and markers:



Original:



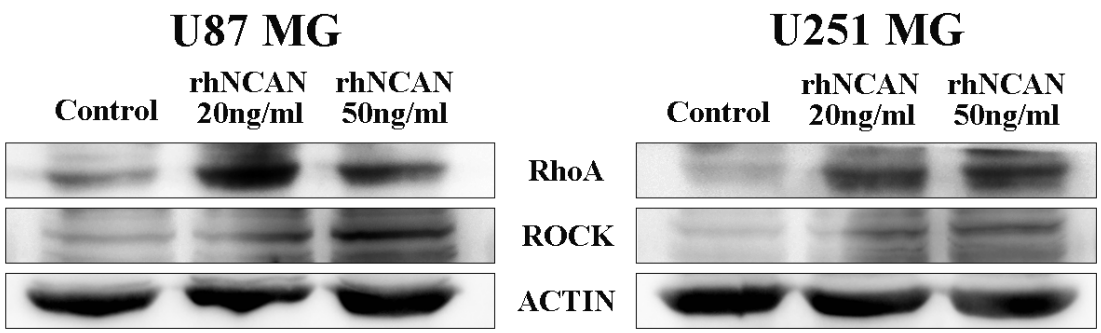




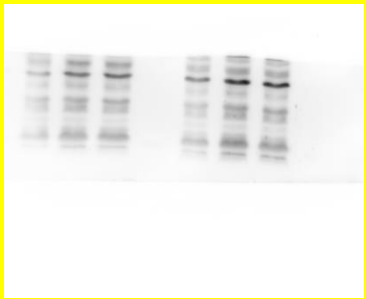
RhoA(21KDa) ROCK(158KDa)

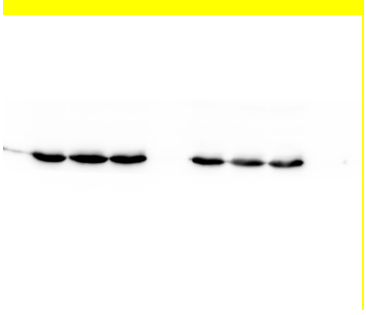
ACTIN(42KDa)

Revised:



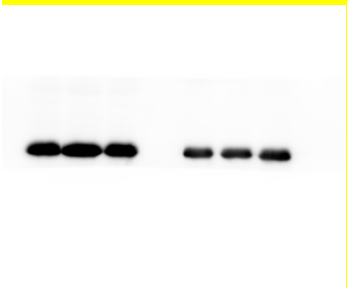
Original-2:





RhoA(21KDa) ROCK(158KDa) GAPDH(37KDa)

Original-3:



RhoA(21KDa) ROCK(158KDa) GAPDH(37KDa)

Clear version: