



BAISHIDENG PUBLISHING GROUP INC

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242 Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com <http://www.wjgnet.com>

Name of Journal: *World Journal of Experimental Medicine*

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Manuscript Type: ORIGINAL ARTICLE

Comments	Response
Reviewer 1	
Authors studied differentiation of hESCs cell line into mature neurons. They observed NPCs and differentiating neurons and optimized culture conditions. Multipotent NPCs differentiated into all three types of cells of nervous system, including neurons, oligodendrocytes and astrocytes. For analyses, authors used phase contrast microscopy. From the view of ethic statement of hESC cultivation, all agents and rules related to hESC maintenance are properly described in the Methodology section. Manuscript is well written, but I missed analysis of some markers, specific for neural differentiation, like III-beta tubulin (as an example) or down-regulation of OCT4. Western blot analysis should be also applied in order to describe induced differentiation pathway properly. In addition, figure description by one sentence is not sufficient and must be improved.	<p>The details for the analysis of markers, specific for neural differentiation, like III-beta tubulin or down-regulation of OCT4 are described in our previous published paper (<i>Shroff G. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738</i>)</p> <p>Figure description is given in detail.</p>
Reviewer 2	
Comment 1: There is no any description of GMP qualified cultivation medium for hESC neither in the submitted paper nor in the cited literature including patents. Author has to provide detailed formulation and growth conditions.	<p>The details can be found from the following link: http://patentscope.wipo.int/search/en/WO200714165 and our published paper: (<i>Shroff G. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738</i>)</p>
Comment 2: There is no any data on hESC line pluripotency neither in the submitted paper nor in the cited literature including patents. Author has to provide detailed information about functional pluripotency of the cell line.	<p>The details for hESC line pluripotency are available from: http://patentscope.wipo.int/search/en/WO200714165 and our previous published paper (<i>Shroff G. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738</i>)</p>

	<i>line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738)</i>
Comment 3: There is no any data on hESC differentiation protocol. Author has to provide detailed description of the neuronal differentiation protocol timeline, reagents and growth factors used and their compliance with GMP, GLP, etc.	Neuronal progenitors are grown in DMEM and do not include any animal products, growth factors or reagents. These cells were grown and every 24 hours media was changed gently. The lab is GMP compliant.
Comment 4: Results section. Fig.1. No neurons are found. Specific cell types have to be confirmed by immunostaining. Fig. 2 no cells are found. Fig.3 looks like fungi DAPI staining or apoptotic nuclear fragmentation. Fig 4 and 5 no cells are found.The overall quality of the data is very poor. Reject.	The figures show cells and neuronal morphogenesis.
Reviewer 3	
<u>General Comments</u> Comment 1: The importance of the research and the significance of the research findings. This research is important in terms of describing about the differentiation of human embryonic stem cells into three types of nervous systems such as neuron, oligodendrocytes and astrocytes.	
Comment 2: The novelty and innovative nature of the research. This is an innovative research describing about the differentiation of human embryonic stem cells.	
Comment 3: The quality of the manuscript's presentation and readability. It is well written, however, some proofreading is needed.	We have again proofread the document carefully.
Comment 4: The ethics-related aspects of the research. The appropriate documents may be needed.	The Institutional Review Board statement (IEC approval) is provided along with the revised manuscript.

<u>Specific Comments</u> Comment 1: <i>Title:</i> It accurately reflects the major topic and contents of the study.	
Comment 2: Abstract: It appropriately describes about the content of the manuscript, however, please specify the abbreviation for NPC firstly described. Introduction: There are some characters that are not shown appropriately. Please correct them.	It has been corrected as described.
Comment 3: <i>Materials and Methods:</i> RNeasy Mini Kit, instead of RNAeasy, is appropriate in RNA extraction and RT-PCR section.	It has been corrected as suggested.
Comment 4: <i>Results:</i> The differences of culture conditions or the culture days in differentiation for three types of the neuronal cells may be described in detail, especially for figure 1. The results of RT-PCR are not described. Please include the result of RT-PCR in Results, or delete the RNA extraction and RT-PCR section from the Materials and Methods.	The results of RT-PCR are described in detail in our previously published paper (<i>Shroff G. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738</i>)
Comment 5: <i>Discussion:</i> The possibility of NPCs differentiated from hESCs may be described more in detail in the 6 th paragraph in Discussion.	The details are added and highlighted in text “Reynolds <i>et al</i> , in their study showed induced <i>in vitro</i> proliferation of striatum isolated cells via epidermal growth factor. These cells were found to account for the morphology and antigenic properties of neurons and astrocytes subsequent to their capacity of division and differentiation into neurons and astrocytes, respectively.” (<i>Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system Science 1992; 255: 1707-1710</i>)
Comment 6: <i>Figure and Table:</i> In figure 2 and 5, the differences of panels in the same day should be indicated.	The differences of panels are being incorporated in the figures.

Comments in edited manuscript (25814)	
Comment 1: Audio Core Tip	It has been provided.
<p>Comment 2:Writing requirement</p> <p>1. <i>Background</i></p> <p>To summarize concisely and accurately the relevant background information so that readers may gain some basic knowledge about your study's relevance and understand its significance for the field as a whole.</p> <p>2. <i>Research frontiers</i></p> <p>To introduce briefly the current hotspots or important areas in the research field as related to your study.</p> <p>3. <i>Innovations and breakthroughs</i></p> <p>To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article.</p> <p>4. <i>Applications</i></p>	<p>Human embryonic stem cells (hESCs) offer unlimited source of cells. They have the capability to renew and differentiate into all cell types. The differentiation may occur at all development stages upon exposure to appropriate signals. These signals act in a hierarchical manner that regulates the development of embryo; thereby, inducing the hESCs to differentiate into specific cell types of three germ layers. The neuron is generated by the combination of various stem cells that align together.</p> <p>The study describes the generation of different neuronal cells from hESC line (SCT-N) during biopsy of blastomeres at 2-celled cleavage stage from a discarded embryo during <i>in vitro</i> fertilization (IVF) process.</p> <p>The hESC lines claim to use defined animal free conditions during derivation and long-term culture which makes it suitable for clinical cell therapy. We were able to show that that neuronal axons and tissues were not generated from single cell differentiation but by joining of multiple cells that communicate and transfer signals to each other, thereby forming neuron tissue.</p> <p>The study provided a system for generation of neural progenitor cells (NPCs) from hESCs. Moreover, the</p>

<p>To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.</p> <p>5. Terminology</p> <p>To describe concisely and accurately any terms that may not be familiar to the majority of the readers, but which are essential for understanding your article.</p> <p>6. Peer-review</p> <p>To provide the major comments from your peer-reviewers that most represent the characteristics, values and significance of your article, and to allow the readers to have an objective point of view regarding your article and research findings.</p>	<p>resulting NPCs from hESCscould serve as an expandable source for neurons production, which could be applied for many purposes such as treatment of neurodegenerative diseases.</p> <p>Neural progenitor cells (NPCs) are multipotent, self-renewable cells that are responsible for forming the main phenotype of the nervous system.</p> <p>Description of the details for the analysis of markers specific for neural differentiation, like III-beta tubuli or down-regulation of OCT4.</p>
<p>Comment 3: References: Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Pleased provide PubMed citation numbers for the reference list, e.g. PMID and DOI, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmedand http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in the E-version of this journal. Thanks very much for your co-operation. Such as: 1 Nayak S, Rath S, Kar BR. Mucous membrane</p>	<p>The references are revised as per the suggestions.</p>

graft for cicatricialectropion in lamellar ichthyosis: an approach revisited. OphthalPlastReconstrSurg 2011: e155-e156 [PMID: 21346670 DOI: 10.1097/IOP.0b013e3182082f4e]	
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