

Asia Pharma 2016 : Rapid generation of sub-type, region-specific neurons and neural networks from human pluripotent stem cells for neurological disorders - Yiling Hong - Western University of Health Sciences

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Human embryonic and induced pluripotent stem cells are of considerable interest in developmental biology and regenerative medicine, representing an enormous opportunity for generating patient-specific cells for screening drugs and cell therapies for various diseases. Stem cell neuronal differentiation has been used as an in vitro model for a number of genetic conditions, such as spinal muscular atrophy and familial dysautonomia, as well as inherited and sporadic forms of various human neurodegenerative conditions, including motor neuron disease, Niemann-Pick disease (NPD), Huntington disease (HD), Parkinson's disease (PD) and Alzheimer's disease (AD). This method involves a combination of supplemented knockout serum replacement medium (SKSRM) with 10% CO₂ and a mechanical procedure termed "AdSTEP," which involves breaking the neurospheres into smaller fragments to increase the efficiency of neuronal production. Furthermore, we injected the fragmented neurospheres into the severe combined immunodeficiency (SCID) mouse brains to investigate the effect of AdSTEP on neurogenesis in vivo, which might have significant impacts on neuronal transplantation and regenerative medicine. Materials and methods include Maintenance of the H9 lines and generation of the human induced pluripotent stem cell (iPSC) line HFS-1, Neuronal initiation to generate neurospheres and neuroectoderm from the h/iPSCs, Neuronal induction of the neurospheres and neuroectoderm, Generation of the neurospherderm from neurospheres using "AdSTEP", Differentiation of neural progenitors, sub-type specific neurons and region-specific neurons, Immunocytochemistry and microscopy, Flow cytometry, Quantitative real-time polymerase chain reaction (qRT-PCR), NanoString CodeSet design and gene expression quantification, Assay of neuronal function with the Fluo-4 Ca²⁺ + fluorescence indicator, Transplantation and histological analysis. The Results includes Ten percent CO₂ facilitated the formation of neurospheres from h/iPSCs ie The procedures and various stages of neuronal differentiation from h/iPSCs with different culture conditions and time courses are shown in. Briefly, the h/iPSCs were exposed to 10% CO₂ in SKSRM for the first 3 days of neuronal initiation. At day 3, distinct round and bright-edged neurospheres were formed. In comparison, unevenly aggregated neurospheres and non-uniform neuroepithelial sheets were formed from the cells cultured with 5% CO₂. The neurospheres were further examined for the expression of neuronal precursor markers (SOX2, NESTIN, PAX6, and FOXG1) via qRT-PCR. The qRT-PCR results showed that with 10% CO₂, the neurospheres expressed twice as much NESTIN, PAX6, and FOXG1 compared with the 5% CO₂ culture condition, although SOX2 changed less significantly. These results indicated that the neurospheres derived with our culture conditions were more stable over a longer period of time compared with the adherent neuroectoderm culture method. This also results in the The AdSTEP mechanical procedure facilitated

the generation of neurospherderm and neural stem cells from the neurospheres ie lumping has always been a challenge for examining neuronal differentiation in neurosphere-derived cultures. To facilitate neurosphere-derived neuronal differentiation, an AdSTEP mechanical procedure was introduced to break the neurospheres into smaller fragments, which were then plated onto Matrigel-coated plates to form a monolayer of neuroepithelial-like cells that were termed the "neurospherderm". In our neuronal culture, the TBR1 cortical neurons appeared within 17 days, and then the deep layer cortical neurons, the FOXP2- and EMX1-positive pyramidal neurons, emerged from the culture at approximately 22–27 days. Finally, the SATB2-positive layer 5 cortical neurons were identified. GABAergic interneurons and excitatory glutamatergic neurons also appeared one week after neuronal induction (17 days) and their populations increased at approximately day 27, which is earlier than that observed in previously reported protocols.

The neurosphere-derived cultures for neuronal differentiation are a valuable model system for studying neurogenesis and understanding the molecular mechanisms associated with neurodegenerative diseases. Recent studies on iPSC-derived neurospheres and 3D cultures showed a significant promise for the development of disease-specific cells with the desired genetic backgrounds, which would facilitate the study of many important diseases, such as Timothy syndrome, Fragile X syndrome or NPD. Here, we present a new defined culture medium and conditions: SKSRM medium and 10% CO₂. This new culture condition doubled the expression of the neuroprogenitor genes NESTIN, PAX6, and FOXG1 compared with the traditional 5% CO₂ culture conditions. The molecular mechanism by which the higher CO₂ levels facilitate neurogenesis is still not clear. It could be due to reduced oxygenation or hypoxia, as previously reported. There are several groups that have reported that hypoxia or reduced oxygenation enhances neural stem cell colony survival and increased NESTIN, SOX1, SOX2, and FOXG1 expression, similar to our study. Furthermore, our neurospherderm not only generate more neuroprogenitors, but it can also give rise to all of the sub-type-specific neurons and synapses, e.g., glutamatergic, GABAergic and cortical neurons. In addition, the neurospherderm-derived neurons also showed stronger spontaneous neuronal activity, as shown by the Ca²⁺ + fluorescence activity, which was accelerated by glutamate in a dose-dependent manner. Therefore, it is clear that the neurosphere-derived neurons respond via glutamatergic neurotransmission, and the enhancement or suppressed electrical activity with glutamate receptor agonists or antagonist, respectively, confirmed the development of an excitatory neural network in vitro. Here, we used calcium-dependent fluorescent indicator dyes that allowed us to measure the synchronized activity across a network of cells. In contrast, it is also possible to determine single-cell resolution neuronal activity using patch-clamp

electrophysiology, but the ability to measure a network is limited to typically one or two neurons. Therefore, the Fluo-4 Ca²⁺ indicator dye was used in this study to identify the neural networks created by the neurospheres-derived neurons. In addition, we also injected the neurospheres into SCID mouse brains. The confocal images showed that the AdSTEP-fragmented neurospheres had abundant PAX6-positive cells in the cerebral cortex that contained larger NTR structures, similar to those observed in vitro. These results suggested that the engrafted neurospheres were further differentiated to neural precursor cells, which further contributed to neurogenesis. Therefore, our AdSTEP neurospheres, generated under defined culture conditions, easily integrated into the mouse brains, demonstrating a great promise for neurogenesis studies and stem cell therapy. Overall, this novel and rapid virus-free method for generating neuronal populations from

neurospheres has many advantages, all of which will have a great impact on our understanding of neuronal identity after neurosphere transplantation as well as the mechanisms of disease.

Biography :

Yiling Hong is an Associate Professor at Western University of Health Sciences. She has received her PhD degree from University of Kentucky in 1997. She had in depth training in molecular biology and stem cell biology. As Principal Investigator of the National Institute of Health grant, her research interest is focus on stem cell differentiation and determination of cytotoxicity and genotoxicity of manufactured nanoparticle in stem cells. She has published over 40 papers.

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