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In vitro culture of human embryonic striatum-derived neural stem cells***★

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Abstract

BACKGROUND: Neural stem cells are always derived from animals, and unsuitable for human transplantation treatment.

OBJECTIVE: To explore the *in vitro* culture methods of human embryonic striatum-derived neural stem cells, and to observe the biological characteristics.

METHODS: The human embryonic striatum were separated from the embryo at a gestational age of 8–16 weeks that received induction of labor with water bag, and then the embryonic striatum was *in vitro* cultured in the serum-free Dulbecco's modified Eagle's medium. The cells were passaged after neurospheres formation, and then the cells were induced to differentiation with the Dulbecco's modified Eagle's medium/F12 containing 10% fetal bovine serum.

RESULTS AND CONCLUSION: The *in vitro* cultured human embryonic striatum-derived neural stem cells grew rapidly and could express nestin. Colony formation assay showed the cell clone formation rate was 6.0%–7.0%. 5-Bromodeoxyuridine incorporation assay showed the cell proliferation rate was 37.9%. Immunofluorescence staining showed that the cells after induction and differentiation could express Tuj-1, glial fibrillary acidic protein and nestin, but not express myelin basic protein. The results indicate that human embryonic striatum-derived neural stem cells cultured in the serum-free medium can maintain their biological characteristics and have self-renewal capacity, and the cells can differentiate into the neurons and astrocytes induced by the fetal bovine serum.

Key Words: stem cells; stem cell culture and differentiation; human embryos; striatum; neural stem cells; culture; differentiation; serum-free; proliferation; fetal bovine serum; biological characteristics; National Natural Science Foundation of China; stem cell photographs-containing paper

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INTRODCTION

Neural stem cells are defined by self-renewing and multi-directional differentiation potentiality, such as neurons, astrocytes and oligodendrocytes which exist in various regions of the central nervous system throughout the mammalian lifespan[1]. The nerve cells never have the reproducibility in traditionary standpoint. That is to say, if the cellula nervosa died because of some endogenous or exogenous factors, the neurologic impairment which originated by the dead cells will never be redeemed. The discovery of neural stem cells will change the viewpoint. Reynolds *et al* [2] were considered as the first man who obtained the neural stem cells from the striatum of mouse fetus. Previous reports have shown that human neural stem cells can be isolated from many regions of the embryo or adult central nervous system such as: the subventricular zone of the lateral ventricle, hippocampus, interbrain, olfactory bulb, cortex and chorda spinalis[3-8]. Recent data indicate that neural stem cells-based therapy has been proved as a potential treatment for once un-treatable diseases including central nervous system injuries

and degenerative disease[9-13]. A major limiting factor in the remedial transplantation of neural stem cells is the difficulty in supplying sufficient amounts of human fetal neural stem cells and the concomitant ethical issues associated with the use of human fetal tissues. A great deal of previous research found that the neural stem cells isolated from the central nervous system of embryo and adult mammalian could be propagated and differentiated *in vitro*[14-15]. Proliferation and differentiation of neural stem cells are affected by the internal and external signals coming from medium components, as well as some complex interactions among cells[16-18]. A reliable source of human neural stem cells would be of immense practical value to both foundation research and clinical neural transplantation trials.

The present study was initiated to observe the growth characteristics and the features of serum induced differentiation of human fetal neural stem cells, and to search a high-performance method that can be used for procuring and propagating neural stem cells *in vitro*, in order to explore a feasible source of suitable cells for neural stem cells-based therapy.

MATERIALS AND METHODS

Design

A cytology experiment *in vitro*.

Time and setting

This study was carried out in the Affiliated Hospital of Medical College, Qingdao University, and Cell Therapy Center of Xuanwu Hospital from August 2009 to April 2010.

Materials

Main reagents and instruments

Reagents and instruments	Sources
Dulbecco's modified Eagle's medium/F12 medium, fetal bovine serum	Invitrogen
Epidermal growth factor, basic-fibroblast growth factor, accutase enzyme	R&D
B27, glutamine	Gibco
Light microscope, confocal laser scanning microscope	Nikon
Paraformaldehyde, mouse anti-5-bromodeoxyuridine antibody	Sigma
Mouse anti-nestin, neuronal Class Ⅲ β-Tubulin, glial fibrillary acidic protein monoclonal antibody	Chemicon
Mouse anti-myelin basic protein antibody	Boster

Brain tissue of human fetus

Brain tissue of induction of labor with water bag human fetus at a gestational age of 8–16 weeks was obtained from the Department of Obstetrics and Gynaecology of the Affiliated Hospital of Medical College, Qingdao University, under aseptic technique. The collection of human fetal tissue and the procedure of our experimentation were approved by the Human Ethics Committee of the Affiliated Hospital of Medical College, Qingdao University, and an informed consent was obtained from the pregnant women.

Methods

Isolation and culture of human fetal neural stem cells

The preparation of brain tissue of induction of labor with water bag human fetus aged 8–16 weeks of gestation was done in accordance with previously published protocols[19-20]. Briefly, human fetal brain tissue was fresh microdissected from fetus hibateral striatum under a microscope, and then washed repeatedly in Hanks solution in order to cleanse blood. Decohesion of meninges and disassociation of ambi-cerebral hemisphere was conducted, and the dislodged striatum. The isolated tissues were mechanically dissociated with eye scissors and a fire-polished Pasteur pipette. And then, the tissues were filtered by a sterile 40-μm stainless steel strainer mesh after 2 minutes standing in ordinary temperature. The viable cells were counted with tryphan blue exclusion and then seeded into a culture capsule at a quantity of 1×10^6 cells in 100 mm culture capsule with 9 mL of a serum-free Dulbecco's modified Eagle's medium/F12 medium consisting 10 ng/mL human epidermal growth factor, 20 ng/mL human basic-fibroblast growth factor, 2% B27 supplement, 1% glutamine and 1% Penicillin-Streptomycin. The cells grew at 37 ℃ in 5% CO_2 and 95% air with saturated humidity.

The cells grew as free floating clusters (neurospheres) after 7–8 days after cultured with semis supersede culture solution every 2 days. The single cells were dissociated by incubation with 300 μL accutase enzyme at ordinary temperature for 5–7 minutes, and triturated with light and soft fire-polished of Pasteur pipettes, and then centrifugation at 1 000 r/min for 3 minutes. Single cells were resuspended and counted with tryphan blue by a microscope and reseeded at a density of 100 000 cells/mL using an improved neubauer counting plate.

Identification of separated and cultured human fetal neural stem cells in vitro

Normal neurospheres of the third or fourth generation were inoculated into the poly-L-lysine-coated 24-well plate containing growth medium and cultured for 24 hours until the neurospheres fixed on the plate. And

the neurospheres cultured in the poly-L-lysine-coated 24-well plate until single cells spread from the neurospheres was considered as the second condition. Following fixation with 4% paraformaldehyde for 40 minutes and washed three times with 1% PBS. The cells were incubated with 5% equinum serum for 1 hour at room temperature and washed three times with 1% phosphate buffered saline. Samples were stained with anti-nestin (1:200) according to manufacturer's instructions. Nestin-positive neurospheres and single cells were observed and the images were processed using the Adobe Photoshop 9.0 software and Spot software.

Cloning efficiency and 5-bromodeoxyuridine proliferation assay of human fetal neural stem cells

In order to demonstrate the multipotency of human fetal neural stem cells, we performed a clonal analysis. Single cells of the third or fourth generation were counted with tryphan blue under a microscope and reseeded at a number of 3 000 cells per well in the flat-bottom of 96-well plates. The growth condition of clone-sphere was observed and the number of clone-sphere was counted. The cloning efficiency was calculated after cells cultured for 5–6 days.

For 5-bromodeoxyuridine proliferation assay, suitable size neurospheres of the third or fourth generation were inoculated into the poly-L-lysine-coated 24-well plate containing normal growth medium, and cultured for 4–6 days until single cells spread from the cluster. The medium was replaced with 0.2 μmol/L 5-bromodeoxyuridine at 24 hours before fixture. Following fixation with 4% paraformaldehyde for 40 minutes, the cells were washed three times with 1% PBS-0.3% Triton X-100 at room temperature. Then the cells were incubated with 4 mol/L HCl (in Distilled water) for 30 minutes at room temperature (DNA denaturation) and washed three times with 1% PBS-0.3% Triton X-100. Then incubated with 5% equinum serum for 1 hour at ordinary temperature and washed three times with 1% PBS-0.3% Triton X-100. Samples were immunofluorescence stained with anti-5-bromodeoxyuridine (1:200) according to manufacturer's instructions. Cellular nucleuses were stained with 4',6-diamidino-2-phenylindole (1:1 000) for 5 minutes. The 5-bromodeoxyuridine fluorescence was observed and the digital images were captured under a confocal laser scanning microscope (TE2000-U). The 5-bromodeoxyuridine-positive cells were counted and the images were processed using the Adobe Photoshop 9.0 software and Spot software.

Differentiation of human embryonic striatum-derived neural stem cells

Suitable neurospheres of the third or fourth generation were inoculated into the poly-L-lysine-coated 24-well plate containing the culture medium with 10% fetal bovine serum but without the growth factors. The culture solution was semis superseded every 2 days. The differentiation procedure was observed under an inversion phase contrast microscope. Our neurospheres were differentiated for 6 days before fixation.

Immunofluorescence labeling

For immunocytochemical characterization, the neurospheres were fixed with 4% paraformaldehyde in 1% PBS for 40 minutes and washed three times with 1% PBS-0.3% Triton X-100 at room temperature. Then, the neurospheres were blocked in 5% equinum serum for 1 hour at room temperature and washed three times with 1% PBS-0.3% Triton X-100. Primary antibodies, diluted 1:200 in 1% PBS, were then applied to the cells overnight at 4 ℃. The primary antibodies and their dilution used in this study were monoclonal mouse anti-nestin monoclonal antibody (1:200), as a marker for identifying undifferentiated human fetal neural stem cells, the monoclonal mouse anti-neuronal Class Ⅲ β-Tubulin antibody (1:250) was used as a marker for identifying neurons, and the monoclonal mouse anti-glial fibrillary acidic protein antibody (1:200) as a marker for identifying astrocytes, anti-myelin basic protein antibody (1:50) was used as a marker for identifying oligodendrocytes. The samples were washed three times in 1% PBS-0.3% Triton X-100. The fluorescein-conjugated goat anti-mouse secondary antibody were used to visualize the signal by reacting with cells for 2 hours at room temperature after washed for three times in 1% PBS-0.3% Triton X-100. Cellular nucleuses were stained with 4',6-diamidino-2- phenylindole (1:1 000) for 5 minutes at room temperature. Immunostained cells were visualized by indirect fluorescence under the fluorescent microscope. Nestin-positive, Class Ⅲ β-Tubulin- positive and glial fibrillary acidic protein-positive cells were observed respectively and the images were showed in the circum-neurosphere where the cells distributed uniformly. All photographes were processed in the Adobe Photoshop 9.0 software and Spot software.

Main outcome measures

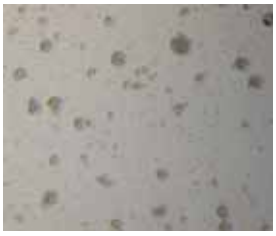
The proliferation and differentiation of the separated and cultured human embryonic striatum-derived neural stem cells were observed.

RESULTS

Culture and identification of human fetal neural stem cells

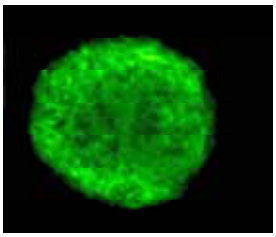
Single cells were isolated from the dissociated striatum

of human 8–16 weeks old fetuses described above. The primary-generation cells were globular, and the percentage of living cells was more than 95% with tryphan blue staining. The cells were continued to proliferate in a serum-free environment, where single cells divided to form small clusters which contained several cells that *in vitro* cultured within 2 to 4 days, and then neurospheres floating within the growth medium were formed. The small neurospheres were irregular round; the volume of them was different as well as its fluffy inter-cells (Figure 1A). At the same time, small proportion of cells was died and the cell debris could be seen in the bottom of the culture dish. Within 8–12 days, large clusters were visible; the neurospheres were buninoid, texture compacted and borderline limpid (Figure 1B).

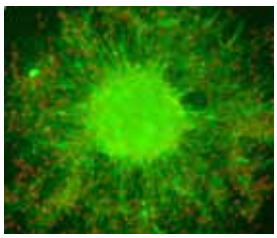


A: The single cells proliferate in a serum-free environment, where single cells divided to form small clusters which contain several cells that *in vitro* cultured within 2 to 4 d, and then neurospheres floating within the growth medium. The small neurospheres were irregular round, the volume of them was different, as well as there are fluffy inter-cells

B: A small proportion of cells died and the cell debris could be seen in the bottom of the culture dish. Within 8–12 d, large clusters were visible, the neurospheres were buninoid, texture compacted and borderline limpid



C: The anti-nestin was used for the assessment of neural stem cells. Immunohistochemical analysis demonstrated that most of the cells in clusters were nestin-positive (green)



D: Immunohistochemical analysis demonstrated that most of the cells in clusters were nestin-positive (green). The cell nucleus were colored to red with 4',6-diamidino-2-phenylindole

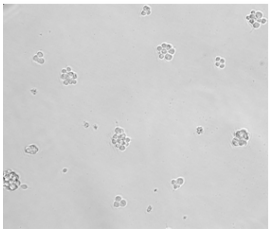
Figure 1 Morphology and identification of human fetal neural stem cells that cultured *in vitro* (×100)

The operation of passage ought to be done when the diameter of clusters about 0.6–0.8 mm. New neurospheres will form after passage. The proliferation was rapid and the passage must to be done in a short time at the beginning culture. This kind of condition was changed after 2 months, the proliferation was slow and the generation time became longer.

The identification of neural progenitor cells was impeded by the shortage of specific markers. So far, the expression of nestin was the only acceptable marker to identify neural stem cells in an early stage of development. So anti-nestin was used for the assessment of the neural stem cells. Immunohistochemical analysis demonstrated that most of the cells in clusters were nestin-positive (Figures 1C, D).

Proliferation of human fetal neural stem cells

Cloning efficiency was used for analyzing the growth rates of human fetal neural stem cells, and the single cells were seeded into a 96-hole plate described above. The small clusters were counted and observed after cultured for 7 days (Figure 2). The average number of the clusters in the 96-hole plate was 180–210, so the cloning efficiency was 6.0%–7.0%.



Single cells were seeded into a 96-hole plate. The small clusters were counted and observed after cultured for 7 d. The average number of the clusters in the 96-well plate was 180–210, so the cloning efficiency was 6.0%–7.0%.

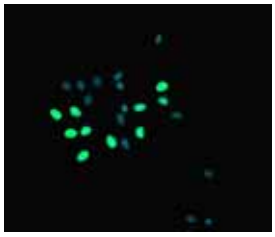
Figure 2 Cloning efficiency of human fetal neural stem cells (×200)

To further detect the proliferation ability of the human fetal neural stem cells, the neurospheres were used for 5-bromodeoxyuridine incorporation assay at 8–9 days after the third passage. The 5-bromodeoxyuridine-positive cells were distributed throughout dispersed unicells. Then, 4',6-diamidino-2-phenylindole staining was used for determining the total count of the dispersed unicells. The percentage of proliferation was about 37.9% (Figure 3).

Differentiation and immunofluorescence labeling of human fetal neural stem cells

The neurospheres were induced to differentiate by transferring into poly-L-lysine-coated 24-hole plate in growth culture medium without growth factors and with 10% fetal bovine serum. The cells rapidly attached to the culture plate and small amounts of cells spread out from the neurospheres after adherent culture for 24–48 hours under the inverted phase contrast microscope. The cells were irregular and have short ecphymas (Figure 4A).

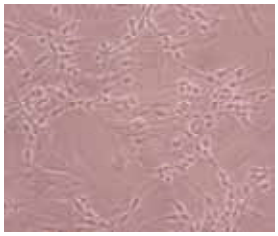
The quantity of cells spread out from the neurospheres was increased with culturing, and the cells with different morphologies were apparent. The differentiated cells spread all over the circum-neurospheres, overlapping meshwork were formed by the ecptoma which spread from the differentiated cells after cultured for 6–8 days (Figure 4B).



The neurospheres were detected with 5-bromodeoxyuridine incorporation assay at 8–9 d after the third passage. The 5-bromodeoxyuridine- positive cells were distributed throughout dispersed unicells. Then, 4',6-diamidino-2-phenylindole staining was used for determining the total count of the dispersed unicells. The percentage of proliferation was about 37.9%.

Figure 3 Proliferation ability of human fetal neural stem cells (×200)

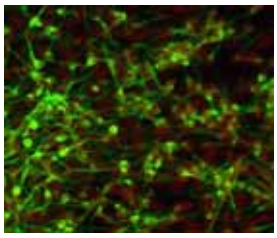
A: The neurospheres were induced to differentiate by transferring into poly-L-lysine-coated 24-hole plate in growth culture medium without growth factors and with 10% fetal bovine serum. The cells were rapidly attached to the culture plate and small amounts of cells were spread out from the neurospheres after adherent cultured for 24–48 h under the inverted phase contrast microscope (×100)



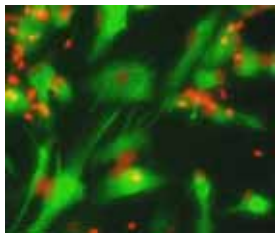
B: The differentiated cells were spread all over circum-neurospheres, and the overlapping meshwork were formed by the ecptoma which spread from the differentiated cells after cultured for 6–8 d (×200)

Figure 4 Morphology of the differentiated cells

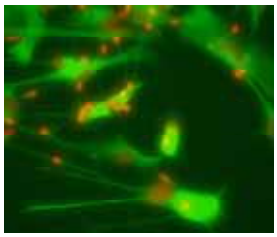
To further detect the differentiation ability of the human fetal neural stem cells, the anti-nestin, anti-myelin basic protein, anti-Class Ⅲ β-Tubulin and anti-glial fibrillary acidic protein were used for immunofluorescence labeling. Differentiated neurospheres were found containing different cell types. Class Ⅲ β-Tubulin-positive cells were smaller and had a clear fringe, the appearance of them was oval-shape or round, and had more than one long ecptoma (Figure 5A). The proportion of Class Ⅲ β-Tubulin-positive in differentiated neural stem cells separated from striatum was about 56.8%. The glial fibrillary acidic protein-positive cells were bigger than the other kind cells, and the appearance of them was irregular with short and thick ecptoma, maybe like the starfish (Figure 5B). The proportion of glial fibrillary acidic protein-positive in differentiated neural stem cells separated from striatum was about 39.8%. The myelin basic protein-positive cells were never seen in the differentiated neural stem cells that separated from striatum in our study. The nestin-positive cells were located on the interior neurospheres, and the number of circum-neurosphere was decreased (Figure 5C). The Class Ⅲ β-Tubulin-positive cells and glial fibrillary acidic protein-positive cells were all nestin-positive cells, but the fluorescence of differentiated cells was dimmish when compared with the cells before differentiation.



A: Class Ⅲ β-Tubulin -positive cells (neurons) were smaller and had a clear fringe, the appearance of them was oval-shape or round, and had more than one long ecptoma (green). The cell nucleuses of neurons were small and hyperchromatic



B: The glial fibrillary acidic protein-positive cells (astrocytes) were bigger than the other kinds of cells, and the appearance of cells was irregular with more short and thick ecptoma, maybe like the starfish (green). The cell nucleuses of astrocytes were big and received dilute staining.



C: The Class Ⅲ β-Tubulin-positive cells and glial fibrillary acidic protein-positive cells were all nestin-positive cells (green)

Figure 5 Expression of Class Ⅲ β-Tubulin, glial fibrillary acidic protein and nestin of differentiated neural stem cells, 4',6-diamidino-2-phenylindole staining was used to disclose the cell nucleus (red) (×200)

The Class Ⅲ β-Tubulin-positive cells had a tendency that they like aggregation when compared with the glial fibrillary acidic protein-positive cells. The glial fibrillary acidic protein-positive cells were hypodispersion in the corona radiata of differentiated neurospheres, but the

Class Ⅲ β-Tubulin-positive cells could form cancellous cells cluster usually. The myelin basic protein-positive cells were unseen.

DISCUSSION

Great interest has been attracted in searching the methods for exploiting the huge potential of neural stem cells in therapeutic and regenerative medicine since 1990s because of its two outstanding features: self-renewal and multi-directional differentiation potentiality. Self-renewal is that the neural stem cells can generate daughter cells which possess all features of the ancestor. And multi-directional differentiation potential is that the neural stem cells can generate two or more than two kinds of functioning cells such as neuron and glia in adequate environment[21-22].

Derive neural stem cells from mammalian animals include human and culture in conditioned medium containing especial growth factors is a common methods to gain plenty of neural stem cells in nowadays[23]. We derived neural stem cells from human fetal striatum and serum-free cultured *in vitro*. The quantities of the cells were increased manifestly and can be propagated, passaged and procured quantum sufficit purpose cells. We used Dulbecco's modified Eagle's medium/F12 as the basal medium to *in vitro* culture the human fetal neural stem cells, Dulbecco's modified Eagle's medium contains the primary nutrient substance and F12 contains basic microelement for cell growth. Growth factor is imperative in serum-free medium to sustain the growth and reproductive activity of neural stem cells[24-25]. The suspended cells will adherence differentiate or apolexis if there is no growth factor or the concentration is too low. Basic fibroblast growth factor and epidermal growth factor are two important broad-spectrum neurotrophic factors which were used in neural stem cells culture investigation. Some microelement and excitatory autacoid such as B27 must be added at simultaneous. All of them are the guarantee for neural stem cells growth *in vitro*.

We used senescence assay, cloning efficiency and 5-bromodeoxyuridine proliferation assay to observe the growth feature of human fetal neural stem cells *in vitro*. The senescence efficiency was heightened along with the prolonging of culture time *in vitro*, while cloning efficiency and 5-bromodeoxyuridine positive cells were degraded. The cycle time of passage manipulation was prolonged from 5 days to 14 days. That may be caused by the characteristics of neural stem cells and the different requirements about growth in different growth stages[26-27].

Passage manipulation is an important procedure in neural stem cells culture, but various methods have be seen in previous reports[28-30]. The time of digestion is difficulty to control in trypsinization and the mechanical digestion is too violence to use. In our experiment, we used the accutase enzyme which is a relatively mild digestive ferment in passage. The size of neurospheres is the fundamental qualification for generation time, 0.6–0.8 mm is the adequate diameter for neural stem cell passage in generally. Activity of neural stem cells would be damaged if digests passage when the clusters are justo minor or justo major. Retrogradation will emerge if the diameter of the neurospheres more than 1 mm, the colouration will change from bright-blance to luteotestaceous or dilute brow and no longer float-growth to say in other words. That is because the infiltration of nutrient substance is difficulty to the central of the clusters and cells in the internal of the neurospheres are sharp-set. Corpuscular condition will deteriorate and it is hard to recuperation if the retrogradation occurrence already. The aged stem cells influence our further study, so we should select after calamity.

Self-renewal is a typical capability of neural stem cells, self-duplication and division growth are the foundation for self-renewal. The 5-bromodeoxyuridine as a ramification of thymine commutability thymine incorporates DNA in S-stage of synthesis period is a common cell marker for generation. We used immunofluorescence staining to assess the generation activity of cells after 5-bromodeoxyuridine added in the culture medium for 24 hours. At the same time, detection β-galactosidase activity is a classic staining to detect senile cell effectively on account of lysosomal enzyme β-galactosidase hyperergy to increase of content in the lysosome, and react with X-gal to display bluish-green[31]. The growth velocity will degrade gradualness along with the frequency of passage accrescence. Some biological substance such as telomerase and the different growing conditions demanded by diverse growth stage changed may be related[32].

The potentiality to differentiate into various kinds of cellula nervosa is another characteristic of neural stem cells. Different programs for differentiation have been used by different researchers[33-35]. We used blood-serum as the revulsivum to induce neural stem cells to differentiation in our experimentation in order to observe the differentiation generate under approximate physio-conditional without other inducing factors. There are miscellaneous physio-factors which affect the differentiation of neural stem cells contained in blood-serum *in vivo*. Nestin, glial fibrillary acidic protein, myelin basic protein and Class Ⅲ β-Tubulin positive

cells were observed under fluorescence microscope with immunofluorescence labeling after 6–8 days differentiate in growth culture medium without growth factors and with 10% fetal bovine serum. Nestin as a kind of filamin and be expressed in non-differentiated cell has been used as a specific marker for stem cell commonly nowadays[36]. Glial fibrillary acidic protein is glial fibrillary acidic protein to mark gliocyte, and Tubulin is neuron-specific Class Ⅲ β-Tubulin to mark neuron[37-38]. Myelin basic protein is myelin basic protein that is a marker protein of oligodendrocyte. Glial fibrillary acidic protein-positive cells distributed steady from borderline of neurospheres to all of the cell monolayer, and they were the first kind of cells differentiated from the clusters in our experiment. Class Ⅲ β-Tubulin-positive cells differentiated from the clusters was later than glial fibrillary acidic protein-positive cells and they were maldistribution and accumulative slightly in some area of the corona-deck. This phenomenon of differentiated cells disposition was stationary but the reason was unknown. That may be related with the different functions of the cells. Sustain, protection and trophism are the main functions of gliocyte, but the action of neurone is neuro-reaction. The percentage of the two kinds of cells was discrepant like in the brain. But the proportion of glial fibrillary acidic protein-positive and Class Ⅲ β-Tubulin-positive cells among the total cells were different in previous reports[39-40]. The reasons may be the different culture and differentiate conditions and the different sources of neural stem cells. The differentiated cells were all nestin-positive demonstration that daughter cells in initial stage of differentiate still express nidogen. And reflect that these daughter cells still have some characteristics of stem cells. The myelin basic protein-positive cells were never seen in the differentiated cells. The possible reasons may be that the differentiation of oligodendrocyte was later than other two kinds of cells, and the number of oligodendrocyte is minor in the central nervous system or the culture environment was unsuitable for oligodendroglial cell differentiation and growth[41-43].

Nowadays, infarctate substructure has been constructed for neurobiology research and nervous system disease through studying the neural stem cells *in vitro*. But there are lots of questions about neural stem cells need us to think and explore, such as how to induce the neural stem cells to differentiate into the object cells which can perform specified function before the extremely character of stem cells and can be effectively controlled and utilized. The objective of our study is to lucubrate and observe the bionomics of neural stem cells in order to provide basis for clinic application.

The neural stem cells isolated from striatum of human fetus can be cultured, proliferated and differentiated *in vitro*. The neural stem cells of the human fetal striatum have self-renewal capacity and can generate into multiple differentiated neural cell types. Neurons and astrocytes are the main kinds of cells differentiated from the neural stem cells with serum. The neural stem cells isolated from the human fetal striatum can maintain their undifferentiated features with self-renewal and multiple differentiated capacities. Neural stem cells isolated from the human fetal striatum can be harvested at a large scale by culture *in vitro*.

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人胚胎纹状体来源神经干细胞的体外培养**★

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文章亮点：

1 实验成功地从经水囊引产的孕 8-16 周人胚胎纹状体分离并体外培养扩增了适用于移植的人胚胎纹状体来源神经干细胞。

2 克隆形成率及 BrdU 掺入实验证实实验培养的人胚胎纹状体来源神经干细胞具有自我更新能力。

3 经胎牛血清诱导后人胚胎纹状体来源神经干细胞可向神经元及星形胶质细胞分化。

关键词：

干细胞；干细胞培养与分化；人胚胎；纹状体；神经干细胞；培养；分化；无血清；增殖；胎牛血清；生物学特性；国家自然科学基金；干细胞图片文章

摘要

背景：目前神经干细胞多由动物获得，不适合人类临床移植治疗。

目的：探索体外环境下人胚胎纹状体来源神经干细胞的培养方法，同时观察其生物学特性。

方法：取经水囊引产的孕 8-16 周人胚胎纹状体，体外用无血清 DMEM 培养基进行培养，待细胞形成神经球后进行传代，并应用含体积分数 10%胎牛血清的 DMEM/F12 培养液进行诱导分化。

结果与结论：体外培养的人胚胎纹状体来源神经干细胞生长迅速，表达神经干细胞标志物 nestin。克隆形成实验显示细胞克隆形成率为 6.0%-7.0%；BrdU 掺入实验显示细胞增殖率为 37.9%。免疫荧光染色显示经诱导分化的细胞表达神经元标志物Ⅲ型 β 微管蛋白、星形胶质细胞标志物胶质纤维酸性蛋白及神经干细胞标志物 nestin，但不表达少突胶质细胞标志物髓鞘碱性蛋白。可见人胚胎纹状体来源神经干细胞在体外无血清条件下可保持其生物学特点，具有自我更新能力，经胎牛血清诱导后可向神经元及星形胶质细胞分化。

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利益冲突：课题未涉及任何厂家及相关雇主或其他经济组织直接或间接的经济或利益的赞助。

伦理要求：研究征得产妇的知情同意，获得青岛大学医学院附属医院伦理委员会批准，符合《医疗机构管理条例》的相关要求。

学术术语：水囊引产-将水囊放置于宫壁和胎膜之间，增加宫内压和机械性刺激宫颈管，诱发和引起子宫收缩，促使胎儿和胎盘排出的终止妊娠方法。

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