Integration of Transplanted Choroidal Plexus Epithelial Cells with Oxygen Glucose Deprived Organotypic Spinal Cord Slices

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Research Article

Keywords: Organotypic spinal cord slices, Spinal cord injury, Choroidal plexus epithelial cells, Cell transplantation, Oxygen-glucose deprivation

Posted Date: March 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1286548/v1

Abstract

This study was undertaken to evaluate the integration of transplanted choroidal plexus epithelial cells with oxygen glucose deprived (OGD) organotypic spinal cord slices. Organotypic spinal cord slices were normally cultured for 14 days, then were OGD for 60 min. They were divided into transplantation group and non-transplantation group. The choroidal plexus epithelial cells, which were cultured for 6–7 days, were labeled by 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanineperchlorate(CM-DiI), and were identified by transthyretin (TTR) in immunocytochemistry. They were adjusted to the density of 0.5–1×10^7/ml. 2μl cells suspension were transplanted into OGD spinal cord slices in transplantation group. Subsequently, the slices were normally cultured for 3 days (NT 3 d group), 7 days (NT 7 d group) and 14 days (NT 14 d group) respectively. Accordingly, in non-transplantation group, 2μl basal medium was dripped to the OGD organotypic spinal cord slices, then the slices were normally cultured for 3 days (N 3 d group), 7 days (N 7 d group) and 14 days (N 14 d group). After 3 days and 7 days of transplantation, the level of LDH in the culture medium was tested by Elisa, and the ratio of caspase 3 active/ caspase 3 was tested by western blotting. After 14 days of transplantation, the differentiations into neurons and astrocytes, and the synapses were identified by immunofluorescence histochemistry. At the same time, the ratios of cell differentiations and synapses in new system, and the changes of MAPK signaling pathway were tested by western blotting. Our result shows that the choroidal plexus epithelial cells were well labeled by CM-DiI and were immune-stained by TTR in immunocytochemistry. The transplantation cells could differentiate into neurons and astrocytes, and there were some synaptophysin positive vesicles between transplantation cells and OGD organotypic spinal cord slices. The level of LDH and the ratio of caspase 3 active/ caspase 3 in the NT 7 d group was significantly lower than which in the N 7 d group ($P < 0.05$), but there was of no significance between NT 3 d group and N 3 d group. The levels of GFAP, TUB-III and synaptophysin in the NT 14 d group were significantly higher than which in the N 14 d group ($P < 0.05$). The ratios of p-JNK/JNK and p-P38/P38 in the NT 14 d group were significantly lower than which in the N 14 d group ($P < 0.05$). But the ratios of p-ERK/ERK in the NT 14 d group were significantly higher than which in the N 14 d group ($P < 0.05$). The transplanted choroidal plexus epithelial cells can integrate with OGD organotypic spinal cord slices into a new system.

Introduction

Hypoxic-ischemic injury, as a pathophysiological change, is common in various spinal cord diseases, and need to be further investigated. Oxygen glucose deprived (OGD) organotypic spinal cord slices not only closely mimic hypoxic-ischemic spinal cord injury (Goncharenko et al. 2014; Liu et al. 2017a; Sypecka et al. 2013), but also preserve the basic tissue cytoarchitecture and complex tissue microenvironment (Cifra et al. 2012; Goncharenko et al. 2014; Liu et al. 2017a; Sypecka et al. 2015; Sypecka et al. 2013). Because of the above advantages, it is an ideal platform for the research of hypoxia-ischemia in spinal cord.

So far, there is no effective and satisfactory method for the treatment of hypoxic-ischemic spinal cord injury. Cell transplantation is a latent attempt for it. Choroidal plexus epithelial cells can be used as seed cells for repairing damaged central nervous system because of stem cells characteristics (Hashemi et al.

2017; Huang et al. 2013b; Huang et al. 2011). They can proliferate and differentiate into neurons and astrocytes (Bolos et al. 2013; Hashemi et al. 2017; Huang et al. 2013b; Kitada et al. 2001). Besides, they can secret many growth factors and neurotrophic factors, such as TGF-β, GDF-15, GDNF, BDNF, NGF, VEGF, etc (Huang et al. 2014; Ide et al. 2016; Thouvenot et al. 2006; Zhao et al. 2018). They can change the host's local microenvironment, which are helpful for nerve tissue regeneration and repairment. Thus the transplantation of choroidal plexus epithelial cells may be a good choice for spinal cord hypoxia-ischemia injury (Ide et al. 2016; Kanekiyo et al. 2016; Sandrof et al. 2017).

Microenvironment regulates seed cells survivals, differentiations, apoptosis and synaptogenesis (Hofer et al. 2012; Shamloo et al. 2015). At the same time, the seed cells change the host's microenvironment through their own activities (Hofer et al. 2012; Jäderstad et al. 2010; Shamloo et al. 2015). They interact and integrate with each other to generate into a new system (Hofer et al. 2012; Jäderstad et al. 2010). In the new system, there are not only behavioral and morphological changes of seed cells, but also behavioral and microenvironmental changes of host's tissue. Under normal physiological conditions, the choroidal plexus epithelial cells could be integrated into organotypic spinal cord slices in our previous study (Liu et al. 2022). But under hypoxic-ischemic condition, because of the changes of local microenvironment, it will produce different effects on the biological behaviors of transplanted choroidal plexus epithelial cells. So the integration between choroidal plexus epithelial cells and OGD organotypic spinal cord slices is different accordingly. By now, the integration between choroidal plexus epithelial cells and OGD organotypic spinal cord slices has not been paid attention, and the particular pathophysiologic changes are unclear.

To better understand the above issues, we conducted the present study. Our goal was to identify the changes of the new system between transplanted choroidal plexus epithelial cells and OGD organotypic spinal cord slices.

Materials And Methods

Ethical statement

Protocols for animal care and experimental management were approved by the Xi'an Jiaotong University Animal Experimentation Committee. Principles of laboratory animal care were followed, and all experimental procedures were conducted according to guidelines established by the National Institutes of Health. All efforts were made to minimize the number of animals used and their suffering.

Animals

Postnatal Sprague-Dawley 5-7 days old rats (n = 36) weighed 22-26 g and neonatal Sprague-Dawley 1 day old rats (n = 6) weighed 5-6 g were supplied by the Center of Experimental Animals at College of Medicine, Xi'an Jiaotong University. The 5-7 days old rats were used for OGD organotypic spinal cord slices, which were divided into transplantation group and non-transplantation group. In the transplantation group, the choroidal plexus epithelial cells which were cultured for 6-7 days and were

labeled by 1,1’-dioctadecyl-3,3,3’,3’-tetramethyl-indocarbocyanineperchlorate (CM-Dil), were transplanted to the OGD organotypic spinal cord slices, then the slices were normally cultured for 3 days (NT 3 d group), 7 days (NT 7 d group) and 14 days (NT 14 d group) respectively. In the non-transplantation group, the same amount of basal medium was dripped to the OGD organotypic spinal cord slices, then the slices were normally cultured for 3 days (N 3 d group), 7 days (N 7 d group) and 14 days (N 14 d group). The 1-day old rats were used for dissociation and primary culture of choroidal plexus epithelial cells.

Materials and reagents

Vibratome (ZQP-86; Shanghai Zhisun Equipment Co. Ltd, Shanghai, China), razor blades , ophthalmic scissors, corneal scissors, microforceps and operating knife blades were used in this experiment. Culture dishes of 35mm, six-well plates, and 0.4-μm-pore polyester membrane inserts (Transwell 3450) were provided by Corning Costar (New York City, New York, USA). Dulbecco's modified Eagle's medium (DMEM/ F12, DMEM/ low glucose, DMEM/ free from glucose), 1,1’-dioctadecyl-3,3,3’,3’-tetramethyl-indocarbocyanineperchlorate (CM-Dil) and fetal bovine serum were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Lactate Dehydrogenase (LDH) Kit was provided by Ray Biotech (Norcross, GA, USA). Rabbit monoclonal anti-GFAP, anti-beta III tubulin (TUB-III), anti-synaptophysin (SYN) and anti-prealbumin (TTR) were purchased from Abcam (Cambridgeshire, UK). Rabbit polyclonal or monoclonal anti-caspase3, anti-caspase3 active, anti-ERK, anti-p-ERK, anti-P38, anti-p-P38, anti-JNK and anti-p-JNK were purchased from Cell Signaling Technology (Danvers, MA, USA). 4',6-diamino-2-phenylindole (DAPI) was purchased from Roche (Basel, Switzerland). Anti-rabbit HRP were purchased from KPL (Milford, MA, USA). RIPA buffer and protease inhibitor cocktail were provided by Cell Signaling Technology (Danvers, MA, USA). BCA assay and ECL were purchased from Pierce Chemical (Dallas, Texas, USA). Fluorescein-conjugated goat anti-rabbit IgG, biotin-conjugated goat anti-rabbit IgG and peroxidase streptavidin were purchased from CWBIO (Beijing, China). Recombinant rat epidermal growth factor (EGF) was obtained from PeproTech (Rocky Hill, NJ, USA).

Primary culture of choroidal plexus epithelial cells

The neonatal 1-day-old rats were used. Primary cultures of choroidal plexus epithelial cells were prepared using the procedures described in our previous study (Huang et al. 2013a). Briefly, 6 rat brains were removed and kept in chilled DMEM/ low glucose medium. The choroidal plexus tissues were extracted from both lateral ventricles, transferred into a beaker containing chilled DMEM/ low glucose medium. The tissue pieces were mechanically triturated by repeated passages through a 1 ml pipette. After centrifugation, the growth medium (DMEM/ low glucose supplemented with 10% FBS and 10 ng/mL EGF) was added. Then, cells suspended homogeneously in the growth medium were seeded in Petri dishes. They were then cultured in a 5% humidified CO_2 incubator at 37°C. The growth medium was changed 48–72h later, then changed every 3 days.

Preparation of organotypic spinal cord slices

5-7 days old rats were used. The organotypic spinal cord slices were prepared according to the following method (Liu et al. 2017b). Lumbar spinal cord was extracted and the meninges were removed. The spinal cord was embedded by two agarose blocks. Then they were transverse sectioned into 350-μm slices. The slices were placed on the surface of the membrane insert and cultured for 14 days at 37 °C in an incubator with 5% humidified carbon dioxide. The growth medium (DMEM/ F12 supplemented with 10% FBS) was changed the day after plating, and then was changed every 3 days.

Preparation of OGD organotypic spinal cord slices

OGD organotypic spinal cord slices were prepared according to the following method (Liu et al. 2017a). Organotypic spinal cord slices, which were normally cultured for 14 days, were washed by PBS twice and placed into a 6-well plate with 1 ml of glucose-free DMEM medium in each well. The slices were then cultured in an anaerobic incubator (N2 90%, H2 5%, CO2 5%) at 37 °C for 60 min. The obtained OGD organotypic spinal cord slices were used in subsequent experimental procedures.

Transplantation of choroidal plexus epithelial cells into OGD organotypic spinal cord slices

Choroidal plexus epithelial cells cultured for 6-7 days were applied. The medium in Petri dishes was discarded, and CM-DiI (1 μg/ml) was added. The choroidal plexus epithelial cells and CM-DiI were incubated together at 37 °C for 30 minutes, then at 4 °C for 15 minutes. The choroidal plexus epithelial cells were washed by phosphate-buffered saline (PBS) for two times, then were mechanically dissociated into cells suspension. Next, they were centrifuged at 2000 ×g for 5 min. The supernatant was discarded, and PBS was added to modulate cells density to 0.5-1×10^7/ml. 2-μl cell suspension was dripped to the OGD organotypic spinal cord slices in transplantation group. Then the slices were normally cultured for 3 days (NT 3 d group), 7 days (NT 7 d group) and 14 days (NT 14 d group) respectively. Accordingly, 2-μl basal medium was dripped to the OGD organotypic spinal cord slices in non-transplantation group, then they were normally cultured for 3 days (N 3 d group), 7 days (N 7 d group) and 14 days (N 14 d group).

LDH quantification

The culture medium in the N 3 d, NT 3 d, N 7 d and NT 7 d groups was collected. The medium in different groups was screened for LDH level with MultiAnalyte ELISArray kits according to the manufacturer's protocol. Briefly, medium was directly added to the ELISArray plates for 2-h binding incubation. After three washes, the plates were incubated for 1 h with detection antibody. After an additional three washes, bound secondary antibody was detected using streptavidin-HRP and quantified with a spectrometer (Thermo, USA) at a 450-nm wave length.

Immunocytochemistry

The choroidal plexus epithelial cells cultured for 6-7 days were fixed by 4% paraformaldehyde for 30 min, incubated with 0.5% Triton X for 15 min, blocked with 0.3% H_2O_2 for 15 min, and 10% normal goat serum for 40 min. They were subsequently incubated with rabbit monoclonal anti-Prealbumin (1:1000) at 4 °C

overnight. After 3 washes in PBS, they were incubated in the biotin-labeled secondary antibody followed by a further treatment of avidin-biotin-peroxidase complex. The nuclei were counterstained with Mayer's hematoxylin. Microscopy was performed (Olympus, Japan). Negative controls were performed, with primary antibodies omitted.

Immunofluorescence histochemistry

The OGD organotypic spinal cord slices in NT 14 d group were fixed with 4% paraformaldehyde for 30 min, incubated with 0.5% Triton X for 30 min and 10% normal goat serum for 40 min. Then they were incubated with rabbit monoclonal anti-GFAP (1:300), anti-beta III Tubulin (1:500), and anti-Synaptophysin (1:100), respectively, at 4 °C overnight. Subsequently, they were incubated with fluorescein-conjugated goat anti-rabbit IgG (1:200) for 2 h. Cell nuclei were counterstained using DAPI. The slices were then rinsed and cover-slipped with fluorescent mounting media. Laser confocal microscopy (Leica, Wetzlar, Germany) was performed. Negative controls were performed, with primary antibodies omitted.

Western blotting

There were 6 samples in each group. Samples in N 3 d, N 7 d, N 14 d, NT 3 d, NT 7 d and NT 14 d groups were collected and centrifuged at 2000 ×g. Then they were homogenized in ice-cold RIPA lysis buffer supplemented with protease inhibitors cocktail. Homogenates were centrifuged at 12,000 × g, and the supernatants were collected and measured by BCA protein assay. Protein samples were normalized and loaded for SDS-PAGE, then transferred to a PVDF membrane. Membranes were then incubated for 2 h in blocking buffer (5% skim milk, tris-buffered saline, 0.1% Tween 20). Subsequently, they were incubated in primary antibodies over night at 4°C: rabbit monoclonal anti-GFAP (1:5000), anti-beta III Tubulin (1:5000), anti-Synaptophysin (1:2000), anti-ERK (1:3000), anti-p-ERK (1:2000), anti-p-JNK (1:1000), anti-P38 (1:2000), and rabbit polyclonal anti-caspase3 (1:3000), anti-caspase 3 active (1:1000), anti-JNK (1:1000) and anti-p-P38 (1:1000) respectively, followed by incubation with HRP coupled anti-rabbit IgG for 1 h. Then they were incubated in ECL solution. The images were taken using Epson V300 camera system (Epson, Japan), and the immunoreactive bands were measured using Alpha view software (Alpha Innotech, San Leandro, CA).

Statistics

SPSS 26.0 software package was used. All data were reported as means ± SD. Group data were compared by Independent sample T-test. Statistical significance was assessed at $P< 0.05$.

Results

The morphology of choroidal plexus epithelial cells

The choroidal plexus epithelial cells were well labeled by CM-DiI. The cytomembrane was emitted strong red fluorescence when observed by fluorescence microscopy (Fig. 1A). And they were well labeled by transthyretin (TTR) in immunocytochemistry. In which, the cytoplasm was manifested as brown (Fig. 1B).

Because TTR is specifically expressed in choroidal plexus epithelial cells (Menheniott, et al. 2010), in our experiment, the cells were identified definitely as choroidal plexus epithelial cells definitely.

The differentiations of transplanted choroidal plexus epithelial cells

The differentiations of choroidal plexus epithelial cells, which were transplanted into OGD organotypic spinal cord slices in NT 14 d group, were identified by immunofluorescence staining. The transplanted cells could differentiate into neurons (Fig. 2C) and astrocytes (Fig. 2F).

The synapses in the transplant new system

The OGD organotypic spinal cord slices in NT 14 d group and transplanted choroidal plexus epithelial cells formed a transplant new system. The synapses in the new system were tested by immunofluorescence staining. The synaptophysin positive vesicles were located between the transplanted choroidal plexus epithelial cells and the OGD organotypic spinal cord slices (Fig. 2I).

The level of LDH

The level of LDH in culture medium was of no significance between N 3 d and NT 3 d groups. The level of LDH in the NT 7 d group was significantly lower than which in the N 7 d group (Fig. 3A), which manifested the decrease of spinal cord injury.

The ratio of caspase 3 active/ caspase 3

The ratio of caspase 3 active/ caspase 3 is positively related with apoptosis. There was of no significance between N 3 d and NT 3 d groups. But the ratio in the NT 7 d group was significantly lower than which in the N 7 d group (Figure. 3B), which indicated the decrease of apoptosis.

The cells differentiations and synaptogenesis in the new system

The ratios of different types of cells in the new system were tested by Western blotting. The levels of GFAP and TUB-III in the NT 14 d group were significantly higher than which in the N 14 d group (Fig. 4A-4C) .The new system promoted the cells differentiations into neurons and astrocytes. The synapses in the new system was also tested by Western blotting. The synaptophysin level in the NT 14 d group was significantly higher as compared with which in the N 14 d group (Fig. 4A, 4D) .There were new synaptogenesis in the new system.

The changes of MAPK signaling pathway in the new system

The ratios of p-JNK/JNK and p-P38/P38 in the NT 14 d group were significantly lower than which in the N 14 d group. The ratio of p-ERK/ ERK in the NT 14 d group was significantly higher than which in the N 14 d group (Fig. 5).

Discussion

OGD organotypic spinal cord slice closely mimic hypoxic-ischemic spinal cord injury, and is suitable for being host tissue for cell transplantation. The choroidal plexus epithelial cells can be used as seed cells. In the present experiment, we investigated the integration between choroidal plexus epithelial cells and OGD organotypic spinal cord slices. We found that the transplanted cells integrated excellently with OGD organotypic spinal cord slices into a new system. The new system promotes transplanted cells differentiating into neurons and astrocytes, and promotes the synaptic connections between transplanted cells and host tissue. In the new system, LDH and caspase 3 active/ caspase 3, as indexes of spinal cord injury and apoptosis, were decreased. And there were changes of MAPK signaling pathway in the new system. The phosphorylation level of MAPK may be related with the cells survival, proliferation, differentiation and apoptosis.

OGD model in long-term cultured organotypic spinal cord slices has been successfully established in our previous study (Liu et al. 2017a). It not only simulates hypoxia-ischemia excellently, but also retains the tissue architecture and microenvironment of in vivo spinal cord (Cifra et al. 2012; Goncharenko et al. 2014; Liu et al. 2017a; Sypecka et al. 2015; Sypecka et al. 2013). This model can be applied to basic investigations, such as neural development, neural function, neural physiology and neural pharmacology. So as an excellent platform, it was used in our experiment.

CM-DiI is always used for fluorescent labeling. The cytomembranes which are dyed manifest stable red fluorescence. In addition, the cellular viability is not affected. In our previous work, it had been confirmed that the choroidal plexus epithelial cells we cultured for 20 days were dyed red fluorescence strongly and continuously, and their viability was excellent (Liu et al. 2021). In the present experiment, the choroidal plexus epithelial cells we cultured for 6-7 days were along with the above studies.

The choroidal plexus epithelial cells, which have characteristics of stem cells, can be induced into neurons and astrocytes under physiological and pathological conditions (Bolos et al. 2013; Hashemi et al. 2017; Huang et al. 2013b; Huang et al. 2011; Kitada et al. 2001). The biological behaviors of transplanted cells were affected by host tissues. In our experiment, the choroidal plexus epithelial cells were induced by hypoxia-ischemia to differentiate into neurons and astrocytes. And a large amount of synapses was located between the transplanted choroidal plexus epithelial cells and OGD organotypic spinal cord slices. The synaptic connections were established between the transplanted cells and the host tissue. The expression of TUB-III, GFAP and synaptophysin in NT 14 d group were increased significantly than which in N 14 d group. The results indicated that the OGD organotypic spinal cord slices promoted differentiations of choroidal plexus epithelial cells into neurons and astrocytes, and promoted the formation of new synaptic junctions.

The OGD organotypic spinal cord slices, as host tissue, are affected by choroidal plexus epithelial cells. The choroidal plexus epithelial cells can secret numerous growth factors and neurotrophic factors, which provide nutrition for the OGD organotypic spinal cord slices (Huang et al. 2014; Ide et al. 2016; Thouvenot et al. 2006; Zhao et al. 2018). The transplanted cells also can alleviate inflammation response, they can help nerve regeneration, repairment, and improve local microenvironment (Hofer et al. 2012; Ide et al.

2016; Jäderstad et al. 2010; Kanekiyo et al. 2016; Sandrof et al. 2017; Shamloo et al. 2015). In our experiment, compare with N 3 d group, LDH in NT 3 d group were neither increased nor decreased. But compared with N 7 d group, LDH in NT 7 d group were decreased significantly. The ratio of caspase 3 active/ caspase 3 in NT 7 d group was significantly lower than which in N 7 d group, but between the groups of N 3 d and NT 3 d, there was no significant difference. Because it required a period for integration of choroidal plexus epithelial cells into OGD organotypic spinal cord slices, LDH as a biochemical marker for spinal cord injury, and the ratio of caspase 3 active/ caspase 3 as a marker for apoptosis, were kept constantly in NT 3 d group, but decreased significantly in NT 7 d group. Neuroprotective effects were occurred 6-7 days after transplantation.

The transplanted cells and host tissue interacted with each other and integrated into a new system (Hofer et al. 2012; Jäderstad et al. 2010). The cell behaviors in new system were dependent on the changes of intra-cellular signaling pathway. Mitogen-activated protein kinase (MAPK) pathway was closely correlated with cell proliferation, differentiation, inflammation and apoptosis (Guo et al. 2017; Sun et al. 2015). It played an important role in the new system. The ERK system was definitely the best characterized MAPK pathway. The ERK pathway was important for integrating external signals from the presence of mitogens such as epidermal growth factor (EGF) into signaling events promoting cell growth and proliferation (Huo et al. 2015; Sun et al. 2017). JNK pathway participated in hypoxic-ischemic-induced cell injury and inflammatory response, and also was important in cell apoptosis and differentiation (Dhanasekaran and Reddy, 2017; Tang et al. 2017). P38 pathway was activated in response to a variety of extracellular stimuli including osmotic shock, inflammatory cytokines, lipopolysaccharides, UV light and growth factors, and which was related with cell apoptosis and inflammation (Corre et al, 2017; Zarubin and Han, 2005;). In our experiment, in the NT 14 d group, the phosphorylation level of ERK increased, and the phosphorylation level of JNK and p38 decreased. The overall effects of the changes of MAPK pathway was the promotion of cells proliferation, differentiation, and the inhibition of cell apoptosis and inflammation. The transplanted choroidal plexus epithelial cells could alleviate hypoxic-ischemic spinal cord injury, which was modulated by the MAPK pathway in the new system.

Conclusion

Transplanted choroidal plexus epithelial cells can integrate with OGD organotypic spinal cord slices into a new system. In the new system, the transplanted choroidal plexus epithelial cells can differentiate into neurons and astrocytes, and the new synaptic junction can be built. And in the new system, LDH and the ratio of caspase 3 active/ caspase 3 decrease significantly. There are neurol protective effects in the new system. The MAPK signaling pathway is changed in the new system, which is related with cells survivals, apoptosis, proliferations and differentiations. But our observation duration for the new system, merely about two weeks, is relatively shorter. And our study for the regulation of the new system is limited in the MAPK pathway. The long-term changes and the detailed regulation need to be fully and completely investigated furthermore.

Declarations

Author contributions

JJL designed the experiments, performed the experiments and wrote the manuscript. XYD performed the experiments and analyzed the data. LX performed data analysis and interpretation. SLH conceived the project and designed the experiments.

Conflict of interest The authors declare no competing interests.

Ethical approval All procedures were approved by the Xi'an Jiaotong University Animal Experimentation Committee. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Acknowledgements This study was supported by National Natural Science Foundation of China (81471247).

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Figures

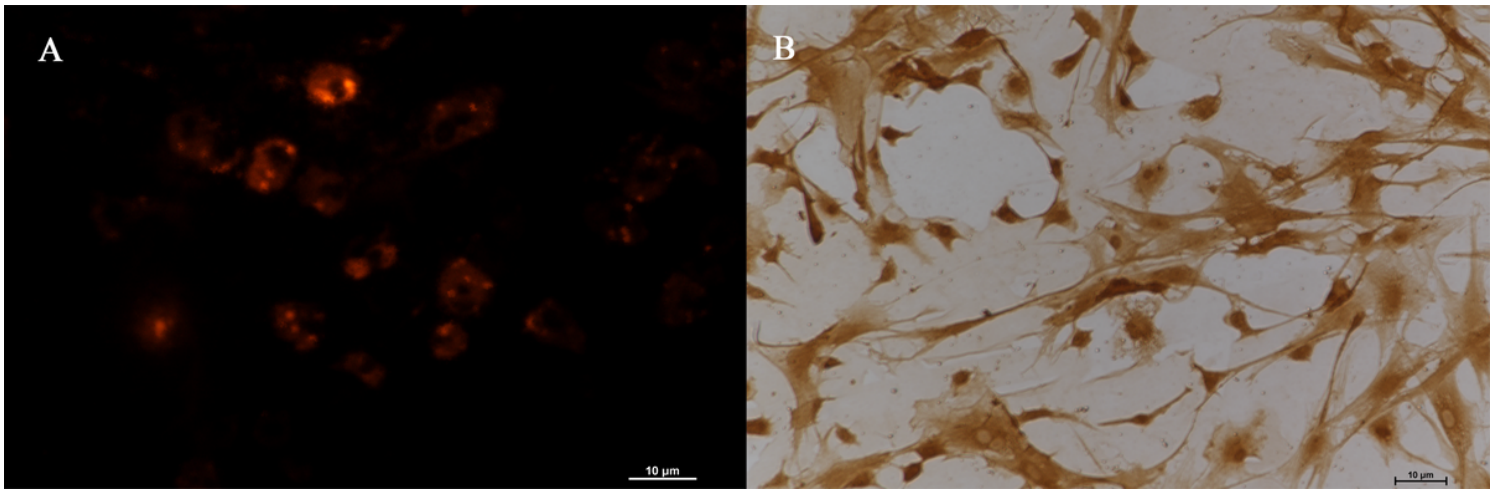

Figure 1

The morphology of choroidal plexus epithelial cells (×200). A) The choroidal epithelial cells were well labeled by CM-Dil, the cytomembrane was emitted strong red fluorescence (Scale bar: 10μm), B) The cells were immune-stained with an antibody against TTR which was specific for choroidal epithelial cells, the cytoplasm was manifested as brown (Scale bar: 10μm).

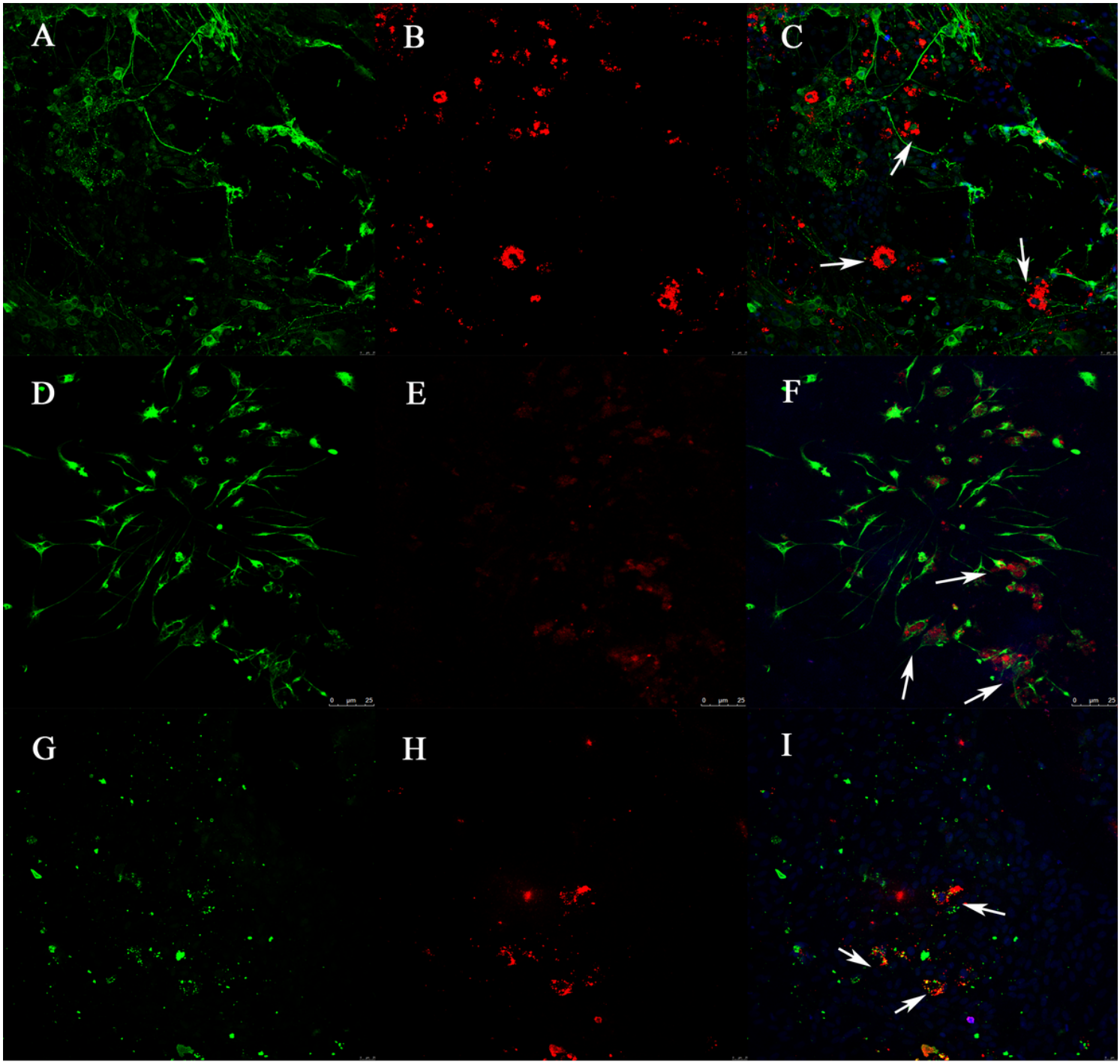

Figure 2

The differentiations of transplanted choroidal plexus epithelial cells and synapses in the transplant new system (×200). A) green: neurons, B) red: choroidal plexus epithelial cells, C) Merged. Part of transplanted choroidal plexus epithelial cells differentiated into neurons (white arrows, Scale bar: 25μm). D) green: astrocytes, E) red: choroidal plexus epithelial cells, F) Merged. Part of transplanted choroidal plexus epithelial cells differentiated into astrocytes (white arrows, Scale bar: 25μm). G) green: Synaptophysin positive vesicles on the OGD organotypic spinal cord slices, H) red: transplanted choroidal plexus epithelial cells, I) Merged. Some of synaptophysin positive vesicles located between the transplanted cells and OGD spinal cord slices (white arrow, Scale bar: 25μm).

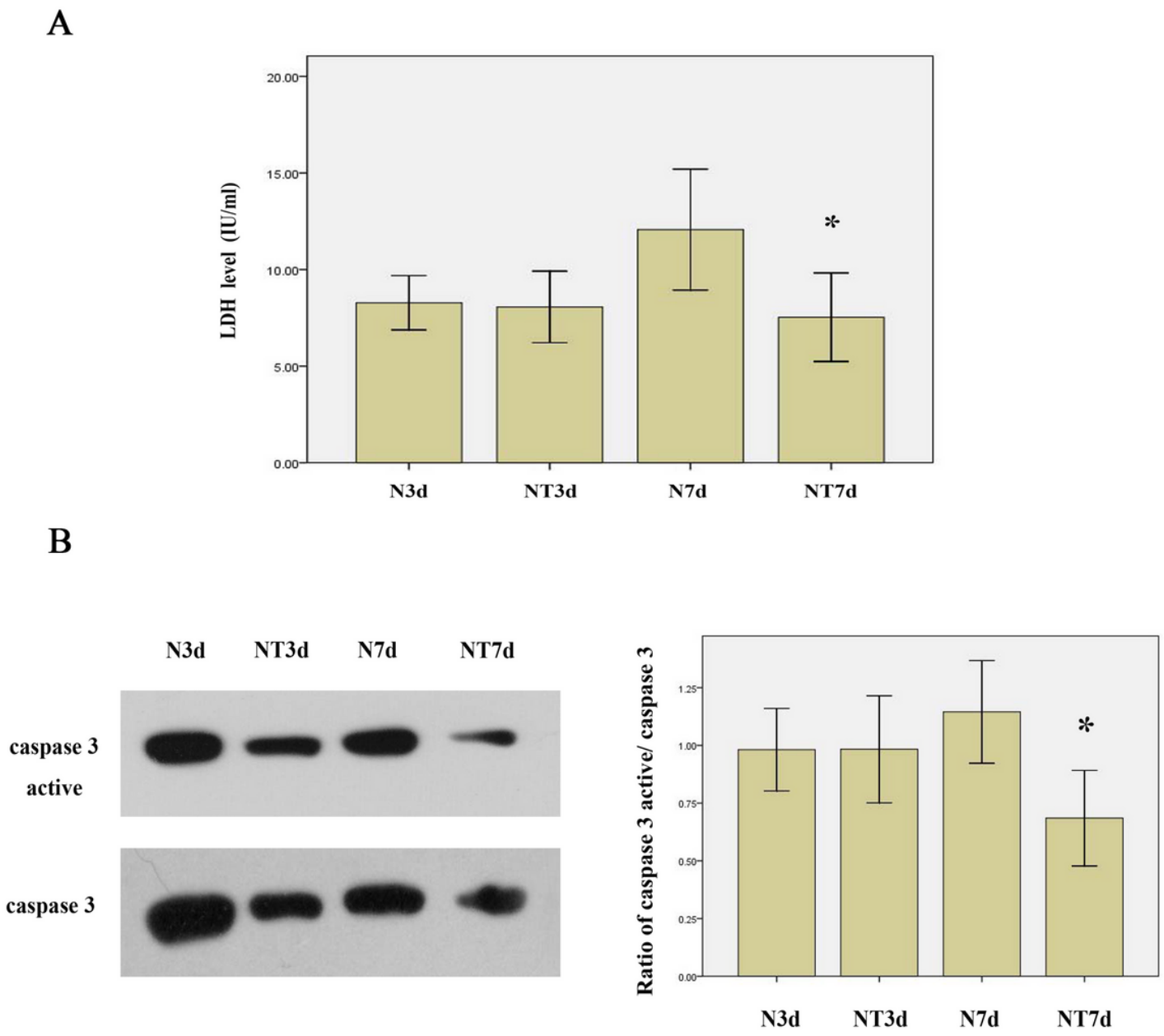

Figure 3

The level of LDH and the ratio of caspase 3 active/ caspase 3. The level of LDH and the ratio of caspase 3 active/ caspase 3 was of no significance between the N 3 d and NT 3 d groups. The level of LDH and the ratio of caspase 3 active/ caspase 3 in the NT 7 d group was significantly lower than which in the N 7 d group. Compare with the N 7 d group, *p <0.05.

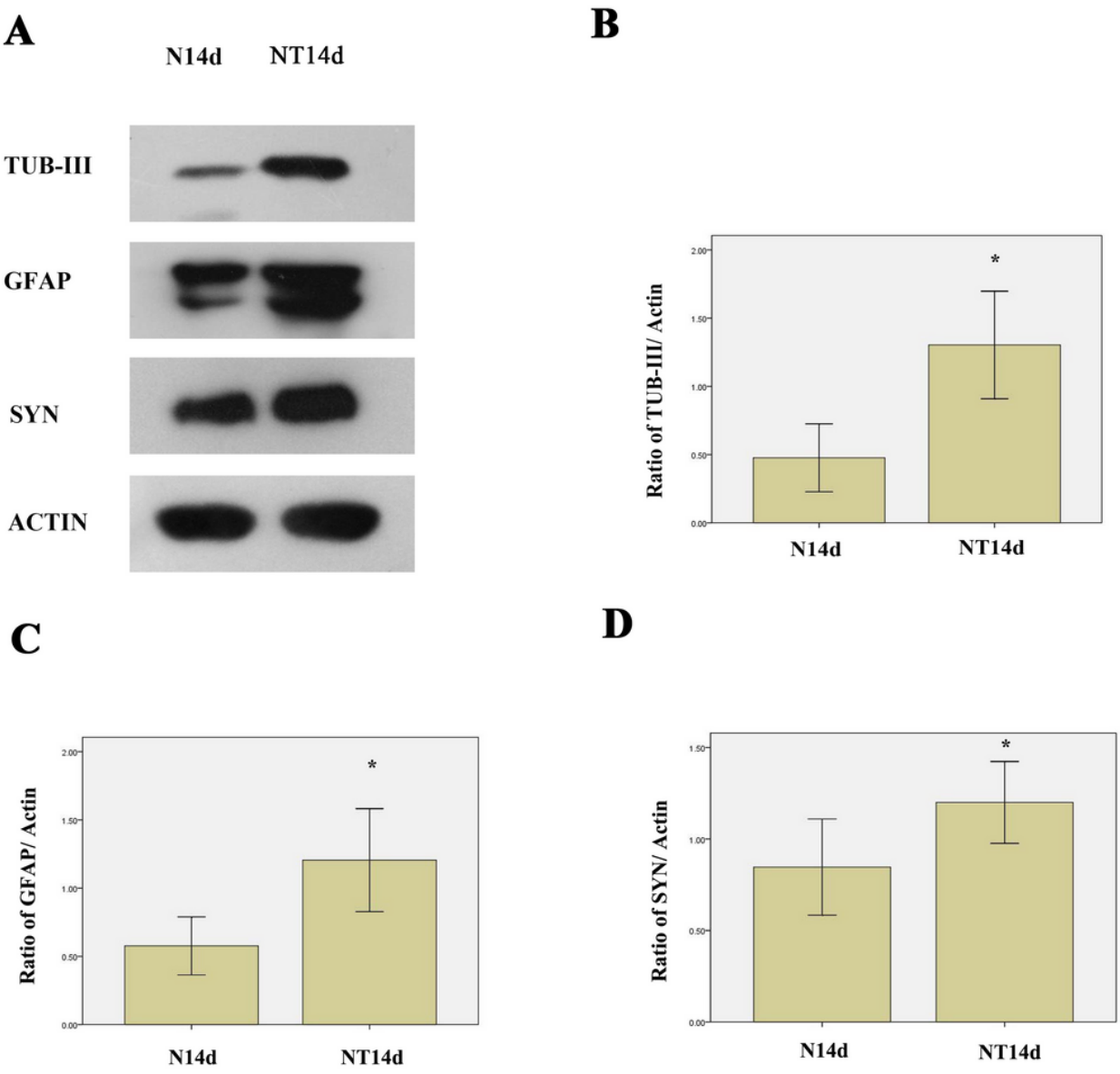

Figure 4

The cells differentiations and synaptogenesis in the new system. The levels of GFAP, TUB-III and synaptophysin in the NT 14 d group were significantly higher than which in the N 14 d group. Compare with the N 14 d group, *p < 0.05.

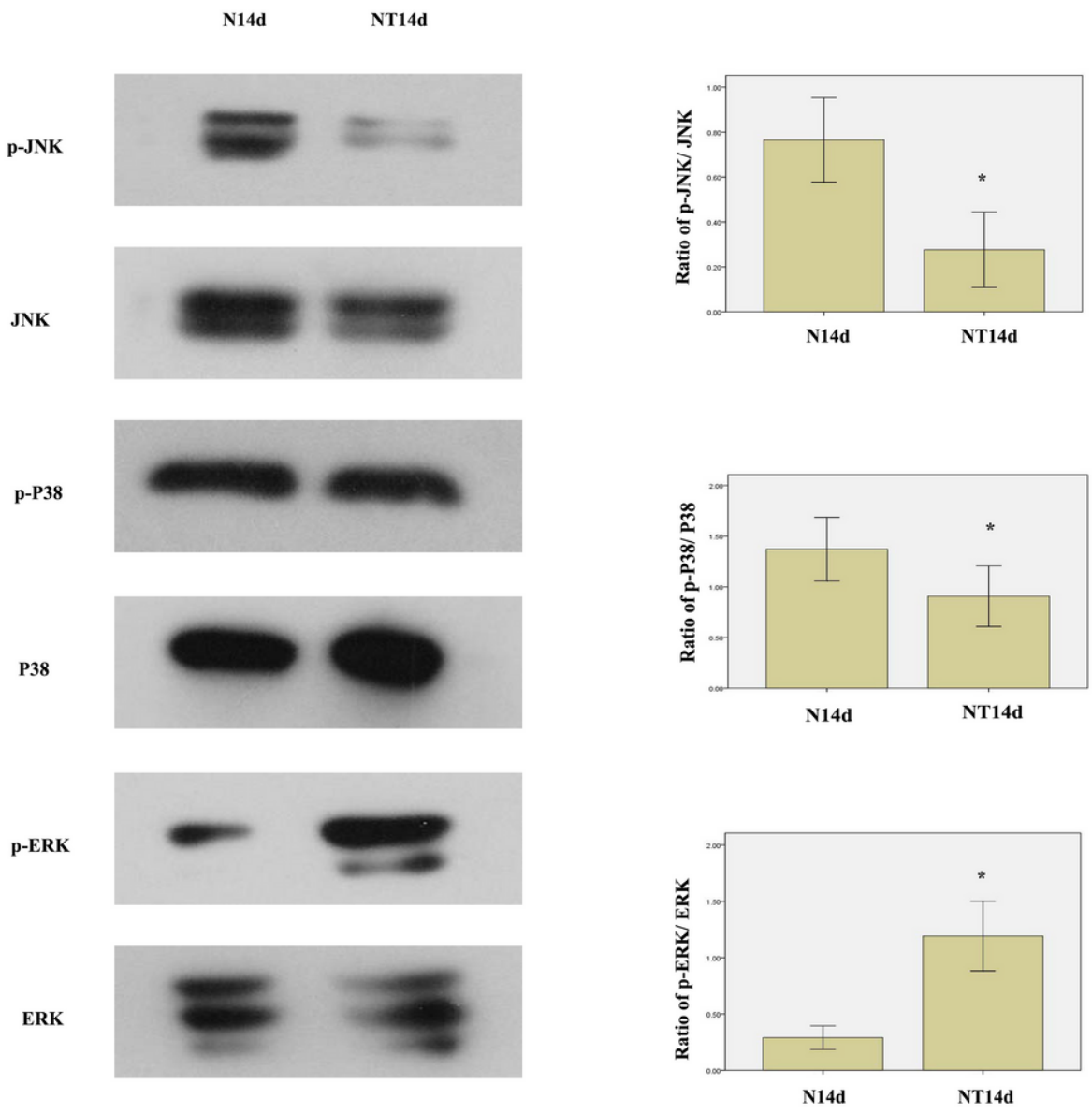

Figure 5

The changes of MAPK signaling pathway in the new system. The ratios of p-JNK/JNK and p-P38/P38 in the NT 14 d group were significantly lower than which in the N 14 d group. The ratio of p-ERK/ ERK in the NT 14 d group were significantly higher than which in the N 14 d group. Compare with the N 14 d group, *$p < 0.05$.