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INVESTIGATIONS INTO THE UTILIZATION OF PLURIPOTENT STEM CELLS FOR EVALUATING NEURODEVELOPMENTAL TOXICITY

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Abstract: Induced pluripotent stem cells are a cell type similar to embryonic stem cells obtained by inducing mature somatic cells to express specific genes [1]. Both iPSCs and ESCs have the ability of unlimited proliferation and multi-lineage differentiation in vitro, and both play important roles in drug screening, cell therapy, etc. However, iPSCs come from a wide range of sources and are easy to obtain, and their acquisition can avoid medical ethical issues. Traditional developmental toxicity research methods not only require a large number of experimental animals, but also have long experimental cycles, cumbersome operations, and species differences. In 1981, Evans and Kaufman [2] first obtained mouse ESCs from the inner cell mass of mouse blastocysts. In 1997, Spielmann et al. [3] established an in vitro alternative model "embryonic stem cell test" by using the characteristics of mouse ESCs to differentiate into the three germ layers, and it became a classic method for in vitro developmental toxicity research. Subsequently, human ESCs and human iPSCs were obtained and used in in vitro surrogate models of developmental toxicity. The application of these human cells solved the problem of species differences [4-6]. The in vitro developmental toxicity model was initially used to evaluate the toxic effects of chemicals through the differentiation of pluripotent stem cells into myocardium. Later, the products of differentiation into other lineages were gradually applied to this model.
Keywords: Stem cells; Nerve; Neurodevelopment; Toxicity model

1 DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO NEURAL LINEAGE

The development process of the nervous system is highly spatiotemporal, and the sensitivity to toxic substances depends on the developmental stage. When in the sensitive stage, even a low level of exposure to toxic substances can cause irreversible brain damage [7]. This kind of brain injury sometimes does not show obvious clinical characteristics, but persistent changes in the nervous system, which is called "silent developmental toxicity" [8]. In addition to the vulnerability of the nervous system itself, humans are also increasingly exposed to developmental neurotoxicity (DNT) substances. According to statistics, from 2006 to 2013, the number of DNT substances increased from 6 to 12 [9]. Therefore, the DNT issue has attracted more and more attention. Currently, the use of pluripotent stem cells to establish in vitro cell models has become an important way to study DNT. Neural lineage cell types differentiated from pluripotent stem cells and DNT assessment methods have become important aspects of this research. This article reviews four aspects: differentiation of pluripotent stem cells into neural lineage, DNT detection indicators and applications, high-throughput technology applications, prospects and challenges.

The use of pluripotent stem cells to establish an in vitro cell model for DNT research relies on the development of technology for the differentiation of pluripotent stem cells into neural lineages. With the development of stem cell culture technology, more and more cell types of pluripotent stem cells have differentiated into the neural lineage. There are two main differentiation methods: adherent method and embryoid body (EB) method.

At present, the neural lineage cell types generated by differentiation of pluripotent stem cells are dopamine neurons, glutamatergic neurons, astrocytes, cochlear spiral ganglion cells, etc. [10-20]. Gradually, the spatial structure has also undergone a transformation from two-dimensional to three-dimensional. For example, Reichman et al. [19] induced human iPSCs to form retinal organs containing ultrastructured photoreceptors; Pamies et al. [21] used iPSCs to differentiate to form a three-dimensional structure similar to the human brain, which is composed of differentiated mature neurons and glial cells. It can simulate synapse formation, neuron spontaneous firing and myelination during neural development, and its myelination can reach 40%. In addition, Schwartz et al. [22] seeded neural progenitor cells differentiated from human ESCs on synthetic polyethylene glycol hydrogel for culture, and added endothelial cells and mesenchymal cells derived from ESCs at a certain proportion during the differentiation process. Mesenchymal stem cells and microglia/macrophage precursors ultimately form a three-dimensional neural structure containing different types of neurons, glial cell populations, and interconnected vascular networks.

In short, using different types of nerve cells generated from pluripotent stem cells is an important basis for in vitro DNT research. Different cell types can also be sensitive to chemicals of different properties. For example, neural progenitor cells are more sensitive to chemicals that induce apoptosis. [23], establishing a specific cell replacement model.

In addition, the three-dimensional neural structure generated by the differentiation of pluripotent stem cells can simulate the interaction between cells and is closer to the neural tissue in the body in structure and function. Application in DNT research can narrow the difference between the cellular level and the in vivo level, further improving Accuracy of DNT assessment.

2 DNT DETECTION INDICATORS AND APPLICATIONS

Neurodevelopmental processes include cell proliferation, apoptosis, differentiation, migration, synapse formation, neurite and network formation, gliogenesis, and myelination. Using pluripotent stem cells to simulate the neural development process and then evaluate the DNT of chemical substances can make up for the shortcomings of traditional experiments. However, how to comprehensively and accurately evaluate the DNT of chemical substances based on cell models is a difficulty in research. At present, the detection indicators for in vitro evaluation of DNT are roughly divided into three categories: nerve cell biological behavior, neuronal function and other indicators.

2.1 Biological Behavior of Nerve Cells

2.1.1 Proliferation and apoptosis

Proliferation and apoptosis occur throughout the entire neural development process. The DNT of chemical substances can be preliminarily evaluated based on the proliferation and apoptosis levels of the cell model. Commonly used related detection methods include cell counting, BrdU staining, CCK8, caspase3/ 7 activity, etc. [23-30].

2.1.2 Differentiation

The differentiation of pluripotent stem cells into neurons with different shapes, structures and functions is essentially the selective expression of genes. Quantitative real-time PCR (qPCR), immunostaining and other methods are usually used to detect the expression levels of specific markers such as glial acidic protein and myelin basic protein to evaluate the impact of toxic substances on the neural differentiation process. Impact [31-32].

2.1.3 Cell migration

Neural crest cell migration is one of the key processes in human fetal development. If this process is interfered with by the external environment or toxic substances, the fetus will develop abnormally. Cell scratch experiments are mainly used in vitro to evaluate the impact of toxic substances on the cell migration process [33-34].

2.1.4 Process growth

Neurons are connected to each other through neurites for information transmission. Ryan et al. [35] used neurons derived from human iPSCs to study and found that some chemicals did not cause cytotoxicity but only selectively inhibited neuronal process growth. It can be seen that protrusion growth can be used as an important indicator to evaluate DNT.

2.1.5 Myelination

The main function of myelin is to provide electrical insulation for axons and accelerate the transmission of electrical signals. Oligodendrocytes are an important cell source of myelin and can be indirectly detected by detecting their precursor cell differentiation, migration and other processes. Evaluate the effects of chemicals on myelination [24, 36-37].

2.2 Neuron Function

Existing research shows that conventional detection methods may not necessarily be able to detect the toxic effects of chemical substances. As the basic functional properties of neurons, electrophysiological properties can be used as functional terminals to evaluate DNT sensitivity. For example, Ylä-Outinen et al. [38] exposed neurons differentiated from human ESCs to 500 nmol/L methylmercury chloride for 72 hours. The electrophysiological signals of the neuronal network were significantly reduced, but cell proliferation and qPCR , immunostaining and other test results did not change.

2.3 Other Indicators

In addition to the above two types of detection indicators, DNT can also be evaluated through other indicators.

2.3.1 Generation of reactive oxygen species (ROS) and oxidative stress

Oxidative stress is an important mechanism by which chemicals cause DNT [39-40]. For example, arsenic, mercury, etc. can cause nerve cells to produce excessive ROS, leading to an imbalance in the oxidation-antioxidation system in the cell, that is, an increase in oxidative stress levels, which in turn can cause mitochondrial dysfunction and cell apoptosis [41-42]. Therefore, the DNT of chemical substances can be evaluated based on intracellular ROS levels, superoxide dismutase and other indicators during the neural differentiation process of pluripotent stem cells [43].

2.3.2 Metabolites

Pamies et al. [43] used non-targeted metabolomic analysis and found that the metabolites of three-dimensional neural structures formed by human iPSCs differentiation differed at different differentiation stages. At the same time, cell

metabolism after treatment with rotenone for 48 hours The product also changed significantly. Therefore, studying the metabolites of cells can help people discover subtle differences from a metabolic perspective and further identify the toxic effects of chemicals.

2.3.3 MicroRNA(miRNA)

miRNA is a small endogenous non-coding RNA molecule that regulates gene expression by targeting and binding to mRNA. At present, it is clear that more than 50% of miRNAs are expressed in the brain and are involved in regulating brain development [44]. Studies have shown that toxic substances can cause the abnormal expression of some miRNAs, and these miRNAs are closely related to the proliferation, migration, and myelination of nerve cells [45].

In short, the neural development process is extremely complex. When using pluripotent stem cells to establish an in vitro cell model to evaluate DNT, it is necessary to combine different detection indicators to make a correct assessment of the DNT of chemical substances. I believe that with the development of science and technology, more sensitive indicators will be Discovered and used in DNT research.

3 DNT HIGH-THROUGHPUT TECHNOLOGY APPLICATIONS

Although using pluripotent stem cells to establish an in vitro surrogate model to evaluate the chemical substance DNT has many advantages over traditional methods, it is also challenging to process and detect a large number of cell samples in different ways. The key to DNT research is to efficiently and accurately predict in vivo results based on in vitro models. Therefore, high-throughput technology is necessary for in vitro DNT research. Currently, there are also some high-throughput technology applications in the use of pluripotent stem cells to study DNT methods.

3.1 High Content Imaging Analysis (HCA) Technology

HCA is an important tool capable of high-throughput imaging and quantitative analysis of image information using multiple models, and can be used for toxicity detection of a large number of chemical substances [46] . For example, a study reported that 80 chemicals were applied to neurons derived from iPSCs for 72 hours, and then HCA technology was used to photograph them and analyze them from four parameters: cell activity, neurite length, neurite number, and branch number of neurons. Image information greatly improves the processing capabilities of image information [35].

3.2 Microfluidic Chip

Microfluidic chip is a micro-platform that integrates cell culture, differentiation, processing and detection and analysis. It can perform two-dimensional and three-dimensional stem cell culture methods, and can be combined with different detection systems to analyze cell samples. Carry out analysis of different indicators [47]. This platform is used in DNT research. It can not only realize the automatic processing of a large number of chemical substances, but also analyze the DNT of chemical substances from different indicators, improving the research efficiency and evaluation accuracy of DNT.

3.3 Microelectrode Array (MEA)

MEA is a non-invasive microelectrode extracellular electrical signal recording technology. It can be used to culture cells for a long time and record the electrical signals generated by different samples in real time. It is a high-throughput detection method for nerve cells. important tool for electrophysiological characterization [48-50].

Although high-throughput technology can detect and analyze a large number of samples to a certain extent, most high-throughput technologies cannot be widely used due to high equipment costs, complex operations, and the need for professional and technical personnel. However, with the advancement of science and technology, it is believed that more and more high-throughput technologies will be further developed and applied, thereby improving the research efficiency and evaluation accuracy of using pluripotent stem cells to evaluate DNT.

4 PROSPECTS AND CHALLENGES

At present, great progress has been made in pluripotent stem cell research. For example, the continuous optimization of pluripotent stem cell culture systems, that is, serum-free and feeder-free culture, has clearer ingredients and is conducive to the realization of standardized production. In addition, the use of pluripotent stem cells to differentiate in vitro to form the diversity of neural cell types, the diversity of DNT detection indicators, and the application of high-throughput detection technology, etc., all help to reduce research costs and shorten the time to a certain extent. DNT research cycle, improve prediction accuracy, etc. However, the use of pluripotent stem cells for DNT research also faces many problems, such as the high cost of stem cell culture, the long differentiation time of neural three-dimensional models, the lack of a standardized evaluation system for neural tissue (or cell) models, and the different exposure methods of chemical substances. (Concentration, action time, etc.), the cell types affected by chemical substances may not be sensitive; the detection system needs to be improved, etc. In addition, although the three-

dimensional model formed by the differentiation of pluripotent stem cells already has preliminary structure and function, there is still a big gap compared with the neural tissue in the human body. Moreover, the current in vitro detection system can only detect the toxic effects of the chemical substances themselves. The toxic effects of the chemical substances in the metabolic process in the body and the composite effects of the placental barrier and the blood-brain barrier in the body cannot be simulated in vitro. This will easily lead to overestimation or Underestimating the DNT of chemicals [51]. In short, although there are still many shortcomings in using pluripotent stem cells to establish cell models in vitro to study DNT, with the maturity and improvement of related technologies, it is believed that pluripotent stem cells, especially iPSCs, will play an irreplaceable role in the future application of DNT in vitro models.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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