WikiLectures:Sandbox

Human Embryonic Stem Cell Differentiation Toward Regional Specific Neural Precursors

Human embryonic stem cells (hESCs) are self-renewing pluripotent cells that have the capacity to differentiate into a wide variety of cell types. This potentiality represents a promising source to overcome many human diseases by providing an unlimited supply of all cell types, including cells with neural characteristics.

Introduction

Human embryonic stem cells (hESCs) have been successfully derived from early preimplantation human embryos and have been shown to have a normal karyotype, express high levels of telomerase activity, and have specific pluripotent intracellular and cell surface markers, and can be propagated for extended periods of time. They are self-renewing pluripotent cells that theoretically have the potential to differentiate into nearly all cell types of the human body. This potentiality represents a promising source to overcome many human diseases by providing an unlimited supply of all cell types, including neural cells and specific subtypes of neural precursors including mature oligodendrocytes, motoneurons, and dopaminergic (DA) cells for future cell-based therapies for neurodegenerative and neurological disorders. The neural differentiating pathway of hESCs can be induced and enhanced under in vitro conditions, and this can be achieved by adding growth factors, growth factor antagonists, and morphogensFile:S1462399405009804sup014.gif. However, the protocol, which includes selection, concentration, and the time point when an exogenous differentiation factor needs to be applied, is a very important issue in targeted differentiation of hESCs and should be considered precisely.

Differentiation of hESCs Toward Oligodendrocyte

Oligodendrocytes are non-neuronal cells located in the white matter and have a vital role in the support and maintenance of the CNS by insulating the axons of the nerve cells. During the process of development, oligodendrocytes originate from the ectodermal germ layer and oligodendrocyte precursor cells (OPCs), which are induced from neuroepithelium. These cells undergo proliferation, migration through the CNS, and finally differentiation toward mature oligodendrocytes. All these processes are exerted by the expression of specific transcription factors and local axonal signals. Oligodendrocytes are very easily identifiable through a number of specific markers. The most important markers of OPCs and oligodendrocytes include NG2, a membrane chondroitin sulfate proteoglycan; platelet-derived growth factor receptor subunit (PDGFR-); galactocerebroside (GalC), the marker for committed oligodendrocytes; myelin basic protein (MBP), the marker of mature myelin; myelin proteolipid protein (PLP), the component of myelin that is expressed on oligodendrocytes and glial precursors; O4, the marker for oligodendrocytes; and finally, oliogodendrocyte lineage genes (OLIG). Oligodendrocyte differentiation factors include ligands that bind the cell surface through nuclear thyroid hormone receptors. It seems that thyroid hormone can induce the expression of RA receptors too. Billon et al. have shown that thyroid hormone receptor -1 mediates normal differentiation and promotes the effect of this hormone on OPCs.

Differentiation of hESCs Toward Spinal Motoneurons

Neurons and glia are derived from the neuroectodermal part of the neural tube during early organogenesis. During development, some morphogens produce a positional code in a concentration gradient manner in different parts of the neural tube (dorsoventral or rostrocaudal) in order to force the cells to differentiate into different neural cells. It has been shown that spinal motoneurons are derived from a single pMN domain during development through the effect of sonic hedgehog (SHH) signaling pathways. In the process of development these motoneurons can acquire different subtypes through a positional identification code in the spinal cord, which in turn is the result of the exposure of different concentrations of SHH and other morphogens and growth factors. Based on these facts, several protocols have been developed combining different morphogens and growth factors in different concentrations in order to obtain spinal cord motoneurons from hESCs.

Differentiation of hESCs Toward DA Neurons

One of the most prominent human neurological disorders is Parkinson’s disease, which is characterized by progressive and selective loss of DA neurons, caused by the insufficient formation and action of dopamine, which is produced in the DA neurons in midbrain substantia nigra. DA neurons play a crucial role in the control of many brain functions, such as voluntary movements and many behavioral processes. These neurons can be identified via the expression of some specific transcription factors, including Engrailed 1 (EN1), PITX3, NURR1, and LMX1b, which are also very important in the development of DA neurons. Differentiation of hESCs toward DA neurons is usually

performed via the formation of EBs. After transfer of EBs from a low attachment plate into a normal adhesion plate, the EBs form neuroepithelial cells that organize into neural tubelike rosettes. After dissociation of neuroepithelial cells and addition of neural differentiation medium, which consists of BDNF, GDNF, AMP, and ascorbic acid (AA), DA differentiation begins 3–4 weeks after the initial treatment of hESCs. The early rosettes differentiate toward late neural tube-like rosettes in the presence of bFGF or FGF8, and after 6 days of exposure to both factors the withdrawal of all morphogens results in the derivation of DA precursors. Cells treated with FGF2 in the early rosette stage form forebrain DA neurons whereas cells treated with FGF8 differentiate toward midbrain DA neurons. The latter treatment results in expression of EN1, OTX2, WNT1, PAX2, and GBX2, which are essential in the patterning of mid–hindbrain junctions.

Differentiation of hESCs Toward Other Neural Cells

- Astrocyte (http://en.wikipedia.org/wiki/Astrocyte)
- Peripheral nervous system (http://en.wikipedia.org/wiki/Peripheral_nervous_system)
- Progenitor cell (http://en.wikipedia.org/wiki/Progenitor_cell)

References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.
2. Buzzard JJ, Gough NM, Crook JM et al. Karyotype of human ES cells during extended culture. Nat Biotechnol 2004;22:381–382.
3. Heins N, Englund MC, Sjöblom C et al. Derivation, characterization, and differentiation of human embryonic stem cells. STEM CELLS 2004;22:367–376.
4. Reubinoff BE, Pera MF, Fong CY et al. Embryonic stem cell lines from human blastocysts: Somatic differentiation in vitro. Nat Biotechnol 2000;18:399–404.
5. Carpenter MK, Inokuma MS, Denham J et al. Enrichment of neurons and neural precursors from human embryonic stem cells. Exp Neurol 2001;172:383–397.
6. Trounson A. The production and directed differentiation of human embryonic stem cells. Endocr Rev 2006;27:208 –219.
7. Polito A, Reynolds R. NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. J Anat 2005;207:707–716.
8. McKinnon RD, Waldron S, Kiel ME. PDGF alpha-receptor signal strength controls an RTK rheostat that integrates phosphoinositol 3�-kinase and phospholipase Cgamma pathways during oligodendrocyte maturation. J Neurosci 2005;25:3499 –3508.
9. Ivkovic S, Canoll P, Goldman JE. Constitutive EGFR signaling in oligodendrocyte progenitors leads to diffuse hyperplasia in postnatal white matter. J Neurosci 2008;28:914 –922.
10. Erceg S, Lainez S, Ronaghi M et al. Differentiation of human embryonic stem cells to regional specific neural precursors in chemically defined medium conditions. PloS ONE 2008;3:e2122.
11. Kang SM, Cho MS, Seo H et al. Efficient induction of oligodendrocytes from human embryonic stem cells. STEM CELLS 2007;25:419–424.
12. Nistor GI, Totoiu MO, Haque N et al. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 2005;49:385–396.
13. Izrael M, Zhang P, Kaufman R et al. Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. Mol Cell Neurosci 2007;34:310–323.
14. Li XJ, Hu BY, Jones SA et al. Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. STEM CELLS 2008;26:886–893.
15. Cho MS, Lee YE, Kim JY et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci U S A 2008;105:3392–3397.
16. Elkabetz Y, Panagiotakos G, Al Shamy G et al. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. Genes Dev 2008;22:152–165.