

Gene correction applications in cell replacement therapy and use in neurodegenerative diseases

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ABSTRACT

Induced pluripotent stem cells offer opportunities for personalized cell replacement therapy due to unlimited self renewal potential and ability to differentiate into different somatic cells. Autologous cell replacement therapy gives opportunity for therapeutic applications with uses of induced pluripotent stem cell technology. As known autologous induced pluripotent stem cells are genetically identical to donor cells. Therefore these cells and their cellular derivatives are not expected to be immunologically rejected. Since 90s, a lot of new technologies have been evolved in molecular biology and genetics. By using these technologies, so many genetic variations have been identified which significantly increase the risks and causes of neurodegenerative diseases in patients. An induced pluripotent stem cell obtained from a fully differentiated cell of a patient carries same mutations in genome which causes disease. Unfortunately, these mutations reduce the dedifferentiation ratio in obtaining induced pluripotent stem cells in laboratory conditions. Also, these mutations affect on induced pluripotent stem cell viability and growth. So, gene correction studies are needed before induced pluripotent stem cell technology for having optimal results in cell replacement therapy. Here in this review, new approaches in gene correction studies including homologous recombination technique were mentioned. The role of direct transdifferentiation technique was explained in obtaining a cell which can be used in replacement therapy successfully. New cell replacement therapy applications were defined especially in neurodegenerative diseases. It seems that, these advanced technologies will be applied widely in permanent therapy of neurodegenerative diseases in recent future.

Cell replacement therapy by using induced pluripotent stem cells; Cell replacement therapy (CRT) is the transfer of healthy cell groups from different human sources (allogeneic transplantation) or from patient himself (autologous transplantation), instead of functionally impaired cells due to disease. For many years, allogeneic cell lines have been used for CRT. In last decade, the allogeneic cell transplantation has left their place to autologous cell transplantation due to problems such as tissue rejection and immune response. The differentiation of stem cell like cells in the same individual from a fully differentiated cell has been enabled by induced pluripotent stem cell (iPSC) technology developed since 2006. In this technology, a fully differentiated cell converts into a dedifferentiated cell type (iPSC) with the transfer of certain genes (Oct3/4, Sox2, Klf4, c-Myc / Nanog genes). These genes have gene control roles especially in embryonic period in genome and act as transcription factors (1, 2). iPSCs have pluripotent ability as seen as in other pluripotent stem cells (eg. Embryonic stem cells-ESC). These pluripotent stem cells can be differentiated into all types of cells present in the original organism in vivo condition. All these steps can be named as reprogramming of a

cell. Reprogramming protocols give chance to obtain desired cell type. In recent articles, GABAergic neurons and Glia cells which can be use in neurodegenerative disease treatment were obtained (3, 4). iPSC technology is expected to be applicable in the treatment of diseases, including neurological disorders, hematological abnormalities, spinal cord injury, heart disease, diabetes, and arthritis (1, 5). Several groups had been generated different iPSCs derived from patients such as amyotrophic lateral sclerosis, familial dysautonomia, spinal muscular atrophy, adenosine deaminase deficiency-related severe combined immunodeficiency (6, 7). In CRT, autologous iPSCs were used in amyotrophic lateral sclerosis and adenosine deaminase deficiency-related severe combined immunodeficiency patients. Preliminary results were hopeful obtained in clinical trials. Autologous transplantation needs less immunosuppressive therapy. Clinical applications have no ethical problems (8).

Neurodegenerative diseases; Neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis are characterized by loss of neurons and/or neuronal functions. The diseases cause severe physical and cognitive disabilities in patients. Dif-

ferent factors are accused of in progression of neurodegenerative diseases. Many of these diseases are genetic. Sometimes the cause is a medical condition such as alcoholism, a tumor, or a stroke. Other causes may include toxins, chemicals, and viruses. In patient's pathology, the excessive accumulation and aggregation of proteins, which cause neuronal dysfunction and neurotoxicity, are important in neurodegeneration. Accumulation of an aggregate-prone neuro-toxic protein is common in all forms of neurodegenerative diseases. Treatment protocols in these cases are generally symptomatic. Permanent treatment conditions are not available now (9, 10).

Among the neurodegenerative diseases, Alzheimer's disease is the most common disease. It was characterized by neuropathological findings due to intracellular neurofibrillary tangles and extracellular amyloid plaques accumulating in certain parts of the brain (10). Parkinson's disease is the second most common neurodegenerative disease in the world. In Parkinson's disease, patients are particularly restricted in body movement. Neurons of the substantia nigra progressively degenerate; as a result, the amount of dopamine available for neurotransmission in the corpus striatum is lowered. Dopaminergic neurons in substantia nigra in midbrain region are the main source of dopamine in the mammalian central nervous system. Their loss is directly associated with Parkinson's disease (11). CRT protocols are mostly being used in Parkinson's disease because of the characteristics of disease. As known, only one type neuron is degenerated (dopaminergic type neuron) and pathology occurs only in midbrain region. The scientists believe that cure totally in Parkinson's disease will be possible with dopaminergic neuron replacement therapy in midbrain region (12, 13).

In 90s', stem cells obtained from a healthy one was differentiated into dopaminergic neurons (redifferentiation). Allogeneic dopaminergic neurons had been used in CRT in Parkinson's disease but the patients had the tissue and cell rejection problems (5). In the last decade, new therapy approaches are improved in cell differentiation and gene correction. iPSCs give opportunity to have pluripotent type stem cells which can be generated directly from adult cells. Gene correction is a technology that gives us the tools for both repairing and mutating DNA, for discovering gene functions and for engineering new genetic variants. By using these two technologies together, a healthy pluripotent stem cell was obtained from a patient. Genetic mutations and/or DNA polymorphisms may cause the disease and/or maybe the reason of genetic predisposition. Genetic alterations cause stress on the reprogramming cells, and force them to the apoptosis. Mutations in a cell decrease obtaining a permanent iPSC that desire to use in iPSC technology. Gene correction studies in cell reprogramming are important for having healthy iPSC (5, 14, 15). On the other hand, new findings also give opportunities which can be used in treatment of neurodegenerative diseases. In a recent manuscript, α -synuclein protein was explained as an initiator factor in Parkinson's disease. As a targeted therapy, a molecule specific for the α -synuclein protein and/or mRNA may inhibit the apoptosis and the degeneration in neurons in Parkinson's disease (16). Such kinds of molecular therapies may be used in combined with CRP in Parkinson's disease (17).

Gene correction by using homologous recombination; In neurodegenerative diseases, mutations and/or polymorphisms are important in disease susceptibility and progression (18). Mutant cells obtained from patients (eg. Huntington's disease,

spinal musculoskeletal atrophy) inhibit cell differentiation and reduce the occurrence of iPSCs (19). Targeted gene correction techniques are used widely in laboratories named as restriction enzyme-mediated integration (REMI); Agrobacterium-mediated transformation (AMT); transposon-arrayed gene knockout (TAGKO); gene targeting technology, mainly about homologous recombination; modern gene editing strategies containing transcription activator-like effector nucleases (TALENs) and a clustered regularly interspaced short palindromic repeat/associated protein system technology (CRISPR/Cas) (19, 20, 21). Among them homologous recombination (site specific gene correction) and CRISPR/Cas techniques are commonly used together. In homologous recombination technology, a normal copy of a mutated gene was directed to a cell by a viral vector to obtain a stable copy of that gene. Normal copy of this gene acts as a template in DNA repairing process. The both allele of mutated gene are destroyed by using site specific restriction enzymes (direct reprogramming). DNA repair enzymes use the normal transferred copy in repair procedure and correct the mutated site (site directed gene correction) (19, 20). Gene correction studies are made especially in ESC phase, in single-gene related diseases such as sickle cell anemia and Thalassemia. Gene correction studies give chances to have genetically modified (engineered) animals which have desired type disease. Mutant zygotes obtained from genetically modified mouse were purified from mutations by using site directed gene correction techniques. The gene corrected zygote was transferred to uterus of a pregnant mouse to have a healthy mouse. Offspring obtained in this technology resembled no disease symptoms (22, 23). This technology is currently being used for having GABAergic neurons which can be used in treatment of Huntington's disease (24).

Direct reprogramming by using transdifferentiation technology; CRT by using transdifferentiation technology is also known as direct reprogramming. Cell is differentiated into a desired cell type by using transcription factors from one lineage to another without using iPSC stage in this technology (25, 26). As known, terminally differentiated cell types have non-dividing structure which maintains long-term stability. Knowing the appropriate transcriptional factors which have role on transdifferentiation step is the key process on that technology. A number of myogenic type cells were obtained by expression of "myogenic factor MyoD" in the literature in 1989. In Myo D expressed conditions, muscle-specific genes were activated in pigment, nerve, fat, liver, and fibroblast cell lines. As far as we know this is the first application of a known transcription factor which derives a cell type lineage to other cell type lineage (27). Neurons and cardiomyocytes were obtained from fibroblasts by using specific transcription factors in literature (28, 29). The cell skips the pluripotent stage and does not develop a unique teratoma in direct reprogramming. As known, teratoma is a specific condition only observed in pluripotent stem cell differentiation in normal condition. Direct reprogramming technology derives the cells to differentiation in a shorter time. This process is cheaper and more effective than iPSC technology (30). Transdifferentiated lineage specific cells (Transdifferentiation-mediated lineage-specific cells) can be inhibited by disease-specific genes. It has been shown in induced neural stem cells (iNSCs) in brain tissues of Parkinson's patients that carry the pathological LRRK2 (G2019S) mutation. Compared to transdifferentiation results in mutated and non-mutated cells, it has been observed that mutated neurons became apoptotic due to the damage in internal nuclear membrane. In this mechanism, disruption of

the integrity of the inner membrane occurs when the mutant LRRK2 (G2019S) protein inhibits the nuclear lamin B1 protein, which plays a critical role in nuclear membrane integrity (31). At the beginning correction of a mutation in a cell and then transdifferentiate this cell into other lineage are essential steps in a perfect CRT (32). So, having a desired cell after gene correction and direct reprogramming is essential in CRT in neurodegenerative diseases. Such kinds of studies will be provided important benefits in treatments of common diseases such as Alzheimer's disease, Parkinson's disease and rare diseases such as Rett's syndrome, Huntington's disease (33).

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Conflict of Interest

The author did not declare anything to disclose regarding conflict of interest with respect to this manuscript.

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