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Narrative Review

Transfection of Exogenous Fibroblast Growth Factor (FGF) on Human Embryonic Stem Cells (ESCs) as a Regenerative Agent for Patients with Type 1 Diabetes Mellitus (T1DM)

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Abstract

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disorder which results in the INS pancreatic β-cell destruction and contributes to around 5 to 10% of all diabetes mellitus cases, especially in children. At the moment, the only treatment for T1DM is by insulin injection, which is injected in order to prevent the complication of T1DM. However, several studies have shown that combination between Embryonic Stem Cells (ESCs) and the Fibroblast Growth Factor 2 (FGF-2) may be a successful modality to treat T1DM. Actually, the ESCs may become the potential therapy in treating T1DM.

Keywords: Type 1 Diabetes Mellitus, Embryonic Stem Cells, Fibroblast Growth Factor-2.

Introduction

Type 1 Diabetes Mellitus (T1DM) and type 2 Diabetes Mellitus (T2DM) are conditions that have always been precipitated with high blood glucose levels (hyperglycemia).[1] The T1DM itself is a condition which is related to T-cell mediated autoimmune disease which results in the destruction of insulin-producing pancreatic β-cells.[2] Majority of the cells which contribute in the destruction are CD8+, CD4+, and other cytokines, for examples are IL-2 and TNF-α.[3] In general, there are around 5-10% people with T1DM comprised of all diabetes mellitus cases worldwide.[4-6] The mortality rate associated with T1DM also differ from each country, however it shows that there is an excess mortality of T1DM.[7]

At the moment, the main choice of treatment is insulin administration.[8] For the reason of mimicking the effect of healthy pancreatic β-cells physiology, dosage of the administered insulin should be done correctly.[8,9] Pancreas transplants have been done in order to restore the production of insulin. Besides that, long term effect of relapse and immunosuppressive agent are the two main issues in pancreatic transplantation.[10,11]

Nowadays, the trend of successful stem cell-derived therapy is being focused on. As the success continues, these cells may replace the β-cells replacement therapy. The combination between Embryonic Stem Cells (ESC) and Fibroblast Growth Factor (FGF) take place in an important contribution that may result in complete curative treatment for T1DM. The transcription of ESC using this exogenous FGF may promote greater chance to succeed the differentiation of ESC. This can help to enable the prospective differentiation of the cells to work functionally.[12,13] Recently, some studies have shown that the combination of exogenous FGF which is related to human ESCs may facilitate the success of β-cells replacement in mice.[14]

The rapid proliferation of the ESCs shows much greater progress than the adult stem cell, however it also carries bigger risks in forming tumors. The isolation of ESCs may induce the functional INS cell that may replace the role of normal β-cells.[14] Including thermo stabilized FGF combined with the small and large molecules can support both differentiation and reprogramming protocols. This may lead to the successful ESCs therapy in children with T1DM.[12,15]

In this review, the author aims to discuss the efficient and therapeutic potential of the combination FGF with ESC and each role related with T1DM condition.

The Pathogenesis of Type 1 Diabetes (T1DM)

The T1DM is a disorder that emerges subsequent to the autoimmune response resulting in the INS pancreatic β-cell destruction. Genetic is one of the factors which may strongly influence the inheritance of this disease. Despite the

hypothesis, the genetic factor does not fit to any pattern of inheritance explanation.[16] Meta-analyses and genome-wide association studies show that T1DM has more than 50 genetic risk loci.[17] Some major genes predisposing T1DM are in the Major Histocompatibility Complex (MHC) region, the Human Leucocyte Antigen (HLA) on the chromosome 6p21 which is usually also termed as Insulin-Dependent Diabetes Mellitus Locus (IDDM1) which is seriously susceptible for autoimmune diseases, including T1DM.[4,16,18]

Insulin genes that are susceptible of T1DM and are related to Variable Numbers of Tandem Repeat (VNTR).[16-18] Other genetic risks associated with the progression of T1DM, such as polymorphism of cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) gene is located on the chromosome 2 in the IDDM12 region.[19,20]

It is believed that an environmental factor may trigger that condition in the early life. From the epidemiological observation, it is proven that viruses represent the most extensive effect.[3,17,21] Another mechanism is called the molecular mimicry, which may trigger the activation of T-cells that aggressively attack the virus that brings the epitope that may replicate the form of β-cells which is similar to GAD.[4,21] Not only does the virus get destroyed, but the pancreatic β-cells are also destroyed.[21] Some studies also indicate that breastfeeding may provide protection against T1DM.[1,3,4,17,22]

Immunological factors also play a role in the progression of T1DM. It actually happens when the T-cells turn to aggressively self-reactive for many tissues, as well as pancreatic β-cells. This immunological factor may happen in both cellular or humoral immunity.[23] In the microenvironment of islet cells, the autoreactive T-cells produce many cytokines that may cause inflammation such as, IL-1, TNF-α (tumor necrosis factor-α) and INF-γ (interferon-γ). The humoral immunity relates with the formation of autoantibodies that may work contrary with GAD65, tyrosyl phosphatase (IA-2), insulin (IAA) and zinc transporter (ZnT8).[4,23]

Overview of Fibroblasts Growth Factor (FGF)

The FGF is known as a protein that is capable to comprise 22 members. The FGF itself has been found in vertebrates and in vertebrates.[24] The FGFs works by binding and activating the Fibroblast Growth Factor Receptors (FGFRs). The FGFRs phosphorylation triggers the activation of downstream cytoplasmic signal transduction called the RAS/MAP kinase pathway.[24-26] The strong interaction between FGFs with Heparan Sulfate Proteoglycans (HSPGs), which contributes in stabilizing FGFs and preventing the thermal denaturation and proteolysis.[24]

Overview of Human Embryonic Stem Cells (ESCs)

Undifferentiated stem cells that may have self-renewal ability and differentiate to any cells are Human Embryonic Stem Cells (ESCs).[27] The ESCs are derived from the embryonic stage 5-6 days after fertilization which is called blastocyst. To get the functional ESCs that may differentiate to any kind of cells, the two parts of the cells must be separated, both the inner and outer layer of the cells.[28] Several assays in vitro and in vivo confirmed the undifferentiated state of ESCs and their development potential to differentiate into any cells.[29]

The pluripotent ESCs have a tremendous potential in dealing with degenerative diseases, which might likely be applied in human beings following the successful transplanting ESCs derivatives to animal models.[30] The differentiated potential to any cells including three germ layers show how promising the ESCs therapy is. By maintaining the right specific culture conditions, ESCs can be transformed into hepatocytes, retinal ganglion cells, chondrocytes, pancreatic progenitor cells, cone cells, cardiomyocytes, pacemaker cells, eggs, and sperms that can be used in the regenerative medicine.[31]

Reconstruction and Administration of Fibroblast Growth Factor (FGF) Combination with Human Embryonic Stem Cells (ESCs)

Pluripotent Stem Cell Derived from Isolated Inner Cell Mass of Human Cells

The ESCs lines the pluripotent precursor of the three embryonic germ layers which can be isolated by using immunosurgery or mechanical methods.[27,32,33] The ESCs lines derived directly from the Inner Cell Mass (ICM). The ICM derived from the human blastocysts which form 5-6 days after fertilization although ESCs lines may also use morula-stage embryos or late stage blastocysts.[28,33] The successful derivation of ESCs itself is highly distinctive, mostly because of the difference in embryo quality and the culture protocols.[33]

To have a successful ESCs derivatives, the isolation of the ICM from blastocysts is the most important step. The majority of all successful ESCs lines are isolated by immunosurgery, which means removing the outer trophectoderm epithelial cell layer from the blastocyst using anti-human whole-serum antibodies and guinea pig complement.[33] The isolated ICM will be placed on the specific substrate and cultured in suitable media. New ESCs lines will be clonally derived from the existing ESCs lines, for the reason of low clonal efficiency, low oxygen level may be considered in the process.[33] Some protocols may also be used to maximize the process.[34] The next step is to do the passaging of ESCs, which can occur by using two pathways between the mechanical or enzymatic.[33,34] The most reliable method for

passaging pluripotent stem cells cultures has been the manual dissection of the colonies.[34] Recent studies have appeared to shown promising results by adding additional steps in its protocols, such as cryopreservation and propagation.[33,34] Some studies have also directly conducted the bFGF to the wound sites to promote regeneration and angiogenesis.[24]

Mechanism of Action and Transfection of bFGF/FGF-2 on ESCs Improves Survival and Efficacy

The FGF-2 gene was firstly knocked down using small hairpin RNA (shRNA) before transfected into the ESCs using Lipofectamine 2000.[13] Following the transfection, ESCs was labeled with 4'6-diamidino-2-phenylindole (DAPI) to detect the expression of FGF-2.[13,35] The examinations were counted on the third, fifth, and the seventh day after the plating. All ESCs were plated in Unconditioned Medium (UM) or Conditioned Medium (CM). To administer bFGF, CM and UM samples were supplied with 4, 24, 40, 80, 100 or 250 ng/ml FGF-2.[13]

Some failures occurred in three plates of UM 4, UM 24, and UM 40 which may not support the ESCs culture. The CM, UM 100, UM 250, and UM 80 showed that capable in helping sustainable of ESCs growth although UM 80 was the least capable in doing so. High pluripotency markers are shown by the cultured of both CM and UM 100.[13] In the early embryonic development of ESCs, FGF-2 is the major signaling member expressed. Adding exogenous FGF-2 is needed to sustain the pluripotency. The MAPK pathway is one of some pathways that may contribute in the self-renewal ability in ESCs. This pathway can positively and negatively regulate the condition of the ESCs. Some studies showed high level activation of MAPK pathway in undifferentiated states of pluripotent stem cells. This pathway was also activated by FGF-2 following the diminutive MAPK pathway following the FGF-2 withdrawal.[35] Related to the PI3K/AKT pathway, which promotes activin A and represent strongly survival pathway in pluripotent stem cell is not significantly affected by the low level of FGF-2.[35,36] This is while p38 MAPK and JNK (c-Jun N-terminal kinase), which control distinctive responses to extracellular stress and mitogenic stimuli can be activated by FGF-2.[35] By getting through these pathways, FGF-2 may help develop β-cells that is functionally active and may support the formation of islet-like cluster from ESCs.[37] The FGF treatment may also provide the expression of endocrine progenitor marker *NEUROG3* in the pancreas, by inducing pancreatic progenitor cells. The ESCs may differentiate into the INS function with some additional protein.[38,39] The FGF-2 also increased the expression of gastrin, which is strongly related to peptide hormone that can be found during the embryonic phase.[38]

Beneficial and Limitation Analysis on ESCs-FGF-2 Combination Inducing β-cells

The FGF-2 is biologically important in improving the regeneration of a cell, cell proliferation, migration, and differentiation. The FGF-2 may promote the proliferation of fibroblast or basically may support wound healing. Additionally, it may help the angiogenesis process where usually found impair in wounded tissue, FGF-1 and FGF-2 are more potent angiogenic factor than vascular endothelial growth factor (VEGF). The FGF-2 also provides and improves cell survival through RAS/MAP kinase pathway and PI3 Kinase/AKT pathway, so that it may support the embryonic development of β-cells.[24] The biological properties of FGF-2 may enhance the formation of islet-like clusters from ESCs which may also differentiate into all three germ layers. Despite those benefits, there are also some limitations towards the complete function of the β-cells. There is still some discussion about the ethical dilemmas, possible immune rejection, possible leading to carcinoma, and genetic instability.[40] The FGF-2 is also thermally unstable. This is why in order to maintain the pluripotency of stem cells, a high level of FGF protein is required.[15] Until now, the latest information regards using these combinations have only been seen in vitro studies detecting the early embryonic development, noticing some pathway mechanisms.

Conclusion

It can be concluded that the ESCs and FGF-2 combination are considered as one of the mechanisms that may become the potential modality in treating T1DM. This combination may overcome the unfinished T1DM therapy. The mechanism of action in this modality includes the ability of FGF-2 to induce the expression of pancreatic progenitor cells through several pathways. Those pathways give significant effect to the regeneration and survival of the ESCs, including proliferation, migration and differentiation. It results on the point where FGF-2 protein may become the choice in completing the ESCs potential therapy for patients with T1DM in the future.

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Authors' Contribution

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