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Stem Cell Research 2018-Umbilical Cord Cells Treatment with Metadichol IRS Proteins and GLUT4 Expression and Implications for Diabetes- Palayakotai R Raghavan- Nanorx Inc

Palayakotai R Raghavan
Nanorx Inc., USA

Introduction

The increased incidence of Diabetes Mellitus (DM) worldwide reinforces the search for new approaches to prevent the progression of diabetes and its complications. Stem cells are the next frontier in medicine. Stem cells are thought to have significant therapeutic and biotechnological potential. Stem cell therapy will not only replace damaged or dysfunctional cells but deliver therapeutic proteins after they have been engineered to do so. This field is paving the way for novel therapeutic interventions through cellular therapies and tissue engineering approaches that are reshaping the biomedical field. The remarkable flexibility of different cell subsets obtained from human embryonic and adult tissues from sources like bone marrow, UC useful in evaluating these cells in the treatment of diabetes and its complications and hold great promise for pancreatic beta cell replacement therapy for diabetes. Umbilical Cord Blood (UBC) contains Mesenchymal Stem Cells (MSCs) that are precursors of certain types of cells (e.g., bone, cartilage, fat or muscle).MSCs are multipotent stromal cells that can differentiate into a variety of cell types which have been shown to improve metabolic control in experimental models of Type 2 Diabetes (T2D). A recently published study by Si showed that infusion of autologous MSCs managed to improve hyperglycemia in T2D rats. Beneficial effects of MSC therapy resulted in enhanced insulin sensitivity via increased signaling of IRS1. Also, AKT (Protein kinase B PKB) phosphorylation leads to translocation of GLUT4 or also known as SLC2A4 (glucose transporter type 4) on cell membrane upon insulin administration in the muscle, liver, and adipose tissue of MSC treated animals. There are several problems that limit the use of MSCs for diabetes therapy. Mottaghi showed that poor engraftment and limited differentiation under in vivo conditions are major obstacles for efficient therapeutic use of MSCs. Hyperglycemia leads to reactive oxygen species overproduction which triggers apoptosis and thereby decreases MSC viability after transplantation. Another large accessible source of adult stem cells is Peripheral Blood Mononuclear Cells (PBMCs). It can be frozen and stored for later use. PBMCs contain many different progenitor cell types and are expandable as they can be cultured and reprogrammed to produce Induced Pluripotent Stem Cells (iPSCs) for clinical applications in regenerative medicine. Blood glucose concentration is tightly regulated in humans. GLUT4 is what maintains whole-body glucose homeostasis. Both insulin and exercise acutely stimulate GLUT4 recruitment to the cell surfaces of muscle and adipose cells independent of transcription or translation. Insulin and IGF signaling requires a family of IRS proteins to integrate extracellular signals into intracellular responses, leading to cellular effects. There are two main IRS proteins in humans, IRS1 and IRS2, that are widely expressed in most human and mammalian tissues. IRS1 and IRS2 mediate the control of various cellular processes by insulin. Insulin binding to the α-subunit of the insulin receptor results in the phosphorylation of IRS1 and IRS2 which leads, via several intermediary steps, to activation of AKT (protein kinase B). Insulin Resistance (IR) can result from deficits in any part of the insulin signaling pathway resulting in inadequate response to insulin.

Treatment for analysis of IRS1, IRS2 and GLUT4 gene expression by semi quantitative RT and RT PCR

The cells were aspirated from the 80% confluence culture flask and centrifuged at 1500 rpm for 5 mins. The cell pellet was then resuspended in 1 mL of complete media and 1 × 106 cells/dish was seeded to each well of the 96 well microtiter plates. After 24 hrs incubation, cells were treated with Metadichol at various concentrations followed by 48 hrs incubation. Post incubation, the cells are harvested for RNA isolation.

RNA isolation and sample preparation

UC cells were washed twice with PBS and to the adherent cells 2 mL of TRIzol (per T25 flask) was

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added and transferred to the tube and vortexed. Samples were allowed to stand for 5 mins at room temperature. Added 0.2 mL of chloroform per 1 mL of TRIzol used. Closed the tube and shaken vigorously for 15 seconds. The tube was allowed to stand at room temperature for 5 mins. The resulting mixture was centrifuged at 10,000 rpm for 15 mins at 4°C. The colourless upper aqueous phase was transferred to a new clean tube. 0.5 mL of isopropanol was added per 1 mL of TRIzol used, mixed gently by inverting the sample 5 times and incubated at room temperature for 5 mins. Then it was centrifuged at 10,000 rpm for 10 mins at 4°C. Supernatant was discarded and the RNA pellet was washed by adding 1 mL of 70% ethanol. Mix gently by inverting the sample a few times. It was then centrifuged for 5 mins at 14,000 rpm at 4°C. Supernatant was discarded by inverting the tube on a clean tissue paper. Later, the pellet was dried by incubating in a dry bath for 5 mins at 55°c. The pellet was then resuspended in 25 µl of DEPC treated water.

Reverse Transcriptase Polymerase Chain Reaction (RT PCR)

A semi quantitative Reverse Transcriptase Polymerase Chain Reaction (RT PCR) was carried out using Techno Prime system to determine the levels of β-actin, IRS1, IRS2, GLUT4 mRNA expressions. The cDNA was synthesized from 2 µg of RNA using the Verso cDNA synthesis kit (Thermo Fischer Scientific) with oligo dT primer according to the manufacturer's instructions. The reaction volume was set to 20 µl and cDNA synthesis was performed at 42°C for 60 mins, followed by RT inactivation at 85°C for 5 mins.

Conclusion

Since adult stem cells are present in PBMCs which have similar expression profiles to UBC one could expect similar actions by Metadichol on these cells leading to observed improvements that we have documented in diabetes patients. The study shows highly related biologically functional gene clusters are the key in targeting diseases. A paradigm shift has been proposed in designing drug design from "one drug one target" to "one drug multiple targets". Our results show that network-based approach clearly seems to be more viable. Our work reveals the unique complex interactions between Metadichol which is a nano formulation of mixture of straight chain aliphatic alcohols and cellular proteins but also the influence of their interactions on the function and behaviour of the system. Metadichol is the first in a class of molecules that targets multiple genes and through multiple pathways and thus multiple disease targets which could be the next wave of the future of drug discovery

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raghavan@nanorxinc.com

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