Development of Plasmid Vectors Encoding Genes Betacellulin and Hepatocyte Growth Factor for Improving Islet transplantation

Rimple patel, Frederick K. Galarza, Yash Desai, Micky Patel, Ravikiran Panakanti.
Roosevelt University College of Pharmacy, Schaumburg IL 60173.

ABSTRACT

Objective:
Human islet transplantation has the potential to replace pancreatic endocrine function in patients with diabetes. The critical challenge here is to improve the survival and functionality of islets for a longer period of time. Betacellulin (BTC) is an epidermal growth factor promoting beta cell (insulin producing cells) proliferation, differentiation and growth. Hepatocyte growth factor (HGF) is a potent mitogen known to improve β-cell growth and function. It also shows anti-apoptotic activity. We aim to develop and assess functionality of the plasmid vectors encoding genes HGF and BTC for improving the outcome of islet transplantation.

Method:
Plasmid vectors were constructed by inserting genes BTC and HGF in the plasmid pcDNA3.1 separately using restriction enzymes Bam HI-XhoI for BTC and NheI-Hind III for HGF. Rat Insulinsoma cells (RIN-5mF) were transfected with plasmid vectors pcDNA3.1-BTC and pcDNA3.1-HGF respectively using lipofectamine 3000 reagent. MTS, Caspase-3 assay and ELISA were done to ascertain the function of plasmid vectors pcDNA3.1-BTC and pcDNA3.1-HGF respectively.

RESULTS:
RIN-5mF cells transfected with plasmid vectors pcDNA3.1-BTC and pcDNA3.1-HGF with lipofectamine 3000 showed less toxicity. There was time dependent increase in expression of HGF and BTC from plasmid vectors. Plasmid vectors especially pcDNA3.1 –BTC showed antiapoptotic effect when transfected with lipofectamine 3000 at 0.15µl.

Materials:
Rat insulinoma cells (RIN-5mF) and RPMI-1640 growth media were obtained from ATCC(Manassas, VA). Lipofectamine(Symbol) 3000 Transfection Reagent, Human HGF ELISA Kit, and Human BTC ELISA Kit were purchased from ThermoFisher Scientific (Waltham, MA). CellTiter-Glo(TM) Luminescent Cell Viability Assay and Caspase-Glo 3/7 Assay were purchased from Promega (Madison, WI). GenElute(TM) Plasmid Miniprep Kit, Dulbecco’s Phosphate Buffered Saline (PBS), and Trypsin was brought from Sigma-Aldrich (St. Louis, MO).

Results:

1. RIN -5mF cells transfected with plasmid vectors pcDNA3.1- BTC and pcDNA3.1-HGF with lipofectamine 3000 showed less toxicity (Fig 2.3 & 4).
2. There was slight toxicity when high concentration of lipofectamine 3000 was used (Fig 5).
3. There was time dependent increase in expression of HGF and BTC from plasmid vectors (Fig 6 & 7).
4. Plasmid vectors especially pcDNA3.1 –BTC showed antiapoptotic effect (Fig 8 & 9) when transfected with lipofectamine 3000 at 0.15µl.

CONCLUSION:

• Plasmid vectors pcDNA3.1 HGF and pcDNA3.1BTC showed good expression of genes BTC and HGF.
• They also protected the cells in the presence of cytokines. More tests need to be done to ascertain the safety and efficacy of these vectors.

References: