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.

ROOSEVELT UNIVERSITY
COLLEGE OF PHARMACY

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 in improving the outcome of islet transplantation.
Method:
Plasmid vectors were constructed by inserting genes BTC and HGF in the plasmid pcDNA3.1 separately by using restriction enzymes Bam HI-XhoI for BTC and Nhe I-Hind III for HGF. Rat Insulinoma cells (RIN-5mF) cells 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.
Implications:
Plasmid vectors pcDNA3.1 HGF and pcDNA3.1BTC showed good expression of genes BTC and HGF. They also showed anti-apoptotic effects by protecting the cells in the presence of cytokines. More tests need to be done to further ascertain the safety and efficacy of these vectors.

Materials

Rat insulinoma cells (RIN-5MF) and RPMI-1640 growth media were obtained from ATCC[Symbol] (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[Symbol] Luminescent Cell Viability Assay and Caspase-Glo 3/7 Assay were purchased from Promega (Madison, WI). GenElute™ Plasmid Miniprep Kit, Dulbecco's Phosphate Buffered Saline (PBS), and Trypsin was brought from Sigma-Aldrich[(St. Louis, MO).

Methods

Plasmid vectors was constructed by inserting genes BTC and HGF in the MCS of pcDNA3.1 plasmids separately by using restriction enzymes Bam HI-XhoI for BTC and Nhei-Hind III for HGF (**fig 1**)

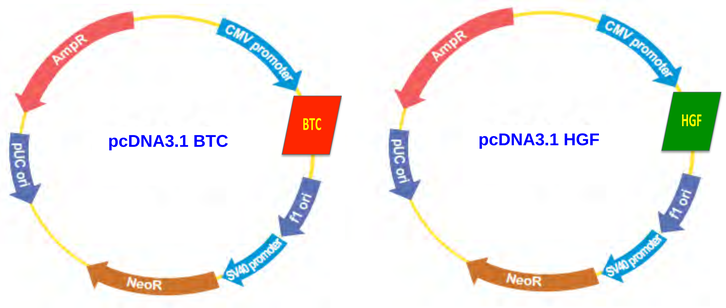

Figure 1: Plasmid Vectors Encoding Betacellulin (BTC) and Hepatocyte growth factor (HGF)

RIN-5mF cells were transfected with plasmid vectors pcDNA3.1-BTC and pcDNA3.1-HGF respectively using lipofectamine 3000 reagent using two concentrations of 0.15µl and 0.3µl recommended by the manufacturer. Caspase -3 levels were measured three days after transfection using non-transfected cells as control.
MTS assay was done on the cells transfected with pcDNA3.1-BTC and pcDNA3.1-HGFy using lipofectamine 3000 reagent using concentration of 0.3µl.
ELISA was performed on the samples collected day1 and day 3 post transfection using lipofectamine 3000 reagent using concentration of 0.3µl.
Following this, cell transfected with the plasmid vectors at two different concentrations of lipofectamine 3000 0.15µl and 0.3µl were also added with growth medium containing cytokines IL-1β and TNF-α (10 ng/mL) to ascertain whether plasmid vectors vectors pcDNA3.1-BTC and pcDNA3.1-HGF could protect the cells from apoptosis.

Results

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

Results

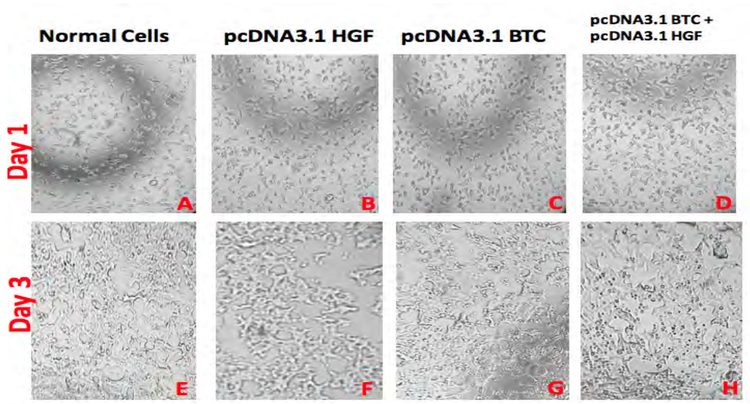

Figure 2: Microscopic Images of Normal and Transfected Cells transfected with plasmid vectors pcdna3.1 BTC and HGF respectively.

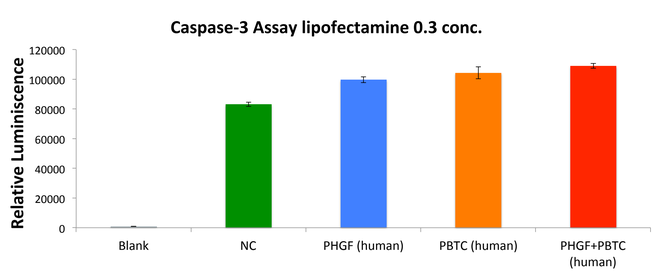

Figure 4: Caspase-3 Assay on RIN-5mF cells transfected with plasmid vectors encoding genes HGF and BTC using Lipofectamine 0.3 µl concentration

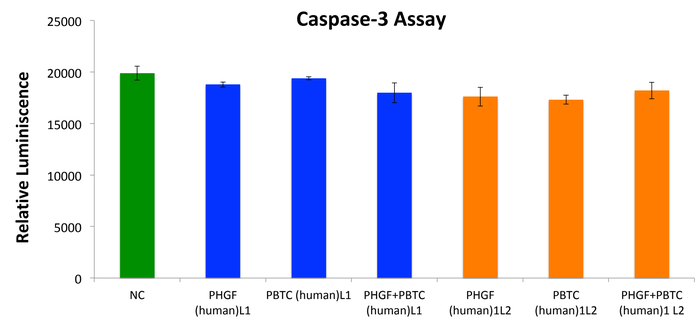

Figure 3: Caspase-3 Assay on RIN-5mF cells transfected with plasmid vectors encoding genes HGF and BTC using Lipofectamine 0.15 µl and 0.3 µl concentration

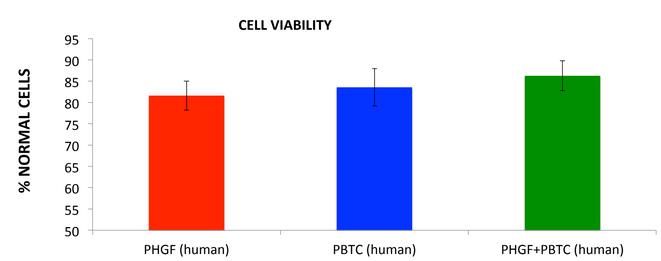

Figure 5: RIN-5mF cells transfected with plasmid vectors encoding genes HGF and BTC using Lipofectamine 0.3 µl concentration. MTS aasay done to assess the safety of the vectors.

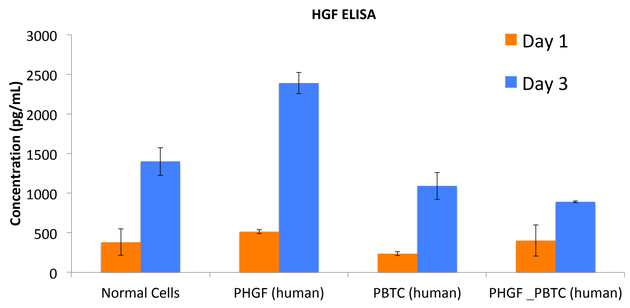

Figure 6: Measuring of protein levels after transfection with plasmid vectors pcDNA3.1-HGF.

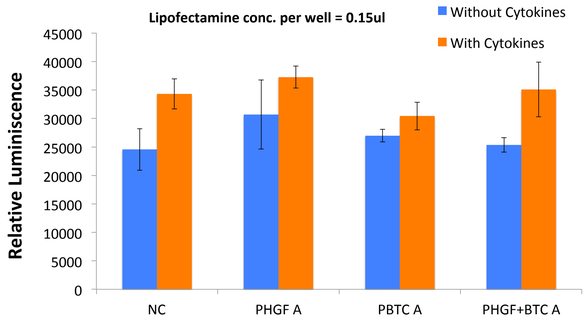

Figure 8: : Caspase-3 Assay on RIN-5mF cells transfected with plasmid vectors encoding genes HGF and BTC using Lipofectamine 0.15 µl concentratioN

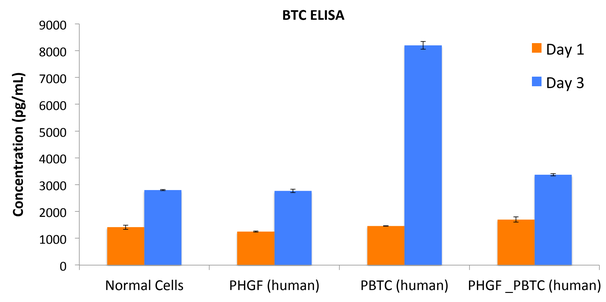

Figure 7: Measuring of protein levels after transfection with plasmid vectors pcDNA3.1-BTC.

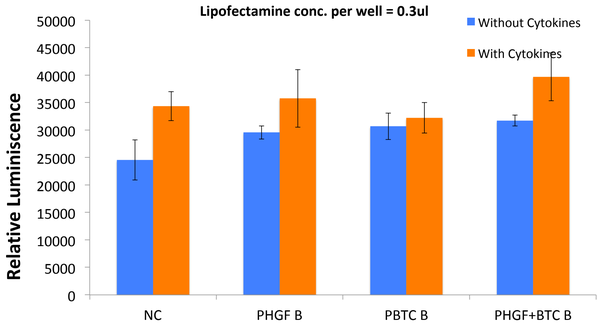

Figure 9: : Caspase-3 Assay on RIN-5mF cells transfected with plasmid vectors encoding genes HGF and BTC using Lipofectamine 0.3 µl concentration

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

1. Oh YS, Shin S, Lee Y-J, Kim EH, Jun H-S. Betacellulin-Induced Beta Cell Proliferation and Regeneration Is Mediated by Activation of ErbB-1 and ErbB-2 Receptors. Maedler K, ed. *PLoS ONE*. 2011;6(8):e23894. doi:10.1371/journal.pone.0023894.
2. Panakanti, R. and R.I. Mahato, *Bipartite adenoviral vector encoding hHGF and hIL-1Ra for improved human islet transplantation.* Pharm Res, 2009. 26(3): p. 587-96.