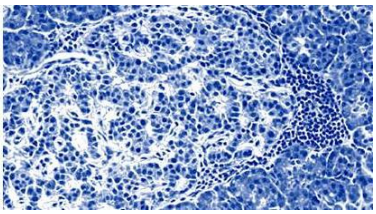
nPOD
Network for Pancreatic Organ Donors with Diabetes



Self-aggregation of stem cell derived beta cells is associated with maturation; mature cells can be identified and isolated using a specific surface marker

AUTHORS

Fiona M. Docherty[1], Kent A. Riemondy[2], Roberto Castro-Gutierrez[1], Jaeann Dwulet[3], Ali H. Shilleh[1], Maria S. Hansen[1], Shane Williams[1], Jay R. Hesselberth[2,4], Richard K. P. Benninger[3], **Holger A. Russ[1]**

1. Barbara Davis Center for Diabetes, Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO
2. RNA Bioscience Initiative, University of Colorado Anschutz Medical Campus, Aurora, CO
3. Barbara Davis Center for Diabetes, Bioengineering and Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO
4. Department of Biochemistry and Genetics, University of Colorado Anschutz Medical Campus, Aurora, CO

PURPOSE

Cell replacement therapy represents a potential cure for type-1 diabetes, as yet *in vitro* differentiation of β-like cells from human pluripotent stem cells results in production of cells that phenotypically and functionally resemble human fetal β cells. The purpose of this study is to establish mature stem cell derived β-like cells (sBC) *in vitro* and investigate potential mechanisms of human beta cell maturation.

METHODS

Direct differentiation of human embryonic and induced pluripotent stem cells into sBCs.

SUMMARY OF RESULTS

We observe that over time immature stem cell derived β-like cells (sBC) self-aggregate in 3D culture forming insulin^{+} 'caps' or self-enriched beta like cells (seBC). Characterization of seBC, by RNAseq, Ca^{2+} signaling, transmission electron microscopy (TEM), hormone content, mitochondrial analysis and global methylation pattern, shows that they are phenotypically more mature than SBC. We demonstrate by single cell RNAseq that seBCs, although already exhibiting improved maturation, compromise a heterogenous cell population displaying sBCs with varying maturity. Interestingly, bioinformatic analyses suggest a developmental trajectory towards most mature β cell phenotype in our culture conditions. Analysis of the mature β cell subset has allowed identification of a specific surface marker that can be used to specifically sort out mature sBCs. Establishing these different models of β cell maturation has allowed us

to begin elucidating the complex mechanisms that drive maturation of human β cells enabling better recapitulation of the process *in vitro.*Finally, taking all of this together, we show that sorting and reaggregation of mature β cells from iPSC derived from type-1 diabetic patients allows production of β-like cells that closely resemble mature human β cells.

CONCLUSIONS

Our results have important implications for current cell therapy efforts by providing a surface marker and critical information on mature stem cell derived beta-like cells.