Differentiation of Canine Embryonic Stem Cells to Insulin-Producing Cells

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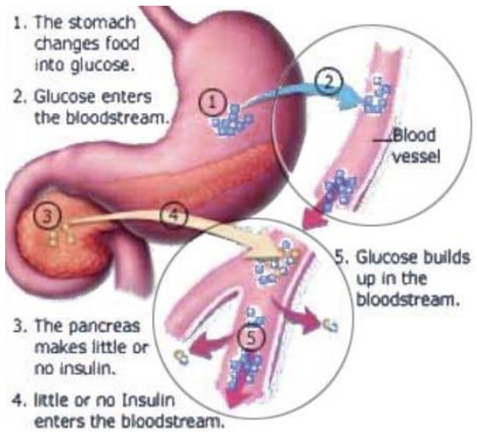
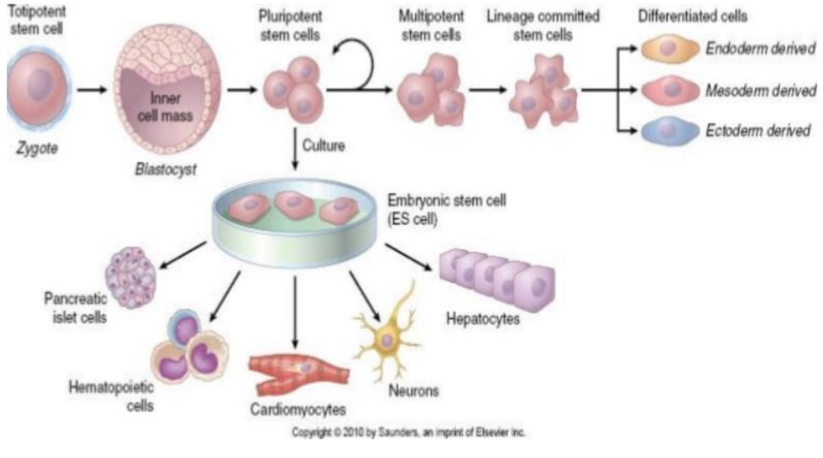
INTRODUCTION

- Canine diabetes mellitus
  - → persistent hyperglycemia due to β cell loss
- Affects 0.6% of dogs in the USA with increasing prevalence
- Clinically managed by life-long daily exogenous insulin injections
- Secondary complications from inappropriate glycemic control
- Currently no cure
- β cell replacement therapy can enable insulin independence
- Embryonic stem cells are an attractive cell source due to its capacity for self renewal and ability to differentiate and form all types of tissue in the body



OBJECTIVE

- Develop a canine-specific beta cell differentiation protocol using a published murine embryonic stem cell protocol



METHODS

- 3 phase protocol
  - Formation of embryoid bodies: standard suspension method vs hanging drop method
  - Spontaneous differentiation from pluripotent embryoid bodies to multilineage progenitors
  - Induction of pancreatic differentiation to islet-like clusters
- Microscopic analysis of cell morphology
- RT-PCR to determine transcript level of pancreas specific genes at Day 14, Day 21, and Day 33

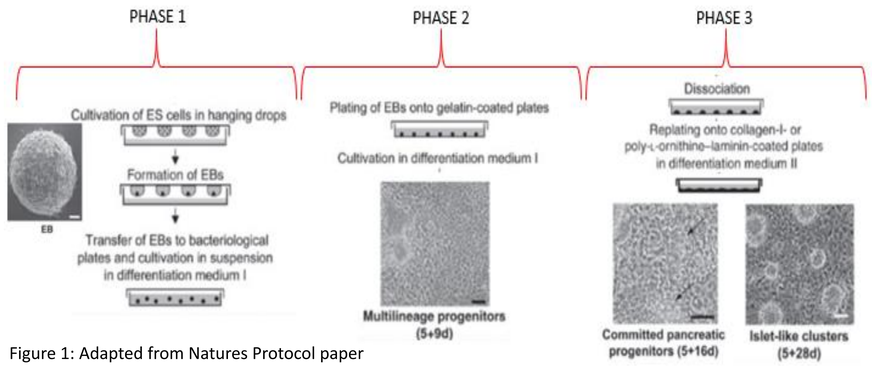
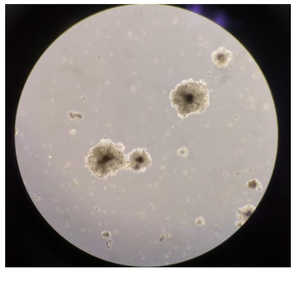


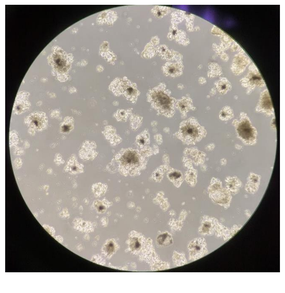
Figure 1: Adapted from Natures Protocol paper

RESULTS

- Phase 1- Growth and Formation of Embryoid Bodies

Hanging Drops:
More uniform in size and shape, but lower yield and overall decreased survival





Standard Suspension:
Larger yield with a heterogenous population

- Induction of Pancreatic Differentiation

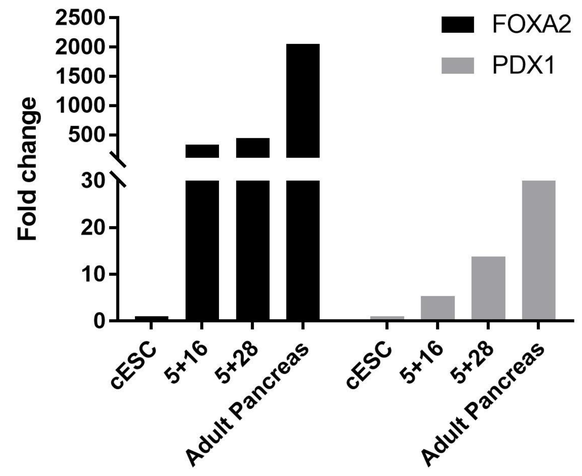


Figure 2: RT-PCR shows upregulation of FOXA2 and PDX1 during the differentiation protocol. PDX1 is a master regulator of pancreas development. FOXA2 is an early marker of endoderm expression. No evidence of islet-cell differentiation such as insulin, glucagon, somatostatin or MAFA expression is detected (data not shown).

CONCLUSION-FUTURE DIRECTION

- Standard suspension yielded more embryoid bodies with longer survival
- Early endoderm markers, PDX1 and FOXA2, were expressed
- Pancreatic specific markers, insulin and glucagon, were not detected
- Protocol needs to be further optimized for cells to express insulin by adjusting the media and the length of time in each phase
- Perform immunohistochemistry to detect whether there are characteristic proteins specific for the pancreatic stage such as insulin and glucagon

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