Characterization of Activated Islet Progenitor Cells (AIPCs) During Maturation to De Novo-Islets

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Introduction and Experiment Design

- AIPCs (Activated Islet Progenitor Cells) are a novel cell population derived from islets and pancreatic tissue using a simple culture protocol that can generate billions of insulin-producing islet cells **(Figure 1)**.
- AIPCs are over 65% insulin-, glucagon-, and CD133-triple-positive, can be propagated for over 200 days in culture, secrete both insulin and glucagon in response to secretagogues, and form cell clusters that represent de novo-islet like structures **(Figure 1)**.
- AIPCs represent not only a novel cell population and a tool to foster studies of islet cell biology, but a realistic cellular approach for the treatment of insulin insufficiency in diseases such as diabetes and pancreatitis.
- To further characterize AIPCs RNA sequencing of pancreatic tissue, AIPCs and de novo-islets from the same deceased donor was performed using the Illumina® NovaSeq™ platform.

Activated Islet Progenitor Cells (AIPCs)

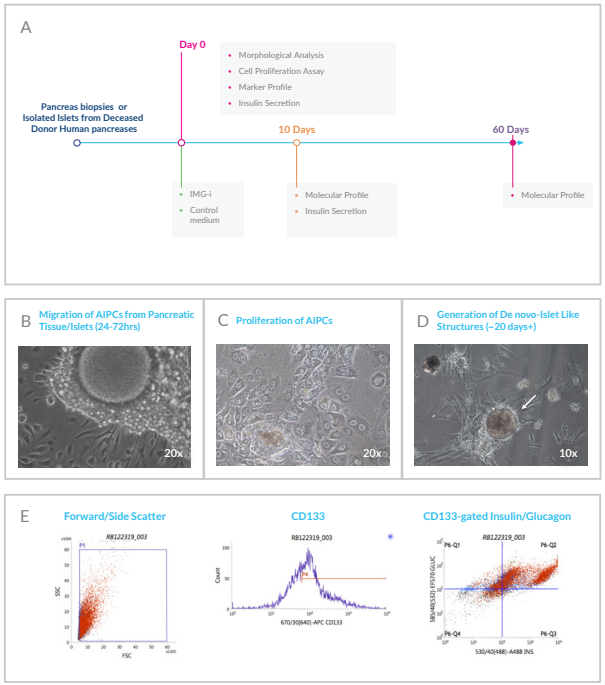
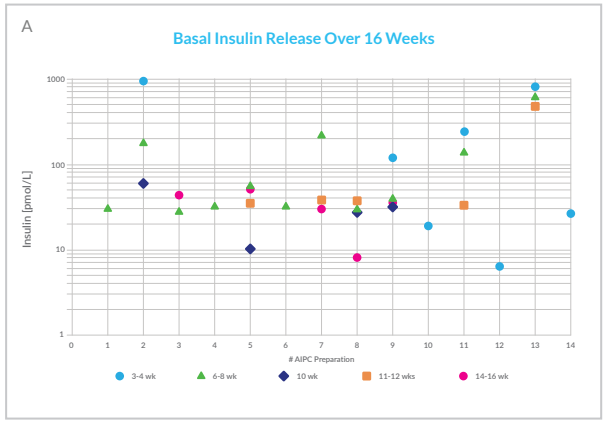


Figure 1. A schematic for the generation of AIPCs **(A)**. IMG-1 activates and mobilizes islet progenitor cells **(B)**. Within 7 days' colonies of AIPCs become established and mono-layer of AIPCs form around day 10 **(C)**. After approximately 7 population doublings and several passages AIPCs begin to spontaneously form de novo-islet like structures, white arrow **(D)**. AIPCs are triple positive for CD133/Insulin and Glucagon **(E)**.

Insulin Secretion of 14 AIPCs Preparations Over 16 Weeks of Culture



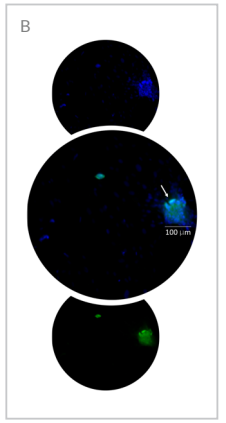
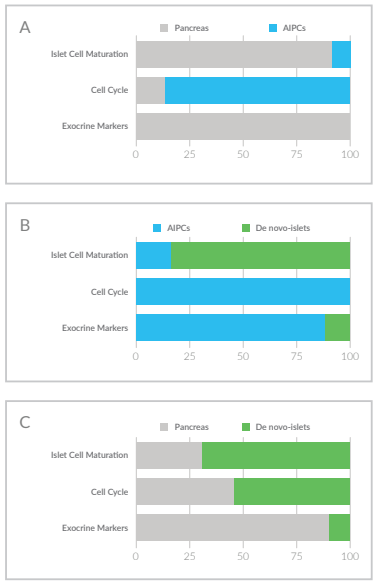


Figure 2. Basal insulin secretion levels were measured in the media of 14 AIPCs preparations randomly over a period of 16 weeks and shown on a scatter plot, (the values were not normalized per cell numbers **(A)**). Representative images of immunofluorescence staining of AIPCs during the process of de novo-islet formation showing Insulin positive cells. **(B)**. Top image shows DAPI, bottom insulin and middle shows merged immuno-florescence.

Comparative Gene Expression of Islet Cell Maturation, Cell Cycle and Exocrine Markers

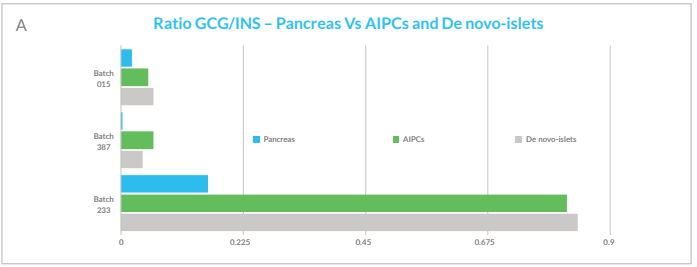


Families of Genes

Islet Cell Maturation	Cell Cycle	Exocrine Markers
INSR	E2F1	PRSS1
INS	BARD1	PRSS2
SST	CHAF1A	CTRC
GCG	CHAF1B	LPL
ISL1	CCNE2	AMY2A
PDX1	CCNE1	CELA2A
NKX2-2	PCNA	AMY1A
NKX6-1	CCND1	
MAFA		
MAFB		
PAX6		
PROM1		
DCLK1		
NEUROD1		
IGFBP1		
IAPP		
PPY		
PAX4		

Figure 3. Comparative gene analysis between AIPCs and Pancreatic Tissue **(A)**, AIPCs and de novo-Islets **(B)** and de novo-Islets and Pancreatic tissue **(C)** looking at genes involved in Islet Cell Maturation **(top)**, Cell Cycle **(middle)** and Exocrine Markers **(bottom)**. To determine differences in gene expression across samples Transcripts per million (TPM) were used. Number of genes are expressed as %.

Ratios of Glucagon/Insulin and MafB/MafA



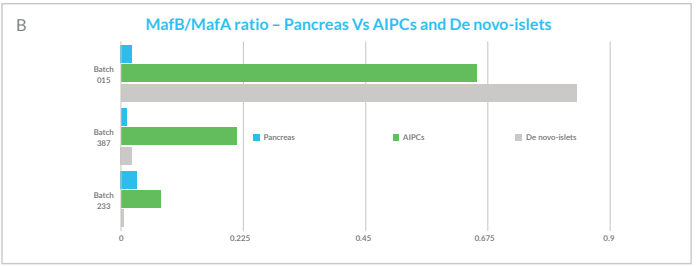


Figure 4. Maturation state of AIPCs and de novo-Islets based on the ratio of glucagon/insulin **(A)** and MafB/MafA **(B)** gene expression in three preparations. Expression of MafB precedes MafA during pancreatic development (Dev Biol. 2006 May 15; 293(2): 526–539.)and adult alpha (glucagon producing) cells can be converted to beta (insulin producing) cells (Cell Metab. 2017 Mar 7; 25(3): 622–634.).

Summary

- AIPCs are an endocrine progenitor cell population that can spontaneously evolve and mature into de novo-islets.
- AIPCs secrete insulin in vitro for over 16 weeks during culture.
- When generating de novo-islets the majority of insulin producing cells migrate to form the cell clusters.
- Microarray analysis demonstrates that AIPCs have more genes expressed related to cell cycle, while de novo-islets express higher levels of genes associated with islet cell maturation, and pancreatic tissue exhibits the highest levels of exocrine marker proteins.
- Ratios of glucagon/insulin and MafB/MafA indicate that AIPCs are very immature pancreatic cells, and AIPCs-derived de novo-islets represent the next stage of development, having a gene profile that is more akin to that of a premature islet.
- AIPCs represent not only a novel cell population suitable to investigate the pathophysiology of diabetes but can function as an islet substitute to treat hyperglycemia.

Acknowledgments

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