Advances in Islet Biology, Keystone 2012 Monterey, CA

Sunday evening – Keynote speaker

**Andrew Hattersley, Peninsula Medical School, Exeter** Human genetics

A human gene KO: no detectable C-peptide, insulin

Advantages in mouse genetics over human genetics; phenotypes humans only.

Improve beta-cell science leads to improved treatment. In there is gene discovery as well. Next generation sequencing, improves gene discovery, which leads to improved treatment.

Neonatal diabetes is likely monogenic. Diagnosed after 6mos is most likely to be T1D.

Mutations in classical glucose metabolism result in neonatal diabetes. KATP channel, Glucokinase. Most common cause of neonatal diabetes is in Kir6.2 K channel subunit. ATP does not close the channel. Kir6.2 is in the brain and muscle, so mutations lead to neurological and muscular defects. Work by Fran Ashcroft

Mutations in SUR1 also cause neonatal diabetes.

How do we move from gene discovery to improved treatment? Sulfonylureas close the channel when ATP does not. 46 year old with Kir6.2 mutation took sulfonylureas all his life. Is insulin treatment better? Some Kir6.2 mutants take insulin for years and then switched to sulfonylureas. Switch works; in fact drugs more effective than insulin to control glucose. Mutation is at the ATP binding site. What site(s) do the drug interact? The fact that the drugs work, suggest different than ATP.

About 50% of neonatal diabetics have mutation in Kir6.2; what are the other genes?

Is GLUT2 important in humans vs mouse? In humans, it looks like GLUT1 and GLUT3 are critical, whereas in rodents it is GLUT2.

However, GLUT2 mutations leads to transient neonatal diabetes.

Beta-cell development and production.

When parents are unrelated, vast majority of cases of neonatal diabetes result from K channel. However, when parents related (e.g, cousins) there are other genes involved.

Examples: Nkx2.2. Homozygous mutations in Nkx2.2 look very similar to the mouse KO for Nkx2.2.

Another: Mnx1; homozygous mutation. Less clear if the Mnx1 KO mouse mimics the Mnx1 mutant human.

Neurod1. Mouse and human look similar when this gene is mutated.

Neurogenin3 deficiency results in neonatal diabetes. Multiple kinds of mutations resulting in altered phenotypes. Ngn3 is an endocrine precursor, thus all endocrine enzymes missing.

Rfx6 mutations look similar in mouse and human.

Mouse KO models have been validated by finding the naturally occurring mutations in humans.

Pdx-1 and tf1a mutations are not found in human. However, in mouse, KO of these leads to no pancreas. In humans without a pancreas, these genes are not mutant.

To identify this, next generation sequencing the child and both parents. Look for the mutation in child but not unaffected parents. Found Gata6 with a new spontaneous mutation. Now know that 56% of apancreas patients have mutations in Gata6. Haploinsuffiency. However, the Gata6 heterozygous mouse has no phenotype at all; homozygous KO of Gata6 is lethal.

Heterozygous transcription factors causing human diseases, but not mouse. Hnf1a, Hnf4a, Hnf1b. Early pancreatic precursor TFs.

Homozygous models fit well. But the haploinsufficiency models do not. Heterozygous mutations is enough in man to stop pancreas development, however, in mouse this is not enough to cause phenotype.

Glucose toxicity: Doesn’t appear to really play a role. Some patients can be hyperglycemic for years due to Kir6.2 mutations, respond very well to tolbutamide. So it doesn’t look like the years of high glucose resulted in beta-cell death.

Monday AM – stem cells as a source of islet cells.

**Signaling pathways controlling differentiation of human stem cells. Ludovic Vallier email lv225@cam.ac.uk.**

Human embryonic stem cells = Hescs. Self renewal into multiple cell types.

Molecular mechanism controlling endoderm development, leading to gut.

Hesc->endoderm->islet cells.

Hesc+CDM+activin+FGF (CDM = chemically defined medium)

Immunostaining for various target cell types.

CDM + activin + FGF2 + BMP4 and then track over several days to see expression of endoderm markers.

Gene expression array on the cells. Artificial differentiation of Hescs mimics natural development in mouse.

Sox17 in human IPS cells.

Ventral forgut endoderm can then be specified into various organs, including pancreatic endoderm, lung endoderm and hepatic endoderm markers.

Pancreatic endoderm. RA + FGF essential for this. Activin and BMP4 are strong inhibitors of this pathway. Pdx1, Hlxb9, Pou5f1, Nkx6.1, Hnf4a and Hnf6 are all used for panc markers. In protocol use a small molecule activin inhibitor.

Can then differentiate these panc endoderm cells into insulin expressing cells, that show gluicose-dependent release of C-peptide. Then transplated these into kidney capsule of NOD mice. Human C-peptide secreted into plasma in response to glucose, so functional.

Human IPS and ESCs can be differentiated into insulin expressing cells using similar protocol.

Activin promotes hepatic differentiation; suppresses pancreatic differentiation. Activin suppresses Mnx1 and induces Hhex, which may explain the panc vs liver specific program respectively.

Hhex is necessary for hepatic specification in human. If knockdown Hhex, loose differentiation into liver cells. Mnx1 similar results; knockdown of Mnx1 leads to loss of Pdx1 during pancreatic differentiation protocol, so Mnx1 is necessary for panc differentiation program.

Beta-cell specification. Small molecules used to screen for what might promote beta-cell lineage. Ngn3 used for beta-cell specification. Wnt pathway molecule increases Pdx1, Ngn3 and C-peptide. Glucose-dependent release of C-peptide, so functional beta-cells.

So, have a stem cell population that can be used to drive into 3 endoderm cell types: lung, liver and pancreas. Wnt pathway small molecule is used to maturate panc beta-cells in vitro and then transplant into kidney capsule. Wnt activator molecule.

 **Generation of self-renewing endoderm stem cells Paul Gadue at Philadelphia.**

Hescs and Hipscs

Difinitive endoderm maintained in culture.

iPS = pluripotent stem cells can be differentiated in various endoderm lineages. Require capability to differentiate stem cells reproducibly into stable mature and functional cell types. How to induce cells to go to a particular cell type. Endoderm forgut can go to liver, pancreas, intestine and lung.

Teratomas: ES/iPS cells can form tumors, as they are pluripotent for a variety of cell types.

Can we culture endodermal progenitors? Endoderm specification. No ectodermal or mesodermal contamination. 3 types: endoderm, mesoderm and ectoderm.

Endoderm progenitors and liver/panc/lung/gut differentiated cell types. What is stable? Maintain progenitors indefinitely, with BMP4, FGF, VEGF and EGF, yield Foxa1 and Sox17 positive and maintainable for up to 1 yr. ALCAM cell surface marker that discriminates endoderm cells from undifferentiated progenitor cells.

Endoderm progenitor line identified and don’t form tumors, whereas other SCs do form tumors. What do these cells spontaneously differentiate into when put in animal? Maintain endoderm cell types; no mesoderm or ectoderm observed. Stain for Foxa1, Hnf4a, Cdx2, Ifabp, Villin1 and Mucin2. Also see AFP and a1anti-trypsin, hepatocyte markers.

What about directed differentiation protocols in vitro for liver, intestine or panc protocols. Went over liver and intestinal differentiation studies. What about pancreas protocol from Nostro et al 2011. Generates beta-cells that are not glucose responsive however.

C-peptide, no Gcg, but do see Pdx1 positive. If see C-peptide, see no Gcg and no Sst expression, so it looks like they’re getting beta-cell specific lineage. What if they differentiate these endoderm progenitors as soon as they are generated, i.e., passage 0 vs passage 6 (over about 1 mo). Compare insulin/c-peptide expression to human islets. In differentiated cells insulin about 20% of that in islets.

Are these cells functional? Yes. They’re derived beta-cell derived from Hescs are glucose-responsive and comparable to primary islet. EP-derived 9gn/100K cells, whereas in islets about 40ng/100K cells. So they are functional by about 20% of that compared to primary islets, so considerable immature for a reason not yet determined.

So, EPs can generate functional beta-cells and don’t form tumors when put in animals.

**Chrstina Nostro from Gordon Keller’s group – short talk.**

Human ES cells to study pancreatic development in vitro. Human pluripotent stem cells used to generate hormone producing cells of pancreas. Pdx-1 positive cells to commit to beat-cell cells, as judged by insulin positivity. What is the basis for this inefficiency?

Block Tgfb with small molecule and Noggin to block Notch signaling. P-SMAD2 blocked via Tgfb inhibition and Notch inhibition. This leads to large increase in insulin expression. The combination of Tgfb and Notch suppression syngergizes.

Wnt3a signaling is critical to establish insulin expressing cells. Not only beat-cells, but see Gcg and Sst expressing cells as well. EM gold staining was used to see that a single cell expresses both Gcg and insulin; a weird granule that has black core and grey hallo.

Ins-GFP reporter line lets them visualize beta-cell differentiated cells. Ins-GFP co-stains with C-peptide. FACS-sort purify these cells. And then transplant in mammary fat pad of NOD mice. 1 mo later, islet like structures are present. Unfortunately none of these clusters express C-peptide or insulin or Nkx6.1, Pdx1. But do expression Gcg, so for some reason dedifferentiated from beta-cell to alpha-cells. No Ki67 staining, so they do not proliferate. Do not know if these alpha-cells are functional.

**Kevin D’Amour – ViaCyte making an encapsulated cell based therapy.**

>750 patients treated with islet transplantation since 2000.

Limitations: transplant site complications, viability of cells,

Renewable stem cells, therapeutic, safe delivery. Pancreatic progenitor, so it has yet to commit to beta-cell lineage. Delivery system is a macro-encapsulation, durable.

Stem cell program. Human ES cells are renewable. Rapid expansion, 10^9 cells in 2 weeks from a single cryovial. Genetically stable. Pluripotent and biology well characterized. GMP compliant cell line. This cell line is the center of the companies technology.

3-7 years of development to optimize the differentiation protocol. 4-stage protocol with ActA, WNT (1), KGF (2), RA+Cyc+Nog (3) and no factors for (4).

CD142 is a cell surface marker of pancreatic progenitor, CD200/318 is an endoderm marker. Transplant these marked cells into animal and get islet like clusters for the Cd142 marked and Gcg positive for the Cd200/318 marked cells.

Suspension culture yields islet like clusters in vitro. Freeze these pro-islet cultures, thaw, formulate, load the delivery device and ship to clinic.

Gene expression for 109 markers, here’s 4: Pou5f1, Sox17, Sox2 and Foxa2. Some go down with time and others go up reproducibly.

ES cells and safety for teratoma AE. High purity endoderm population is needed to avoid teratoma. No mesoderm or ectoderm tolerable. Chga, Pdx1, Nkx6.1 are all pancreatic progenitor markers.

Device program. Macro encapsulation of islet like clusters. Excludes Host immune cells, but allow O2, nutrients and proteins. Biostable, so retrievable from patient. Retains grafted cells; excludes host cells. Facilitates microvascularization.

Combination product: subQ transplant. Device is vascularized. Cells modulate foreign body response. Transplanted mice immune to STZ treatment, remove grafts and then mice become hyperglycemic. Without transplanted device, STZ treatment yields immediate elevated glucose. So STZ does not kill grafted cells; Why? Mice receive overdose of islets: about 80K IEQ/Kg, yet no hypoglycemia.

Cells survive in capsulated device and do not proliferate, so effect is coming from that which was transplanted. Most of the progenitors adopted an endoderm lineage, 80-90% endocrine. Remaining 10% thought to be ductal in nature. Stain for insulin, gcg, sst, ghrelin, pp. minimal exocrine as judged by ihc and rna profile. If spike in undifferentiated hEScells, get teratoma.

Not yet tested in humans. Cell composition. Cryopreserved. Product encapsulated to can be retrieved if needed. One encapsulation device is about the size of a credit card. Think that about 10 of these would be needed to satisfy the insulin needs of a patient. Seeking FDA approval now.

**Raphael Sharffman – France, pancreatic beta-cell development, transferring data from rodent to human.**

To understand how human beta-cells develop. Work out this developmental program in mouse and transfer to human development.

Rodent fetal pancreas and grown in culture, over time will give rise to beta-cells. Development a number of markers for this process: Pdx1->Ngn3->NeuroD->Pre-beta->insulin. In response to Fgf7,10.

Small molecules that inhibit Cftr. Expression of Cftr and Ngn3 are co-expressed. Is Cftr suppressing Ngn3 expression? Cft4 inhibitors. Glibenclamide (100uM) increased Ngn3 expression. Is this molecule a Cftr inhibitor? GlyH101, another Cftr inhibitor yields the same result. GlyH101 increases beta-cell development.

This is interesting. Why is Cftr negatively regulating beta-cell function/proliferation. Cftr KO mice have increased beta-cell mass. Why? As I recall, Cftr is a Cl- channel. What’s it doing in beta-cells; what’s the level of expression in islets?

In vivo effect of GlyH101, a CFTR inhibitor. Increased beta and alpha cell mass.

What is GlyH101?

Transfer to human beta-cell development. Immature human fetal pancreas. In culture, cells are positive for Pdx1, Sox9 and Nkx6.1.

Human fetal pancreas and transplant into kidney capsule of immune-compromised mice. 3 mos later, Ngn3 expression is high. Exocrine marker, CPA. Endocrine differentiation from insulin, sst, gcg appear in small clusters, as if the pancreas continued its normal developmental program. Completely rescues hyperglycemia from alloxan treatment, which is lost with kidney removal.

Human beta-cell lines to study beta-cell proliferation and function. Rare human lines, while a large number of rodent beat-cell line. Rodent lines generated by transgenesis of targeted oncogenesis. Did this with human fetal pancreas. Lentiviral gene transfer of oncogene under the control of RIP driving SV40 LT antigen. Results in insulinomas in 6 mos.

Starting from human islets, do not get the same result. So human fetal pancreas has a potential that human islet do not. Markers, Pax4, Pax6, NeuroD, Rfx6, Nkx2.2, Ins1. Harvested these cells from insulinomas to isolate beta-cell line clones; have about 30 or so of these clones. DNA methylation at the insulin locus to assess which are best; high DNA methylation indicates transcriptional regulation. This is high in fetal pancreas and low in non-beta cell tissues (done by Yuval Dor).

Best lines show nice GSIS and dose-response to GLP-1. So SV40LT generates a human functional beta-cell line. Working now to generate a 2nd generation beta-cell line with floxP sites flanking SV40LT gene so this can be removed from beta-cell line with Cre. Not sure how this will work? Inducible; stop proliferation?? In these clones, SV40LT high and then greatly reduced after Cre expression via adenovirus expressing Cre. Ki67 before and after SV40LT excision; this also decreases after Cre introduction. Unexpectedly, insulin expression greatly increased after Cre, suggesting SV40LT suppressed insulin. So, SV40LT and Ki67 goes down with Cre; Insulin goes up, as does insulin protein content.

Drug-based regenerative medicine as new approach for beta-cell therapy.

**Short talk – Directed differentiation of patient-specific IPs cells. Diego Balboa Alonso, University of Helsinki**

Permanent neonatal diabetes mellitus = PNDM, 1:120K people with diabetes diagonised <6mos. Mutations known in Abcc8, Kcnj11 and Insulin. Other genetic etiologies in Finnish population. 24 PNDM patients identified in Finland.

Stem cell center at Biomedicum. Patient-specific iPSC lines. HEL22 – PNDM iPS clone, as an example. Capable of differentiating into primary dermal layers. With directed differentiation, can get hepatocytes, beta-cells and intestinal cells. These cells could be used for graft back into donor for liver, pancreas, or intestinal diseases.

HEL11.4 pancreatic differentiation protocol. Now, all appear to be freeder free protocols, as these are animal based cell lines that must secrete some trophic factors, but is undefined. ActA+NaButyrate; Nog+CyclopamineRA,FGF…

Beta-cell markers: Pdx1, Nkx6.1, Ngn3. Hormone: insulin, Gcg increase with Noggin treatment. What did we see for Noggin expression in the TC study; Enpeng was excited about this. People all agree here that Noggin treatment of iPS cells is necessary to promote beta-cell differentiation. I think Enpend found that it was expressed in Sst cells; we stopped there because we didn’t have any way to get at delta-cells, despite a floxed allele that was available in SF lab.

HEL38: negative for mutations in Sur1, Kir6.2, Gck, Ins and Pdx1. What is it then?

Na-butyrate – what is this doing and why does it promote beta-cell differentiation. This is added in stage1 of the differentiation protocol with ActivinA. Na-butyrate is known to inhibit HDACs, and therefore, may assist in chromatin unfolding for key gene expressions.

Monday eve – mechanisms of beta-cell replication

**Andy Stewart – Induction of proliferation in human beta-cells.**

Mouse vs human islet architecture distinct.

Replicative capacity of rodent and human beta-cells are also different.

Glucose, ffa, insulin, igf1/2, gh, glp1 ex-4 promote rodent beta-cells; none work for human islets.

Human beta-cells very refractory to replication in response to challenges that are very effective to promote proliferation in rodent islets. Human beta-cell proliferate early in life, but not in adulthood. Kassem glaser 2000; meier, butler 2008. So replication does occur embryonically and neonatally, but falls very quickly after birth.

Nuclear proteins that control G1/S control molecules; pocket proteins bind E2F transcription factors which is regulated by kinases and cyclin dep kinases. Series of inhibitors kip/cip and ink4 family. Block activity of cyclins and cdks.

Surveyed these molecule via western blots in rodent and human islets: islet G1/S protein. Rodents don’t have Cdk6. Rodents also have high levels of cell cycle inhibitors. We don’t really want to enter this field; we want to know what is upstream of these molecules and regulating them in a physiological manner.

Human islets: p16 and cip/kip family known to be present in human islets. Human islets lack Ccnd2 but have Cdk6. In rodents Ccnd2 is abundant. Significance of this species difference unclear. Overexpression studies with adenoviruses and tested them in human islets to determine potential to drive proliferation. Showed that Rb is phosphorylated, in parallel with induced proliferation as judged by BrdU incorporation. Combination studies: D2 + C4, D1+C6 and D3 + C6 all yield greater proliferation than either alone. Is Ccnd3 in rodent appear irrelevant though.

C6 and D1 treated human islets transplanted into kidney capsule of NOD mouse rescues STZ diabetes. 1500 islets of these is as effective as 4000 islets not transduced with any gene. BrdU labeling of these shows proliferation which is greatly increased in the D1/C6 treated islets over control human islets. This all published.

Cyclins D, A and E all effective to promote proliferation. Using adeno-shRNAs to knockout the various inhibitors to see if that is effective. Not easy.

What’s present in human beta-cells rather than human islets. “Human beta-cell G1/S atlas” using immunohistochemistry. Beta-cells have: Rb, p107/p130 (which are in the cytoplasm), Cdk4/6 (cytoplasmic), Cyclins D1 and D3 (cytoplasmic), A, E, Cdk1/2 (also in cytosol), Ink4 family and Cip/Kip, p21 in nucleus and p57 in nucleus, whereas others in cytosol, E2f family, in cytosol. Andy didn’t believe it, so did sub-cellular fractionation. With this: Rb, E2F3, E2F7, p57 and a little p21 in nuclear fraction; all others were found in cytosolic fraction. Essentially IHC studies were confirmed by cellular fraction studies. What are they doing in the cytoplasm?

What happens to these molecules if D3/C6 overexpressed? Basically, Ki67, Cdk6, p21, p27, D3, p16 become nuclear, whereas p57 translocates to the cytosol. Force proliferation in human beta-cells, p16, cip/kip become nuclear, while p57 goes out. Trafficking? Adeno-Cdk6-GFP is not nuclear, unless you drive proliferation with D3/C6 overexpression. Movies. Thinks this G1/S trafficking is likely an important regulatory process.

 Note: should have put the IIDP as a funding agency for the JDRF project.

For IHC studies, used enzymatic dispersal and plated beta-cells on cover slips prior to infection with adeno. Kushner has shown that the D cyclins are also in the cytoplasm of rodent beta-cells. Glucose infusion promotes Ccnd2 translocation from cytosol to nucleus, in parallel to increased BrdU incorporation. Why are inhibitors also moving into nucleus in response to overexpression of D3/C6. Significance – cells that have p27 nucleus are not positive for Ki67.

**Sueng Kim – Calcineurin signaling in beta-cells.**

How does a beta-cell mass get established once derived from Ngn3 precursors are formed? Embryogenesis, maturation, proliferation in post-natal period, and then adulthood.

Ca signaling promotes beta-cell proliferation, perhaps during post-natal period of proliferation. This may reflect glucose metabolism via GK as shown by Yuval Dor.

Increased beta-cell proliferation in juvenile human islets. Juveniles < 10 yrs.

A role for Cn/NFAT in the pancreatic beta-cell. FK506 and CsA (cyclosporine). 10-30% patients develop post-transplant diabetes when they take these drugs following organ transplant. CnA/CnB dephosphorylate NFATc, allowing it to move to the nucleus to function as TF. FK506 and CsA inhibit the activity of Cna/b and thus leave NFAT phosphorylated in cytosol. Gsk3b re-phosphorylates NFAT, moving it from nucleus to cytosol. Cn/NFAT required for maintaining b-cell growth in mouse.

Ca activates Cna/b, leading to nuclear NFATc. KO’d out Cnb in mice, which should impair NFAT dephosphorylation. At birth beta-cell mass unaffected, however, post-natal diabetes develops after 20d, which is lethal. Serum insulin loss. Thus, Cnb required during post-natal period.

GSIS severely reduced in response to glucose and Arg. Insulin content is also down in Cnb Kos, mRNA for Ins down. Ins2, Pdx1, Glut2 and Gck down as well. If there is a reduction in insulin, is there a reduction in other components of the dense core vesicles? Eg. ChgA/B and other components of the insulin granule. ChgA and ChgB, IAPP and IA2, Ins1, Ins2 are all down in Cnb KO islets.

What genes does NFATc regulate. ChIP to determine targets. Found NFATc to sit on promoters of ChgA/B and IA2. IAPP was not found, so that may be indirectly regulated by NFAT. What about in human islets? FK506 treatment of human islets leads to similar results in human islets as found in mouse islets. ChIP studies in human islets confirm NFAT targets found in mouse. Summary: Cn/NFAT is required for beta-cell maturation.

Beta-cell proliferation reduced in Cnb KO islets. NFATc targets Ccna2, Ccnd2 and FoxM1. Goodyear and Kim 2012. Cyclins A and D2 and FoxM1 expression are all down in Cnb KO islets. So Ca may mediate post-natal period of beta-cell proliferation in response to glucose metabolism leading to activation of Calcinuerin (Cn) dephosphorylation of NFAT.

Does GK regulate NFATc in beta-cells? What are the signaling pathway upstream of NFAT? GK activator induces expression of NFAT and NFAT target genes in a Cn-dependent manner. Idea: Li works to promote proliferation in human islets. Is this due to suppression of Gsk3b, leading to increased nuclear localization of NFAT?

Acknowledged Fernandez for isolation of human islets.

**Short talk – cell cycle regulation with core molecules. Annicotte from France.**

An E2F1/Cdk4/Cdkn2a pathway controlling beta-cell proliferation and function.

E2F1 KO have reduced beta-cell proliferation, leading to impaired GTT kinetics. Kir6.2 is greatly reduced in E2F1KO islets. Adeno-E2F1 rescue of KO islets restores Kir6.2 expression and normal GSIS.

Kir6.2 expression regulated by glucose and insulin but not Dzx, ChIP studies shows E2F1 bound to Kir6.2 promoter in response to glucose or insulin.

Cdk4 inhibitor results in impaired GTT and Kir6.2 expression in islets. New mouse model, Cdk4(R24C) yields to inactivation of Cdk4, leading to uncontrolled beta-cell proliferation. Massive islets which improves glucose kinetics during GTT. Hyperinsulinemia. Upon deletion of E2F1, the Cdk4(R24C) effect was lost, thus, E2F1 required for defective Cdk4 to promote beta-cell proliferation. Islet hyperplasia lost in double transgenic model.

Also did Cdkn2a (p19/ARF/p16/Ink4a) and E2F1 double transgenic; no time, however, to cover results. Will do so at poster #107.

Cdk4(R24C) also expressed in brain.

**Ernesto Bernal-Mizrachi – integration of Pi3K, mTOR and Gs3K in beta-cell proliferation.**

mTORC1 integrates nutrient/metabolic state. Regulation of beta-cell mass as well. Akt signaling appears to integrate several signaling pathways that regulate proliferation, including mTORC tsc1/2 signaling. Mutations in Tsc1/2 induce disease in humans with tumors. Akt phosphorylates Tsc2, leading to activation of mTORC and increased beta-cell proliferation. mTORC1 has raptor; mTORC2 has rictor.

Constiutive active Akt leads to beta-cell hyperplasia. Conditional KO of Tsc2 yields the same. Rapamycin leads to reduced beta-cell proliferation. Rapamycin inhibits S6K phosphorylation. To test mTORC more directly, deleted Raptor in beta-cells, a component of the mTORC1 complex.

Beta-RaKO, leads to reduced S6K. No weight phenotype, fasting, fed insulin and glucose unchanged. No change in GTT at 2mo, 4mo or oGTT. No change in ITT or insulin content in islets. Surprised. Possibilities: mosaic CRE expression, or compensatory mechanisms during development. Crossed mice to Rosa reporter mice, showing good CRE expression. Inducible deletion of Raptor in adult mice lead to loss of Raptor and loss of S6K phosphorylation.

Inducible Raptor Kos, had improved GTT at 5wks and hyperinsulinemia. Surprised again. GSIS in vivo had elevated insulin secretion. GSIS in vitro Raptor induced KO islets have increased insulin secretion. Beta-cell mass increased in Raptor KO islerts, but no increase in Ki67; studying apoptosis in these mice.

HFD challenged mice. This now showed impaired glucose and insulin values in response to HFD. GTT for HF fed mice shows effect. However, have improved glucose tolerance due to elevated insulin secretion. oGTT vs IP-GTT shows improved phenotypes; not due to increased insulin sensitivity. Beta-cell mass increased and not due to increased proliferation as judged by Ki67 stain.

FFA-induced insulin secretion vs glucose dependent insulin secretion. So is Raptor involved in FA-induced insulin secretion? Glucose vs Glucose + palmitate. With palmitate or glucose alone, Raptor KO islets have elevated insulin secretion. However, with glucose+palmitate, insulin secretion reduced in Raptor KO iselts.

Fatty acid effects on insulin secretion. Metabolomic studies with Raptor KO islets with high gluc or high gluc + palmit. Beta-oxidation greatly reduced in Raptor KO islets, however, LC-CoA levels way down in Raptor KO islets. Now this doesn’t make sense.

mTORC1 is not necessary to beta-cell mass and proliferation. Does play a role in beta-cell adaptation to HFD. mTORC1 regulates LC-CoA levels. Speculate that there is a defect in FFA transport or reduced ACSL activity. Have not considered LC-carnitines.

**Short talk – Jeffrey Tessem, Duke, the Role of Nkx6.1 in mediating beat-cell proliferation.**

We in the Newgard Lab have asked whether stimulation of adult beat-cells is a viable option to promote an increase in beta-cell mass in mouse. Gene targets of Nkx6.1 at 48h. Nr4a1/Nr4a3. Orphan nuclear receptors. Deletion induces obesity and insulin resistance. SNPs enhance GSIS. Nr4a’s induce cellular proliferation in non-beta-cells.

Adeno-Nr4a1/3. Effective to promote proliferation at 48h and 72h, whereas Nkx6.1 requires 72h. Think that Nkx6.1 targets Nr4a’s.

Necessary for Nkx6.1 mediated proliferation. shRNAs against Nr4a’s block Nkx6.1 effect. Dom-neg mutant blocks Nkx’s effect as well. So the Nr4a’s are necessary and sufficient for Nkx6.1 to work.

Is there an in vivo phenotype for the Nr4a’s. Nr4a1/3 Kos have loss of beta-cell mass. Ki67 and pH3 stain is also down in Nr4a Kos.

What are the down-stream targets of the Nr4a’s? Microarrays. Ube2c, Ube2s and Cdc20 are members of the APC. E2F1 and Ccne. These induced by Nr4a’s. What is Ube2c? The E3 ligase of the APC, which targets p21 degradation.

Nkx6.1->Nr4a->Ube2c degrades p21; Nkx6.1 also induces Ccne which drives proliferation.

Nkx6.1 also induces p21 as a braking mechanism. However, the increase in Ube2c leads to p21 degradation. E2F1 and Ccne1 expression induced as part of the Nr4a increase leading to increased proliferation.

Nr4a’s and Nkx6.1 do not induce proliferation in aged islets. They’re going to combine our Ezh2 virus with their viruses to ask if this permits their genes to now work in the aged islets.

Translates to humans? Nr4a’s can sporadically induce proliferation in some preps of human islets. Nkx6.1 is ineffective in humans islets. Not sure why?

Adenovirus mediates non-physiological levels of gene expression. Would this yield off-target effects due to such high levels of expression?

**Monday night poster**

Poster from Al Powers’ group, Danielle. Gcg receptor KO mice show dramatic alpha-cell proliferation. I don’t think this is new, as D Steiner showed this many years ago. What they now have is liver-specific Gcg Kos result in alpha-cell hyper-proliferation. Further, if WT islets are put into kidney capsule of liver-specific Gcg R Kos, alpha-cells proliferate. Suggests a hepatic circulating factor responsible for driving alpha-cell proliferation. Using metabolomics of plasma from Gcg R Kos to identify factor(s). Why is it specific for alpha-cells? Not glucagon, b/c whole-body Gcg R Kos still show proliferation. Is it insulin? Is insulin different in WT vs GcgR KO? GcgR antagonist currently in drug trials. Are humans undergoing alpha-cell hyperplasia?

Tuesday AM – reprogramming efforts

**Mimo Acilli – Foxo transcription factor KO in gut cells produces functional insulin producing cells.**

Appearance of bona fide beta-cells in the gut in response to Foxo KO. Ngn3-cre and Foxo flox/flox results in Ngn3+ progenitor pool of cells in gut. Foxo1 ablation results in expanded enteric Ngn3+ cells. This also results in appearance of insulin positive cells in the Ngn3-Cre Foxo Kos.

Pictures of 1d old mice. With age, the relative number of insulin producing cells, diminish, which may reflect reduced proliferation of these cells compared to others. These gut Ins+ cells arise from recombined (i.e., Foxo1 KO) cells. Used a Rosa-GFP reporter to identify these. Also saw Gcg and PP, Syp, Gck, Pcsk2, C-Pep + cells. The entire pancreas appears in the Foxo KO gut cells.MafA, Pdx1 and Nkx6.1 also seen.

Generation of Ins+ cells in adult mice following tamoxifen-induced foxo1 KO. Ins, Pcsk2 and c-peptide cells result. So this can occur in adult mice.

Do they really act like functional beta-cells? Glucose dose-dependent insulin and c-peptide secretion from NKO gut. Gligenclamide and Dzx sensitive insulin producing cells. Isolated gut cells used for this. Acid-EtOH extracts from the gut of these mice can be injected into WT mice and result in lowered blood glucose.

STZ model. FoxoKO result in lowered blood glucose in STZ model, giving glucose of about 250mg/dl for fed glucose levels. Insulin and c-peptide levels in FoxoKO measured 20d post STZ rises, for fed and fasted insulin and c-peptide. Renegernation of gut Ins+ cells post-STZ than pre STZ.

Foxo inhibition in the gut of larger, non-human primates could possibly lead to reactivation of pancreatic program to yield a new population of insulin + cells.

Evidence of active Wnt signaling in NKO gut. Aes mRNA levels up in KO. Model: Foxo represses pancreatic lineage in gut cells. Loss of Foxo de-represses these genes, to reactivate the pancreatic program and possibly used to treat T1D.

Part2: Stages in the progression of beta-cell failure. Islet IHC samples of WT, euglycemic insulin-resistant and diabetic. Increase in islet mass in Ins resistant. Changes in Foxo1 activity during diabetes progression. In early onset of diabetes, Foxo1 goes nuclear in punctate staining, at the same time insulin + cells lost. What is the role of nuclear Foxo in this pathogenic process. Thinks Foxo in the nucleas leads to beta-cell de-differentiation.

Reduced b-cell and increased alpha-cell mass in aging and multiparous IKO mice. InsCre LoxP-ROSA-GFP reporter line for lineage tracing studies. These lineage tracing studies demonstrate that diabetes arises from b-cell dedifferentiation, not beta-cell loss due to apoptosis. Loose MafA and Pdx1 expression. How do they know dedifferentiation rather than de-granulation? EM studies show not degranulated; also no Pdx1 and MafA expression. Pcsk2, Glut2, C-peptide, Pcsk1 and Gck are also lost. So really dedifferentiated beta-cells in response to the loss of Foxo1.

Reactivation of Ngn3+ cells occur with Foxo1 loss; this also coincides with expression of Hdac1, L-myc and loss of MafA, insulin, Pdx1. Hyperglucagonemia in FoxoKOs. Beta-cells transdifferentiate in alpha-cells. Lineage tracing studies again used to demonstrate ins-cre Rosa-GFP to now show these strain for Gcg.

Dedifferentiation in insulin-resistant diabetes; not apoptosis is the main cause of beta-cell failure. Foxo1 is required for b-cell lineage maintenance. Dediff beta-cells are prone to converting into alpha-cells. T2D treatments should aim to restore b-cell dedifferentiation. Inhibition of Wnt and/or Notch signaling might be helpful in this to promote redifferentiation of beta-cell.

This reminds me of the “trans-differentiation” phenom we saw with Phil in the obese mice for CCK-promoter LacZ cells. In lean mice, LacZ predominantly stained alpha-cells. In obese mice, LacZ stained beta-cells. Did the alpha cells become beat-cells? Mimo is arguing that beta-cells dedifferentiate and/or trans-differentiate into alpha-cells with Foxo1 loss. What is the relationship between obesity induction of CCK and Foxo expression?

**Matthias Hebrok – cellular plasticity in pancreas.**

There is plasticity of the beta-cell in the pancreas. Role of pancreatic mesenchyme. Hypoxia in beta-cells.

Mesenchym sits on top of the pancreatic epithelial structure. Does the mesenchyme secrete factors that influence pancreatic endodermal differentiation? Laser capture excision to profile mesenchyme and endoderm analysis to see what genes are expressed. Found 12 secreted factors specifically expressed in mesenchyme tissue.

Does these factor affect expression of beta-cell differentiation in vitro; yes. Induce Pdx1. Nkx3.2;Rosa26LacZ specifically marks mesenchymal cells. So used this system to isolate later stage mesenchymal cells via FACS isolation. Doing this as a function of age and beta-cell challenge, determine what genes are expressed, which are encoding secreted factors and use these molecule in in vitro differentiation protocols. Searching for these molecules now.

Mesenchymal cells surround islets in adult mice; a thin net of mesenchymal cells surrounding the islets. Nkx3.2-Cre;DTR to specifically ablate these mesenchymal cells. PdgfRalpha expression to specifically measure mesenchyme levels. This results in hyperglycemica. GTT intolerant. No change in ITT. Isolated islets for GSIS shows reduction. Doesn’t lead to disruption of islet architecture or loss of beta-cell mass. May have small increase in TUNEL.

PECAM1 to stain endothelial; these do not overlap with mesenchyme. DT does not lead to loss of blood vessels. Ins1/2, Glut2, Kir6.2, Sur1, Pdx1 and MafA all down in DT treated mice. Why is mesenchyme affecting these islet genes?

DT treatment of naïve islets after isolation: this leads to loss of insulin expression. Suggesting that mesenchyme regulates islet gene expression in islets cultured.

Hes1 expression goes up with DTR treatment to ablate mesenchyme. Hes1 is in the notch signaling pathway. Beta-cells have plasticity. Von Hippel-Lindau complex and hypoxia. Under noroxygenic conditions, VHL mediate decrease in Hif1a. Under low oxygen, Hif1a translocates to nucleus and binds to HRE site on target genes.

Hypoxia = Hif1a. What are the target genes? Pro-inflammatory cytokines (Il-1b and Tnf1a) also activates Hif1a in the absence of hypoxia.

Pdx1-creER and VHL flox/flox. Glucose metabolism dysregulated in VHL KO islets. LDHa and Mct4, Pgk, Pfk and Glut1 expression are up in VHL KO. Islets behave as though they are hypoxic, given they lack VHL. In the absence of VHL, Hif1a is stabilized, enters nucleus and induces targets genes.

Ins-Cre VHL flox/flox leads to diabetes in 8mo old mice. Fed and fasted glucose elevated. GTT intolerant. Fed and fasted phenotypes for glucose levels change over time. In 1st several weeks to months, normal glucose; at about 40 wks of age, completely lost. No change in ITT.

Is the beta-cell differentiation state compromised in the VHL mice? Ins-Cre/VHL flox/flox mice. Ins, Glut2, Nkx6.1 and MafA expression all down. Further, Sox9, beta-catenin expression goes up in Ins+ cells. Hes1, Notch1/2, Hey1/2/L, Axln2/ Fcf1, Fcf3, and Tcf4 are all up. Notch and Wnt pathway signaling components UP.

Sox9. Cells having high Sox9 have lower Ins expression. Sox9 expression comes up prior to loss of insulin loss, so not a consequence but may be a cause of dedifferentiation. Sox9 elevated expression precedes hyperglycemia, loss of Ins, Pdx1, etc. Hnf6 parallels Sox9. Is So9 a driver of the sub-set of phenotypes in the VHL mice.

Ectopic expression of Sox9 via Pdx-Cre flox/floxSTOP-Sox9. Sox9 transgenic leads to loss of insulin and GTT intolerant. Does hypoxia affect beat-cell function in culture? Ins-1 cells with 20% O2-> 2% O2 leads to induction of Sox9.

Hypoxia promotes de-differentiation in cultured beta-cell lines. Mouse islets and human islets behave the same way. Hypoxic conditions leads to upregualtion of Sox9 and downregulation of mature beta-cell markers.

Beta-cell 🡪 hypoxia 🡪 de-differentiation. Sox9 comes up and Sst expression comes up, so speculating that beta-cell de-diff into delta-cells.

**Chris Wright, Vanderbilt – lineage interconversion between pancreas cell types with Pdx1 overexpression models**

Positioning Mnx1 as a beta vs delta cell determinant in endocrine lineage diversion.

Lineage competent study of Ptf1a expressing cells. Partial ductal ligation induces mutipotency from adult Ptf1a expression exocrine cells type.

Organogenesis comprises linked sub-modules. Acinar, ductal, endocrine sub-sets. But what about interconversion between beta and delta and beta and alpha? Acinar to beta and vise versa; organ fates: liver and pancreas.

Nat Genetics 2002. Ptf1a distinguishes pancreas from other organ fates. Ptf1aCreER x flox/floxSTOP-ROSA26REYFP

Allows lineage tracking of Ptf1a expressing cells. Add TM at different time points to probe when Ptf1a is expressed and which cell types. Ptf1a is initially multipotent cells to give rise to acinar, duct and endocrine; later Pftf1a is expressed in acinar primarily.

IHC to identify Ptf1a+Sox9+Hnf1b+ cells: these mark the residual multipotency population that can give rise to all 3 compartments – acinar, duct and endocrine. Ngn3+ to mark endocrine; Sox9/Hnf1a to mark duct and Ptf1a only to mark acinar.

“Distant cousin reprogramming” can you convert acinar to beta-cells. Partial duct ligation (PDL) induces expression Ngn3. PDL spares islets, causes loss of acinar, which convert to beta-cells. The acinar cells reprogram to beta-cells via expression of Ngn3. Ptf1aCreERxR26YFPR will label acinar only; never labels islets. 1mo post-PDL find Ptf1a lineage cells in new ducts, as judged by CK19+ stain.

2mo post-PDL, many Ptf1a lineage cells in ducts. Post-PDL 60d you see Ptf1a lineage cells in islets, about 1% of islets label. Some of these Ptf1a stain for insulin. Why so slow to occur and why so few?

Is there a block to cramming more endocrine cells into the islets? Recall the islets were spared from PDL, so they may not have room to add more endocrine cells. What about STZ treatment for PDL model? If 1st STZ and then PDL, you see many many more Ptf1a lineage cells show up in the ducts; very very rare that you find them in islets. So not enough room hypothesis wrong.

New endocrine cell access to islets is “unpreferred”. Adult + PDL leads to Ptf1a lineage cells showing up in the ducts, however, cannot get into islets, but remain near. What about combining Pdx1 overexpression (OE) with PDL model when monitoring Ptf1a lineage tracing study? Call this the Pdx1 boost. This is the Ptf1a-CreERxSTOPflox/floxPdx1/ROSA26GFP.

Pdx1 boost results in more GFP + cells showing up in ducts; ongoing to see if they appear in islets. Induction of anomalous intra-islet sprouting.

PDL many islet become these Ptf1a complex in and near ducts. Ngn3 activated. After a long time, some enter islets. STZ accelerate this process, but doesn’t lead to more of them moving into the islet. Inhospitable model.

Melton: acini +adeno-pdx1/ngn3/mafa 🡪 beta-cells. What controls this plasticity and the relation to Ptf1a.

Inherent plasticity with PDL model without exogenous TFs as in Melton.

**Short talk – Acinar to endocrine reprogramming from Magnusson, Ngn3 reprograms acinar to endocrine. Jud Schneider.**

Ngn3 is a specific marker for endocrine cells. MafA/Pdx1/Ngn3 yields beta-cells from acini (Melton).

What about Ngn3 alone in adult acinar cells. Ptf1aCreERxNgn3/GFP-diallele, drives Ngn3 and GFP in Ptf1a expressing only. Yields acinar specific expression of Ngn3. About 1mo, 80% of cells expressing Ngn3 also express Ghrelin; sst was the next most expressed hormone. Did not ever see insulin stain in Ngn3 + cells.

FACsort GFP + cells to show that Ngn3 induction leads to decreased expression of Amy, Trypsin, Ptf1a and Rbpjl. Ghrl, ChrA, N-Cad and Rfx6 go up. The Ngn3+ cells show increased proliferation via Ki67 stain. Also get increase in vascularization as judged by PECAM stain; co-localize with Ngn3+ cells. Islets change composition: Ngn3+ cells migrate into existing islets. 80% of the islets at 1mo contain at least one Ngn3+ cell.

Overall, Ngn3 OE in Ptf1a cells lead to Ghrl cells (epsilon cell).

No functional studies done yet. All data based on IHC and lineage tracing studies with GFP expression from ROSA line.

Mice don’t have any obvious phenotypes, e.g. appetite, body weight.

OE Ngn3 in Acini 🡪 Ghrl cells 🡪 what’s next? OE of Ngn3 in liver cells yields beta-cells, so the effect of Ngn3 to reprogram depends on what cell type it is being OE.

**Short talk – transition from beta to duct cells. Christine Beamish in David Hill’s lab, Canada.**

What about making beta-cells from pre-existing beta-cells. De-differentiate beta-cells into duct cells, expand and then redifferentiate duct cells into beta-cells.

Whole islets from 7d mice. Dediff beta-cell into duct cells, via promotion of ductal epithelial. Track beta-cell lineage via Ins-GFP reporter. Islet put on collagen coated plates and result in a monolayer after 1mo.

Mature islet markers diminish during this transition in culture. Ductal markers go up (e.g., CK19). 2wks in culture results in no Insulin expression and about 50% express CK19. 1mo in culture stable CK19 expression. In addition to CK19, see Hnf6, Hnf1b, Sox9 and CAII.

RIPCre;Z/AP+/+ islets.

Insulin + stem cells which are Glut2 negative. These appear to be pluripotent beta-cells that give rise to CK19 population in the monolayer. So the CK19 duct cells derive from these rare beta-cells. A mature beta-cell expresses both ins and Glut2. The Ins+/Glut2- cells are the putative progenitors of the duct cells; these considered immature beta-cells.

Glut2 expression defines a wide spectrum of b-cell maturation states.

**Luke Baeyens – Cytokine signaling in beta-cells.**

Acinar cells + EGFR signaling -> dedifferentiate into a beta-cell.

Transplant into kidney capsule can correct hyperglycemia.

Reactivation of Ngn3 mediates the transdifferentiation.

Treat short and long-term hyperglycemia.

Alloxan injection, 24h later, inject EGF+CNTF from osmotic pump.

Long-term, same but 35d between alloxan and oxmotic pump.

TM-responsive Cre models; TM injection 24d prior to study. ElaCreERT-ROSALacZ, RipCreERT-ROSA26LacZ, Hnf1bCreERT-ROSA26LacZ for acinar, beta and duct lineage respectively.

EGF+CNTF treatment of alloxan treated mice lowers blood glucose in about 50% of the mice; the remaining 50% are non-responsive. What are the origin of these cells that underlie the recovery? About 80% of the insulin positive cells, were pre-existing beta-cells.

What about the Ela model which tracks acini lineage? A minority of the insulin positive originate from acinar cells.

Pro-endocrine activation in ALX/EGF-CNTF treated mice. Ngn3, Pdx1 up.

For the chronic model, wait 35d before providing EGF/CNTF, which results in vast majority of ALX mice recovering normal glycemia. What are the origins of these guys? Minority of insulin + cells originate from pre-existing beta-cells. Not beta and not duct cells. So this means they must have come from acinar? Yes; a very high proportion of insulin+ cells contain the acinar specific lineage label. Why the shift from acute vs chronic hyperglycemia model from beta to acinar to origins of beta-cells in response to EGF/CNTF? In the acute model, 50% of beta-cell remain and these may be preferentially rescued by treatment, giving rise to more beta-cells. In the chronic model, >95% of beta cells are killed by ALX, shifting to an acinar population for that which repopulates the beta-cell mass. Rescue beta-cells not killed by acute treatment; reconstitution of beta-cell mass from acinar pool.

Islet architecture in chronic model is weird: alpha cells in the middle and beta cells on outside. In this model, alpha cells left behind in little balls, beta cells coming from acinar dedifferentiation, migrate toward these balls and organize around the outside.

In acute model, those animals that repond to the EGF/CNTF maintained euglycemic out to 35d. The chronic hyperglycemia model was the same, remaining stable out to 150d.

EGF/CNTF treatment of acute and chronic hyperglycemia restores euglycemia. In acute, ins+ cells originate from pre-existing beta-cells, whereas in chronic model, they derive from acinar.

Preferred ALX over STZ.

Monday afternoon workshop#1

8 talks for 2 hrs

**1. Targeting Gpr119 to stimulate beta-cell proliferation.** Gpr119 expressed in intestinal L cells resulting in Glp-1 release. Activating Gpr119 on beat-cells results in insulin secretin. Does the Gpr119 agonist result in increased beta-cell proliferation in vitro and in vivo? Gpr119 agonist for 3d and BrdU O/N for in vitro assay. Insulin/BrdU co-stain. Oleoylethanolamide (OEA). PSN632408. 10uM for both. Confocal images of intact islets and BrdU stain. OEA and PSN compound have increased BrdU+ cells than control.

In vivo model: STZ induced diabetes. Insulin treat for 2 wk and then use WT islets into kidney capsule transplant. Gpr119 agonist for 4wks, put BrdU in water and look for proliferation in kidney graft. OEA treatment accelerates the time needed for the graft to normalize glucose resulting from STZ. 4wks post-transplant, remove kidney containing graft, resulting in rapid increase in glucose. Insulin/BrdU co-stain in islet graft in kidney. OEA treatment increases BrdU/insulin + cells in graft. PSN compound yields the same result. OEA and PSN treated mice have 5-fold increase in circulating GLP-1.

**2. Conditional expression of EGFR in beta cells.** Tyr kinase receptor. Binding of EGFR, dimerizes the receptor and activates MapK pathway and Pi3K pathway. DomNeg EGFR results in reduced beta-cell mass in response to HFD challenge. This work is to activate EGFR in beta-cells and ask if that protects from STZ and result in increased beta-cell proliferation. Inducible E858R-EGFR on Ins-rtTA line. E885R is constituitively active mutant. Dox induced increased expression of E885R mutant receptor. After 1-3mo of treatment, no change in beta cell proliferation of mass. However, 3D reconstruction of whole pancreas suggests increased insulin + volume. However, these mice were not challenged.

STZ model. Dox treated mice were protected from STZ. EGFR mice also had increased survival from STZ. 40d post STZ, beta-cell mass was increased 3x over control mice. Dox induced expression of constit EGFR protects against STZ induced beta-cell death and diabetes.

Have not tried co-treatment with gastrin and EGFR.

**3. Rescue of serca2b expression improves beta-cell survival.** ER important for Ca storage. ER [Ca] is about 250nM, about 200-fold higher than cytosol. SERCA ATPase is a Ca pump transporting Ca from cytosol into ER lumen.

11 isoforms; 3 in beta-cells. Serca2a, b, and 3. B isoform is highest and has highest affinity for Ca. Serca2b is downregulated in islets of T2D donors. Also down in islets from db/db mice. Basal Ca in cytosol is elevated and glucose-stimulated rise in Ca blunted in db/db islets.

IL-1b and high glucose result in reduced expression of Serca2b. GSIS also reduced in response to this treatment. Overexpression of human Serca restores normal GSIS in these Ins-1 cells.

Islets from whole body Serca2b het mice. GSIS is impaired. Adeno overexpression of Serca2b in Ins1 cells. FACS flow of Ins1 cells. Stress treatment of Ins1 cells reduced G2 and increased G1. Ad-Serca2b reverses this effect. Stress dependent activation of Caspace-3 is blocked by Ad-Serca2b. CHOP also blocked in response to rise in TM. Reducing ER stress, and apoptosis, while enhancing proliferation. Loss of Serca2b results in loss of ER Ca.

**4. Mette Jensen Pyruvate cycling regulates Kv2.2 and GSIS.** Pyr-ICD cycling. aKG and NADPH are potentially linked to insulin secretion. Can NADPH regulation Kv channels? Focus on Kv2.1 and Kv2.2 because believed to be involved in GSIS. siCIC and siICD results in reduced expression of Kv2.2. Lipid-induced suppression of Kv2.2 expression. Kv2.1 is unchanged.

Impaired insulin secretion shouldn’t result from reduced Kv2 expression. siRNA against Kv2.2 results in diminished GSIS. Overxpression of Kv2.2 rescues the inhibitory effect of knocking down ICDs. Overexpression of Kv2.1 does not mimic this effect of Kv2.2

When done in islets: ad-siICD results in downregualtion of GSIS, which is reversed by overexpression of Kv2.2 What is the mechanism?

Outward K+ currents are increased in 832/13 cells with lowered ICDs and Kv2.2 expression. If outward currents through K channels are elevated, this would hyperpolarize the membrane and antagonize GSIS.

Thus, one function of the Pyr-isocit cycle is to maintain Kv2.2 expression, which functions as an inhibitor of other K channels. Knockdown of Kv2.2 results in higher outward K current, elevated hyperpoloarization, and reduced insulin secretion.

**5. Role of activin and Fstl3 in alpha cell expansion and transdifferentiation.** Fstl3 KO mouse phenotype – Tgfb superfamily of growth factors activate Actr2b. Fastl3 is a regulator of Activin, A&B, myostatin.

Fstl3KO yield increased islet area. Why. Gene expression analysis of Tgfb superfamily in mouse and human islets. Tgfb2 and Fstl3 is elevated in T2D cultured islets. Where is the high activinA in mouse islets located? Alpha cells.

ActivinA is a ligand for Tgfb receptor. Adeno-activinA overexpression results in increased alpha-cells in mouse and human islets. ActivinB on alpha-cell line increases proliferation. An activinB KO mouse: number of alpha-cell/islet reduced; also have reduced beta-cell mass. Think that reduced alpha cell leads to reduced beta cells due to loss of transdifferrentiation from alpha to beta.

Model: Fstl3 from beta cells regulates activinA in alpha cells.

Many are talking about an alpha to beta transdifferentiation phenomenon. Again, this speaks to what we potentially saw in the CCK-LacZ knockin mouse, where we saw peripheral labeling in lean but beta-cell staining pattern in obese islets. Was that a trans-differentiation or was CCK expression simply switching from alpha to beta cells? I think we would need an alph-cell lineage reporter strain to address this question.

**6. SMAD signaling regulates islet cell proliferation and development**. What is the source of new beta cells? How do we generate more beta-cells from existing beta-cells in patients. Beta-cell self-duplication according to Melton and Dor is the source of new beta-cells. PDL results in beta-cell dedifferentiation into an immature beta-cell, replicates and then redifferentiates into mature beta-cells.

Tgfb signaling pathway. SMAD2/3 required for this pathway. SMAD7 suppresses. SMAD6 also inhibits. However, Pdx-driven SMAD7/6 yield different results in pancreas.

In vitro SMAD2/3 knockdown leads to increased beta-cell proliferation. SMAD7 knockdown gives the opposite results. SMAD2 knockdown in mouse, leads to increased beta-cell mass. SMAD7 KO leads to the opposite effect, exactly in parallel to in vitro work. Partial pancreatectomy between 50-70% removed. pSMAD2/3 is reduced in response to PartPanctectomy, leading to BrdU staining.

PartPanc leads to upregulation of SMAD7 which colalize with PP in the islet periphery. Lineage tracing studies show that these PP cells originally were beta-cells that have dedifferentiated. These SMAD7/PP+ cells are the dedifferentiated beta-cell, they proliferation and then redifferentiate into mature beta-cells.

Should look at SMAD 2, 3 and 7 in the islets from our mice.

**7. Identification of circulating factors involved in beta cell adaptation to pregnancy.** Effect of pregnancy serum on human islet cell proliferation. BrdU incorporation in human islets. Serum from 3rd trimester preg women. Leads to 4x increase in BrdU incorporation. Liraglutide and GH Plus 5% human preg serum gave highest results. 40nM liraglutide and 20nM human GH. Effect was greater at 11mM than 5mM glucose.

Effect of preg women serum on rat islets as a function of gestational age. 34wks preg showed the greatest effect. To identify factor(s) fractional serum from preg women using HPLC and then screened fractions on Ins1 cells. Size fractionation was the 1st used. There were fractions that contained positive factors as well as those that inhibited Ins1 proliferation. Not clear why they moved to Ins1 cells, nor what their assay was. Active fractions were then HPLC and peptides identified via MS/MS. Complement C3 factor was shown to be an inhibitor; this also inhibits insulin secretion. How it is related to C1Ql3?

Positive factors: HM-kininogen, fibrinogen, alpha-1 antitrypsin, apoA1 and angiotensinogen.

Now collecting serum from preg T1D as well as preg T2D and gestational diabetes. Identification of differences may help narrow the list of candidates.

**8. Regulation of beta-cell proliferation perinatlly by osteocalcin signaling.** Relationship between energy metabolism and bone metabolism. Insulin signaling in the bone and osteocalcin signals in islets; osteocalcin is specifically from osteroblasts. Osteocalcin enhances insulin secretion and sensitivity.

Binds to Gprc6a. Upregulates expression of Ins1, Ins2, Cdk4, Ccnd1 and Ccnd2. Long term injections of osteocalcin increases beta-cells mass. Osteocalcin expression occurs at E16, so late in embryogenesis. Hypothesizes that this late onset is ivolved in the beta-cell proliferation burst perinatally.

Hyperglycemia and decreased in beta-cell proliferation in osteocalcin KO mice.

Gprc6a expressed in lung, muscle, islets and min6. Highest in lung and islet. Osteocalcin put on WT islets induces Ins1 expression; this doesn’t occur in Gprc6a KO islets.

PdxCrex flox/flox Gprc6a to give beta-cell specific KO (really panc specific). Gprc6a KO islets show reduced proliferation specifically on beta-cells; not alpha or other cell types. Leads to reduced beta-cell mass and beta-cell area in panc. This causes GTT intolerance. Reduced insulin secretion during GTT.

Tuesday evening – epigenetic regulation in beta-cells

**Jorge Ferrer – long non-coding RNAs in islets.**

 DNA🡪RNA🡪protein as a reminder of the dogma

Non-coding short RNAs: miR, piR, snoR, PARS < 200 nucletodies

Non-coding long RNAs >200 nucleotides.

XIST is a LincRNA that mediate x inactivation.

Eric Lander’s paper knockdown about 100 LincRNAs in pluripotent cells.

John Rinn LincRNA RoR to program iPS cells.

Ignasi Moran in Jorge’s lab has done RNAseq in moue and human islets. Linking non-coding RNAs to beta-cell.

Human islets >90% purity (more than 100 samples now) and done RNAseq on islets and acinar PolyA. Also did ChIP-seq analysis of histone methylation. Catalog of active human islet cell genes.

Example, SUR1 (aka ABCC8). H3k4me3 marks active promoter. Most abundant expressed genes: Ins1, Gcg, Sst, etc. known beta-cell genes.

15% of reads aligned to exons; 66% intronic and 19% non-genic (at least 1kb away from known gene). Yielded 1128 human islet LincRNAs, >200bp in length. Antisense, intergenic, convergent.

Islet lncRNAs are often islet-specific. E.g., lnc12, lnc77, lnc78, lnc94. Intergenic, antisense.

Derivation of human beta-cells from hESCs. See these lncRNAs induced in the functional maturation stage of the ESCs in vivo (put in the kidney capsule).

Are they regulated in the adult islet in response to glucose? Some show glucose responsiveness; all going upregualtion with 11mM glucose. So some are dynamically regulated by glucose, much like coding RNAs, e.g., insulin.

Islet lncRNAs are typically located within large intergenic region. Are they located within these islet specific open chromatin regions? Yes. They are very often located within large domains of islet specific chromatin and adjacent to islet specific genes. Suggested to be co-regulated or perhaps regulating coding genes in cis or trans.

Evolutionary conserved? Is somewhat limited, yet detectable. Much less than coding genes, but more than random sequence. Are they present in mouse islets for that found in human islets?

Orthologous sequences for linc RNAs from humans found in mouse. Did RNAseq in mouse islets. 50% are found in mouse. RNAseq was much deeper in human islets than mouse islets. These orthologous RNAs are also islet specific by 4-5 times.

In mouse developmentally regulated by comparing in fetal pancreas vs adult islets. Neonatal islets vs adult islets. Many are induced upon maturation into islets. Glucose regulated as found in human; not the same between the species, but the phenom is reproduced. Regulation of ob/ob islets are different between ob/ob vs lean.

Li-Lnc25 is specific for islet cells and located in a gene desert adjacent to MafB. Depletion of Lnc25 in human beta-cell (EndoC-b-H1) leads to decreased expression of Glis3 mRNA. Used hairpins against Lnc25 that achieves efficient knockdown of Lnc25. Hnf4a, Pdx1, Nkx6.1, Ki67 were unaffected in response to Lnc25 knockdown. Glis3 is knocked down about 25%. Don’t know the significance. Function like a miRNA.

Human GWAS: Ide-Kif11-Hhex locus is about 300Kb. Many of the SNPs from GWAS are in non-coding regions. Islet Lnc RNAs enriched for variants associated with T2D. MAGENTA. Intergenic Lnc RNAs are significantly enriched for SNPs associated with T2D and fasting glucose. Many of the genes identified as hits for human GWAS, the actual lead SNP falls right on a LncRNA rather than the nearby gene identified.

What are in our region on Chr 17? Is there one near Zfand3?

Fantastic!

Mechanism of action? Vast majority not known. Formation of ribonuclear complexes; decoys for miRNAs.

**Klaus Kaestner – global epigenetic analysis in islets.**

miRNA function be a factor in islet failure in T2D?

epigenetic basis for the apparent transdifferentiation?

epigenomics provide clues for reprogramming?

In human GWAS, genes only explain small fraction of diabetes risk. What is the difference b/t normal and diabetic islets?

Epigenetic changes contribute to the onset of T2D: DNA methylation, histone modification and miRNA profiling as it differs between islets from controls and T2D islets.

Short RNAs 19-25 nucleotides. Argo/RISC complex that regulates target mRNAs.

Determine the miRNA transcriptome of the human islet from healthy and T2D donors. RNAseq of the short RNAs. Total atlas of miRNAs.

What is different between control and T2D samples. Some up and some down in diabetic islets.

7, 655, 656, 487a, 487b, 485, 369 are different.

How to predict targets? Potentially a miR targets 100s to 1000s mRNAs.

HITS-CLIP. Highthroughput sequencing of cross-linked RNAs. Cross linked Argo protein with UV all for miR/mRNA target. IP Argo complex and then sequence miR and piece of mRNA co-IP’ed. This gives you pieces of mRNA that are found complexed with the Argo complex and you get miR sequences. You ask which coincide?

Which are differentially regulated in normal vs T2D islets?

miR-30a was upregulated in T2D isles. This guy targets CPE in the 3’UTR. CPE process pre-pro-insulin to mature insulin. Mutations yield hyperglycemia. Another example, Rfx6 also is targeted by miR-30a. This one is a TF; Rfx6 null lack islet cells. Overexperssing miR-30a in Ins1 cells impairs GSIS.

Model: upregulated miR-30a in T2D islets leads to downregulation of Rfx6 and CPE.

Epigenetic basis for apparent transdifferentiation potential of mature beta-cells. Human islets disrupted, stained for surface markers for various cell types, FACS sorted types for alpha, beta acinar and ductal and then determine transcriptome from RNAseq and Epigenome for DNA methylation.

H3K4me3 = mark of active transcription. H3K27me3 is the repressor mark; these are inactive genes. Gcg and Ins make total sense in terms of which fraction they appear to be active vs inactive. Pdx1 had active marks in beta and alpha and acinar cells (acinar leakage known, but not known in alpha). Why is it active in alpha? However, along with the active histone mark was the inactive histone mark in alpha. The inactive mark was not present in beta cells. This phenom in alpha cells known as bivalent marks and the repressor mark wins. This gene is poised for activation, so once the repressor marks removed the gene is turned on. It is as though the alpha cell looks like a beta cell for the activation marks, however, has the bivalent repressor marks to keep it an alpha. If these repressor marks de-repressed, transdifferentiates into beta cells.

In alpha cells there are about 3000 genes with these bivalent marks. About 50% of these bivalent alpha cell marks remain so in beta cells; i.e., in beta cells these epigenetic marks are much more defined than in alpha cells.

Bivalent in alpha, but repressed in beta cells. Early developmental regulators. Epigenetic plasticity in alpha may underlie the multipotent differentiation potential of alpha cells.

50uM Adox inhibits histone methyl transferase enzymes. 72h culture and then analyze histone tracks. Adox results in bi-hormonal cells that stain for Gcg and Ins.

Genetic lineage tracing with GcgCrexROSA-YFP reporter will show up labeling alpha cell lineage. Then put Adox on these mouse islets and this shows alpha cell lineage that become bihormonal to express Gcg and Ins.

**Anil Bhushan – epigenetic mechanisms that regulate beta-cell identity.**

Pdx-1 🡪 beta cells during embryogenesis, are post-mitotic. And then there is a burst of proliferation and there is a decline in capacity to proliferate as a function of age.

Mechanism of age-dependent decline in potential? How do we rejuvenate the capacity of beta-cells to replicate like they did post-natal.

P16/Ink4a locus is regulated by polycomb genes. Age dependent decreased in beta cell proliferation correlate with p16/Ink4a/Bmi1 locus.

In young, Ezh2 🡪 H3k27me3 on the Ink4a/Arf locus; and then the second complex contains Bmi1/Ring1b Ub H2a-ub.

In aged cells, MLL sits on the Ink4a/Arf locus to activate the expression of this cell cycle repressor. Ezh2 transgenic mice to increase expression in beta-cells to ask if this will rejuvenate capacity to replicate by repressing Ink4a/Arf locus. In 2mo old mice, this yields increased beta-cell proliferation (Ki67), which translates into doubling of the beta-cell mass. P16 levels are reduced in response to Ezh2 overexpression in the 2mo mice. But in 8mo old mice, this procedure does not work. P16 levels don’t change in response to Ezh2 overexpression. What happened in 2mo that doesn’t happen in 8mo mice at the Ink4a/Arf locus?

What about STZ challenge and then overexpress Ezh2 in 8mo old mice. In 2mo old mice, this works; it the 8mo old mice, STZ challenge still doesn’t yield Ezh2 induced proliferation.

Ink4b, Arf, Ink4a locus. ChIP for Ezh2 binding to this locus. In 2mo Ezh2 binds to the locus; at 8mo it is not getting to the locus. But the H3k4me3 mark, 2mo it’s up, in 8mo it isn’t. Hypothesis: MLL is bound in old islets and keep the locus active; this is not displaced even with overexpressed Ezh2. How about knockdown of MLL and Ezh2 OE for a 2 hit? This now leads to increased proliferation, even in islets of 8mo old mice.

MLL needs to be dislodged and Ezh2 needs to be overexpressed. This leads to reduced p16 protein levels. MLL knockdownis not enough alone. Ezh2 is not enough alone. With decreased MLL binding, get increased Ezh2 binding.

Ezh2 overexpression in combination with MLL siRNA knockdown is sufficient to drive proliferation in old MEFs (p13).

What about the Pdgf signaling? Did that lead to increased Ezh2 and MLL knockdown? PDGF leads to MLL dissociation and Ezh2 recruitment. What is the signal that leads to aging? One is the loss of PdgfR expression. Tgfb signaling induces p16. Tgfb leads to dissociation of Ezh2 from p16 locus, whereas it promotes recruitment of MLL to the locus, leading to increased p16. SMAD2/3 binds to the p16 locus, which might be the signaling molecule of the Tgfb signaling pathway.

MLL-JmjD3-Brg1 is the trithorax complex which get recruited to p16 locus by Tgfb singaling, leading to increased p16 expression. If you inhibit Tgfb signaling with inhibitor, causes increased cellular proliferation; this done in aged MEFs. This Tgfb inhibitor leads to about a 3-fold increase in proliferation in aged mouse islets. The Tgfb inhibitor also promotes proliferation in human islets. Called it inhibitor X. Tgfb inhibitor dislodges MLL from p16 locus. Tgfb inhibitor yields increased proliferation in mice as well.

Tgfb🡪 Smad3🡪 trithorax epigenetic activator🡪 p16 locus🡪 reduced beta cell proliferation.

**Short talk – cis-regulatory variation in islet dysfunction and diabetes.**

GWAS hits in non-protein coding regions of the genome for SNPs associated with T2D. GWAS variants alter islet regulatory elements leading to islet dysfunction. Epigenetic approach to see if these regions overlap identified SNPs in the GWAS studies.

Profiled DNAse cut sites and histone modifications for active and inactive tracks. H3K4me3 marks promoters; H3k4me3 marks repressors. Active from poised elements come from different histone modification tracks.

C2cd4a/b/Vps13c locus on Chr 15.

H3k27ac and H3k4me1 show active transcription. H3k4me3 appears to indicate inactive transcription. P300 mediates the H3k27ac marks.

C2cd4a/b and Vps13c locus control region. C2cd4a/b induced by IL-1beta, while the Vps gene is unchanged. Thapsigargin induces the C2cd genes in Min6.

80% of glucose and insulin related traits from GWAS overlap open regulatory regions in human genome in islets.

**Short talk – Neurog3 acts as a polycomb response element in vivo.**

Want to understand the in vivo control of Neurog3 expression during pancreagenesis. Enhancers will provide cell type specific control of gene expression. Neurog3 regualtory elements mapping of enhancers.

Sequence comparison in different species for exons as well as the 5’ promoter region.

1. Does L798 compound effect glucose uptake in 3T3L1 or muscle cells? This would essentially ask if Ptger3 has beneficial or deleterious effects on tissues other than islets.
2. Is Ptger3 expressed and have effects in heart?
3. In vivo validation studies are the next step for our program with Ptger3 to progress toward Pharma partner. What animal is the best one to consider: BTBR-ob/ob, PANIC mice, STZ, rip-HuDTR, NOD, all, others?
4. Working up the cAMP effects of Ptger3 activation/inhibition in T2D islets will confirm mechanism – Michelle wants to focus on this work. This would confirm our model for how Ptger3 is antagonizing GLP-1 pathway.
5. Jin Shang asked for copy of poster; Bei Shan (from Lily) asked for the same thing. Takeda wanted to take pics.
6. Jeff in Phil’s lab will repeat dimerzer study in PANIC mice, this time taking the islets when mice hyperglycemic and asking if Ptger3 expression elevated.
7. Write Jorge next week to make plans for sharing the RNAseq data in mouse and human islet covering our region. Contact Gary Churchill to get advice on how to identify syntenic regions in human for Mouse Chr 17 20-50Mb. Jorge’s reads in human islets much deeper than in mouse islets, so that might be more reliable data on what is expressed within this region.
8. Seung Kim – would like to talk to us about gene regulatory network construction, mouse genetic stuff and our observations with Li to induce proliferation in human islets. Li probably doesn’t simply inhibit Gsk3b. Does Li lead to increased NFAT signaling in response to its dephosphorylation? Michelle has done this as well; did she add all the other things Mary did, or just Li to human islets.
9. People take LiOH for depression. Are these people at reduced risk for developing diabetes, due potentially to increased beta-cell proliferation.
10. Biomarkers was a big deal during the JDRF lunch on Wed. Could we do a non-targeted MS-based survey of plasma metabolites in B6-ob vs BTBR-ob, say 1st at 10wk when diabetic and then at 4wk that precedes diabetes. Presumably, there will be difference as a function of diabetes, but which of these differences are present pre-disease. If we had peaks from the MS that showed this pattern, we could then propose to identify these and characterize them as markers that would predict diabetes. Collaboration with Newgard for something that we could propose to do for renewal.
11. Send Tom Wilke and Andy Stewart the CMAP compound that came from the 3 list search. Several compounds on that hit list were discussed today for their potential to promote proliferation in rodent and human beta cells. Andy agreed to look at my compound list on dissociated young rat islets. Tom is going to ask if they modulate Rgs16 expression in his PDAC cells.
12. Tom is going to send me a list of genes up/down regulated in activated vs non-activated PDAC cells. Upload these to the CMAP program at the Broad to see which compounds may have mimicked the gene expression signatures and which, if any, overlap with that I did for the other lists.
13. Talked to Mike Stitzman in Collins’ lab. Would like to collaborate with mouse islets for DNA histone methylation analysis. Right now they’re looking in human islets, but not as a function of disease state. They are correlating the histone marks with loci identified in human GWAS for T1D and T2D. Could we collaborate to do this kind of analysis on control vs T2D isles at 10 and 4wks of age. Doing it at 4wk would potentially identify epigenetic changes that precede disease state and thus consistent with cause rather than effect.
14. Had a long discussion with Vincenzo Cirollo. Nobody is investigating the genetic dependence the maturation process of iPS or ES cells to form mature/functional beta-cells. Vincenzo can make iPS cells from primary mouse beta cells. Proposal would be to do so from the 8 CC founder stains that represent 90% of the genetic diversity present in humans. These iPS cells would be studied in vitro for their differentiation potential and eventually would be transplanted into CC RI strains to determine the genetic factors mediating differentiation program. Send Vincenzo the GTT curves and photo of the 8 founder strains.

Wednesday AM – Immunopathology of T1D.

**Matthias von Herrath – Novel insights into the immunopathogenes of human T1D.**

3 autoantibodies which appear to be additive for susceptibility to develop diabetes. Do they occur concurrently or is it staged in terms of when the antibodies appear? ICA512, mIAA, IA2ic.

MHC class 1 upregualtion in islets from T1D, also see CD8+ and CD4+ cells. These T-cells infiltrate into islet interior. HLA-ABC staining is also seen.

When looking at whole pancreas, see this phenom occurring in waves, appearing in lobes of the pancreas; not uniformly present.

Is the MHC class I a reflection of a viral infection? This never occurs in T2D, so probably isn’t related to hyperglycemia.

What about the CD8+ cells?

**Jerry Nepon Therapeutic options for T1D.**

Autoimmunity susceptibility🡪autoimmune triggering🡪clinical autoimmunity.

Biomarkers: presence of susceptibility genes; islet auto antibodies and t-cells; metabolic and immune markers.

Immune memory is very persistent; refactory to regulation. Innate responses set the tone of the system. Adaptive regulation is precarious/balanced.

Trials: block inflammation (anti-IL1); ablate or redirect effector populations, re-present antigen in a tolerogenic form; support beta-cell survival/function/regeneration (Glp-1).

Repeat dosing of anti-CD3 (T-cell compartment) antibody. Give for 1wk and repeat 12mo later. This results in a delay for the loss of C-peptide, but following the delay the kinetics of loss is the same without treatment.

Anti-CD20 (B-cell compartment). This results in a delay in the loss of C-peptide, very similar to the therapy directed at T-cell suppression. Following delay, parallel loss in C-peptide as untreated group.

Abatacept - blocks interaction between T and B cells. Same result as two previous. This drug is used in rheumatoid arthritis and is powerful immune suppression.

Immunomodulation is effective, but is not durable; it transiently delays loss in c-peptide. Auto-immune memory may underlie the non-durable nature of these therapies.

Glutamate decarboxylase (GAD) – one of the beta-cell antigens.

IL-2 + Rapamycin treatment, a mechanism to suppress T-cell activity.

Single autoantibody usually does not result in diabetes; however, these people at risk. 2nd antibody pushes you forward and 3rd antibody yields diabetes. So treating the people with 1 or 2 antibodies may be more effective as a therapy than trying to treat the people with 3 antibodies and full-blown diabetes.

14% of people that receive pancreas transplant have recurring diabetes. What was it about the 85% of those that do not have recurring disease? Trying to look into these people to see what was it that allowed them to receive the pancreas without mounting another autoimmune attack.

**Dale Greiner – Humanized mouse for diabetes study; study functional human cells in vivo.**

Mice grafted with functional primary human cells and tissues. Human beta-cells, islets and immune systems. Immunodeficient mice: Prkdc/scid mouse; Rag1/null; Rag2/null. These mice have compromised T-cell and B-cell function. Radiosensitive.

Nude mice lack T-cells, but have B-cells. CB17 and NOD mice have scid mutation inserted. NOD/scid mice are very receptive to human grafted tissue. Mice don’t develop autoimmune reaction, because they don’t have functional immune system.

NOD/scid/IL-2Receptor-null mice. Why is the IL-2r gamma chain so important. IL-2, 4, 7, 9, 5 and 21 cytokine signaling mediated through IL-2R for innate immunity. NOD/scid die within 5-7mos.

NOD/scid/IL2gamma-null and Rag1null/IL2rgamma-null, = NSG and NRG respectively.

Put these models into Akita, RIP-DTR and ob/ob backgrounds.

Grant human islets into mice, GTT and then measure human-specific insulin and c-peptide levels. Can also image human islets within live mice.

Human islets put into Akita/NRG mice result in beta-cell proliferation, about 1%. The same thing with mouse islets would be about 10-15% proliferation rate of beta-cells.

NSG+RIP-HuDTR model. Human DT receptor expressed in mouse beta-cells will result in DT-induced death. If not expressing Human DTR, this doesn’t work. Titrate DT down such that mouse beta-cells expressing HuDTR without killing human islets grafted into kidney. Low dose DT will kill mouse beta-cells without harming human islets.

Allo and Auto-immune response. Three human immune system models established in these mouse models.

Pancreas, thymus and bone marrow all needed to fully establish human T1D model. Marrow for HSC progenitors for T and B-cells. Thymus to “educate” the T/B cells and pancreas in order to present antigen to T/B cells.

**Pere Santamaria – beta-cell regeneration in the NOD mouse.**

A new paradigm in the progression of autoimmunity: the diabetogenic autoimmune response generates pathogenic and counter-pathogenic t-cell circuits for each autoantigenic specificity. Low avidity for specificity (memory autoregulatory); high avidity for specificity 1 (effector). These effector T-cell circulate through body, recognize their antigen and kill cells.

Multiple pathogenic and counter-pathogenic circuits simultaneously. Memory autoregulatory are counter-pathogenic; effectors are pathogenic and outnumber greatly the counter guys. Trying to induce expression of the memory autoregulatory ones, so they are boosted to down regulate the effector T-cells. A cell known as the “professional antigen presenting” cell, educates the T-cells for what antigen they recognize in the body. Expanding the negative feedback regulatory ones, leads to blunting of the activation and recruitment of effector T-cells.

**Short talk – Non-evasive imaging of the NOD mouse, from PO Bergrenn’s group.**

Islet transplants into human recipients results in allo-immune rejection. Pancreatic islet transplantation into the eye. Engraft on top of the iris and become revascularized. If enough islet put into eye, can correct STZ induced diabetes. Due to being put into eye, allows for non-evasive imaging of the individual islets.

STZ treated NOD/scid receiving human islets into eye.

**Short talk – Novel Pdx1 target, Clec16a.**

Pdx1 important for mito metabolism and GSIS in adult islets. Microarrayed adult islets and found KIAA0350 down in Pdx1 het islets. T1D GWAS hit

C-type lectin domain family 16, member A = Clec16a.

Highly conserved to worm, for structural domains. What does it do? Not much known; only one.

Ema, an ortholog in fly, is an endosomal protein. Ema interacts with Vps-C HOPS complex for late endosomes, a gatekeeper for endosome maturation and fusion with lysosomes.

Enrichment of Clec16a expression in islets. Hypothesis: Pdx1 target Clec16a to regulate endosome maturation.

siRNA in Min6 cells leads to late endosome accumulation. siRNA knockdown also reduces GSIS. Using seahorse O2 consumption measure, see reduced mito function with siRNA against Clec16a.

Does Pdx1 regulate Clec16a. knockdown of Pdx1 reduces Clec16a. ChIP show Pdx1 sitting on Clec16a promoter.

Working on a beta-cell KO of Clec16a. All of this started with a microarray of WT vs Pdx1 het islets. What is the relationship between it regulation of endosome function and mitochondrial respiration?

**JDRF workshop on beat-cell regeneration; Wed noon.**

Bushan, Powers, Kim, Dor, Wright, Killian, Sussel, Ferrer, Bonner-Wier, Bernal-Mizrachi, Urano, Hebrok, Stewart, Stein, van Herrath, Rakeman, Greiner, Johnson, others…. (we are the only mouse geneticists in the crowd).

Beta-cell survival and restorative therapies are two primary goals. Identification of biomarkers of early beta-cell stress. Looking at this as a drug discovery program to translate targets into a pipeline for small molecule programs.

Physiological mimetics for beta-cell replication like obesity, etc. Our Ptger3 program would be well suited as a combined therapy for proliferation in combination with Glp-1 therapies. We need to show that Ptger3 antagonism promotes beta-cell proliferation.

**Seung Kim short talk; Ideas about regeneration and biomarkers.** Multiple approaches are important; developmental biology, epigenetics, etc. Not just beta-cells; not just regulation. Emphasis on conserved mechanisms; how to find these? Focus more on human islets and pancreas studies. Cis-regulatory elements are likely to be conserved.

More on conserved mechanisms; look at flies, humans, mice – should more appreciate conservation to indicate importance. Human islets should be more available. Attention to targeting the beta-cell or pancreas; how? Surgical, vascular, pancreas, or islet singularity, beta cell singularity; the group is very very human-centric, which means for us, we have to link our studies back to human islets.

Biomarkers: more molecular markers becoming available all the time. How to assess them? And their use? Molecular biology and biochemistry. Triggers: pathogens, markers of risk or inception of destruction. Outliers: resistance to T1DM of those at risk. Discovery and development of biomarkers of beta cell stress and dysfunction that would facilitate clinical studies and disease diagnosis. Could we do a metabolomics or other kind of plasma survey to look in 4wk old BTBR ob/ob mice to identify markers that are different than 4wk old B6 ob/ob mice which may portend diabetes?

Tension: how much discovery science can be supported without specific deliverables? Juvenile/fetal islets are not widely available, yet can be had. Shouldn’t have multiple cottage-type efforts, rather than broadly distributed. What specifically should JDRF cover? Should be more cross-talk between people that work on T2D and T1D.

**Yuval Dor; T1DM regenerative therapy.**

A drug combination that increases functional beta cell mass in patients while preventing autoimmunity. Spontaneous in rodents, not sure yet in humans. Evidence for human beta cell potential: monogenic PHHI. Human beta cells are one AA away from non-malignant expansion. Productive beta cell replication; embryonic embryogenesis.

1. Age-related decline of beta cell replication; what is the basis for this?

2. Pathways for targeting replication. Cancer can teach us: Men1 induces beta-cell replication in adults. Glucose metabolism is a key endogenous stimulus in mouse and human; druggable (GKA, SU, Calcineurin). Highly toxic; must understand and uncouple expansion/death. Multiple reports showing beta-cell replication that ignore resulting cell death.

3. Endogenous progenitors/facultative stem cells. Why do ducts stop generating Ngn3+ cells around birth? Neogenesis in adult life. Acinar and duct cells biology?

4. Reprogramming. What happens spontaneously and/or in response to injury? Molecular mechanism; triggerable with small molecules; competence in other tissues?

Thomas Edison, “To have a good idea, it is good to have many ideas”.

Therapies, small molecule or biological that promote replication of beta-cells in the pancreas.

**Dale Greiner – When do human beta cells proliferate? What happens to human beta cells in the presence of inflammation during autoimmune destruction? What is the role of environmental factors on non-beta cells in human beta cell proliferation? Are we looking at the right cell?**

Can they proliferate? Age association; fetal do; juvenile may, adults don’t. Can you induce it? Yes in response to chronic hyperglycemia; don’t proliferate, but do incorporate BrdU (need to be careful here).

If we do promote proliferation, how do we stop auto-immunity? Necessary interface between beta-cell biologist and immunologist. nPOD samples.

Inflammation and role for non-beta cell in inducing proliferation of beta-cells. Endothelial vasculature also is lost when beta-cells die. Bone marrow enhances mouse beta-cells recovery from STZ. HSC don’t become beta-cells, but do become endothelial lining of islet during beta-cell replication underlying recovery.

Role of mesenchyme in promoting beta-cell proliferation; are we looking at the right cell for what is needed to promote beta-cell proliferation. Mesenchym, endothelial cells, supporting environment to nurse recovery process.

Rodent vs human beta cell proliferation capacity? Most use young rodents and compare that to older aged human islets. If one used older rodent islets, you will see loss of capacity in rodent as well; so are humans and rodents really that different in their capacity?

Goals from the JDRF for advancing beta cell regeneration:

1. Enhance distribution of human cadaveric islets.
2. Juvenile islets.
3. Embryonic pancreas.
4. Non-human fetal and neonatal pancreas.
5. Methods to isolate populations of pure human beta cells.
6. Unequivocal methods for assessing human beta cell proliferation in vitro.
7. Doing the same as #6 in vivo, both independent of markers.

A – Challenges in translating beta cell biology to therapeutic strategies for regeneration.

B – Challenges in regenerating beta cells in the context of ongoing autoimmunity.

Powers:

1. Mechanisms of funding
2. Science
3. Create resources that move field forward
4. Niche in funding/science/resources from JDRF with limited budget

**Do we have a good target in Ptger3? Anil has shown Tgfb inhibitor promotes proliferation of rodent and human and in mouse in vivo.**

**Wed eve – cell to cell communication Ondine Cleaver.**

Overall aim is to understand an ES cells takes on the step wise series of inductions/decision to become a beta-cell. Directed differentiation of ES or iPS cells. This hasn’t been established yet; why? Is 3D architecture of the microenvironment in the pancreas important; and how do blood vessels influence this cell fate decisions?

Beta cells are born within a tubular epithelial structure in pancreagenesis. Pancreas epithelium has a defined 3D conformation. Pancreas epithelial closely associated by blood vessels. How does the pancreatic epithelium develop? Pancreatic branching displays predictable, but not stereotyped branch formation and ultimate morphology. Branches occur all over the pancreas silmultaneous.

“tip” is the first sign of a branch. Lineage trace these tips, they give rise to the entire pancreas. Branch morphogenesis does not occur by tip extension. Tips don’t link, but the number of tips increase over time. Branches occur with repeated tip formation all over the epithelium. Blood vessels tightly associated with central epithelium between tips. There are microlumes within the branched epithelium. All of this occurs in the embryo during pancreas generation.

Hypothesizes that beta-cell progenitors are located within these microdomains of the pancreatic branches.

Capillaries to endocrine cells during this branching period. Dynamic blood vessel signaling. Candidate blood vessel signals. Microarrays done by Melton and Dor. Looked for secreted or cell-tethered in the vessels and cell surface in the epithelium. Found Ephrins (from aorta) and Ephrin receptor (in endoderm). EphrinB and EphrinBreceptor. EphBR KO (both B2 and B3, double KO): have a branching mutation. They don’t form the pancreatic branches. EphB mutants have reduced E-cadherin and beta-catenin expression. They also show reduced Ptf1a expression in pancreatic buds. All of this results in disrupted islet architecture and impaired GTT. EphB is cell tethered, so mediates a cell cell contact rather than secreted locally.

**Vincanto Cirulli: cell cell interaction and cell matrices.**

Panc1 = human pancreatic cell line.

Gcg and PP cells enriched for NCAM expression. If you disrupt islets, FACs sort beta from alpha cells and then put them back into the dish they will self-assemble into islets with a normal architecture of beta interior and alpha mantle. If you use an anti-NCAM antibody, this self-assembly is disrupted such that the alpha and beta are intermixed in the ball.

Integrins. A very high level of functional redundancy. B1-integrin is expressed all over the pancreas. Conditional KO of b1-int using rip-cre; did rip-cre x rosa26 to verify recombination. Beta-cells now lose >90% of b1-int. Take islets and put them in culture; they don’t do anything, whereas WT islets stick down and spread out over the dish with time. In pancreas islets are smaller and have reduced beta-cell mass with no change in alpha cell amount. Why a lack of beta-cell expansion in b1-int KO?

At E17.5 already see a reduction in beta-cell mass; at P4, even more evident. Run a microarray to identify DE and see enrichment in cell cycle genes. Ccnd1 is reduced and p21 is very high. Why is b1-int required for beta-cell proliferation?

Alpha-tubulin was greatly reduced in b1-int KO; is this involved in driving cell cycle?

Netrins; chemoattractants highly homolgous to laminin. was greatly reduced in b1-int KO; is this involved in driving cell cycle?

Netrins; chemoattractants highly homolgous to laminin.

Catenins, alpha. Pdx-cre results in loss of insulin, gcg, sst, pp, Ghr, and increase in Sox9. The Sox9+ cells show high PCNA, so appear to be upregulated for growth.

Thinks that the a-catenin KO upregulates Sox9 population, they hyperproliferate and then provide a source of new beta-cell precursor. They can rescue the loss of beta-cell phenotype in the a-catenin KO with inhibitors of the hedge hog pathway.

**Peter Zandstra – Endoderm development.**

Tissue engineering strategies to mimic the cell-to-cell interactions that occur during pancreagenesis. Niche related signals in Discher, Mooney and Zandtra, 2009.

Can we control endogenous signaling with local niche engineering?

How can important niche signals be engineered?

What are the key inductive factors that regulate tissue development and disease progression?

Engineering toolbox in high dimensional formats for HTS programs, e.g., microfluidic chips.

Gdf3 regulates SMAD1/3 signaling.

This was the 1st talk that was essentially incomprehensible; at least to me.

**Short talk – from Al Powers group: VEGF-a direct autonomic innervation of the islet.**

Islets are highly vascularized and richly innervated. TUJ1 is a neural marker. Nerve fibers close associated with intra-islet microcappilaries. Shared signaling molecules in “mutual guidance” of peripheral nerves and blood vessels. VEGF-A.

Vegf-a signals both endothelial cells and schwann cells.

Pdx1-cre and Vegfa-fl/fl or the rip-rtTA;Tet-O-vegfa+dox for KO and uprgualtion respectively.

Vegfa KO leads to loss of blood vessels and innervation. Vegfa up results in hypervscularization and hyperinnervation.

More specific innervation classes:

vAchT is parasympathetic nerve fibers. Saw same thing as before.

TH is sympathetic innervation. Vegfa KO increases TH expression. Vegfa up animals lose TH.

TH = tyrosine hydroxylase.

Neural crest derived cells do not express Vegfa receptors in postnatal islets. So Vegfa effects may not be a direct effect on nerve cells.

Vegfr2 or Nrp1, two Vegfa receptors.

Vegfa recruits endothelial cells for microvascularization. Neural crest cells associate with these blood vessels. In Vegfa KO, lose endothelial recruitment. Don’t think the Vegfa effect is directly influencing neural crest cells.

**Short talk – Glial derived neurotrophic factors, David Cano.**

Neural crest migration in mouse islets. Nerve cells migrate into the pancreas around E10 during pancreagenesis.

GDNF, glial cell line derived neurotrophic factor. Binds NCAM. LacZ GDNF knockin mouse line.

E11.5 see GDNF activation. Co-localize with Ngn3+ cells, but not with acinar markers in early pancreas. In P7 pancreas, GDNF not expressed. So involved in pancreatic development.

Pdx-cre and Gdnf-fl/fl. Does not yield a phenotype in islet formation. However, does result in loss of neural crest migration. Islet innervation is also lost. TH not effected. vAChT is lost, so specific for parasympathetic innervation.

Gdnf activation peaks at E12.5 and E15.5 in embryo. Gdnf is a chmoattractant factor for neuronal precursor cells in pancreas. Inactivation of Gdnf in pancreatic epithelium abolishes migration of neural precursors.

Gdnf is secreted in early pancreas and is a trophic factor for migrating neural crest cells involved in parasympathetic system. So Gdnf is expressed in endocrine precursor domain.

GTT in Gdnf-PdxCre mice: no phenotype at 3.4mos, however, improved GTT kinetics at 6.5mos.

Thursday morning –

**Bridget Wagner from the Broad; HTS for small molecules in Ins1 cells and human islets. Ins1 cells for protection from apoptosis and human islets for replication.**

Small molecule that increase beta-cell proliferation. Human islets, BrdU markers. Screen for proliferation, transdifferentiation, and protection from cytokine-induced apoptosis. Will talk about this last effort.

Tnfa, IL-beta, IFNgamma converge on NfKb and stat1 signaling that lead to cell death.

Histone deacetylase inhibitors protect beta-cells from cytokine induced apoptosis. Looking for which HDAC isoform is the target.

Ins-1 cells: ATP levels, caspace3 activity and nitrite secretion are all modulated by cytokines. Cytokines lead to a drop in cellular ATP by about 50%. Measure ATP content in cells in response to cytokines with and without screening compounds. Screening 300K compounds, 6B cells, 30L media, 2000 384-well plates. Ran compounds in duplicate.

Found hits that did better than a pan-HDAC inhibitor. Took some of the hits and did focused SAR. Compounds selective for cytokine induced apoptosis; didn’t suppress apoptosis in response to glucose, thapsigargin. Effective in human islets in response to cytokine effects on apoptosis and GSIS.

OK, have a compound with good potency, selectivity and cross-reactivity with human islets. What is the target? Gene expression analysis, determination of binding partners, RNAi screening modifier and computational predictors.

Gene expression profiling in Ins-1 cells with cytokines with and without compound. Used CMAP to look for other compounds that mimicked transcriptional signatures. Found interferon-gamma responsive genes set. Compound alone results in only 10 genes changing expression. Compound blocks the change in gene expression for many in response to cytokine.

Compound blocks cytokine induced stat1 phosphorylation. Is it a JAK inhibitor? Tested the compound against a panel of kinases, including JAK1 and didn’t change anything, so not a JAK kinase inhibitor.

Determination of binding partners. Used SILAC and modified the compound with a linker group attached to a primary alcohol on one end. PEG-amine linker moiety, which retains activity to block cytokine effects. SILAC; light and heavy media with and without small molecule with linker. Did this and found a candidate list of 17 peptides enriched for binding to the molecule. Usp9x found to bind the compound. siRNA against Usp9x mimicked the effect of the compound on blocking cytokine induced apoptosis.

Deubiquitinating enzyme Usp9x, aka DUBs. So a DUB enzyme when suppressed, blocks cytokine induced apoptosis. That suggests that cytokines are promoting the ubiquitination of something involved in cell death. What is ubiquitinated? Did thermal binding assays to show Usp9x directly binds their compound.

Usp9x stabilizes MCL1 and promotes tumor cell survival.

siRNa modifier screening. Will focus on the DUB class of genes and ask if these knockdowns protect cells from cytokine induced cell death. If so, do they mimic the compound effect such that added compound doesn’t add. Cytokines increase the proteosomal activity of cells. Compound does not affect proteosomal activity.

Summary: phenotypic cell based screening approach to a specific enzyme for further discovery.

**Bryan Leffitte, – Novel therapeutic approaches at GNF; Novel therapeutics for T1D. 2.4M compound library.**

Also did a protection from cytokine induced beta cell death. Identification of molecules that promote proliferation.

RT71 beta-cell line. Take hits and then put them on mouse islets. Rip-DTR model for compound and human islets put indo kidney capsule of NOD/scid mice.

Diarylurea series. EdU assay in rat islets co-stained with insulin. Compound stimulates 3-fold increase in proliferation with about 1uM in vitro. Did in vivo with rip-DTR model and then inject compound into mouse. Compound improved GTT and fed glucose levels. Results in increase in Ki67 stain at 10d but not 42d. Compound was not very effective in human islets, so dropped.

Another compound was identified that was effective in human islets. Dissociated human islets 1-10uM compound yields increased Ki67 of insulin+ cells without disruption of function. Human islets treated with compound for 6d. Took 2000 islets and put them into kidney of NOD/scid. Compound treated 2000 much better at correcting STZ glucose than 2000 of non-treated.

Target id? Gsk3b inhibition is effective to promote proliferation in rat and human islets. Gsk3b phosphorylates TF beta-catenin and actiation of the Wnt pathway. Found compound to be Gsk3b inhibitor. Compound results in nuclear beta-catenin. Overexpression of Gsk3b with Adeno blunts effect of compound in rat islets.

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Want something that does not activate the Wnt pathway. Have other compounds that promote EdU incorporation into rat and human islets. Don’t know if this EdU is actually proliferation though. Compounds also lead to reduction to Casp-3 activation. Currently making linker compounds for pull-down assays to identify target(s).

Compounds improve GTT kinetics with rip-DTR in response to DT. Resulted in increased Ki67 ins+ cells.

Also screened for compounds that can protect from cytokine induced cell death. ATP levels used for assay. Tested in vivo in multiple low-dose STZ models. Compounds protect beta cells from STZ killing.

**Yuval Dor – Glucose control of beta-cell replication and survival:**

Can beta-cell regenerate from a diabetogenic injury? Cellular origin of new beta cells in adult life? And Singals that control beta-cell regeneration, mass?

Blood glucose via b cell glycolysis is a key physiological regulator of b cell replication beta cell mass homeostasis.

Glucose🡪GK🡪ATP🡪membrane depola🡪 Ca influx🡪 Calcineurin🡪 NFAT🡪beta cell replication.

When insulin secretion insufficient to maintain normal glucose, beta cells sense a chronic increase in rate of glycolysis and some beta cells switch from G0 to G1 and replicate. Drugs that activate GK should enhance glycolysis as a means to boost insulin secretion; double % replicating beta cells.

GK activator injected into mice makes them hypoglycemic and results in 2-fold increase in Ki67 in islets. In humans, PHHI is a mutation in GCK have large well-structured islets that are very large. Persistant hyperinsulinemic hypoglycemia of infancy, necessitating pancreas removal.

In PHHI patient there was increased Ki67 of Ins+ cells. Also see increased TUNEL; net effect is a hyperplastic islets.

Mouse model of PHHI disease. Active mutant of GK-STP-fl/fl and then conditional inducible cre to remove STOP sequence. ROSA locus, so get weaker expression than RIP promoter. ROSA locus yields about 2-fold increase in expression of mutant GK.

Seahorse shows increased O2 consumption of mutant islets. Doubling Ki67 in mutant islets. Reduced phopho-AMPK. Hypoglycemia transiently occurs in these mice, however, then animal revert and become hyperglycemia. 4d after transgene induction see increased TUNEL resulting in reduced beta-cell mass. If they use rip-Cre, get a much more dramatic phenotype resulting in complete loss of beta-cell mass due to high activation of mutant GK.

GKA dose drugs into mice results in increase in % Ki67 in beta cells. Put in food, don’t see any increase in beta cell mass. Clinical trials suggest only a transiet improvement of diabetes traits. GK activity is both mitogenic and cytotoxic; why?

Mechanisms of GK toxicity. CHOP levels go up in GK mutant islets, suggesting severe ER stress. However, p-H2AX levels go up, suggesting DNA damage. Upregulation of p53, which is executor of cell death. P53 is the tumor suppressor. GK activation results in oxidative stress, which leads to loss of MafA and insulin. Can we rescue GK induced beta cell toxicity? Yes.

Conditional allele for p53 crossed into GK mutant mice: Ins-Cre-GK and p53-fl/fl partially prevents the hyperglycemia and preserves islet mass. P53 deficiency prevents death of transgene+ beta cells. However, these rescued beta-cells are not functional.

 About GLP-1 pathway to relieve ER stress resulting from GK activation? Liraglutide – eliminates CHOP stain, reduced DNA damage and partially rescues apoptosis resulting from GK activation. Liraglutide improves islet function in GK islets. Suggests a combination approach with GLP-1 agonists and GK activators.

pH2AX levels were up in the pancreas of PHHI pancreatic sample.

So GK activation results in a pro-beta cell replication pathway as well as a death pathway via oxidative stress. GLP-1 pathway may be a way to suppress to the toxic piece.

P53 is induced in the islets of db/db mice. So it may be that p53 mediated DNA damage and cell death is the basis for glucotoxicity.

Similarity between T2D and PHHI? Initial event is increased glycolysis. Increased secretion, beta cell replication. Reduced blood glucose. Beta-cell decompensation.

Does the GLP-1R expression change in response to GK activation? GLP-1 has been shown to increase GK expression. Not sure if the receptor changes in GK mutant.

Working with Keastner now to get a knockin of the mutant GK using the normal regulatory pathways for GK expression and to yield a null for WT GK allele.

Can metabolic stress result in direct DNA damage? Hyperglycemia appears to result in p53 induction and p-H2AX levels. Is this a direct effect or indirect.

**David Hill – Pathways to regeneration. Statins, augurin and immunization with mycobacterial adjuvant.**

Bone marrow progenitors that are Cd4+/cKit+ were injected into STZ treated mice resulted in improved glycemia and increased beta-cell mass. The marrow derived cells surrounded islets to nuture recovery, but did not themselves become beat-cells. Cells instead differentiated to endothelial cell characterstics. Think this was the mechanism behind transplanted marrow effect. What about endogenous marrow cells, following STZ damage to host beta-cells.

Host marrow cells also invade into the pancreas and surround the islets. They appear to accumulate around the islets during a recovery period following multiple low dose STZ treatments. None become insulin+, however, they do turn into endothelial cells, much like what was seen for the transplanted marrow cells.

The cells also become macrophages. But they stain for Pdx1, but never for insulin.

Statins: they increase circulating endothelial number. Increase EPC differentiation. Inhibit T-cells. Statins have been shown to delay the onset of diabetes in response to STZ treatment. This effects appear to be independent of HmgCoA suppression. Statins delayed the loss of beta-cell function in newly diagnosed T1D patients as judged by c-peptide levels. What are statins doing in beta-cells?

Gavaged mice with statins that also got STZ injection. Statins increased PCNA+ cells in islets and increased beta-cell mass. Done islet size measures and see effect in the small class of islets. Statins improve GTT curves. Angiogenesis and beta-cell mass increased in response to statins in normal and STZ treated mice.

Mycobacterial adjuvant: Complete Freund’s Adjuvant (CFA), blocks diabetes in STZ mice. CFA injection upregualtes Reg2 gene and protein expression. Reg2 mRNA and protein in the pancreas of prediabetic NOD mice following CFA treatment. Reg2 is the only one up; not other isoforms. Reg2 IHC shows in beta-cells.

Amplification of CFA-induced Reg2 in pancreas by STZ.

What is this doing to host gut microbiome?

What is the relationship between CFA and Reg2? Autoimmune damage to beta cells is delivered by both Th1 cells and Th17 cells. Subset of highly inflammatory CD4+ T-cells. Cytokines induce these guys, which leads to IL-17 and other ligands.

IL-22 mRNA expression in the pancreas or spleen of pre-diabetic and diabetic NOD mice following treatment with saline or CFA. Ability of IL-22 to regulate Reg2 expression in isolated islets. So CFA🡪IL-22🡪Reg2; things other than IL-22 are also involved, as IL-22 alone only partially mimics effect of CFA.

Phase I trial completed using BCG vaccine to treat subjects with established T1D with the goal of reducing auto-reactive T cells. BCG is similar to CFA. Pro-biotics? Microbiome. Interact with mesenteric lymph nodes leading to activation of T-cells to stimulate IL-22?

Ecrg4 🡪 leads to Augurin protein. Normally tethered to cell membrane, but can be cleaved by proteases. Ecrg4 highly expressed in pituitary, adrenal and pancreas. STZ treatment results in reduced expression in islets. CFA induces expression of Ecrg4. Downregualted in islets of diabetic mice.

Augurin is found in alpha-cells; is also in duct. STZ leads to loss of Augurin in alpha-cells. In islets isolated from NOD, Pdgf induces expression of Ecrg4. A sentinel that signals inflammatory mechanisms? A dynamic beta cell suppressor removed consequent to beta cell injury?

**Fred Levine, Sanford-Burnham,San Diego Induction of beta-cell replication by Hnf4a antagonist.**

Diabetogenic actions of antipsychotics that are Tgfb angonists.

Did a HTS looking for molecule to stimulate insulin expression. Found one that was the target for this compound. Binds to the LBD of the Hnf4a crystal. Is the MODY1 gene.

Hnf4a- compound binds with kd of 12nM. FA’s inhibit insulin secretion and inhibit Hnf4a and insulin expression. Think that FA’s bind the LBD domain of Hnf4a.

Hnf4a antagonist induces beta cell replication in 4mo old mice. Compound inject daily for 14d with BrdU in water over same time. Beta-cells show replication, whereas alpha and delta cells don’t replicate. Compound induces the replication of alpha, delta and beta cells when coupled with STZ treatment. Islet architecture is not preserved however, as the cells are all mixed up.

Mechanism? p16/ink4a levels down with Hnf4a antagonist, as well as p57 and p21. These done in vitro with human cell line. What about in vivo? In 4mo old mice, high levels of nuclear p16; with compound nuclear p16 levels go down.

Human data. Not yet.

Rabbits. Injected into rabbits and get BrdU increase in acinar and islets.

Potent Hnf4a antagonist. Inhibit CDKI’s including p16. Induce beta-cell replication in vivo in mice and rabbits.

Compounds is highly metabolized in liver, so don’t see much of an effect of the compound in liver. There is a little hepatosteatosis.

Hnf4a KO have the opposite phenotype. So why is this antagonist giving increased beat-cell replication. Embryonic effect in Kos, whereas, if you do conditional KO (at least in liver) you don’t see this. So the difference may be embryonic development vs adulthood. Jorge Ferrer has done Hnf4a KO in adult islets and don’t see increased proliferation; suggest that this compound may be targeting something else to underlie the effects on proliferation.

**Olav Anderson – chemical screen in zebrafish idenfies adenosine signaling in beta cell regeneration.**

Beta cell regeneration model using tissue specific expression of nitroreductase (NTR). In the fish, there is only one islet; if treat with NTR the islet is killed. Screen by adding drugs to water and look for regeneration following NTR challenge. 10K compounds.

Zardaverine, cilostamide. These compounds inhibits PDE3-4. I think these compounds were identified in my query of the broad CMAP database. Further evidence that approach might work to identify novel compounds. Did it also identify adenosine signaling compounds? Need to return to that lsit of compounds and ask if we should test some of the novel compounds for their ability to promote beta cell proliferation. We need a positive control compound, like Forskolin and/or Pde inhibitors.

Adenosine signaling compounds also identified. Adenosine receptors module cAMP levels. NECA is the compound.

NECA compound is the best adenosine receptor compound found so far. When they put this on the fish with EdU, they get doubling of proliferative rate in the islet following NTR treatment during regeneration.

Morpholino knockdown of the adenosine receptor, A2aa, the effects of NECA is blunted.

NECA has been shown to improve glucose in STZ treated mice. The same thing has been seen in NOD mice. Both of these effects were attributed to anti-inflammatory effect, not beat-cell replication

Mice islets in vitro + NECA leads to increased Ki67.

High STZ injections + NECA lowers glucose and prevents them from dying. NECA treatment in STZ mice have increased beta-cell mass and proliferation Ki67+/Ins+cells.

Effects were mediated by A2aa receptor.

NECA and Cilostamide appear to be synergistic.

Receptor activation leads to AC activation, cAMP production and beta-cell proliferation. Cells secrete ATP and become adenosine outside. Does the A2aa receptor KO have a beta-cell replication phenotype?

Thursday afternoon workshop #2: Beta cell survivability

**Brandon Taylor from Sander lab; Nkx6.1 is required for beta-cell function and maintenance.**

Beta-cell specific KO of Nkx6.1. Pdx1-cre TM inducible. This results in elevated blood glucose as early as 1wk after TM. At 2mos blood glucose is about 500mg/dl. Impaired GTT at 1wk. Insulin values during GTT have essentially no insulin secretion.. IHC shows huge loss of insulin staining in islets of KO. Number of beta cells appear normal, but amount of insulin expression 90% down. Ins1/2 expression normal. Proinsulin and insulin protein levels down, so post-transcriptional regulation in response to loss of Nkx6.1.

Microarray of WT vs KO islets. Insulin processing genes down. PC1, Ero1b and Slc30a8 all down. Ccnd2 down; Ccnb1, 2 and a2 not changed. Ki67 down. Is the effect of Nkc6.1 loss on Ccnd2 direct or indirect? Glut2 down in Kos, so this may lead to a loss of glucose uptake and glycolysis. GKA and BayK compounds to try and restore Ccnd2 levels. GKA did not rescue, but this may reflect poor glucose entry due to loss of Glut2. However, BayK compound did restore Ccnd2 levels in KO islets. Suggests that Nkx6.1 effect is indirect.

**Ja Young Kim-Muller from Mimo’s lab – Triple Foxo KO in beta cells.**

The role of Foxo in making and regulating beta cell function. Intermediate glucose levels leads to nuclear localization of Foxo. Nuclear Foxo leads to beta cell death.

Foxo1,3,4 fl/fl x rip-Cre. All 3 because of functional redundancy and compensation among them. 4-5mo old KO mice. Mild hyperglycemia under both fasting and fed conditions. 3 Ko have impaired GTT kinetics; no change in ITT. Due to normal insulin secretion and not whole-body insulin sensitivity changes. Clamp studies: reduced GSIS in vivo. Glibenclamide injection or Arg injection into mice and have insulin curves for each. 3KO mice have reduced insulin secretion for both. This is nice and shows in vivo secretion potential to these segretagues.

Isolated islet studies: 3KO islets secretion reduced insulin in response to glucose and KCl. IHC studies to look at islet architecture. Glut2, Kir6.2 and Sur1 down in Kos. Microarray WT vs 3KO islets. V-gated Ca channels, PKc and IP3 and PLc all down in 3KO islets; all are Ca dependent proteins. Tested BayK, PMA, Forsk, IBMX and Carbazol. BayK, PMA and Glibenclamide all down in Kos. Forsk, IBMX and Carb unaffected.

**Archana Kapoor - MafA expression is sufficient to overcome problems in Pdx1 haplo-insufficient mide.**

Pdx1 heteros have impaired GTT response. TetON promoter for MafA-myc introduced into Pdx1 hets. If you drive MafA embryonically, you don’t get a panc. Turn on MafA expression with DOX right at birth, you can then reverse the dysfunction caused by Pdx1 het. Dox-induced MafA-myc expression 2-4-fold in islets. If pulse the Dox from birth to 1mo, sufficient to restore insulin expression to normal levels from that reduced in the Pdx1 hets. MafA overexpression enhances Pdx1 expression.

**Brian O’Sullivan - WFS1 variant enhances beta cell viability and function. From Fumi Urano.**

ER stress 🡪 UPR, if mild survival; if sever and chronic, dysfunction/death.

ATF6, Perk and Ire1 mediated the UPR. Inverse relation between ER stress and beta-cell viability.

WFS1 (DIDMOAD): diabetes, optical atrophy, renal, ataxia, and other symptoms.

>100 mutations in WFS1; R611 if His-11 are susceptible; if Arg-611 are protected from developing diabetes.

WFS1 localizes to beta-cells to ER or secretory granules. Alleviation of chronic UPR conditions; coregulation of TF involved survival; granular acidification and maturation of secretion and SNARE complex formation for secrtion.

Studied Arg and His variants at 611 position and overexpressed these in Ins1 cells. Viability and function studied. Dox-induced WFS1 overexpression and thapsigargin treat for ER stress. Did a FACS study for mitoprobe stain (for mito function) vs PI stain for apoptosis. H611 is protective while R611 is even more protective.

WFS1 overexpression lead to increased GSIS and higher insulin content; no difference between the two alleles. Microarray Ins-1 cells expressing one or the other allele.

Netrin-1 signaling. R611 has a higher induction of this gene than H611 allele. Netrin-1 expressed in fetal and panc rengeneration follow PDL. Netrin-1 prevent caspace activation in Ins1 cells.

WFS1🡪Netrin-1🡪anti-apoptotic

**Luke Berchtold - Integrative approach identifies Panx-2 as antiapoptotic gene in beta-cells.**

Integration of GWAS data with protein-protein interaction networks.

Identified in human islets: Cd276, Panx2, Cxcr7, Cd83, IL27ra, Ccr7

siRNA against Panx2 leads to increased cytokine induced apoptosis.

Pannexins have structural similarity with the gap junction family, the connexins. Pass molecules upto 1kD.

**Young-mi Song – Inhibition of autophagy by increased ER stress via glycated albumin induced ROS apoptosis.**

**Mark Huising – Urocortins promote beta cell survival and can prevent STZ induced diabetes.**

Urocortins are small neuropeptides. Belong to the family of CRF, Ucn1,2,3. Activate family of GPCRs. CRF added to islets promotes GSIS, cAMP, essentially everything that one gets from GLP-1.

Beta-cells secrete Ucn3 in response to glucose. Ucn3 colocalizes with insulin only.

Mip-H2b-mCherry allows one to FACS sort beta cells from primary islets. Used these to show co-expression of insulin and Ucn3.

In human islets, Ucn3 co-label alpha cells.

Stimulation of CRFs induces neonatal beta cell proliferation. These at 7TM GPCRs. Ucn’s promote survival in beta cells and human islets by inducing Akt-phosphorylation and protect from cytokine induced apoptosis.

Beta cell specific inducible mouse for Ucn3. Multiple low dose STZ model. Induction with Dox for Ucn3 transgene is complete insensitive to STZ induced diabetes.

**Jung - Suppression of Autophagy.**

Auto = self; phagy = eating. Used to maintain cellular homeostasis. Type 2 programmed cell death. Role of autophagy in beta-cells. Crinophagy from Rhodes.

Autophage deficient mouse model. Atg-fl/fl mouse. Is autophage mediated by ER stress? Yes; sometimes called ER-phagy.

In autophage deficient beta cells ER stress is elevated and beta cells die.

Perk deletion induces diabetes due to elevated ER stress. Perk het mice are only slightly glucose intolerant in GTT. When Perk hets are cross with Atg7KO, glucose GTT improved.

Thursday eve plenary – maturation and maintenance of beta cell function.

**Lori Sussel – induction and maintenance of beta cell fate identity/maturation.**

Specification and maintenance of cell fates into the variety of cell types in the pancreas. Lineage decisions made that are not irreversible; there remains high degree of plasticity.

Transcriptional regulation of cell fate decisions. Endo🡪 liver or panc🡪exo or endo🡪alpha or beta.

Nkx2.2 expressed at early pancreas progenitor stage with Ptf1a/Pdx1+cells. These cells lead to Ngn3+ cells and then the various endocrine cells of the islet.

Nkx2.2 promotes alpha and beta and suppresses ghrelin differentiation. Nkx2.2 KO have no beta and no alpha and replaced by ghrelin population. Nkx2.2 can function as repressor or as an activator during different stages of panc development.

Protein structural domains and how they are involved in these different lineage processes. Domains: TN, DNA binding domain, NK domain. TN = tin man domain that mediate interaction with groucho proteins to mediate transcriptional repression.

Made mouse with Nkx2.2 with inactivating mutations in TN domain. Mice live, but have greatly reduced number of beta cell and hyperglycemic. Pathway: Pdx1🡪Ngn3🡪sst, gcg, ins, ghr or pp cells. In Nkx2.2-TN/TN transgenic, large increase in ghr cells and loss of beta cells; also see increase in gcg cells. Were surprised to see increase in alpha cells.

Arx goes up which is a TF for alpha cells. Master regulator of alpha cells. Ngn3🡪alpha if Arx is on; if Arx off goes to beta. If beta cell miss-express Arx, transdifferentiate into alpha cells. In Nkx2.2-TN/TN, Arx is upregulated in beta cells, which become alpha cells. Nkx2.2 normally suppresses Arx expression via the TN domains.

 Nkx2.2 recruits a repressor complex to the Arx promoter to normally suppress Arx suppression in beta cells. The TN domain on Nkx2.2 is essential for this recruitment. Lineage tracing method to show these alpha cells in Nkx2.2 mutant had a beta cell lineage. Ins-Cre;ROSA:Tomato. You see Tomato stains in Gcg cells in Nkx2.2 mutant.

Beta cells transdifferentiate into alpha cells. Is the reverse true? If you shut off Arx, does an alpha cell become a beta cell? An Arx-fl/fl mouse was used with rip-Cre (normally Arx isn’t expressed in beta cells). In this system, no longer see the conversion of beta to alpha in the Nkx2.2 mutant. However, this still did not rescue the beta cell number. What about doing the alpha-cre with the Arx-fl/fl allele; do these become beta or something else?

TN groucho interaction domain is important in the beta vs grh cell choice. What about the NK/SD domain? Another knockin mouse with a mutation in the NK/SD domain. These mice were severely hyperglycemic. Loose beta cells and alpha cells and see strange ductal morphology. Alpha cell numbers remain unchanged with this domain mutation. Ghr cells did transiently increase. Co-labeling of the different hormones showed PP/Gcg/Ins or Sst/Gcg/Ins in one cells. These polyhormonal cells were seen as early as E12.5, with about 30% of the cells expressing multiple hormones. Overall, a general confusion called “schmendocrine” cells.

NK/SD domain also involved in beta and ghr cell lineage decision. However, also involved in maintaining islet cell identity.

Model: progenitor🡪pre beta-cell🡪beta-cell during this process the DNA methylation increases. Cellular plasticity increases with reduced DNA methylation.

Reprogramming event: In Nkx2.2-TN/TN mutant, the mis-expression of Arx reprograms a beta cell to become an alpha cell.

**Roland Stein – Islet transcription factors are principle targets of oxidative stress in T2D.**

Loss of TFs under oxidative stress lead to beta cell dysfunction. Hyperglycemia🡪ROS🡪loss of TFs and then loss of MafA, Pdx1 and Nkx6.1.

Oxidative stress 🡪 principle mediator of beta cell dysfunction. TF signature in response to this stress. H2O2 are very destructive at high concentrations.

Glutathione peroxidase expression in beta cells rescues hyperglycemia in db/db mice. In the diabetic db/db mouse, MafA is lost in nucleus of beta cells. When they neutralize ox stress with GPx, MafA returns to the nucleus in beta cells of db/db mice.

Develop a beta cell line to recapitulate this phenotype with a beta cells containing cytosolic MafA. Ox stress induces MafA translocation to cytosol. MafA is heavily phosphorylated, 20-25 phosphorylation sites throughout protein. In N terminal Gsk3b mediate phosphorylation. Beta cells lines exposed to 50uM H2O2, MafA becomes dephosphorylated which is blocked by oakadaic acid. When dephosphorylated, MafA translocates from nucleus to the cytosol. Foxo1 does the reverse movement upon ox stress. Dephosphorylated MafA does not dimerize, so can’t bind DNA, which it only does with homo-dimerized.

However, Oakadaic acid blocks dephosphorylation of MafA, but does not block its movement from nucleus to cytosol in response to ox stress. Site direct mutation of all the phospho sites does not prevent its movement to cytosol with ox stress. PP2a results in the depho of Foxo1, which also dephospho of MafA. Are all of these candidate phosphor-proteins substrates for Cip2a binding.

Ox stress also stimulate translocation of Nkx6.1 from nucleus to cytosol. Model: Ox stress🡪 MafA out🡪 Nkx6.1 out. But Pdx1 does not get pushed out; ChIP for Pdx1 or MafA in response to H2O2. Loss of DNA binding under ox stress for both of these TFs. Pax6 is not effected by ox stress.

What about nucleus/cytosol location of Nkx6.1, MafA, Foxo1 in db/db islets. Nkx6.1 and MafA get excluded from the nucleus as a function of diabetes in db/db. Whereas, Pdx1 does not change in nuclear localization. This can be corrected by reducing ox stress with overexpression of GPx (anti-oxidant enzyme, glutathione oxidase).

Human T2D islets. T2D islets have compromised GSIS in perifusion assay in response to glucose and glucose+IBMX. In T2D islets, MafA/B, Pdx1, Nkx6.1, Ins, Glut2 are all down in T2D vs normal islets.

Are MafA and Nkx6.1 expression levels down regulated in BTBR-ob/ob islets?

**Susie Bonner-Weir - maturation of beta-cell function.**

Fetal and neonatal islets are glucose unresponsive. Perifused rat islets from 7d, 14d, 21d and adult islets, show a dramatic difference in GSIS potential. This is interesting because it reminds me of the loss of GSIS with aged adulthood.

Laser-capture microarray analysis. Almost 600 genes DE between P1 vs adult, about 50% higher in neonate. Many specialized beta cell genes were down in neonate islets. Glp1R, Ins, Glut2 and GK are all very low in neonate. Between 7 and 9 days there was a sharp rise in GK, whereas the others, go up as well, but not as sharply inclined. Looked at TFs and saw large differences as well.

IHC in very young vs older islets. Pdx1 goes from cytosolic to nucleus, as does MafA. So got Adenos for Pdx1 and MafA and accelerate the maturation of these very young islets. If you add too much of a TF, you have problems. They titrated these TFs such that they saw 2-3 fold increase. MafA works, however, Pdx1 doesn’t change GSIS potential of neonate islets.

Reverse hemolytic plaque assay to measure secretion of individual beta cells. MafA essentially makes the neonate beta cells behave as though they were adult beta cells. How long do they wait after MafA overexpression before testing for GSIS?

Thyroid hormone change dramatically at around 7d post weening. Serum T4 peaks at about 13d. Thyroid hormone A is at p7, but goes away in adult. Thyroid A has the opposite pattern. Took 7d islets and cultured them in T3, resulting in MafA expression and GK expression. T3 enhanced GSIS in 7d islets. Thra binding sites within the MafA promoter. T3 effects are lost if overexpress a dom-neg form of MafA. Model: Thyroid hormone drives maturation via inducing expression of MafA.

Hyper-thyroid animal model. Treat mice with T3 injections induced nuclear MafA in 7d islets. With T3 treated mice, MafA was upregulated. However, in whole animal did see much of an effect. T3 accelerates the development of the adrenal gland, giving almost double the level of corticosterone circulating. Glucocorticoids suppress effects of T3 in p9 islets, both on gene expression and glucose responsiveness.

**Barak Blum, from Melton’s Lab, functional beta cell maturation.**

HESCs in vitro to HESCs transplanted. Non-functional beta-cell like insulin expressing cells. Study functional maturation process for these HESC-derived in NOD/scid mouse when transplanted.

Function based on GSIS.

Find markers that distinguish mature vs immature beta cells.

Use these markers to screen vis HTS for maturation factors.

P1-P2 islets (immature) vs P9-Adult (fully mature) GSIS potential, huge difference in secretion potential.

Young islets have a relatively high level of basal secretion. Had a very nice biorad device for perifusion studies. Might want to ask him about the specifics.

Microarrayed these young vs old islets. Facs sorted beta cells from Pdx1-GFP expressing mice to profile just beta-cells. E18.5+p1 vs P10+adult islets. No traditional beta cells genes showed up as key markers for this initial comparison.

Unc3 was one that was up in adult islets. Sharp increase between p1 vs p10. Urocortin3. Specific to beta cells. Beta cell progenitors from HESC put into mouse and asked if their maturation process was different than if left in vitro. In vitro cells are Ins+ but Ucn3-, whereas in the mouse the cells are Ins+ and Ucn3+.

Unfortunately, Ucn3 is dispensable for beta cell maturation.