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SOCIEDADES Y REUNIONES CIENTÍFICAS

Reunión anual del grupo de trabajo «islotes pancreáticos» de la Sociedad Española de Diabetes

Annual meeting of the working group ''pancreatic islet'' of the Spanish Society of Diabetes

Juan R. Tejedo[a*] y David A. Cano[b]

[a]*Centro Andaluz de Biología Molecular y Medicina Regenerativa, Universidad Pablo de Olavide, CIBERDEM, RED TERCEL, Sevilla, España*

[b]*Servicio de Endocrinología y Nutrición, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, España*

[*] Correo electrónico: juan.tejedo@cabimer.es; jrtejhua@upo.es

El grupo de trabajo de islotes pancreáticos de la Sociedad Española de Diabetes (SED) está formado por investigadores cuyas líneas de trabajo están relacionadas con el estudio del islote pancreático a nivel fisiológico, celular y molecular, tanto en condiciones normales como en situaciones patológicas como la diabetes.

En la actualidad el grupo de islotes pancreáticos está compuesto por 36 miembros. Las áreas de investigación desarrolladas por los miembros del grupo incluyen un espectro muy amplio, pudiendo mencionarse entre otras el crecimiento y diferenciación, la inmunología, el metabolismo y función de la célula beta o el trasplante de islotes. Desde el punto de vista experimental las estrategias utilizadas por los componentes del grupo de trabajo incluyen aproximaciones *in vitro* (islotes pancreáticos aislados y líneas celulares), la utilización de modelos animales de diabetes (por dieta y genéticos) y el estudio en sujetos humanos sanos o afectos de diabetes.

Los objetivos generales del grupo de islotes pancreáticos son:

1. Facilitar y agilizar el intercambio de información relacionada con sus respectivas líneas de investigación con el fin de avanzar en el conocimiento de la biología del islote pancreático y fomentar la traslación de este conocimiento al ámbito clínico.
2. Estimular la realización de proyectos de investigación comunes.
3. Optimizar e incrementar los recursos existentes en los distintos grupos de investigación que componen el grupo de trabajo.

Para alcanzar estos objetivos una de las herramientas fundamentales son las reuniones anuales del grupo. Las reuniones del grupo se estructuran en 2 grandes bloques. Por una parte, tiene lugar una asamblea de todos los miembros asistentes donde se proponen y discuten las diversas actividades del grupo. Por otra parte se organiza una reunión o simposio científico donde todos los miembros del grupo tienen la oportunidad de presentar sus resultados más recientes en forma de comunicaciones orales. Los miembros interesados en presentar sus datos deben enviar previamente un resumen de la comunicación antes de su aceptación por el coordinador del grupo.

Las reuniones anuales del grupo de islotes pancreáticos suelen realizarse en el contexto del congreso de la SED. Este año, sin embargo, por diversas cuestiones organizativas no se pudo realizar en Pamplona, y se decidió trasladarlo a octubre y realizarlo en el auditorio de la sede de la SED, en Madrid. Para esta ocasión se presentaron 19 comunicaciones orales cuyos resúmenes incluimos en este artículo especial. Creemos que estas comunicaciones ilustran perfectamente la complejidad del estudio de la biología del islote pancreático, así como el excelente estado actual de esta área de investigación en nuestro país.

PAX4 IS CONFINED TO AN ISLET β-CELL SUBPOPULATION SUSCEPTIBLE TO PROLIFERATION

Lorenzo PI, Jiménez-Moreno CM, Fuente-Martín E, Herrera I, Cobo-Vuilleumier N, Delgado I, Rojas A, Soria B and Gauthier BR

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Department of Stem Cells, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Seville, Spain

Background and aim: Pax4 is an important factor for adult β-cell plasticity. Ectopic expression of Pax4 was shown to confer protection against apoptosis and increase proliferation of adult islet cells. However, sustained expression of this factor results in β-cell dedifferentiation and hyperglycemia. These results raise the paradigm of how, under normal physiological conditions, Pax4 expression is maintained in β-cells without causing dysfunction. Herein we characterized endogenous Pax4 expression pattern in postnatal islets using a novel Pax4 promoter/GFP reporter transgenic mouse model. We also establish whether Pax4 expression correlated with the proliferative status of β-cells and identified downstream molecular targets.
Materials and methods: Using a transgenic mouse model (pPAX4/GFP-CRE) expressing GFP and CRE, under the control of the 409 bp pancreas specific PAX4 promoter, we analyze the expression pattern GFP/Pax4 in islets during postnatal development as well as in pregnant mice. In order to follow the fate of Pax4/GFP expressing cells over time, lineage tracing analysis was performed by crossing pPAX4/GFP-CRE mice to Rosa26R/lacZ reporter mice. Proliferation of islet cells was assessed by immunostaining using anti Ki67 sera along anti-GFP, -glucagon and -insulin sera. DNA microarray analysis using the transgenic mice Pax4-RIP-rtTA that conditionally express Pax4 in β-cells after doxycycline treatment was conducted for the identification of Pax4 target genes.
Results: In neonatal animals a period at which islets exhibit a high proliferation rate, GFP/Pax4 expression was detected in the majority of islet cells. The abundance of GFP/Pax4+ cells decreased gradually during postnatal development reaching approximately 25% of islet cells by 13 weeks. This GFP/Pax4+ subpopulation was predominantly confined to insulin+ cells and distributed randomly within islets. In more mature animals (1 year) in which β-cell proliferation is low, the abundance of GFP/Pax4+ cells decreased even further, being detected in approximately 10% of islet cells. As expected, lineage tracing analysis revealed that the majority of islet cells were derived from Pax4 expressing cells during development. Yet, sustained GFP expression was detected in only 30% of β-Gal positive cells in adults suggesting selective repression of the transgene in 70% of islet cells. Interestingly, the abundance of GFP/Pax4+ cells was transiently increased in islets of pregnant females, a physiological condition known to stimulate β-cell replication. Consistent with GFP expression, endogenous Pax4 transcript levels were also transiently increased during pregnancy. The proliferation marker Ki67 was preferentially increased in GFP/Pax4+ cells of pregnant females. Consistent with the latter, KEGG analysis of the transcriptome of Pax4-overexpressing islets as compared to control islets revealed enrichment in the cell cycle pathway. Intriguingly, both cell cycle activators and inhibitors were simultaneously induced in islets overexpressing Pax4.
Conclusion: Our data suggest that in adults Pax4 is confined to a subpopulation of β-cells prone to proliferation. Nonetheless, microarray profiling suggests that a molecular brake is imposed on β-cell replication which is unleashed upon a physiological stimuli requiring increased insulin output such as during pregnancy. The existence of a Pax4-expressing proliferation prone subpopulation allows islet expansion whilst glucose homeostasis is secured by non-Pax4 expressing β-cells.
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REGULATION OF ZIC1 GENE EXPRESSION BY NITRIC OXIDE AND ITS ROLE IN THE REGULATION OF SONIC HEDGEHOG PATHWAY IN MOUSE EMBRYONIC STEM CELLS

Beltran-Povea A[a], Salguero C[a], Tapia-Limonchi R[a], Hitos AB[a], Díaz I[a], Cahuana GM[a,b], Bedoya F.J[a,b], Tejedo J.R[a,b]
[a]*CABIMER (Andalusian Centre of Molecular Biology and Regenerative Medicine). University of Pablo de Olavide, Seville*

[b]*CIBERDEM, Instituto de Salud Carlos III, Madrid 28029, Spain*

Background and aims. The mESC grown with high concentrations of Nitric oxide increases the expression of several differentiation genes, such as Zic1, a marker of differentiation into ectodermal, becaming in a study target in the process of differentiation into endoderm. Zic1 is essential to the regulation of Sonic Hedgehog (Ssh) pathway in neural development, and it physically and functionally interacts with Gli proteins. The aim objective of this study is to determine if Zic1 is regulated by NO, and if it has a role in the regulation of Ssh in mESC related with endoderm development.
Methods. mESC R1/E were cultured in presence of LIF and in absence of LIF to induce spontaneous differentiation. After 3 days of culture they were exposed to a NO donor diethylenetriamine NO adduct (DETA-NO) at 500 μM during 19 hours. Adult pancreatic cells INS-1E and MIN6, and human neural HS-SY5Y were used as control conditions. Zic1 gene and protein expression was measured by real-time PCR, Western-blot and immunofluorescence assays. DNA methylation state was analyzed by a MSP assay. Zic1 regulation by Egr1 transcription factors was analyzed by a chromatin immunoprecipitation assay. The role of Zic1 in Ssh was studied developing Zic1 gain and loss of function assays.
Results It has been shown that in presence of Nitric Oxide Zic1 expression was increased, at the same time that Pdx1 expression, and both genes coexpressed in these cells. However significant changes on DNA methylation were not observed in Zic1 promoter, indicating that Zic1 regulation by NO is not methylation dependent. Furthermore we proved that Egr1, a transcription factor with a binding site on Zic1 promoter, could act as an activator of Zic1 expression after NO treatment. Then we proved that Zic1 was expressed in adult pancreatic cells, and that coexpressed with Pdx1 in adult pancreatic cells and in pancreas tissue. Finally, we determined that NO treatment suppresses Ssh signaling pathway, decreasing the expression of it targets genes, which is promoted in part by Zic1. Last we tested that Zic1 was expressed in mouse adult islets, whereas Ssh target

genes weren't expressed, reveling thus the possible role of Zic1 in Ssh signaling pathway inhibition and differentiation process toward endoderm in mESC.

Conclusion Zic1 is being regulated by NO differentiation treatment in mESC, increasing considerably its expression after the treatment, being this modifications methylation-nondependent. The Egr1 was concluded to act as an activator, increasing Zic1 expression after the treatment with NO. The expression of Zic1 in adult pancreatic cells, and the coexpression of Zic1 with Pdx1 observed in mESC treated with NO and in adult pancreatic cells are enough evidence to conclude that Zic1 may be involved in the process of differentiation to endoderm. The treatment with high concentrations of NO in mESC is inhibiting Ssh pathway, reducing the expression of its target genes, which have been seen to be silenced in islets and adult pancreatic cells. We have seen that this inhibition is promoted in part by Zic1, whose overexpression induce a decrease in the expression of these genes, what will be corroborated by silencing Zic1 by a siRNA. Finally, to test whether Zic1 has a role in Ssh signaling pathway inhibition and differentiation process, we pretend to performed loss of function assays in adult cells and check if expression of Ssh target genes and proliferation are promoted.

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LA HAPLOSUFICIENCIA DE PDX1 CAUSA LA FORMACIÓN DE TUMORES DE ISLOTES PANCREÁTICOS EN RATONES QUE SOBREEXPRESAN EL ONCOGÉN C-MYC

Álvaro Flores[a,b], Alejandro García-Núñez[a,b], José Luis Muñoz-Bravo[a,b], David A. Cano[a,b]
[a]Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, España
[b]*Unidad de Gestión de Endocrinología, Hospital Universitario Virgen del Rocío, Sevilla, España*

El proto-oncogén c-Myc es un regulador clave de la proliferación y apoptosis celular, entre otros procesos biológicos. Así, la sobrexpresión de c-Myc puede inducir efectos antagónicos que dependen del contexto celular, nivel de sobrexpresión y actividad de otros oncogenes y genes supresores de tumores. En el caso de las células beta, varios estudios han indicado que la activación de c-Myc resulta en dediferenciación celular y muerte por apoptosis. Sin embargo, un estudio reciente realizado en líneas celulares e islotes humanos en condiciones proliferativas ha identificado un patrón común de expresión de varios genes relacionados con ciclo celular incluido el gen c-Myc. Estos resultados indican que un aumento moderado de los niveles de c-Myc (lejos de los niveles observados que causan apoptosis) puede inducir la proliferación en células beta sin causar apoptosis.

Los ratones Ins-myc^{ER} portan una versión modificada del gen c-Myc, de tal manera que la proteína cMYC solo se encuentra activa en presencia de un ligando del receptor del estrógeno. El tratamiento con un ligando como el tamoxifen en estos ratones Ins-myc^{ER} induce una apoptosis generalizada de las células beta, y los ratones desarrollan una severa hiperglucemia. Hemos observado que los ratones Ins-myc^{ER} muestran una actividad residual del transgén myc^{ER} incluso en ausencia del ligando. Este ligero aumento de niveles de cMYC causa un moderado aumento de la proliferación de las células beta, pero sin inducir apoptosis. Estamos utilizando este modelo animal para determinar cómo cMyc regula la proliferación de células beta. El análisis de la expresión génica de las células beta de los ratones Ins-myc^{ER} ha mostrado una disminución de varios genes relacionados con el mantenimiento de la identidad celular de células beta, como el gen Pdx. La disyuntiva entre el mantenimiento de la identidad celular y la proliferación parece ser un problema generalizado en células maduras.

Nos hemos planteado determinar si la modificación de esta identidad celular en células beta puede afectar a la proliferación celular en varias condiciones pro-proliferativas, incluida la activación de c-Myc. Para ello hemos generado ratones transgénicos dobles que portan el transgén myc^{ER} y una mutación en heterocigosis del gen Pdx. Los ratones Ins-myc^{ER}Pdx$^{+/-}$ jóvenes mostraron una mayor tasa de proliferación de las células beta que se traducía en un aumento de la masa de islotes pancreáticos. Sin embargo, en animales mayores hay una disminución de esta masa de células beta y los ratones, de hecho, se vuelven diabéticos. De forma sorprendente, hemos observado la presencia de tumores de islotes pancreáticos en ratones Ins-myc^{ER}Pdx$^{+/-}$ de edad avanzada. Estos resultados apuntan a una compleja interacción entre Pdx y cMyc en la regulación de la proliferación e identidad cellular.

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AN OPTIMAL AND REPRODUCIBLE PROTOCOL FOR LENTIVIRAL TRANSDUCTION OF INTACT HUMAN AND MOUSE PANCREATIC ISLETS

Carmen Jiménez-Moreno[a], Irene de Gracia Herrera[a], Petra Isabel Lorenzo[a], Livia López-Noriega[a], Domenico Bosco[b], Benoit Gauthier[a], Alejandro Martin-Montalvo[a]
[a]*Department of Stem Cells and, CABIMER-Sevilla*

[b]*Cell Isolation and Transplantation Center, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland*

Background and aims: The determination of the potential benefits of gene expression modulation in islet physiology is imperative to identify novel 'druggable' targets for the treatment of pancreatic endocrine pathologies. However, achieving high levels of either gene expression or repression in intact islets composed of approximately 3000 cells compacted together in a 3-dimensional conformation has been a major challenge for scientists in this field. Despite advances in the development of gene transfer techniques on islet cell populations, the efficiency and difficulty of current protocols, which are based on electroporation, gene gun particle bombardment, cationic liposomes and polymeric particles among others, have been a limiting factor to universalize a reliable protocol for this approach. Here we describe a

simple, reproducible and easy-to-use lentiviral transduction protocol that allows ∼80% islet cell transduction for intact human and mouse islets.

Materials and methods: An optimal infection protocol was established using isolated mouse and human islets and a lentiviral construct expressing the Green Fluorescence Protein (GFP) under the Ubiquitin constitutive promoter. GFP expression was determined by flow cytometry, live imaging and immunohistochemistry analyses. Subsequent to transduction islet viability and functionality was assessed by the MTT assay, apoptosis induction by ELISA and glucose-induced insulin secretion (GSIS), respectively.

Results: Two mutually non-exclusive parameters were considered for the development of an optimal viral transduction protocol: 1) Islet cell accessibility or permeability to viral transduction without compromising islet architecture and 2) Multiplicity Of Infection (MOI) without compromising islet viability. We observed that freshly isolated and non-trypsinized islets exposed to increasing MOI up to 100 resulted in higher GFP expression levels in cells predominantly located at islet periphery. Of note, a MOI 100 caused massive cell death and islet dismay. In contrast, standard cell culture trypsinization caused islet disaggregation independent of viral dosage. Mild trypsin treatment preserved islet architecture while promoting GFP expression in cells throughout islets including the core of the micro-organ with increasing MOI (0 to 20). Islet exposed to viral particle up to MOI 20 exhibited a 2 to 3 fold increase in apoptosis as compared to non-infected cells. Nonetheless, cell metabolic health as assessed by the MTT assay revealed no differences among the various experimental groups including controls. Consistent with the latter GSIS was as robust in MOI 20 transduced islets as compared to non-infected islets. Taking into consideration the various experimental conditions, we achieved GFP expression in 80% of islet cells preserving integrity and performance using a protocol of mild trypsinization prior to transduction with MOI 20.

Conclusion: We have developed a reliable easy-to-use protocol for mouse and human islet transduction. Our protocol will allow the implementation of mechanistic studies aiming to decipher possible beneficial effects of gene expression modulation in intact islets. Potentially this protocol could be implemented to transduce islets with factors implicated in survival or performance prior transplantation.

β-CELL DEDIFFERENTIATION AND REDUCED β-CELL NEOGENESIS CONTRIBUTE TO IMPAIRED β-CELL MASS EXPANSION IN AGED RATS

Noèlia Téllez[a,b], Yasmina Martí[b], Marina Vilaseca[b], Arturo Pla[b] and Eduard Montanya[a,b,c]

[a] *CIBERDEM*

[b] *Laboratory of Diabetes and Experimental Endocrinology, Clinical Sciences, IDIBELL-University of Barcelona*

[c] *Endocrine Unit, Hospital Universitari de Bellvitge*

Aging is a non-modifiable risk factor for the development of diabetes. The contribution of impaired beta cell regeneration to this increased risk remains unknown. In young rats, 90%-pancreatectomy (Px) results in beta cell mass regeneration which is further enhanced with gastrin treatment. The aim of our study was to investigate the β-cell regeneration potential of aged rats using the 90%-Px model and gastrin treatment.

Material and methods: 1 and 12 month-old Wistar rats underwent 90%-Px and were treated from the day of surgery with [15leu] gastrin-17 (150 μg/kg · 12 h, Px+G, n=21) or with vehicle (Px+v; n=21). A group of sham-operated rats treated with vehicle was included for each age group (S+v; n=18). Pancreatic remnants were harvested on days 3 and14 after surgery for morphometric, immunohistochemical and gene expression analysis.

Results: Young Px rats showed increased β-cell mass that was further increased with gastrin treatment. Gene expression and nuclear immunolocalization of nkx6.1 in ductal cells, and the percentage of extra-islet β-cells (indirect markers of β-cell neogenesis) were also increased in gastrin-treated young Px rats.β-cell apoptosis was similar among groups, and β-cell replication and size were similarly increased in gastrin and vehicle-treated young Px rats.in aged rats, β-cell mass was not increased in any of the Px groups, despite the increased β-cell replication and individual β-cell size. Gene expression and nuclear immunolocalization of nkx6.1 in ductal cells, and the percentage of extra-islet β-cells were not increased in aged gastrin- or vehicle-treated Px rats. The dedifferentiation-related transcription factors Neurog3 and Sox9 were significantly upregulated in islet β-cells from aged Px rats.

Conclusion: The potential for compensatory β-cell hyperplasia and hypertrophy is retained in aged rats. In contrast, impaired β-cell neogenesis along with beta cell dedifferentiation may contribute to the limited beta cell regeneration in aged rats

LA SUPRESIÓN DE BACE2 ALIVIA LA DISFUNCIÓN DE LA CÉLULA β-PANCREÁTICA INDUCIDA POR LA SOBREEXPRESIÓN DE AMILINA (IAPP)

Gema Alcarraz-Vizán, Carlos Castaño, Lisa Cadavez, Montse Visa, Joel Montane, Joan Marc Servitja, Anna Novials
Laboratorio de Investigación en Diabetes y Obesidad, Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), IDIBAPS, España

Introducción y objetivo *β-site APP-cleaving enzyme 2* (BACE2) es una proteasa que ha sido encontrada en el cerebro, donde se cree que desempeña un papel en el desarrollo de la enfermedad de Alzheimer (AD). Se ha localizado también en el páncreas, donde parece desarrollar un importante papel fisiológico, ya que ratones deficientes de BACE2 muestran una mejor tolerancia a la glucosa que sus compañeros control de camada. Las enfermedades amiloidogénicas, incluyendo el AD y la diabetes tipo 2 (T2D), comparten la acumulación de proteínas que se pliegan de forma anómala o son insolubles y que interfieren con la función celular. En el caso de la T2D los depósitos de amilina (IAPP) representan una característica clave de la enfermedad. El objetivo del presente estudio fue investigar el efecto

de la modulación de BACE2 en las alteraciones sobre la célula β inducidas por la sobreexpresión de IAPP en un modelo *in vivo*.
Materiales y métodos Ratones heterozigotos que sobreexpresan el IAPP humano (Tg-hIAPP), ratones deficientes de BACE2 (BACE2-KO) y sus respectivos controles fueron utilizados para analizar su fenotipo después de 16 semanas alimentados con dieta rica en grasa (HFD). A continuación, estos 2 modelos animales fueron cruzados con la intención de analizar el impacto de la supresión de BACE2 en las alteraciones de la célula β observadas en los ratones Tg-hIAPP. Se realizaron test de tolerancia a la glucosa (GTT) y a la insulina (ITT) para evaluar el fenotipo metabólico, y se calculó el área bajo la curva (AUC) del GTT como medida de la homeostasis de la glucosa. Se cuantificó la capacidad de secretar insulina en respuesta a la glucosa (GSIS) con un kit de ELISA. La proliferación fue estimada mediante tinción inmunológica de Ki67 y la masa β-celular mediante tinción inmunológica de insulina.
Resultados El GTT de los ratones Tg-hIAPP después de 16 semanas de dieta estándar reveló intolerancia a la glucosa respecto a los animales control. Estos animales mostraron un aumento de 1,3 veces ($p < 0,05$) en la masa β-celular, y sin embargo la respuesta secretora de insulina se vio reducida después de la inyección de glucosa (25% de disminución $p < 0,05$ vs. animales control de la misma camada). La alimentación con HFD durante 16 semanas indujo resistencia a la insulina e intolerancia a la glucosa tanto en animales Tg-hIAPP como en animales control. Por otro lado, los animales BACE2-KO presentaron una mayor homeostasis de la glucosa que sus compañeros control, cosa que correlaciona con un incremento de la respuesta secretora de insulina en respuesta a la inyección de glucosa ($p < 0,05$) y con un incremento en la masa β-celular y en la proliferación de la célula β. Además, los animales BACE2-KO alimentados con HFD presentaron una reducción del 18% en el peso corporal ($p < 0,05$) y una mejor homeostasis de la glucosa que los animales control (28% de disminución en el AUC del GTT), indicando que la deleción de BACE2 protege contra la HFD. Los animales cruzados (Tg-hIAPPxBACE2-KO) presentaron una mejora en la tolerancia a la glucosa comparados con los animales Tg-hIAPP (18% de disminución en el AUC). Esta mejora se atribuye a un incremento en la respuesta de secreción de insulina en respuesta a una carga de glucosa, indicando un efecto beneficioso de la deleción de BACE2 sobre la función β-celular.
Conclusión La inhibición de BACE2 compensa los defectos en la tolerancia a la glucosa inducidos por la sobreexpresión de hIAPP en la célula β. Por tanto, la disminución de BACE2 puede representar una buena estrategia terapéutica para mejorar la función β-celular en la T2D.
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THE LRH-1 AGONIST BL001 RESTRAINS DEVELOPMENT OF HYPERGLYCEMIA IN THE RIP-B7.1 MOUSE MODEL OF EXPERIMENTAL AUTOIMMUNE DIABETES

Cobo-Vuilleumier N[a], Jimenez Moreno CM[a], Perez-Fernandez MM[a], Pozo D[b], Lorenzo PI[a], Gauthier BR[a]

[a]*Department of Stem Cells, Unit of Pancreatic Islet Regeneration and Development, CABIMER, Seville*

[b]*Department of Cell Signalling, Advanced therapies in neuroprotection and immune-regulation CABIMER, Seville*

Background and aims: We have previously shown that the orphan nuclear receptor LRH-1 is expressed in human and mouse islets and that its over expression protects β-cells against stress-induced apoptosis. We have also demonstrated that activation of LRH-1 using a small synthetic agonist codenamed BL001 protects islets against cytokine-induced apoptosis and rescues islet function from T2 diabetic donors. Herein we evaluated whether BL001 could prevent or revert hyperglycemia in the RIP-B7.1 mouse model of experimental autoimmune diabetes (EAD).
Methods: RIP-B7.1 mice were treated with BL001 (10 mg/Kg body weight) or vehicle once daily for 5 days prior (prevention) or after (reversion) preproinsulin cDNA immunization. Animals were then injected (prevention and reversion) daily for up to 8 weeks with BL001/vehicle. Blood glucose levels were measured weekly. Pancreata were extracted at various time points to perform histological studies.
Results: Approximately 75% of animals immunized and treated or not with vehicle developed hyperglycemia subsequent to immunization. In contrast, approximately 35% of BL001 pre or post-treated mice developed hyperglycemia subsequent to immunization. Hematoxylin and eosin staining revealed massive lymphocyte infiltration (insulitis) in immunized and vehicle pre-treated RIPB7.1 that developed hyperglycemia whereas normoglycemic BL001-pre-treated and immunized animals revealed marginal to no insulitis. Immunohistochemical analysis of insulin and glucagon confirmed complete destruction of β-cells in islets of vehicle-treated animals 8-weeks post immunization. Astonishingly, islet architecture comprising β-cells in the core and α-cells at the periphery of the microorgan was completely preserved in BL001 and immunized mice that remained normoglycemic, similar to vehicle or BL001 non-immunized RIP-B7.1 mice.
Conclusion: The LRH-1 agonist BL001 reduced by 50 percent the development of hyperglycemia in RIP-B7.1 mice. Concomitantly, insulitis was abolished in the BL001-treated and immunized mice suggesting a potential immunomodulatory effect of the compound. Cytokine profile are currently being conducted.
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LIPOSEME-BASED IMMUNOTHERAPY FOR TYPE 1 DIABETES

Irma Pujol-Autonell[a], Arnau Serracant-Prat[a], Mary Cano-Sarabia[b], Rosa M. Ampudiaa, Manuel Puig-Domingo[a], Daniel Maspoch[b], Joan Verdaguer[a], Marta Vives-Pi[b]

[a]*Institut de Recerca Germans Trias i Pujol, Badalona, Barcelona, España*

[b]*Institut Català de Nanociència i Nanotecnologia, Bellaterra, Barcelona, España*

The development of new therapies to induce self-tolerance has been an important medical health challenge in type 1 diabetes. An ideal immunotherapy should inhibit the autoimmune attack, avoid systemic side effects and allow β-cell regeneration. Based on the effect of apoptosis and efferocytosisin immunological tolerance induction, we hypothesized that apoptotic mimicry can help to restore tolerance lost in autoimmune diabetes. In an attempt to investigate this latter aspect, the present study evaluated the effects of phosphatidylserine (PS) liposomes, a well-characterised membrane component expressed during apoptosis, containing autoantigens in experimental type 1 diabetes.
PS-liposomes filled with insulin peptides were prepared, in the size range for an efficient phagocytosis and with multivesicular vesicles morphology, with advantages in terms of encapsulation. The effect of antigen-specific PS-liposomes in the re-establishment of peripheral tolerance was assessed in a spontaneous model of the disease, the Non Obese Diabetic(NOD) mouse.
We have shown that PS-liposomes loaded with insulin peptides induce tolerogenic dendritic cells and impair autoreactive T cell proliferation. When administered to NOD mice, liposome signal was detected in the pancreas and draining lymph nodes. Moreover, this immunotherapy arrests the autoimmune aggression, reduces the severity of insulitis and prevents type 1 diabetes by apoptotic mimicry.
Since antigen specific PS-liposomes resemble apoptotic cells in inhibiting maturation and immunostimulatory function of dendritic cells, we believe that liposomal microparticles could be optimum vehicles to restore tolerance to autoantigens thus constituting a promising strategy for autoimmune diseases.
Email: mvives@igtp.cat
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UNA DIETA HIPERCALÓRICA E HIPERLIPÍDICA BASADA EN ACEITE DE OLIVA ES CAPAZ DE REVERTIR UNA DIABETES TIPO 2 INDUCIDA POR UNA DIETA OCCIDENTALIZADA

Enrique Jurado[a], Antonio Cárdenas[a,b], Genoveva Berná[a,b], Bernat Soria[a,b], Franz Martín[a,b]
[a]*Department of Stem Cells, Andalusian Centre of Molecular Biology and Regenerative Medicine. University of Pablo de Olavide, Seville (CABIMER-UPO), Sevilla, Spain*

[b] CIBERDEM, CIBER of Diabetes and Associated Metabolic Diseases, Instituto de Salud Carlos III, Madrid 28029, Spain

Objetivo Desarrollar un modelo animal de diabetes tipo 2, lo más humanizado posible, y testar los efectos de un aceite de oliva rico en compuestos polifenólicos y fracción insaponificable.
Material y métodos Noventa ratones C57BL6J machos de 5 s se alimentaron durante 6 meses con: a) dieta estándar (Ct; n = 30); b) dieta alta en grasas saturadas (48% grasa animal) (WHHSF; n = 30) y c) dieta WHHSF durante 3 m y después la misma dieta hiperlipídica, pero con el aceite de oliva (HHOO; n = 30) durante otros 3 m. Se midió y calculó a los 0, 30, 60, 90 y 180 d el peso, la glucemia, la insulinemia, el colesterol total, HDL-colesterol, LDL-colesterol, interleucina-6, interferón gamma, test de tolerancia intraperitoneal a la glucosa y a la insulina (ITT), índice HOMA y secreción de insulina inducida por glucosa.
Resultados Todos los grupos comieron igual. Tras 90 d WHHSF desarrolló obesidad y diabetes y niveles altos de citocinas. Tras 180 d el peso, la glucemia y las citocinas de HHOO disminuyeron significativamente. Las HDL-colesterol fueron estables todo el estudio, en los 3 grupos. A los 180 d no se encontraron diferencias en LDL-colesterol entre Ct y HHOO, mientras que los valores de WHHSF fueron significativamente superiores. El test de tolerancia intraperitoneal a la glucosa, el test de tolerancia a la insulina y el HOMA mostraron que WHHSF desarrolló resistencia a la insulina. La secreción de insulina inducida por glucosa mostró una reducción significativa en la secreción de insulina y en la sensibilidad a la glucosa en WHHSF. Estos parámetros se recuperaron en HHOO.
Conclusión Una dieta hipercalórica e hiperlipídica, basada en aceite de oliva virgen extra rico en polifenoles y compuestos menores, es capaz de revertir las alteraciones fisiológicas y metabólicas de una diabetes tipo 2 inducida por una dieta occidentalizada. Por lo tanto, además de la cantidad de grasa de una dieta, su calidad también es importante.

CHAPERONES AMELIORATE BETA CELL DYSFUNCTION AND AMYLOID FORMATION ASSOCIATED WITH HUMAN ISLET AMYLOID POLYPEPTIDE OVEREXPRESSION

Joel Montane[a,b], Lisa Cadavez[a,b], Carlos Castaño[a,b], Anna Novials[a,b]
[a]*Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi iSunyer (IDIBAPS), Hospital Clínic de Barcelona, Spain*

[b]*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Spain*

Background and aims: In type 2 diabetes and islet transplantation beta-cell dysfunction is thought to be due to several causes, one being the formation of toxic protein aggregates called islet amyloid, formed by accumulations of misfolded human islet amyloid polypeptide (hIAPP). The process of hIAPP misfolding and aggregation is one of the factors that may perturb the endoplasmic reticulum (ER) homeostasis, leading to beta cell dysfunction and ultimately, cell death. Molecular chaperones have been described to be important for regulation of ER response to ER stress by stabilizing protein conformation and improving ER capacity. The aim of the present work is to determine whether chaperone treatment is able to counteract hIAPP-induced beta cell dysfunction and, ultimately, diminish amyloid formation and cell death in pancreatic islets.
Material and Methods: hIAPPTg islets andINS1E cells, stably expressing hIAPP (hIAPP-INS1E) were cultured with 16 mM or 25 mM glucose and 400 μM palmitic acid and treated with chemical chaperones taurine conjugated ursodeoxycholic

(TUDCA) and phenyl butyric acid (PBA) or endogenous chaperones BiP/GRP78 (BiP) or protein disulfide isomerase (PDI). Islet function was determined by glucose-stimulated insulin secretion and gene expression and protein levels of ER stress markers (CHOP, ATF3 and spliced XBP1) were analysed by real-time RT-PCR and Western blot. Wild type and hIAPPTg islets were cultured for 7 days at 16 mM glucose and treated with TUDCA or PBA. Amyloid formation was determined by Thio S staining.

Results: hIAPP-INS1E cells exposed to high glucose and palmitic acid showed an increase in ER stress when compared to INS1E cells expressing rat IAPP or INS1E control cells. Treatment with chaperones BiP, PDI, TUDCA or PBA alleviated ER stress and increased insulin secretion in hIAPP-expressing cells. Treatment of wild-type and hIAPPTg islets with BiP, PDI, TUDCA and PBA increased insulin output in basal conditions after a glucose-stimulated insulin secretion. When hIAPPTg islets were exposed to high glucose and palmitic acid, chaperone treatment was able to revert beta cell dysfunction by restoring insulin secretion. Moreover, when hIAPPTg islets were cultured for 7 days at 16 mM glucose, amyloid plaques were formed throughout the islet engaging 18.2±2.3% of the insulin positive area. TUDCA and PBA treatment was able to diminish amyloid formation of hIAPPTg islets to 5.3±0.9% and 1.2±0.4% respectively, indicating that chaperones may play an important role in preventing beta-cell dysfunction and amyloid formation associated to T2D.

Conclusion: Chaperones ameliorate induced ER stress, increase insulin secretion and ultimately, diminish amyloid formation in a context of hIAPP overexpression. These innovative approaches could reveal new therapeutic targets and aid in the development and evaluation of strategies to diminish ER stress and limit the damaging amyloid observed in islets before transplant or in type 2 diabetic patients.

HMG20A IS EXPRESSED IN ISLETS AND MAY REGULATE THE MODY6 GENE

Mellado-Gil J.M.[a], Fuente-Martin E.[a], Bosco, D.[c], Rivero, S.[b], Reyes, J.C[b]. and Gauthier B.R.[a]
[a]*Department of Stem Cells and* [b]*Molecular Biology, CABIMER-Avenida Américo Vespucio, 41092, Sevilla*

[c]*Cell Isolation and Transplantation Center, Department of Surgery, Geneva University Hospitals and University of Geneva, Geneva, Switzerland*

Background and aims: Common variants in the HMG20A gene have recently been associated with increased risk of Type 2 Diabetes Mellitus (T2DM) and potentially obesity in various ethnic populations. An islet associated-function for this 'diabesity' gene encoding for a factor that binds chromatin and exerts global genomic changes is far from evident. Nevertheless, HMG20A was shown to promote neuronal differentiation by activating NEUROD, a transcription factor also essential for islet development and mature function. Indeed, NEUROD is important for proper insulin secretion and mutations in this gene cause MODY6. Thus, HMG20A-mediated regulation of NEUROD could potentially be a key molecular hub essential for β-cell function and alteration in the expression of either factor could lead to T2DM. Herein we aimed to establish whether HMG20A is expressed in islets and assess whether lipids as well as glucose regulate levels of this factor. Furthermore, we determined the potential regulation of NEUROD by HMG20A in islets.

Materials and Methods: Human and mouse islets were cultured under various experimental conditions such as high glucose and lipids. Quantitative PCR (QT-PCR) was then used to estimate HMG20A transcript levels. Mouse liver, muscle and fat were also used as control tissues. HMG20A islet levels were also assessed by immunocytochemistry. HMG20A-mediated regulation of NEUROD was evaluated by RNA interference in the mouse MIN-6 insulinoma cell line.

Results: We demonstrate that mouse islets express high levels of HMG20A as compared to muscle and fat whereas the transcript is most abundant in the liver. In parallel, HMG20A protein was detected in mouse and human β-cells as well as in other islet cell types. Human islet cultured in the presence of high glucose concentrations exhibited a transient increase in HMG20A transcript levels reaching a maximum of 2-fold at 72 hours. In contrast palmitate inhibited HMG20A in mouse islets by 48 hours. Depletion of HMG20A by RNA interference in MIN6 cells resulted in a concomitant 50% repression of NEUROD mRNA levels.

Conclusion: HMG20A is expressed in islets and is modulated by diabetogenic conditions (high glucose and lipids). More importantly, the MODY6 gene, NEUROD appears to be a target of HMG20A suggesting that polymorphisms associated with this gene may be implicated in impaired insulin secretion.

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PROTECTIVE EFFECTS OF EPOXYPUKALIDE ON GLUCOSE METABOLISM AND PANCREATIC BETA-CELLS IN STZ-INDUCED DIABETIC MICE

José F. López-Acosta[a], Jennifer Muñoz-Barrera[a], Ana R. Díaz-Marrero[b], Mercedes Cueto[c], Germán Perdomo[d] and Irene Cózar-Castellano[a*]
[a]*Instituto de Genética y Biología Molecular (University of Valladolid-CSIC), Valladolid, Spain*

[b]*Departamento de Química Orgánica, Instituto Universitario de Bioorgánica ''Antonio González'' -CIBICAN, University of La Laguna, Spain*

[c]*Instituto de Productos Naturales y Agrobiología (CSIC), La Laguna, Tenerife, Spain*

[d]*Facultad de Ciencias Ambientales y Bioquimica, University of Castilla-La Mancha, Toledo, Spain*

Aim/hypothesis: Diabetes is a chronic disease leading to high mortality and morbidity in humans. We recently demonstrate that epoxypukalide (Epox) is a natural compound with beneficial effects on primary cultures of rat islets. In this study, we extend our previous investigations to test the hypothesis that Epox protects β-cells and improves glucose metabolism in STZ-induced diabetic mice.

Methods: We used 3-months old Swiss OF1 male mice that were treated with Epox at low-dose (100 μg/kg body weight) and high-dose (200 μg/kg body weight). Diabetes was induced by multiple intraperitoneal low-doses of streptozotocin (STZ) on 5 consecutive days. Glucose homeostasis was evaluated measuring fasting and non-fasting blood glucose levels, plasma insulin levels, and glucose tolerance. Histomorphometry was used to quantify the number of islets, the number of pancreatic β-cells of each islet and infiltrates within pancreatic islets.
Results: Epox treatment (100 μg/kg or 200 μg/kg) did not alter glucose metabolism in non-STZ diabetic mice. In contrast, Epox treatment (200 μg/kg) significantly reduced fasting blood glucose levels and improved glucose tolerance. These metabolic changes were associated with a marked increase in the number of islets (∼60%) and similar increment in the number of pancreatic β-cells per islet. In addition, Epox treatment lowered by ∼50% inflammatory infiltrates within pancreas.
Conclusion: Epox did not exhibit any beneficial effect on glucose disposal rates in normal mice. However, Epox improved glucose homeostasis and ameliorates pancreatic β-cell toxicity in STZ-induced diabetic mice.

TAURINE TREATMENT PREVENTS DISTURBANCES IN CIRCADIAN RHYTHMS CAUSED BY HIGH FAT DIET FEEDING

Elaine Vieira[b], Ana Lucia C. Figueroa[a], Hugo A. Figueiredo[b], Sandra A. Rebuffat[b], Ramon Gomis[a,b,c,d]
[a]*Diabetes and Obesity Laboratory, IDIBAPS, Barcelona, Spain*

[b]*CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Spain*

[c]*Department of Endocrinology and Nutrition, Hospital Clinic. Universitat de Barcelona, Spain*

[d]*Faculty of Medicine, University of Barcelona, Spain*

Background and aims: Obesity and type 2 diabetes is associated with disruption of circadian rhythms. Strategies that correct disturbances in circadian rhythms could be good candidates to prevent these diseases. The amino acid taurine has been shown to prevent obesity and ameliorate metabolic dysfunction in animal models of high fat diet and also to increase sleep and shifts the diurnal locomotor activity in drosophila. However, nothing is known whether taurine can regulates circadian rhythms in mice. Therefore, we aim to study whether long term taurine treatment can ameliorate disturbances in circadian rhythms caused by high fat diet feeding.
Material and methods: Ten weeks old male C57BL/6 mice were divided in 4 groups. Control (C): mice fed with chow and Control+taurine (C+T): mice fed with chow and 2.0% of taurine in the drinking water. HFD (HFD) fed with 45% of fat and HFD+taurine (HFD+T) treated with 2.0% taurine in the drinking water. Mice were sacrificed at different times of the day (6:00, 12:00, 18:00, 24:00). Plasma insulin and leptin levels were measured by ELISA. The expressions of clock genes in different tissues were measured by RT-PCR.
Results: High fat diet increased food intake during the day and night time compared to chow fed mice ($p<0.001$). The increase in food intake was followed by an increase in plasma insulin secretion ($p<0.05$) during day and night time in the HFD group. HFD+T treatment prevented the increase in food intake and plasma insulin during the day and night time. HFD increased visceral fat ($p<0.05$) whereas HFD+T group had a decrease in visceral fat ($p<0.05$). Plasma leptin levels were decreased at 12 h in chow fed mice ($p<0.01$) and increased at 24 h. HFD treatment disrupts circadian leptin levels compared to control group exhibiting highest level at 18 h and lost the peak of leptin levels at 24 h. Taurine reduced plasma leptin levels at 12 and 24 h and prevented the disruption of circadian leptin levels caused by HFD. HFD treated group exhibited high insulin levels through out the 24 h whereas taurine treatment decreased plasma insulin levels and restored the circadian pattern of insulin. The expression of Clock was upregulated in the hypothalamus from HFD fed mice and it expression was enhanced in HFD+T group at 24 h. In pancreatic islets, the expression of Rev-erb alpha, Bmal1 and Per1 were downregulated by HFD and taurine prevents this effect only in the case of Per 1 expression.
Conclusion: Taurine treatment prevents the disturbances in the circadian levels of leptin, insulin and food intake and caused by HFD feeding. These results suggest that taurine could be a potential candidate to prevent disturbances in circadian rhythms and a potential target to treat diseases associated with disturbances of the biological clock.

FUNCIÓN DEL FACTOR DE TRANSCRIPCIÓN GATA6 EN LA REGENERACIÓN PANCREÁTICA

Manuel Carrasco[a], Irene Delgado[a], Alejandro García-Núñez[b], Bernat Soria[a], Franz Martín[a], David Cano[b], Anabel Rojas[a]
[a]*Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Sevilla, España*

[b]*Insituto de Biomedicina de Sevilla (IBiS)-Hospital Universitario Virgen del Rocío (HUVR), Sevilla, España*

Antecendentes Resultados recientes de nuestro grupo y otros han mostrado que los factores de transcripción GATA4 y GATA6 tienen un papel redundante en el desarrollo embrionario del páncreas. Mientras que la inactivación de GATA4 o de GATA6 no tiene ningún efecto aparente en la formación del páncreas, la inactivación simultánea de ambos genes produce agénesis pancreática, como consecuencia de defectos en proliferación y diferenciación de los progenitores pancreáticos. Estudios de secuenciación masiva en humanos han revelado que mutaciones en el gen GATA6 es la causa más común de agenesia pancreática asociada a diabetes neonatal. Más aún, mutaciones en GATA6 causan un amplio abanico de fenotipos extra e intrapancreáticos, que van desde total ausencia de tejido pancreático hasta desarrollo de diabetes en estadios posnatales con o sin suficiencia exocrina. Estos datos sugieren que GATA6, además de desempeñar un papel importante en el desarrollo

pancreático, también pudiera ejercer una función en la célula beta adulta.
Objetivo Determinar el papel de GATA6 en la función de la célula adulta.
Materiales y métodos Para estudiar el efecto de la función de GATA6 en islotes de ratón se ha generado un mutante condicional Gata6$^{flox/flox}$; Pdx1-Cre (Gata6 cKO). El páncreas de los ratones Gata6cKO se ha analizado mediante técnicas de histología e inmunohistoquímica. Se han realizado ensayos de intolerancia a la glucosa (IPGTT) y ensayos de intolerancia a la insulina (ITT) en ratones Gata6 cKO y controles a las 8 semanas y a los 7 meses de edad. Por último, se han realizado experimentos de inducción de diabetes inducidos por estreptozotocina (STZ).
Resultados Los análisis histológicos de los islotes Gata6 cKO muestran una morfología similar a los islotes controles, tanto a las 8 semanas de edad como a los 7 meses. En ambas edades los ratones son normoglucémicos, sin embargo, mientras que los ratones jóvenes Gata6 cKO no muestran intolerancia a la glucosa, a los 7 meses desarrollan intolerancia a la glucosa. Más aún, análisis inmunohitoquímicos usando varios marcadores específicos de célula beta muestran una reducción en la acumulación del transportador de la glucosa Glut2. Estos resultados pudieran explicar, al menos en parte, la intolerancia a la glucosa observada en ratones Gata6 cKO adultos. Los ensayos de inducción de diabetes mediante tratamiento con STZ muestran un retraso en el desarrollo de la diabetes en ratones Gata6 cKO que en ratones control, lo que refuerza la idea de una disminución de GLUT2, transportador de la glucosa y de STZ en los ratones Gata6 cKO.
Conclusión Nuestros resultados muestran que la inactivación del gen Gata6 en progenitores pancreáticos produce defectos en la tolerancia a la glucosa en ratones adultos, indicando que GATA6 ejerce un papel importante en la función de la célula beta.
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USO DE INHIBIDORES EPIGENÉTICOS PARA LA MEJORA DE LA DIFERENCIACIÓN ENDOCRINA *IN VITRO*

Marta Fontcuberta[a], Sara Cervantes[a,b], Ramon Gomis[a,b,c], Rosa Gasa[a,b]
[a]*Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, España*

[b]*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, Barcelona, España*

[c]*Universidad de Barcelona, Hospital Clínico, Barcelona, España*

Antecedentes y objetivo Durante el desarrollo embrionario, el programa de diferenciación celular está controlado por factores de transcripción específicos de linaje y por mecanismos epigenéticos. El factor de transcripción bHLH Neurogenina3 (Neurog3) promueve el programa de diferenciación endocrina en el páncreas. Estudios preliminares de nuestro grupo muestran que la activación de genes endocrinos en respuesta a Neurog3 está asociada a la pérdida de la marca epigenética represiva trimetilación de la lisina 27 de la histona H3 (H3K27me3) en las regiones promotoras de estos genes. Estos resultados sugieren una estrecha relación entre Neurog3 y los reguladores epigenéticos de esta marca. En el presente estudio nos hemos propuesto estudiar el efecto de la inhibición de la metilasa (EZH2) y las desmetilasas (JMJD3/UTX) de H3K27 en la activación del programa endocrino en respuesta a Neurog3.
Materiales y métodos Hemos usado la línea ductal mPAC como modelo de diferenciación endocrina dependiente de Neurog3. Hemos utilizado los inhibidores químicos GSK126 y EI-1 (inhibidor de EZH2)y GSK-J4 (inhibidor de JMJD3 y UTX). La expresión de genes se ha estudiado mediante *real time* PCR y el enriquecimiento de marcas epigenéticas de interés mediante ChIP. Los niveles totales de H3K27me3 se han analizado por western blot.
Resultados Nuestros experimentos muestran que los inhibidores de las desmetilasas JMJD3/UTX y de la metilasa EZH2 provocan un incremento y una disminución significativa de los niveles totales de la marca H3K27me3 en las células mPAC. Además, la adición del inhibidor GSK-J4 bloquea significativamente la inducción promovida por Neurog3 del programa endocrino en las células mPAC. Por el contrario, los inhibidores EI-1 y GSK-126 potencian este programa. En cualquier caso, la pérdida de la marca H3K27me3 sin la presencia del gen inductor Neurog3 no es suficiente para activar transcripcionalmente los genes silenciados, a excepción de genes bivalentes que muestran expresión a nivel basal (por ejemplo Atoh8).
Conclusiones La manipulación de la actividad de reguladores epigenéticos de la marca H3K27 podría ser útil para diseñar protocolos de diferenciación endocrina *in vitro* más eficientes. Actualmente estamos aplicando estos inhibidores a cultivos de explantes pancreáticos embrionarios.
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DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TOWARDS PANCREATIC BETA-CELL SURROGATES THROUGH REGULATION OF PDX-1 BY NITRIC OXIDE

Salguero-Aranda C[b],Tapia-Limonchi R[b], Cahuana GM[a], Hitos AB[a], Diaz I[a], Hmadcha A[b], Fraga M[c], E[a], Martín F[a], Soria B[b], Tejedo JR[a], Bedoya FJ[a*]
[a]*Andalusian Center for Molecular Biology and Regenerative Medicine-University Pablo de Olavide, CIBERDEM, RED-TERCEL, Seville, Spain*

[b]*Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER) -Fundación Progreso y Salud, CIBERDEM, RED-TERCEL, Seville, Spain*

[c]*Department of Epigenetics, Oncologic Institute of Principado of Asturias, Oviedo 33006, Spain*

Background and aims. Pancreatic and duodenal homeobox 1 (Pdx1) is a transcription factor that regulates the embryonic development of the pancreas and the differentiation towards beta cells. An appropriate regulation of its expression must be essential during the generation of insulin producing cells derived from pluripotent stem cells. Our main objective is to study Pdx1 regulation in mouse embryonic stem cells (mESC) after DETA-NO treatment and to develop a differentiation protocol to insulin-producing cells.
Methods: mESC, D3 and R1-E, were used to study Pdx1 gene regulation and to design a differentiation protocol to insulin producing cells. INS-1E cells were used as control of Pdx1 expression. To analyze Pdx1 expression real time-PCR and western blot were employed, and flow cytometry and immunocytochemistry were used to quantify and localize Pdx1 expression. Bisulfite Sequencing PCR (BSP) and bisulfite pyrosequencing were carried out to analyze the methylation of Pdx1 promoter on treated and untreated cells. To analyze the epigenetic histone marks on Pdx1 promoter, chromatin immunoprecipitations to H3 acetylated, H3K4me3 and H3K27me3 were developed. An immunoprecipitation with anti-Jarid2 antibody was made to determine the interaction between Polycomb Repressive Complex 2 (PRC2) proteins and P300. Finally, a differentiation protocol from mESC to beta cells was designed using DETA-NO, valproic acid, P300 inhibitor and a last step of suspension culture. The results were analyzed by RT-PCR and immunocytochemistry.
Results: We have shown that exposure of mESCs to high concentrations of DETA-NO promotes the expression of Pdx1 and that is associated with the release of PRC2 and the histone acetyltransferase P300 from its promoter. These events are accompanied by changes in bivalent marks of histone H3k27me3 and H3K4me3, site specific changes of DNA methylation, and no change in H3 acetylation of Pdx1 promoter. Based on these results, we developed a protocol to differentiate mES cells towards insulin producing cells. This protocol succeeds in obtaining cells that express pancreatic beta cell progenitor markers such as PDX1, INS1, GCK and GLUT2.
Conclusion: In this study, we show that Pdx1 is highly expressed after DETA-NO treatment and the relation with site specific changes of methylation, changes in bivalent marks of histone H3k27me3 and H3K4me3 and release of PRC2 and P300 from its promoter. The knowledge of Pdx1 regulation in mESC has allowed the development of an efficient differentiation protocol for generating insulin−producing cells.
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MICRORNAS MODULATE DIFFERENTIATION IN MOUSE AND HUMAN EMBRYONIC STEM CELLS INTO INSULIN-PRODUCING CELLS AND CONTRIBUTES TO MATURATION

Javier López-Beas[a], Bernat Soria[a,b] and Abdelkrim Hmadcha[a,b]

[a]*Department of Stem Cells, Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER)-Fundación Progreso y Salud, Sevilla, 41092, Spain*

[b]*CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, 08036, Spain*

MicroRNAs constitute a class of molecules regulating gene expression in many different cell types and can be identifed two types of miRNAs, universally miRNAs and specific miRNAs of development stage, so functions of miRNAs could be linking to the biologies of the tissues in which expressed. Some miRNAs are specifically expressed in stem cells and a few miRNAs have been identified to be expressed preferentially in islets, miR-375 and miR-7 are two of the most abundant miRNAs in pancreas, this miRNAs are necessary for proper pancreatic islet development. To determine the functional role of miR-7 in the endocrine lineage, the currently most widely used miRNA mimic strategy for miRNA overespression is based on mimics RNAs chemically synthesized, which mimic mature endogenous miRNAs after transfection into cells. We hypothesized that miR-7 have important role in our experimental induction of pancreatic β cells, our study provides evidence the role of miR-7 in improve insulin secretion and regulate differentiation of pancreatic β cells during development. The results provide new insight into the identification of specific miRNAs implicated in pancreatic differentiation.

JARID2 MODULATES PANCREATIC ENDOCRINE CELL DIFFERENTIATION

Sara Cervantes[a,b], Marta Fontcuberta[a,b], Ainhoa García[a,b], Anna Tutusaus[a], Young Sook Lee[c], Ramón Gomis[a,b,d], Rosa Gasa[a,b]

[a]*Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*

[b]*Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, Barcelona*

[c]*Department of Cellular and Regenerative Biology, University of Wisconsin-Madison, Madison, Wisconsin, USA*

[d]*University of Barcelona, Hospital Clínic, Barcelona*

Background and aims: Pancreas development and endocrine cell formation result from the coordinated actions of embryonic signaling pathways and transcription factors that dynamically change gene expression patterns. These changes are intimately associated with alterations in chromatin structure by the addition/removal of covalent modifications. Among them, changes in the methylation state of histones and in particular, the trimethylation of histone H3 at Lysines 4 and 27, have emerged as important modulators of developmental decisions. The Polycomb Repressor Complex 2 (PRC2) carries out repressive functions by catalyzing the addition of methyl groups to Lysine 27. Jumonji (Jmj), also known as Jarid2, is a catalytically-death histone

demethylase that regulates PRC2 activity and targeting, and is essential for mouse embryonic development. In this study, we aim at determining the potential role of Jarid2 in pancreatic endocrine cell differentiation.

Material and methods: We have generated Pdx1-Cre; Jarid2$^{lox/lox}$ mice, which lack Jarid2 specifically in pancreatic cell progenitors and their descendants, and have investigated their embryonic and adult phenotypes by immunofluorescence stainings and gene expression profiling.

Results: Our experiments revealed that ablation of Jarid2 in Multipotent Pancreatic Progenitor Cells alters expression of endocrine and exocrine differentiation markers. Importantly, loss of Jarid2 results in a 40% decrease in insulin positive area during the secondary transition. Although endocrine specification is not altered in Pdx1-Cre; Jarid2$^{lox/lox}$ pancreas, the expression of a subset of key endocrine transcription factors, including that of Mafa and Mafb, is perturbed by loss of Jarid2. These defects in gene expression lead to a reduction in β-cell mass at birth and glucose intolerance in adult mice.

Conclusions: Jarid2 expression increases during endocrine differentiation and is required for implementation of the pancreatic endocrine cell differentiation programme.

RESVERATROL AMELIORATES THE MATURATION PROCESS OF β-CELL-LIKE CELLS OBTAINED FROM AN OPTIMIZED DIFFERENTIATION PROTOCOL OF HUMAN EMBRYONIC STEM CELLS

Daniela Pezzolla[a], Abdelkrim Hmadcha[a,b] and Bernat Soria[a,b]

[a]*Department of Stem Cells, Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER)-Fundación Progreso y Salud, Sevilla, 41092, Spain*

[b]*CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, 08036, Spain*

Human embryonic stem cells (hESCs) retain the extraordinary capacity to differentiate into different cell types composing the adult body, including pancreatic β-cells. However, for this particular lineage, a gold-standard differentiation protocol has not yet been achieved. Besides, recent studies evidenced the impact of resveratrol (RSV) on insulin secretion, even though the mechanism by which this polyphenol potentiates glucose-stimulated insulin secretion (GSIS) is still not clear. The aim of this study was to optimize an efficient differentiation protocol that mimics in vivo pancreatic organogenesis and to investigate whether RSV may improve the final maturation step to obtain functional insulin-secreting cells. Our results indicate that treatment of hESCs (HS-181) with activin-A induced definitive endoderm differentiation as detected by the expression of SOX17 and FOXA2. Addition of retinoic acid (RA), Noggin and Cyclopamine promoted pancreatic differentiation as indicated by the expression of the early pancreatic progenitor markers ISL1, NGN3 and PDX1. Moreover, during maturation in suspension culture, differentiating cells assembled in islet-like clusters, which expressed specific endocrine markers such as PDX1, SST, GCG and INS. Similar results were confirmed with the human induced Pluripotent Stem Cell (hiPSC) line MSUH-001. Finally, differentiation protocols incorporating RSV treatment yielded numerous insulin-positive cells, induced significantly higher PDX1 expression and were able to transiently normalize glycaemia when transplanted in streptozotocin (STZ) induced diabetic mice thus promoting its survival. In conclusion, our strategy allows the efficient differentiation of hESCs into pancreatic endoderm capable of generating β-cell-like cells and demonstrates that RSV is a critical compound ameliorating the maturation process.