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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

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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

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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

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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