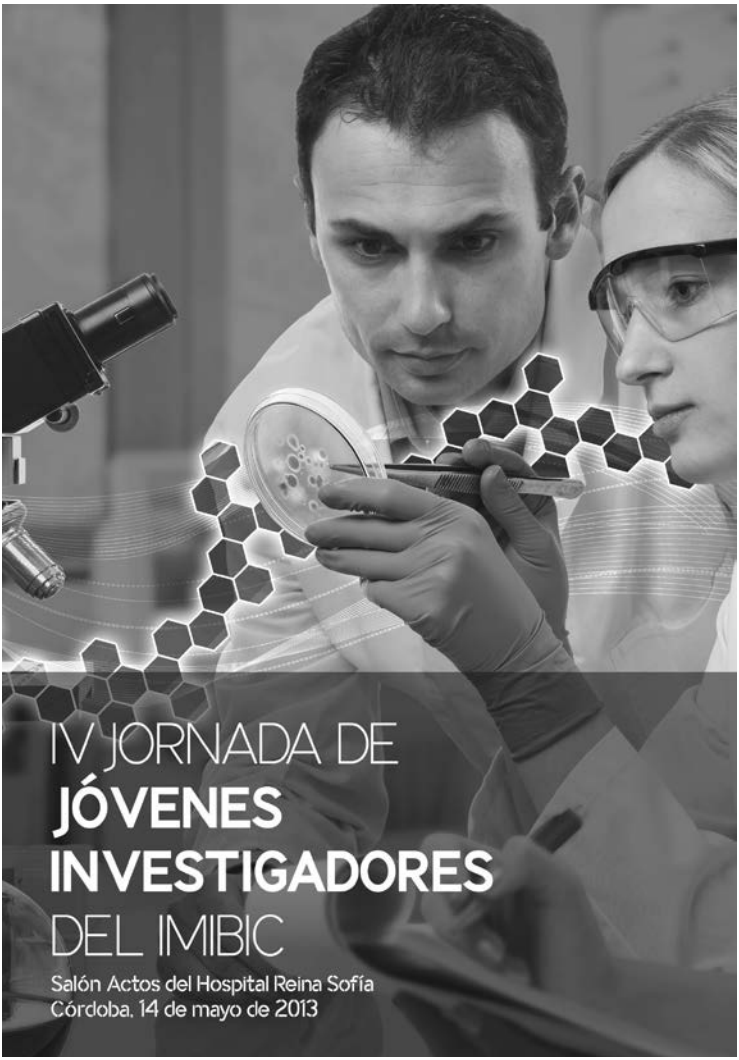
IV JORNADA DE **JÓVENES INVESTIGADORES** DEL IMIBIC

Salón Actos del Hospital Reina Sofía
Córdoba, 14 de mayo de 2013



IV JORNADA DE **JÓVENES INVESTIGADORES** DEL IMIBIC

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PROGRAMA DE LA JORNADA

9:00 - 9:30	**Inscripción y colocación de pósters**
9:30 - 10:00	**Acto de Inauguración**
10:00 - 12:45	**Sesión I. Enfermedad Cardiovascular. Obesidad y Síndrome Metabólico. Enfermedades hepáticas y digestivas. Enfermedades renales y nefrourológicas.**
I.a 10:00 - 10:15	Comparative effect of dietary fat in the expression of oxidative stress related genes in adipose tissue and peripheral blood mononuclear cells from metabolic syndrome patients. Patricia Judhit Peña Orihuela.
I.b 10:15 - 10:30	Effects of parenteral nutrition formulas on plasma lipid profile in children with bone marrow transplantation. Mercedes Gil Campos.
I.c 10:30 - 10:45	Obesity-induced hypogonadism in the male: premature reproductive senescence and contribution of Kiss1-mediated mechanisms. Miguel Ángel Sánchez-Garrido.
I.d 10:45 - 11:00	The small GTPase Rab18 in the regulation of lipid droplet dynamics in adipocytes. Yoana Rabanal Ruiz.
I.e 11:00 - 11:15	Magnesium increases osteogenic differentiation of rat mesenchymal stem cells through notch signaling activation. Juan Miguel Diaz Tocados.
11:15 - 11:45	**Café. Sesión de Pósters**
I.f 11:45 - 12:00	Neuroproteomic approaches to identify novel kisspeptin-regulated pathways in the hypothalamus: studies in Kiss1 ko mice. Antonio Romero-Ruiz
I.g 12:00 - 12:15	Effects of mediterranean diet supplemented with coenzyme q10 on metabolomic profile in elderly men and women. Lorena González de la Guardia
I.h 12:15 - 12:30	Proteomic profiling of adipose tissue subcutaneous in obese patients before and after bariatric surgery. Natalia Rocio Moreno Castellanos
I.i 12:30 - 12:45	Metabolic control of puberty: essential roles of alpha-msh signaling in mediating the permissive effects of leptin on puberty onset. María Manfredi Lozano.
I.j 12:45 - 13:00	Calcium deficiency reduces circulating levels of fibroblast growth factor 23. María Encarnación Rodríguez Ortiz
13:00-14:00	**Conferencia Plenaria** **Dr. Óscar Fernández-Capetillo**
14:00-16:00	**Almuerzo**

16:00-18:15	**Sesión II. Oncología y Oncohematología. Enfermedades Inflamatorias Crónicas y Enfermedades Infecciosas. Senescencia.**
II.a 16:00 - 16:15	Role of carbamylated high density lipoprotein on EPCs selection in vitro. Carlos Luna Ruiz
II.b 16:15 - 16:30	Combination of multitarget kinase inhibitor AEE788 and cyclooxygenase-2 inhibition for the treatment of colorectal cancer: a preclinical study. Araceli María Valverde Estepa
II.c 16:30 - 16:45	Regulation of alternative splicing of ghrelin gene by the antisense strand gene GHRLOS. David Rincón Fernández-Pacheco
II.d 16:45 - 17:00	AGTR-1 as a biomarker of response to treatment with the antiangiogenic drug bevacizumab in solid tumors. Francisco Manuel Conde Pérez
17:00-17:30	**Sesión de Pósters**
II.e 17:30 - 17:45	The ubiquitin ligase SIAH2 as a prognostic and predictive marker in lung cancer. Paula Moreno Casado
II.f 17:45 - 18:00	Recruitment of pluripotent “side population” cells in non-malignant reactive human lymph nodes. Vianihuini Figueroa
II.g 18:00 - 18:15	Role of new components of somatostatin and ghrelin systems on the proliferation of pancreatic neuroendocrine tumor cell lines. Alicia Villa Osaba
18:15-19:00	**Entrega de premios y Acto de Clausura**

SESION I

- HEART DISEASE
- OBESITY AND METABOLIC SYNDROME
- HEPATIC AND DIGESTIVE DISEASES
- RENAL AND NEPHROUROLOGICAL DISEASES

01 COMPARATIVE EFFECT OF DIETARY FAT IN THE EXPRESSION OF OXIDATIVE STRESS RELATED GENES IN ADIPOSE TISSUE AND PERIPHERAL BLOOD MONONUCLEAR CELLS FROM METABOLIC SYNDROME PATIENTS

Author/es: Patricia Peña Orihuela, Javier Delgado Lista, Antonio Camargo, Eliana Meza Miranda, Ana Ortíz, José López Miranda.
IMIBIC Group: B02.Nutrigenómica. Síndrome metabólico.
Oral Communications

Background: Oxidative status may be modulated by the diet in Metabolic Syndrome (MetS). We studied the effect of 4 diets with different quantity and quality of dietary fat on postprandial expression of gp91phox, p47phox, CAT, GPx1 and TXNRD1 in adipose tissue (AT) and peripheral blood mononuclear cells (PBMC) from MetS patients.
Study Design: A randomized, controlled trial (LIPGENE study) MetS patients were assigned to 1 of 4 diets (12 wk each): (i) high-saturated fatty acids (HSFA), (ii) high-monounsaturated fatty acids (HMUFA), (iii) low-fat, high-complex carbohydrate diet supplemented with n-3 polyunsaturated fatty acids (LFHCC n3), and (iv) low-fat, high-complex carbohydrate diet with placebo (LFHCC). A meal reflecting the fatty acid composition as the original diets was conducted post-intervention.
Results: The postprandial mRNA levels of gp91phox and p47phox increased after HSFA meal in both AT and PBMC (all $p<0.05$). The postprandial expression of CAT, GPx1, and TXNRD1 decreased after HSFA meal ($P<0.001$, $P=0.001$ and $P<0.001$) in AT, and the expression of CAT and TXNRD1 after HSFA meal was lower than after the HMUFA, LFHCC and LFHCC n-3 meals (all $p<0.05$). By contrast, in PBMC, the expression of CAT, GPx1 and TXNRD1 increased after HSFA meal. The postprandial CAT and TXNRD1 mRNA levels were higher after HSFA meal than after LFHCC ($P=0.039$ and $P=0.013$) and the LFHCC n-3 meals ($P=0.011$ and 0.033). The GPx1 ($P=0.044$) mRNA levels were higher after HSFA meal than after LFHCC n-3 meal. The GPx1 and TXNRD1 mRNA levels at 2 h after HSFA meal intake were higher than after the HMUFA ($P=0.019$ and $P=0.021$), LFHCC ($P=0.038$ and $P=0.002$) and LFHCC n-3 ($P=0.001$ and $P=0.001$) meals.
Conclusion: The consumption of HSFA diet increase oxidative stress as consequence of the reduction of antioxidant genes expression in AT whereas the PBMC, increase the expression of the antioxidant defences as consequence

Keywords: Metabolic Síndrome, Oxidative stress, Diet, Adipose tissue, PBMCs

NOTES:

02 EFFECTS OF PARENTERAL NUTRITION FORMULAS ON PLASMA LIPID PROFILE IN CHILDREN WITH BONE MARROW TRANSPLANTATION

Author/es: M Gil-Campos, MA Baena-Gómez; FJ Llorente-Cantarero; JL Pérez Navero, MJ de la Torre Aguilar.
IMIBIC Group: BE07. Metabolismo infantil.
Oral Communications

Background: In children undergoing bone marrow transplantation (BMT) it is often required parenteral nutrition (PN).
Objective: This study compares plasma lipid and fatty acids profiles after a fish oil or soybean PN in BMT children.
Methods: 14 children with BMT and requiring PN for at least 10 days were selected. They were randomized to use a lipid emulsion with fish oil, or soybean oil.
Blood samples before starting and at the end of the PN were taken to analyze lipids. Results: Similar changes in plasma levels of cHDL, cLDL, triglycerides (TG) and apolipoprotein A were detected with both formulas. TG levels increased more after the administration of the emulsion of fish oil although levels of cHDL, and apo A decreased more after administration of soybean oil.
There were no differences by groups in plasma linolenic acid levels although in both formulas, levels increased after 10 days of PN. Eicosapentaenoic acid increased in fish oil formula group respect to soybean fed; their levels were higher at the end of the supplementation compared with the basal levels.
Linoleic acid did not differ between the two groups. Also, arachidonic acid levels in fish oil formula reduced their levels at 10 days respect to basal time and when PN last 21 days, this acid decreased in patients with both formulas.
Conclusion: PN for 10 days are effective and safe in children. There are initial changes in plasma lipid profile more dependent on the duration of PN, than the type of lipid formula itself. However, plasma fatty acid profile changes are related with the omega fatty acid composition of the formulas. Other paediatric studies are necessary in order to establish the importance of the duration of interventions and the use of non-traditional lipid composition of PN formulas.

Keywords: bone marrow transplant, parenteral nutrition, fatty acids.

NOTES:

03 OBESITY-INDUCED HYPOGONADISM IN THE MALE: PREMATURE REPRODUCTIVE SENESCENCE AND CONTRIBUTION OF KISS1-MEDIATED MECHANISMS.

Author/es: M.A. Sánchez-Garrido, F. Ruiz-Pino, M. Manfredi-Lozano, S. León, D. García-Galiano, A. Romero-Ruiz, J.M. Castellano, C. Diéguez, L. Pinilla, M. Tena-Sempere.
IMIBIC Group: B03. Regulación hormonal del balance energético, la pubertad y la reproducción.
Oral Communications

Reproduction is sensitive to insufficient body energy reserves, especially in females. Metabolic regulation of male reproductive axis is less obvious, and the impact of conditions of persistent energy excess has received moderate attention. Yet, the escalating prevalence of obesity and the clinical evidence of its deleterious effects on male fertility have raised considerable concerns. We report here phenotypic and mechanistic studies of the reproductive impact of conditions of postnatal under- or over-nutrition, coupled to high fat diet (HFD) after weaning. Metabolic and hormonal analyses in young (4-mo) and middle-aged (10-mo) animals revealed that HFD caused profound metabolic perturbations, which were worsened by precedent postnatal overfeeding; these were detectable already in young males but aggravated in 10-mo-old rats. Impairment of reproductive parameters took place progressively, and HFD alone was sufficient to explain many, but not all these alterations. In young males, testosterone (T) levels and steroidogenic enzyme expression were suppressed by HFD, without compensatory increases of LH levels, which were in fact partially inhibited in heavier males. In addition, obese males displayed suppressed hypothalamic Kiss1 expression despite low T, and HFD inhibited LH responses to kisspeptin. Overweight anticipated some of the reproductive effects of ageing, such as the suppression of hypothalamic Kiss1 expression and decline in serum T and LH levels. Nonetheless, HFD per se caused a detectable worsening of key reproductive indices in middle-aged males, such as basal LH and FSH levels, as well as LH responses to kisspeptin. Our study demonstrates that nutritional stress, especially HFD, has a profound deleterious impact on metabolic and gonadotropic function, as well as on Kiss1 system, and precipitates reproductive senescence in the male.

Keywords: Early programming, postnatal overfeeding, postnatal underfeeding, high fat diet, obesity, gonadotropins, testosterone, kisspeptins, male rat

NOTES:

04 THE SMALL GTPASE RAB18 IN THE REGULATION OF LIPID DROPLET DYNAMICS IN ADIPOCYTES

Author/es: Rabanal-Ruiz Y, Almabouada F, Palomo ME, Guzmán-Ruiz R, García-Navarro S, Vazquez-Martínez R, Malagón MM
IMIBIC Group: BE06. Metabolismo y diferenciación adipocitaria. Síndrome metabólico.
Oral Communications

Adipocytes store excess energy in lipid droplets (LDs). Lipid storage and mobilization in LDs is regulated by proteins that associate to the LD surface, including, among others, several members of the Rab family of small GTPases that regulate intracellular trafficking, such as Rab18. We have recently shown that Rab18 regulates both insulin-mediated lipogenesis and β-adrenergic-induced lipolysis in adipocytes. Herein, we examined the relationship of Rab18-labeled LDs with organelles involved in lipid management and evaluated its interaction with the cytoskeletal components responsible of organelle transport. Confocal microscopy revealed that both insulin and isoproterenol increased Rab18 recruitment to LDs and the rapprochement of LDs to endoplasmic reticulum (ER) membranes. However, only insulin enhanced the colocalization between Rab18- LDs, peroxisomes and mitochondria. These data suggest that Rab18 might provide physical and metabolic linkage of LDs to these organelles. Interestingly, LDs are surrounded by microtubule bundles, which, partially, coincide with patches of Rab18 labeling, suggesting that LD linkage to other compartments might be mediated through the interaction of this GTPase with the microtubule cytoskeleton. In line with this, immunoprecipitation assays identified MAST3, a microtubule associated kinase, as a putative Rab18 interacting protein. Other components of the Rab18 interactome (identified by Yeast Two-Hybrid) include LPPR3, which has been proposed to interact with ER enzymes involved in triglyceride synthesis, further supporting a functional, Rab18-mediated, association between LDs and the ER. In all, our results suggest that, through its interaction with the microtubule network, Rab18 may help maintain or retain LDs at specific positions in the cell to favour lipid transfer between LDs and other intracellular organelles. This role for Rab18 in lipid management in adipocytes is further supported by our findings on the enhanced expression of this GTPase in adipose tissue of obese human.

Funding: MINECO/FEDER (BFU2010-17116), J. Andalucía/FEDER (CTS-6606), and CIBERobn (ISCIII), Spain

NOTES:

05 NEUROPROTEOMIC APPROACHES TO IDENTIFY NOVEL KISSPEPTIN-REGULATED PATHWAYS IN THE HYPOTHALAMUS: STUDIES IN KISS1 KO MICE.

Author/es: A. Romero-Ruiz, L. Steffanini, S. León, M. Manfredi, D. Garcia-Galiano, L. Pinilla, W.H. Colledge, M. Tena-Sempere.
IMIBIC Group: B03. Regulación hormonal del balance energético, la pubertad y la reproducción.
Oral Communications

Solid experimental evidence has documented the role of kisspeptins as major gatekeepers of the reproductive brain, as well as his sensitivity to different forms of metabolic stress by transporting the metabolic cues to the centers controlling puberty in the hypothalamus. However, while much has been learnt on the upstream regulators of Kiss1 neurons and the intracellular signaling cascades recruited upon Kiss1 receptor activation, limited knowledge is still available on the final targets of such cascades. To obtain an integral view of the pathways activated by kisspeptins in the hypothalamus, we have applied proteomic analyses to hypothalamic tissue of a Kiss1 KO mouse, where kisspeptin signaling was activated by injection of kisspeptin-10 (Kp-10). As complementary approach, neuroproteomics was also employed in a mouse model of acute Kiss1 suppression, due to short-term (24-h) fasting at puberty. Adult male Kiss1 KO mice were orchidectomized (ORX) before the experiments to avoid the fluctuations of endogenous sex steroid levels after Kp-10 injection. In keeping with previous references, ORX did not induce changes in circulating LH in Kiss1 null animals, whereas Kp-10 injection evoked robust LH secretory bursts, thus proving the validity of our model. Proteomic analyses identified >30 proteins that were differentially expressed in the hypothalamus of Kiss1 KO mice after Kp-10 stimulation, including elements of the synaptic machinery, metabolic pathways, structural proteins and glial factors. In parallel, proteomics was also applied to mouse models of fasting and re-feeding. As validation of the model, fasting for 24-h was shown to induce a significant suppression of hypothalamic Kiss1 mRNA expression coupled to lowering of serum LH levels; both responses were reverted by re-feeding. In this model of transient suppression of the endogenous Kiss1 tone due metabolic stress, initial proteomic analyses identified up to 5 different targets that are differentially expressed in the hypothalamus of fasted animals. Interestingly, two of the five hits, synapsin-1 and glial fibrillary acidic protein (GFAP), were also found to be differentially expressed in Kiss1 KO experiments. Validation analyses by western blots have confirmed that both, Kiss1 KO mice after kp10 injection and fasting models, present increased levels of GFAP and phosphorylated synapsin-1 proteins in the hypothalamus. Our results suggest the involvement of neuronal as well as glial pathways in mediating hypothalamic responses to kisspeptin and conditions of metabolic stress. Further analyses and validation assays (by expression analysis and neuroanatomical studies) are in progress in our laboratory.

Keywords: Kisspeptin, Puberty, Metabolic Stress, Neuroproteomic, Glial Cells.

NOTES:

06 EFFECTS OF MEDITERRANEAN DIET SUPPLEMENTED WITH COENZYME Q10 ON METABOLOMIC PROFILE IN ELDERLY MEN AND WOMEN.

Author/es: González-Guardia L,Yubero-Serrano EM,García-Ríos A, Nieves Delgado-Casado, Roche H, Pérez-Jiménez F, López-Miranda J
IMIBIC Group: B02. Nutrigenómica. Síndrome metabólico.
Oral Communications

Introduction: Prospective studies have demonstrated the beneficial effects of Mediterranean diet consumption due to its great antioxidant capacity. Coenzyme Q10 (CoQ) is part of the mammalian mitochondrial electron transport chain and a potent lipid soluble antioxidant. Metabolomics focuses on the interaction between the products of metabolism and dietary intake, identifying which metabolites are responsible for the effects of different diets. The aim of this study was to determine whether diets with different fat quality and supplementation with CoQ affect the metabolomic profile in urine from elderly people.
Materials and methods: 5 men and 5 women were randomly assigned to receive, in a crossover design, 4 isocaloric diets for 4-week periods each: (1) Mediterranean diet supplemented with CoQ (Med+CoQ diet; 200 mg/day), (2) Mediterranean diet, (3) Western diet rich in saturated fat (SFA diet), (4) Low-fat, high-carbohydrate diet enriched in n-3 polyunsaturated fatty acid. Urine samples corresponding to baseline and 12 h fast after each dietary intervention were analyzed by 1H-NMR spectroscopy.
Results: Multivariate analysis showed increased urinary levels of hippurate ($p = 0.037$) after Med+CoQ diet intake and increased urinary levels of phenylacetylglycine ($p = 0.049$) after SFA diet, in women. Following consumption of Med+CoQ diet, we observed positive correlations between hippurate and CoQ ($p=0.038$) and β-carotene plasma levels ($p=0.023$). In addition, we found that hippurate was inversely related to Nrf2 gene expression ($p=0.004$), antioxidant enzymes gene expression as thioredoxin ($p=0.004$), superoxide dismutase 1 (SOD1) ($p=0.030$) and gp-91phox subunit of NADPH oxidase ($p=0.039$). After SFA consumption phenylacetylglycine was inversely related to CoQ plasma levels ($p=0.039$) and positively correlated with isoprostanes ($p=0.013$).
Discussion: Our results show increased hippurate urinary levels and decreased phenylacetylglycine urinary levels after Med+CoQ diet consumption compared to SFA diet. The association between hippurate and antioxidant biomarkers and, in contrast, phenylacetylglycine and oxidant biomarkers, suggests that this diet could be beneficial to avoid processes linked to oxidative stress such as cardiovascular, neurodegenerative diseases and aging.

Keywords: Coenzyme Q10, Mediterranean diet, Metabolomics

NOTES:

07 PROTEOMIC PROFILING OF ADIPOSE TISSUE SUBCUTANEOUS IN OBESE PATIENTS BEFORE AND AFTER BARIATRIC SURGERY

Author/es: Moreno N , Guzmán-Ruiz R Diaz-Ruiz A, García-Navarro S, Vázquez-Martínez R, Malagón MM.
IMIBIC Group: BE06. Metabolismo y diferenciación adipocitaria. Síndrome metabólico.
Oral Communications

Obesity is tightly linked to insulin resistance, which represents a major risk factor for the development of type 2 diabetes and cardiovascular disease. In obese patients resistant to conventional treatments for weight loss, bariatric surgery (BS) is an effective alternative for reducing the incidence of diabetes. Herein, we have characterized the proteome of subcutaneous adipose tissue (AT) of morbidly obese subjects before and after BS in order to identify candidate biomarkers associated with the loss of fat mass and the improvement of insulin sensitivity. AT from obese patients (BMI=50.2±1.2 kg/m2), including normoglycemic (NIR) or insulin resistant (IR) patients, were collected before (PRE) and after (POST) (13.3±0.37 months) BS. Comparative proteomic analyses of AT samples were carried out by 2D-DIGE/MALDI-TOF/TOF. This enabled the identification of 61 differentially expressed proteins among the different groups. Pathway analysis software revealed that proteins were related to insulin signaling and belong to three major functional groups: metabolic and cellular processes, and immune response. Specifically, BS decreased inflammation, as assessed by pJNK/JNK ratio, and improved the glycemic profile and adipokine pattern in morbidly obese patients. An increase in the expression of metabolic enzymes (DLDH, RALDH1) was also observed in both NIR and IR patients after BS. Moreover, BS also evoked adaptive changes in cytoskeletal (vimentin, septin11 and TCP1beta) and mitochondrial proteins (mitofilin), which were more noticeable in NIR patients. Finally, an effect of BS on the expression of angiogenic markers (TrpRS, VEGF), which were differentially expressed between NIR and IR subjects, was also observed. In sum, our data indicate that NIR and IR patients differed in their AT proteomic profiles before BS and, although to a lesser extent, also after BS, and suggest that BS could be more effective if carried out before the development of IR.
Funding: MINECO/FEDER (BFU2010-17116), J. Andalucía/FEDER (CTS-6606), and CIBERobn (ISCIII)

Keywords: Keywords: Bariatric surgery, obesity, adipose tissue, insulin resistance.

NOTES:

08 METABOLIC CONTROL OF PUBERTY: ESSENTIAL ROLES OF ALPHA-MSH SIGNALING IN MEDIATING THE PERMISSIVE EFFECTS OF LEPTIN ON PUBERTY ONSET

Author/es: Maria Manfredi-Lozano, Silvia Leon, Francisco Ruiz-Pino, David García-Galiano, M.J. Vázquez, Miguel A. Sanchez-Garrido, Leonor Pinilla, Juan Roa, Manuel Tena-Sempere
IMIBIC Group: B03. Regulación hormonal del balance energético, la pubertad y la reproducción.
Oral Communications

Melanocortins (MC), which include alpha-, beta- and gamma-melanocyte stimulating hormones (MSH), are products of the proopiomelanocortin (POMC) gene that operate via five different MC receptors and are involved in a wide variety of biological functions. A prominent population of POMC neurons in the hypothalamic arcuate nucleus is known to synthesize alpha-MSH that acts via MC3R and MC4R to suppress food intake. Indeed, alpha-MSH plays a key role in mediating the anorectic effects of leptin. In addition, alpha-MSH pathways seem to be involved in the central control of reproduction, although variable results have been reported. Yet, recent data in the ewe suggested a potential interplay between alpha-MSH and Kiss1 circuits in the regulation of the reproductive axis. However, whether alpha-MSH signaling is involved in the control of puberty onset remains largely unexplored.

In this work, we aimed at investigating the putative function of alpha-MSH signaling in the control of the gonadotropic axis at puberty and explored potential leptin/alpha-MSH/ kisspeptin interactions. The alpha-MSH agonist, MT-II, injected centrally elicited robust LH responses in pubertal male and female rats, even against unfavorable metabolic conditions (i.e., short-term fasting), while infantile animals were non-responsive. Using selective MCR agonists, we documented that this effect is mediated by MC4R, but not MC3R, activation. Chronic blockade of MC3/4R by the administration of the antagonist, SHU9119, during the pubertal transition disrupted the normal timing of puberty, as demonstrated by delayed vaginal opening. In addition, while leptin conducted a positive effect on puberty onset in females subjected to chronic sub-nutrition during the pubertal transition, the effect of leptin was blocked by central co-administration of SHU9119. Finally, to elucidate potential alpha-MSH/kisspeptin interactions, we tested the ability of kisspeptin-10 to elicit LH secretion after effective blockade of MC3/4R, and explored LH secretory responses to MT-II in mice with congenital inactivation of the kisspeptin receptor, Gpr54. While MT-II effects on LH secretion were abrogated by SHU9119 pre-treatment, blockade of MC3/4R did not affect LH responses to Kp-10. In turn, although net LH responses to MT-II were markedly attenuated in Gpr54 null mice, LH secretion was modestly but significantly stimulated by the alpha-MSH agonist in the absence of kisspeptin signaling. Complementary analyses are in progress in our laboratory addressing potential changes in the hypothalamic expression of Kiss1 gene following central blockade of MC3/4R.

In sum, our results are the first to document an essential role of alpha-MSH, acting likely via MC4R, in the control of female puberty. Preserved alpha-MSH signaling seems indispensable not only for the proper timing of puberty but also for conveying the permissive effects of leptin. While our data suggest that some of the stimulatory effects of alpha-MSH are kisspeptin-dependent, they also point out the existence of kisspeptin-independent effects of alpha-MSH on gonadotropin secretion, whose relative importance in the metabolic control of puberty merits future investigation.

Keywords: alpha-melanocyte stimulating hormone; metabolism; puberty; MC3-R; MC4-R; leptin, kisspeptin

NOTES:

09 CALCIUM DEFICIENCY REDUCES CIRCULATING LEVELS OF FIBROBLAST GROWTH FACTOR 23

Author/es: Encarnación Rodríguez-Ortiz, Ignacio López, Juan R. Muñoz-Castañeda, Julio M. Martínez-Moreno, Alan Peralta-Ramírez, Carmen Pineda, Antonio Canalejo, Philippe Jaeger, Escolástico Aguilera-Tejero, Mariano Rodríguez, Arnold Felsenfeld, and Yolanda Almadén
IMIBIC Group: D06. Metabolismo del calcio. Calcificación vascular
Oral Communications

Introduction: Fibroblast growth factor 23 (FGF23) is a hormone which mainly acts on kidney regulating phosphorus (P) and calcitriol (CTR) levels. CTR production is a protective physiological mechanism to avoid the deleterious effects of low calcium (Ca). However, the organisms might be unprotected if FGF23 inhibits CTR production in a state of hypocalcemia. Thus, the aim of our study was to evaluate whether Ca level affects FGF23 secretion.
Methods: Adult male Wistar rats were randomly allocated into several groups: control, low Ca low vitamin D diet (LCa-LD) and low Ca low vitamin D high P diet (LCa-HP-LD). Blood samples were collected to measure plasmatic concentrations of Ca (automatic analyzer), P and creatinine (spectrophotometry), PTH and FGF23 (ELISA), and CTR (RIA). Additionally, parathyroidectomized (PTx) rats were infused with calcium gluconate (10 mg/kg/hour for 6 hours) or fed with a high Ca diet to determine whether Ca directly regulates FGF23 secretion.
Results: Ca levels were lower in LCa-LD and LCa-HP-LD than in control group (3.24±1.16 and 3.80±0.88 mg/dl respectively vs. 4.80±0.12, $p<0.05$). As expected, PTH secretion was also increased in animals fed with Ca-deficient diets (1510±1072 and 825±944 pg/dl vs. 57±21 pg/dl, $p<0.05$), as CTR was (427±47 and 452±43 pg/ml vs. 296±106, $p<0.05$). However, despite high PTH and CTR levels, FGF23 remained low in LCa-LD group (37±56 pg /ml) vs. control (163±51 pg/ml, $p<0.05$). Calcium infusion produced an increase in FGF23 (218±62 vs. 101±5 pg/ml in non-treated rats, $p<0.01$) and in Ca concentration (4.12±0.32 vs. 2.36±0.20 mg/ml, $p<0.01$). In a similar way, rats fed with high Ca diet had higher FGF23 (194±73 vs. 94±9.6 pg/ml, pmenor0.05) and Ca (3.04±0.36 pg/ml vs. 2.28±0.16 mg/dl, $p<0.01$) than rats fed with a normal content Ca diet.
Conclusion: Our results suggest that extracellular calcium modulates FGF23 production as a physiological mechanism to avoid hypocalcemia.

Keywords: FGF23, mineral metabolism, vitamin D, calcium, phosphorus, PTH

NOTES:

SESION II

- ONCOLOGY AND ONCOHEMATOLOGY
- CHRONIC INFLAMMATORY DISEASES AND INFECTIOUS DISEASES
- SENESCENCE

10 ROLE OF CARBAMYLATED HIGH DENSITY LIPOPROTEIN ON EPCs SELECTION IN VITRO.

Author/es: Carlos Luna, Andrés Carmona, Paula Buendía, Mª Rosa Moyano, Mª José Jiménez, Alejandro Martín-Malo, Pedro Aljama, Julia Carracedo, Rafael Ramírez.
IMIBIC Group: A06. Daño celular en la inflamación crónica.
Oral Communication

Introduction: EPCs (Endothelial Progenitors Cell) are involved in the balance cell damage / repair of vascular endothelium. Recruitment from the bone marrow and its function may be altered in uremia. Uremic toxins produced carbamylation of proteins, it is unknown whether high-density lipoprotein (HDL) modified by carbamylation may be involved in the growth and function of EPCs.
Aims: Assess the effect of chemical modification on HDL by carbamylation in EPCs growth in culture.
Materials and Methods:We developed an in vitro carbamylation method analogous to physiological process that occurs in patients with CKD and uremia. Carbamylation in vitro was carried out by contacting the Normal lipoprotein (nHDL) with a buffer of NaBO 0.1M + KOCN 0.5M during 48h at 37 ° C, transforming it into cHDL. In a primary culture of EPCs isolated from peripheral blood of healthy subjects we were monitored the formation of CFUs (Colony Forming Units) by microscopy. (Control - Control +, + nHDL, + cHDL at concentration 100ug/ml). We were assessed by flow cytometry the phenotype of EPCs I, EPCs II and endothelial adhesion markers (CD54, Tie-2).
Results: It was observed that in the presence of nHDL, the number of CFUs increased significantly (91.8 ± 10.80) compared to Control - (55.5 ± 8.20) and cHDL (56.2 ± 9.6), ($p < 0.001$). No significant changes were observed in the number of CFUs in cultures with cHDL compared to the Control -. Relative to EPCs phenotype, were observed in cultures with nHDL the reduction of % EPCs I and the increase of % EPCs II. As for adhesion markers there is a trend of increasing both (CD54 and Tie-2) in culture compared with control - and nHDL. (See table).
Conclusion: cHDL function on the proliferation of EPCs is decreased in relation to nHDL. Our study indicates that protein carbamylation by a method analogous to that which occurs in uremic patients, can't allow the benefitial function on the recruitment of EPCs in patients with CKD.

Keywords: Endothelial Progenitors Cell, High-Density Lipoproteins, Uremia, Carbamylation, Proliferation, CKD, Endothelial Disfunction.

NOTES:

11 COMBINATION OF MULTITARGET KINASE INHIBITOR AEE788 AND CYCLOOXYGENASE-2 INHIBITION FOR THE TREATMENT OF COLORECTAL CANCER: A PRECLINICAL STUDY

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IMIBIC Group: A08. Nuevas terapias en cáncer.
Oral Communication

Introducción: KRAS and BRAF mutations are common in CRC and confer resistance to anti-EGFR therapy. Combined therapies may provide improved therapeutic responses over monotherapies. We examined the efficacy of AEE788 and cyclooxygenase-2 inhibition as anti-tumor treatment in a preclinical model of colorectal cancer.
Material and Methods: The human colorectal cells lines SW48 (KRAS/BRAF non-mutated), Caco-2 (BRAF V600E), HCT116 (KRAS G13D) were exposed to the indicated treatments and cell proliferation, apoptosis, EGFR, Akt and ERK 1/2 activation, VEGF expression/production, angiogenic and colonosphere formation capacities were determined.
Results: AEE788 inhibited EGFR phosphorylation in all three cell lines, although the more effective inhibition of EGF-dependent growth was observed in BRAF mutated Caco-2 cells. In these cells, AEE788 inhibited the activation of intracellular kinases Akt and ERK1/2, efficiently induced apoptosis and caused cell cycle arrest at G2/M transition. Furthermore, AEE788 reduced in Caco-2 cells both mRNA VEGFA164 expression, VEGF production, VEGF-dependent growth and reduced the endothelial tube formation. These anti-tumoral effects were associated with the inhibition of ERK1/2 phosphorylation by AEE788 treatment and the high expression of COX-2 observed in Caco-2 cells. The addition of a specific cyclooxygenase-2 inhibitor (celecoxib) significantly enhanced the antiproliferative effect of AEE788 in Caco-2 cells but not its antiangiogenic effects. Notably, the combined treatment of Caco-2 or HCT116 (KRAS G13D) cells impaired their potential to form colonospheres, which is a functional assay for the renewal capacity of cancer stem cells (CSCs). Changes in morphology and size of colonospheres were also observed.
Conclusion: Our results support that AEE788 therapy may be effective in the treatment of colorectal cancer. Besides, its antitumoral efficacy may be potentiated by combination with COX-2 inhibitors, and this combined therapy may also be effective to specifically target CSCs in colorectal cancer, even in a context of K-RAS mutation.

Keywords: AEE788, KRAS, COX-2, CCR

NOTES:

12 REGULATION OF ALTERNATIVE SPLICING OF GHRELIN GENE BY THE ANTISENSE STRAND GENE GHRLOS

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IMIBIC Group: B01. Hormonas y cáncer.
Oral Communication

Ghrelin is a pleiotropic hormone encoded by a gene (GHRL), which can also originate several splicing variants associated with diverse neuroendocrine pathologies. Specifically, our group has recently identified a new splicing variant named In1-ghrelin, which is differentially expressed in several tumoral pathologies, where it could be directly related to their tumorigenicity. However, the molecular mechanisms responsible for the appearance of this variant and its regulation are unknown. In this regard, many studies point out the important role of non-coding RNAs in the regulation of alternative splicing processes. Particularly, it has been recently described the existence of a non-coding gene (GHRLOS) in the antisense strand of GHRL. Specifically, GHRLOS presents six known splicing variants, which could be involved in regulating the alternative splicing of GHRL. Some of those variants overlap with the intron retained in the In1-ghrelin mRNA variants and could, therefore, be associated with its generation. Hence, we aimed to determine 1) the relationship between the expression ofeach GHRLOS variant, ghrelin and In1-ghrelin in human tissues and in endocrine-related tumors; 2) the regulatory capacity of GHRLOS variants on ghrelin and In1-ghrelin expression in tumoral cell lines; and 3) the effect of GHRLOS variants overexpression on functional parameters, such as proliferation, in tumoral cell lines. Our data demonstrate that the expression of some GHRLOS variant is differentially correlated with the expression of the ghrelin gene variants (i.e, the correlation between the expression levels of GHRLOS-2 and GHRLOS-3 with In1-ghrelin is R2=0,964 and R2=0,807, respectively, in human tissues). Additionally, overexpression of GHRLOS variants can induce increased proliferation rate in tumoral cell lines, similar to that shown for In1-ghrelin overexpression. Altogether, our data suggest an involvement of GHRLOS variants in the alternative splicing of the GHRL and, consequently, in the dysregulation of the ghrelin system observed in many endocrine-related cancers.

Keywords: opposite strand gene, lncRNAs, splicing regulation, ghrelin, In1-Ghrelin, GHRLOS, cancer.

NOTES:

13 AGTR-1 AS A BIOMARKER OF RESPONSE TO TREATMENT WITH THE ANTIANGIOGENIC DRUG BEVACIZUMAB IN SOLID TUMORS

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Oral Communication

The antiangiogenic drug bevacizumab is a humanized monoclonal antibody against human vascular endothelial growth factor (VEGF), which has shown efficacy for the treatment, among others, of metastatic colon and breast cancer. However only few patients get a huge benefit of this antiangiogenic therapy and there is an imperative need for reliable predictive biomarkers of response. Significantly, hypertension secondary to antiangiogenic therapy is a marker of treatment efficacy. Angiotensin II receptor 1 (AGTR1) is a component of the renin-angiotensin system (RAS) which controls blood pressure and cardiovascular homeostasis. There is experimental evidence that AGTR1 is involved in processes of carcinogenesis, invasion, metastasis and angiogenesis, and our group has previously shown that the response to bevacizumab in breast cancer is significantly higher in tumors expressing AGTR1. Therefore, this study was aimed to analyze the role of AGTR1 in the response of tumor cells to bevacizumab.

Antibody array experiments showed that angiotensin II treatment of BT549 breast cancer cells, which express AGTR1, induced phosphorylation of ERK 1/2 and Akt. Furthermore, ERK1/2 phosphorylation was impaired by pre-treatment with the AGTR1 antagonist losartan. However, angiotensin II had no effect on BT549 proliferation, although bevacizumab slightly reduce cell growth in a dose-dependent manner either in the presence or the absence of angiotensin II. When AGTR1 was transiently over-expressed in MCF-7 breast cancer cells a proliferative effect of angiotensin II was observed compared to non-transfected cells. Furthermore, an antiproliferative effect of bevacizumab was observed in the presence of angiotensin II, mainly in AGTR1 transfected cells. Notably, similar results were obtained with a stably AGTR1 transfected MCF-7 cell line.

In conclusion, our data indicate that antitumoral effects of bevacizumab in breast cancer cells depends on AGTR1 expression and angiotensin II levels, supporting the potential use of AGTR1 and/or angiotensin II as predictive markers of response to antiangiogenic therapy

Keywords: Bevacizumab, VEGF, AGTR1, intracellular signaling, tumor proliferation and invasion.

NOTES:

14 THE UBIQUITIN LIGASE SIAH2 AS A PROGNOSTIC AND PREDICTIVE MARKER IN LUNG CANCER.

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IMIBIC Group: A05. Inflamación y cáncer.
Oral Communication

Background: Lung cancer is the leading cause of cancer-related deaths worldwide, and this high mortality partly reflects the limited efficacy of the currently available therapies. In the context of a project to identify new targets for lung cancer treatment, we decided to study the possible role of seven-in-absentia homologs 2 protein (SIAH2), an ubiquitin ligase which is able to modulate signalling pathways key in the development of lung cancer as RAS, p53 and hypoxia pathway.
Methods: One hundred and thirty-one samples from patients treated surgically for primary lung cancer from January 2011 to December 2012 were obtained for the study. Genic and protein expression levels, as well as an immunohistochemical evaluation of SIAH2 was used to determine the expression of SIAH2, and compared with clinic-pathologic variables, in the overall series and among the 2 major NSCLC histological subtypes (adenocarcinoma and squamous-cell carcinoma-SCC).
Results: Changes in SIAH2 gene expression were not detected. However, protein expression was increased significantly in tumor samples compared to normal lung samples, both in the overall series (0.6 vs 0.44, p=0.011) and when analysing the 2 main histological subtypes separately (adenocarcinoma: 0.59 vs 0.38, p=0.02; SCC: 0.65 vs 0.49, p=0.02). Furthermore, immunohistochemical evaluation revealed a strong nuclear staining in all cases (mean total score of 6,52), and that the intensity of the staining was higher among poorly-differentiated tumors. SIAH2 expression by immunohistochemistry correlated positively with SIAH protein expression in lung tumors (0.32, p=0.02).
Conclusions: This is the first study that shows the role of SIAH2 on human lung cancer. We observed that SIAH2 expression is enhanced significantly in adenocarcinoma and squamous cell lung cancer. No significant changes at a genic level were observed, thus suggesting a post-traductional control mechanism. An increased correlation of SIAH2 expression with grade of differentiation was detected, suggesting that this protein could be used as a prognostic biomarker to predict lung cancer progression. These results, together with the oncogenic functions of SIAH2, confirm the potential clinical applications of targeting SIAH2 in lung cancer therapy.

Keywords: Lung cancer, SIAH2, hypoxia, Prognostic marker

NOTES:

15 RECRUITMENT OF PLURIPOTENT "SIDE POPULATION" CELLS IN NON-MALIGNANT REACTIVE HUMAN LYMPH NODES.

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IMIBIC Group: C03. Biología celular en hematología.
Oral Communication

Background: Side Population (SP) cells are stem cells with pluripotent capacity that have been found in many normal tissues and are characterized by a high capability to efflux the dye Hoechst 33342 (Ho342) due to an efflux pump called ABCG2 (ATP-binding cassette G2). Interestingly, SP cells are also present in haematological malignancies being considered as a cancer initiating stem cell. However, the detection and characterization of SP in lymph nodes suspected of lymphoma has not been addressed so far.
Methods: We analyzed sixty-nine removed lymph nodes suspected of malignant lymphoma). Final pathologic diagnoses were: 35 (50.7%) Reactive Lymphadenitis (RL), 20 (29%), Non-Hodgkin Lymphoma (NHL) 12 (17.4%) Hodgkin Lymphoma (HL) and 2 metastasis lymph nodes (ML)
Results: We did not detect measurable amounts of SP cell in lymph nodes form NHL or ML identified by their profile of Ho342 dye efflux after excitation at 350 nm ultra-violet light. Strikingly, SPHo342Low in 82.3% of RL samples (mean 1.89±1.17%; range 0-39.12%) and in 50% of HL samples (mean 0.66±0.63%; range 0-7.69%). 98% of cases had a surface phenotype CD45+/CD34-/CD19-/CD20-/CD-133Low/neg and 2% were CD45+/CD34-/CD19+/CD20+CD133neg. SP cells from RL were sorted on complete medium with IL-3, IL-6 and SCF and cell colonies were visible after 5 days acquiring hematopoietic phenotype CD34+/CD133+. IHC analysis disclosed high level of ABCG2 protein in germinal centers in 95% of Reactive Lymphadenitis samples polarized to para-cortex area. In 30% of Hodgkin Lymphoma samples were positive for ABCG2 protein on Reed-Stemberg cells and non-tumoral cells. After mRNA extraction from paraffined samples, NANOG and OCT-4 and ABCG2 were quantified by PCR . We found a significant increased gene expression of ABCG2 in Reactive Lymphadenitis samples compared to other groups ($P<0.01$) and were also statistically correlated with the detection of SP cell in lymph node. NANOG and OCT-4 were detectable at low levels and no statistical differences were found when comparing LR with HL or NHL.
Conclusions: Pluripotent SP cells are present in enlarged lymph nodes in reactive processes and in reactive component of HL, but they are lacking in NHL and metastatic lymph nodes. This finding was correlated with the expression of ABGC2 at protein and gene level. This pluripotent SP cell is likely to be recruited after inflammatory stimuli in non-malignant processes.

Keywords: Side population cells, lymphoma, cancer-initiating cells

NOTES:

16 ROLE OF NEW COMPONENTS OF SOMATOSTATIN AND GHRELIN SYSTEMS ON THE PROLIFERATION OF PANCREATIC NEUROENDOCRINE TUMOR CELL LINES.

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IMIBIC Group: B01. Hormonas y cáncer.
Oral Communication

Somatostatin (SST), cortistatin (CORT), their receptors (sst1-5), ghrelin and its receptors (GHSRs) comprise two interrelated systems that regulate multiple cell functions from hormone secretions to proliferation rate. Some components of these systems are co-expressed in several tissues, such as the pancreas. Specifically, SST, ghrelin and some of their receptors (sst1, sst2, sst5 and GHSRs) are expressed at pancreatic level. Recently, our group has identified new functional components of these systems originated by alternative splicing as it is the case of two truncated variants of sst5 (sst5TMD5, sst5TMD4), as well as an alternative ghrelin gene isoform, named In1-ghrelin. These new components of SST and ghrelin systems have been found to be alteredin several endocrine-related pathologies including pituitary tumors and breast or prostate cancers; where their overexpression was associated with an increased malignancy or tumorigenesis. The fact that we have observed that the expression of these splice variants is also altered in neuroendocrine tumors (NETs) compared to normal tissue led us to hypothesize that these new variants could be implicated in malignancy of pancreatic neuroendocrine tumors. In the present study, we analyzed the mRNA expression profile of both axes in cell lines derived from pancreatic NETs (BON-1 and QGP-1) and determined the functional consequences of the over-exposition to these new variants (In1-ghrelin and truncated sst5) on cell proliferation. Our results showed that the majority of components of SST/CORT and ghrelin systems are present at detectable levels in BON-1 and QGP-1, although at variable expression levels. Interestingly, overexpression of In1-ghrelin, sst5TMD5 or sst5TMD4 markedly enhanced the proliferation rate in both cell lines, suggesting that these new functional variants could play a relevant role in the pathophysiology of pancreatic NETs.

Keywords: neuroendocrine tumors, In1-ghrelin, somatostatin, ghrelin, truncated receptor

NOTES:

POSTER SESION

01 CD8+ T CELLS POLYFUNCTIONALITY: EFFECT OF CMV AND AGE

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IMIBIC Group: A01. Inmunosenescencia T y NK. Respuesta inmune antiviral.
Poster

CMV chronic infection plays an important role in the development of T cell age-associated changes (immunosenescence). It has been described a clonal expansion of CMV-specific CD8+ T cells in the elderly, with limited functional capacities. Furthermore, CMV infection also has a deleterious effect on the efficacy of influenza vaccination in the elderly, suggesting that CMV chronic infection in old age restricts immunological diversity and impairs the immune system functionality.

A single T cell can produce simultaneously multiple cytokines of the same type, commonly referred as polyfunctional. Several publications have shown that a higher number of polyfunctional T cells is correlated with a better prognosis during HIV infection and that the quality of the response, i.e. polyfunctionality, is predictive of control of the infection following challenge. Moreover, polyfunctional T cells produce higher amounts of cytokines than monofunctional T cells, i.e., polyfunctional cells are more efficient. Thus, the efficiency of the T cell response is associated with the capacity of responding cells to produce several cytokines ("polyfunctionality" as a marker of quality) rather than with the percentage (quantity) of specific T cells.

For that reason, we analyze the effect of CMV infection on CD8+ T cells polyfunctionality —degranulation (CD107a) and/or cytokines co-production (INFg and TNFa) —, in a cohort of young (10 CMV-seropositive and 10 CMV-seronegative) and middle age healthy donors (all of them where CMV-seropositive, n=12), in response to a nonspecific antigen (SEB). The results obtained show that the percentage of SEB-responding CD8+ T cells increases with the combination of both ageing and CMV infection but not with age alone. We also find an increase of polyfunctional CD8+ T cells in young and middle age CMV-seropositive individuals when compared to young CMV-seronegative. However, such difference is not found between young and middle age CMV-seropositive individuals. Thus, CMV infection increases the polyfunctionality of CD8+ T cells. When the effect of age on CMV-seropositive donors is analyzed, we observe an increase of TNFa monofunctional or IFNg/TNFa bifunctional CD8+ T cells, whereas the percentage of polyfunctional cells (IFNg/TNFa/CD107a) remains the same.

Therefore, here we demonstrate that CMV infection improves the polyfunctionality and consequently the quality of CD8+ T cells and this improvement is not significantly affected by age. On the other hand, age in combination with CMV contribute to the inflammation found in senescence by increasing the percentage of cells producing pro-inflammatory cytokines.

Keywords: CD8+ T cells, Polyfunctionality, immunosenescence, CMV infection.

NOTES:

02 STREPTOCOCCUS PNEUMONIAE MEMBRANE-DERIVED VESICLES INDUCE HOST CELL DEATH AND PROINFLAMMATORY RESPONSE IN MACROPHAGES

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IMIBIC Group: DE07. Identificación de proteínas antigénicas para el desarrollo de nuevas vacunas
Poster

Pneumococcal infections are a major cause of morbidity and mortality worldwide, being a major public health problem. Clinical manifestations of this include invasive pneumococcal infections, such as pneumonia, meningitis and febrile bacteremia; and non-invasive manifestations such as otitis media, sinusitis and bronchitis. Although there are vaccines against Streptococcus pneumoniae, they are not fully effective and have a high cost of production, which limits its application to international routine vaccination programs, especially in developing countries, where is most needed.
It has been recently demonstrated that Gram-positive organisms release membrane-derived vesicles (MVs) which, analogously to outer membrane vesicles (OMVs) of Gram-negative bacteria, can play a role in delivering virulence factors to host cells. Last year, we showed that pneumococcus produces these MVs and that these are biochemically different from cellular membranes. In this study we show that five pneumococcal strains, with different clinical manifestations pattern, produce MVs by scanning and transmision electron microscopy (SEM and TEM respectively) in cell preparations and after their purification in a density gradient with Optiprep by TEM. When J774 macrophages are infected with MVs, these induce a transitory dose-dependent cell death in MTT assays and a transitory pro-inflammatory response, as shown by measuring IFN-gamma, IL-10, IL-12p70, IL-1beta, IL-6, TNF-alpha and mKC. In addition, we show that these MVs contain pneumolysin, a virulence factor whose route for delivery is still unknown. Finally, we show that some proteins from these MVs are recognized by empyema-derived human sera. Because all of this, we think that MVs could be a good way to obtein an improved vaccine or even a faster diagnosis tool.

Keywords: Streptococcus pneumoniae, Membrane-derived Vesicles, MTT assay, Cytokines, Sera.

NOTES:

03 CAVEOLAR NECC2 AND INSULING SIGNALLING IN ADIPOCYTES

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IMIBIC Group: BE06. Metabolismo y diferenciación adipocitaria. Síndrome metabólico.
Poster

Afferent reception involves the participation of different signaling systems that mediate the correct communication between the extracellular and intracellular compartments, which initiates at specific membrane domains wherein receptors and signaling intermediates accumulate. Among these domains, there are the caveolae, flask shape invaginations enriched in cholesterol and sphingolipids covered with oligomers of caveolin. In adipocytes, caveolae harbour the insulin receptor (IR), the glucose transporter, GLUT4, and cortical actin filaments in close relationship with the IR. It has been proposed that caveolae are crucial for insulin action by acting as signaling platforms that assemble the molecular components of IR transduction. We have recently identified a novel protein, neuroendocrine long-coiled coil protein (NECC2), which contains a putative caveolin-binding domain and displays structural characteristics of a scaffolding factor (i.e., several coiled-coil domains spanning almost the entire sequence). In the present study, we evaluated the intracellular localization, regulation, and function of this protein in murine 3T3-L1 adipocytes and also examined the expression of NECC2 in human adipose tissue under different metabolic conditions. Our studies show that NECC2 distributes in the cytosol and in close apposition to the cell surface, wherein it colocalizes with the caveolae marker caveolin-1, cortical actin, and the IR under resting conditions. Interestingly, NECC2 is not internalized with the IR upon insulin stimulation but remains close to the plasma membrane; in fact, the quantity of NECC2 at the cell surface increases in response to insulin. Finally, the expression of NECC2 is up-regulated during adipocyte differentiation as well as in obesity, especially in conditions of insulin resistance, thus suggesting an adaptive role for this protein to increased adiposity. Altogether, our findings support the view that NECC2 could serve as a tethering factor or as a molecular scaffold to regulate insulin signaling in adipocytes.
Funding: MINECO/FEDER (BFU2010-17116), J. Andalucía/FEDER (CTS-6606), and CIBERobn (ISCIII), Spain.

Keywords: Caveolae, adipocytes, insulin, signalling, NECC2

NOTES:

04 STUDY OF ENDOTHELIAL DAMAGE MARKERS IN CDK IN HEMODIALYSIS

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IMIBIC Group: A09. Nefrología.
Poster

Introduction: Patients with chronic kidney disease (CKD) have a higher mortality from cardiovascular events (CVD) than the general population. During the process of reendothelialization and endothelial activation, events occur involved in endothelial dysfunction and its subsequent progress in a process of atherosclerosis. Endothelial microparticles (EMP) are considered markers of endothelial damage and are increased in patients with CVD, CKD and diabetes mellitus (DM) type II.
Objectives: To evaluate EMP levels and angiogenic factors in HD patients with and without DM.
Materials and Methods: 160 patients with CKD on HD RED Biobank of Nephrology Kidney. We selected two groups: Group 1, 80 patients and Group 2 DM, 80 DM patients of whom 17 had DM 63 DM type I and type II. EMP apoptotic (CD31+Annexin V+) were quantified by flow cytometry and VEGFR2, Ang1 and Ang2 were determinated by ELISA.
Results: The plasma concentration of VEGFR2 (pg/ml) was similar in both groups of patients. The ratio Ang2/Ang 1 showed a statistically significant decrease in patients with DM compared with the group without DM. The EMP apoptotic show a statistically significant increase in patients with DM compared to non-DM.
Conclusion: The ratio of Ang2/Ang1 together with EMP apoptotic HD CKD patients, with and without DM, both of them could be used as a prognostic biomarker for the progression of CVD.

Keywords: angiogenic factors, cronic kidney disease, diabetes mellitus, endothelial microparticles, endothelial damage.

NOTES:

05 SAMPLE PREPARATION AND UNTARGETED METABOLOMICS ANALYSIS OF EXHALED BREATH CONDENSATE

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IMIBIC Group: D04. Metabolómica. Identificación de componentes bioactivos.
Poster

Exhaled breath condensate (EBC) has been widely used for analysis of inflammatory and oxidative stress markers in humans and it enables the study of the early effects of different diseases or exposures on the lung and upper airways. EBC is usually sampled by cooling expired air using a condenser (–20 °C) and it is composed by volatile compounds and liquid aerosol droplets. The latter are constituted by water and a mixture of semivolatile and nonvolatile compounds.

Gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) were used for analysis of EBC samples. In this study, the former was selected for untargeted metabolomics analysis. Due to the low concentration of the analytes in the samples and the incompatibility between the sample matrix and the selected analytical technique, sample preparation must be considered as the key step of the analytical process. Two sample separation techniques such as liquid–liquid extraction and solid-phase extraction were evaluated, operating under optimal conditions, in order to find the most adequate for sample preparation. In addition, as volatility of the target analytes was required for analysis, most of the semivolatile and nonvolatile compounds were transformed into volatile derivatives via derivatization reactions such as silylation, methylation or acylation. Identification was achieved by comparing the GC retention indices and the mass spectra provided by the detector with the NIST-MS and the Golm metabolome database reference libraries. The reliability of the identified compounds was ensured by the use of spectral match (match ≥ 875) and retention index values (I-difference ≤ 50).

The developed methodology was applied to the analysis of EBC samples collected from healthy volunteers. The number of identified compounds in the samples was higher than 450. Differences in the composition of the analyzed samples due to the sample preparation were evaluated using statistical tools.

Keywords: Exhaled breath condensated, Untargeted metabolomics analysis, Sample preparation, Gas Chromatography- Mass Spectrometry.

NOTES:

06 PROTHROMBOTIC PROTEINS DECREASED IN PATIENTS WITH METABOLIC SYNDROME AFTER LONG-TERM INTAKE OF MEDITERRANEAN DIETARY PATTERN.

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IMIBIC Group: B02.Nutrigenómica. Síndrome metabólico.
Poster

Introduction: The metabolic syndrome (MetS) is a disorder in which converge different conditions and its etiology is the result of a complex interaction between genetic, metabolic and environmental factors including eating habits. The beneficial effect of the Mediterranean dietary pattern has been demonstrated. The aim of this work is to identify the changes that are induced in the proteome of peripheral blood mononuclear cells (PBMC) of patients with metabolic syndrome after long-term intake of a Mediterranean dietary model.
Materials and Methods: We isolated the proteic fraction of PBMC to 12 patients with MetS, participants in the LIPGENE study, at times 0 hours and after 12 weeks of the intake of a typical Mediterranean dietary pattern. Proteome changes were identified by 2D-PAGE electrophoresis analysis.
Results and Discussion: Four proteins that decrease their concentration after the long-term intake of a typical Mediterranean dietary pattern were identified: thrombin (F2), fibrinogen-β (FGB), Talin-1 (TLN1), capping protein-Z (CAPZA1). The analysis of metabolic pathways using Ingenuity Pathway shows that these proteins are associated with coagulation mechanisms and the induction of chemotaxis between neutrophils, production and release of proinflammatory molecules. Our results show that the decrease in the concentration of the proteins identified, which are directly related to a prothrombotic state, may have a beneficial effect induced by the long-term intake of the Mediterranean dietary pattern, by reducing the inflammatory status and the risk of developing atherosclerosis in patients with metabolic syndrome.
Conclusion: Proteomic changes identified in the PBMC proteome after long-term intake of a typical Mediterranean dietary pattern suggest that this nutritional model reduces the prothrombotic status in patients with MetS.

Keywords: Mediterranean diet, metabolic syndrome, proteomics

NOTES:

07 DIFFERENTIAL EXPRESSION OF MICRORNAS IN MONOCYTES AND NEUTROPHILS FROM PRIMARY ANTIPHOSPHOLIPID SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS. POTENTIAL VALUE AS BIOMARKERS OF ATHEROTHROMBOTIC DISEASE.

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IMIBIC Group: A07. Enfermedades autoinmunes sistémicas e inflamatorias crónicas del aparato locomotor y tejido conectivo.
Poster

Background: No study has evaluated the expression profile of miRNAs associated with the cardiovascular and the atherothrombotic risks observed in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS).
Objective: To identify the miRNAs involved in the regulation of pro-inflammatory, prothrombotic and oxidative status in SLE and APS patients.
Methods: Neutrophils and monocytes were isolated from 11 APS patients, 17 SLE patients and 26 healthy donors. Selected miRNAs were quantified by RT-PCR. The expression of proinflammatory and prothrombotic proteins was evaluated by flow cytometry. Antioxidant enzymatic activity was also quantified. Carotid intima-media thickness (CIMT) was measured as a marker of early atherosclerosis.
Results: In silico search reported miR-124a, -125a, -125b, -146a, -155, and -222 as candidates. The expression levels of these miRNAs appeared significantly decreased in neutrophils from SLE and APS patients compared to healthy donors. Only the miR-124a was found reduced in monocytes. The expression levels of the miRNAs analyzed in SLE patients negatively correlated with the disease activity (SLEDAI) and the anti-dsDNA titers. In addition, an inverse correlation between anticardiolipin antibody titers and miRNAs expression was found in APS patients. Moreover, significant negative correlations between miRNAs expression and oxidative stress parameters were observed in APS monocytes and neutrophils. Inverse correlations were found with VEGF-R1, IL-8 and PAR2 in APS, while in LES significant correlations were related to IL-2, IL-6, IL-10 and MCP.1 Low levels of miR-146a in APS and SLE neutrophils, and miR-155 in APS neutrophils, were associated with pathological CIMT. The presence of thrombotic events was associated with low levels of miR-146a and miR-155 in neutrophils and monocytes.
Conclusions: miRNAs differentially expressed in monocytes and neutrophils from APS and SLE patients correlate with markers of autoimmunity, inflammation, thrombosis and oxidative stress, and are associated with atherothrombotic processes. Therefore, these miRNAs might be considered potential biomarkers of atherothrombotic disease.

Keywords: MicroRNA, Lupus, Antiphospholipid syndrome, atherothrombotic disease, inflammation and oxidative stress.

NOTES:

08 INFLUENCE OF AGE AND PRO-INFLAMMATORY STATE ON THE FREQUENCY OF NK CELLS SUBPOPULATIONS

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IMIBIC Group: A01. Inmunosenescencia T y NK. Respuesta inmune antiviral.
Poster

Several previous studies have show that NK cells are affected by the process of immunosenescence. In this study, we analyzed the effect of age and pro-inflammatory state on the frequency of NK cells subpopulations in elderly donors. We have also studied if CMV-infection affects the distribution of these subpopulations in young donors.
A total of 30 young donors (16 CMV-seropositive and 14 CMV-seronegative; age range 19-35) and 26 elderly donors (age range 72-91) were included. NK cell subpopulations defined by the expression of CD56 and CD16 were studied by multiparametric flow cytometry. CMV serostatus and the levels of C-reactive protein (CRP) were studied.
Our results did not show significant different in the frequency of NK cells from CMV+ and CMV- young donors, indicating that CMV infection seropositivity does not affect the frequency of NK cells in healthy young individuals.
An increased percentage of total NK cells was observed in elderly individuals compared with young. The percentage of CD56dimCD16+ subpopulation was maintained while the percentage CD56-CD16+ subpopulation was increased with age. The frequency of the CD56brightCD16- and CD56brightCD16low subpopulations was decreased. The frequency of CD56brightCD16- subpopulation in the elderly was inversely associated with the levels of CRP, indicating that the frequency of NK cell subpopulations changes with age and that these changes are more pronounced in elderly donors with pro-inflammatory state.

Keywords: Immunosenescence, NK cells, CMV-infection, CRP levels

NOTES:

09 THE OVER-EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE INDUCES MITOCHONDRIAL DISFUNCTION AND CELL DEATH IN HEPG2 CELL LINE

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IMIBIC Group:A02. Estrés oxidativo y nitrosativo en hepatopatías agudas y crónicas.

Poster

Introduction: Inducing nitric oxide (NO) production has proven to be a potential therapeutic strategy for the treatment of experimental hepatocarcinogenesis. We have recently proved that stable over-expression of endothelial nitric oxide synthase (NOS-3) increases expression of CD95 in the human hepatocarcinoma cell line HepG2, making the cells to be more susceptible to anti-Fas-induced cell death. In addition, NOS-3 has been localized in mitochondria and it has been related to cellular oxygen consumption through inhibition of the oxidative phosphorylation system. The objective of the present study was to determine the effect of NOS-3 over-expression in anti-Fas-induced cell death and at mitochondrial level.

Material and Methods: The study was conducted from HepG2 cell line stably transfected with plasmid pcDNA4/TO containing the cDNA of NOS-3. Cell death was induced by anti-Fas agonist and was evaluated by caspase-3 and caspase-9 activation, and cytochrome c release into the cytosol. Under these conditions, we studied the cellular localization of NOS-3 by confocal microscopy, and by western-blot in mitochondria isolated by ultracentrifugation. Similarly, we evaluated the activity of the electron transport chain, ATP levels and cellular oxidative stress, by polarography and spectrophotometry. The post-translational modifications were assessed by western-blot analysis of the mitochondrial fraction.

Results: The over-expression of NOS-3 was associated to mitochondrial biogenesis increase, higher activity of the respiratory complexes II+III, reactive oxygen species production, ATP generation, and cytochrome c release. It was also observed an increase of protein nitration related to NOS-3 over-expression. Immunolocalization experiments showed that NOS-3 is mainly found in plasma membrane and perinuclear area, but also in the outer mitochondrial membrane of the transfected cells. In the presence of anti-Fas, it was detected an cellular increase of NOS-3 expression/stability that coincided with a lower localization of the protein in the mitochondrial fraction. Similarly, the higher caspase-3 and-9 activities in the presence of anti-Fas were associated to NOS-3 over-expression.

Conclusion: According to previous studies in endothelial cells, NOS-3 was located in the mitochondrial outer membrane of human hepatocarcinoma cells. Over-expression of NOS-3 was associated to mitochondrial activity increase and susceptibility to cell death in the presence of anti-Fas.

Keywords: nitric oxide;oxidative stress; apoptosis; nitric oxide synthase; HepG2

NOTES:

10 DIETARY FAT MODIFIES THE INFLAMMATORY STATE IN METABOLIC SINDROME PATIENTS WITH INSULIN RESISTANCE: THE LIPGENE STUDY

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IMIBIC Group : B02.Nutrigenómica. Síndrome metabólico.
Poster

The metabolic syndrome (MetS) is a cluster of metabolic abnormalities leading to increased risk for cardiovascular disease and diabetes type 2. The obese, insulin resistance (IR), pro-inflammatory states are central to the disease process.
Our aim was to determine whether the long-term consumption of four isoenergetic diets with different fat contents has a selective influence on the expression of pro-inflammatory genes in peripheral blood mononuclear cells of MetS patients according the HOMA index (Homeostasis model assessment of IR).
75 patients were divided into two groups according the HOMA index: HOMA<3.2 and HOMA≥3.2 and randomly assigned to one of four diets: high saturated fatty acids (HSFA); high monounsaturated fatty acids (HMUFA) and two low-fat, high complex carbohydrate diets, supplemented with long-chain n-3 polyunsaturated fatty acids (LFHCC n-3) or placebo (LFHCC), for 12 week each. Fasting blood samples were taken in post-intervention period (week 12). Expression levels of MMP (metalloproteinase)-9, TNF (factor necrosis tumoral)-α, IL (interleukin)-6, IL-1β and IL-8 were measured by RT-PCR. The data were analyzed using univariate general linear model and one-way ANOVA.
MMP-9 gene expression was reduced after intake of HMUFA diet compared with HSFA diet in patients with HOMA≥3.2 ($p<0.05$). Furthermore, the consumption of HSFA and LFHCC diets induced an increase of TNF-α mRNA levels respect to HMUFA diet in patients with HOMA≥3.2 ($p<0.05$). Finally, we observed an increase of IL-8 gene expression after intake of LFHCC and LFHCC n-3 diets compared to HMUFA diet in patients with HOMA≥3.2 ($p<0.05$). There were no significant differences in IL-6 and IL-1β mRNA levels among the four diets.
The long-term consumption of HMUFA diet attenuates the inflammatory state associated with MetS in patients with IR. These findings support recommendations to consume this dietary pattern as useful preventive measure against the chronic inflammation and IR that underlies in MetS patients.

Keywords: Metabolic Syndrome, Insulin resistance, Inflammation, Monounsaturated fatty acid

NOTES:

11 THE SMALL GTPASE RAB18 MODULATES NEUROENDOCRINE SECRETION BY INTERACTING WITH COMPONENTS OF THE MICROTUBULE-BASED SECRETORY GRANULE TRANSPORT MACHINERY

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IMIBIC Group: BE06. Metabolismo y diferenciación adipocitaria. Síndrome metabólico.
Poster

Several Rab proteins control hormone release by regulating the activity of different components of the secretory granule transport machinery. Particularly, Rab18 inhibits secretory granule movement, which leads to reduced neuropeptide and hormone secretion in neuroendocrine cells, but how this GTPase accomplishes its role and the identity of the proteins that regulate Rab18 activity (effectors) remain unknown. In this work, we searched for the Rab18 effectors that ultimately determine its participation in neuroendocrine secretion. Time-lapse video-microscopy revealed that a functional microtubule-based cytoskeleton network is necessary for Rab18 to anchor to secretory granules and inhibit their movement. Furthermore, yeast two-hybrid experiments allowed us to identify several putative Rab18 interacting proteins, among which it is worth to highlight a kinesin-1 for its well-known role in anterograde secretory granule transport. We confirmed Rab18/kinesin-1 interaction by Fluorescence Resonance Energy Trasnfer (FRET), which also showed that such an interaction only occurs with the active, GTP-bound conformation of Rab18. Furthermore, FRET also revealed that huntingtin (HTT), a protein that modulates intracellular membrane trafficking, associates with the inactive, GDP-bound form of Rab18. HTT overexpression increased Rab18 association to the surface of secretory granules in PC12 cells, which suggests that HTT could facilitate exchange of GDP by GTP in Rab18. Altogether, these results suggest that Rab18 reduces neuroendocrine secretion by interacting and regulating the activity of various components of the microtubule-based transport apparatus. We have also extended our studies to humans by analyzing the gene expression fingerprint of patients with different types of pheochromocytoma and found a strong positive correlation between Rab18 and GDI2 expression, a factor that inhibits GTP binding and activation of Rab18. This and the fact that Rab18 expression is abnormally reduced in pheochromocytomas could contribute to the hormone hypersecretion characteristic of these tumors.

Funding: MINECO/FEDER (BFU2010-17116), J. Andalucía/FEDER (CTS-6606), and CIBERobn (ISCIII), Spain.

NOTES:

12 RECONSTRUCTING A VOLUMETRIC MUSCLE LOSS BY ADIPOSE TISSUE IMPLANTATION. AN EXPERIMENTAL STUDY.

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IMIBIC Group: BE05. Estrés oxidativo y nutrición.
Poster

Skeletal muscle (SM) regeneration is a powerful process of tissue reconstruction that occurs after muscle injury. However, when there is a substantial loss of muscle mass the volume deficit is replaced with fibrous tissue.
This study examines the possibility that the implantation of adipose tissue (AT) can contribute to the reconstruction of an important volumetric muscle loss by the participation of AT-derived stem cells in muscle regeneration. We based on the fact that AT has been identified as a source of pluripotent cells; that can be converted to a myogenic phenotype when cultured in vitro in a myogenic environment. Moreover, the regenerating SM environment in vivo is capable of inducing uncommitted progenitors to terminally differentiate.
Wistar rats were anaesthetized and a cylindrical piece (6 Ø x 5 mm length) from central portion of anterior tibial muscle was removed. Inguinal subcutaneous AT of the same rat was inserted in the gap; as control group frozen AT was implanted. Rats were sacrificed 7, 14, 21, 28 and 90 days postimplantation; muscles were removed and analyzed by light microscopy using histological, histochemical and inmunohistochemical techniques.
Our observations show that implanted AT is replaced with new SM tissue. The newly muscle fibers were formed from the regenerative response of surviving muscle host, but the observation of desmin-positive mononucleated cells in the implantation zone suggest that it can also result from undifferentiated multipotent mesenchymal cells of AT. However the new muscle tissue showed several cyto-histoarchitectural abnormalities indicating inadequate innervation and mechanical reintegration.
Implantation of AT to reconstruct important volumetric muscle loss may be an alternative, although the new muscle does not reach its original structure, not having evaluated its function yet. Our results can be interesting to evaluate histologically SK designed by tissue engineering, although they have similarities, but not completely replicates the normal characteristics.

Keywords: Skeletal muscle; muscle regeneration; adipose tissue; mesenchymal stem cells

NOTES:

13 SPATIOTEMPORAL EVOLUTION AND GENOTYPIC CHARACTERIZATION OF A NOSOCOMIAL OUTBREAK OF KLEBSIELLA PNEUMONIAE PRODUCING KPC-3.

Author/es: Irene Gracia-Ahufinger, Rocío Tejero-García, Lorena López-Cerero, Manu Causse, Marcelino González-Padilla, Fernando Rodríguez-López, Francisco Solis, Manuel Casal.
IMIBIC Group: A04. Enfermedades infecciosas.
Poster

Introduction:Nosocomial infections with KPC producing Klebsiella spp. are due to blaKPC genes transfered by plasmids, that produce a pattern of multidrug resistance with few therapeutic options. We describe an outbreak of KPC-3 producing Klebsiella pneumoniae (KPC-3-Kpn) in non-endemic area, originated by a patient transfered from an Italian hospital.
Material and Methods: For 31 weeks, KPC-3-Kpn isolates were recorded. The identification and sensitivity testing was performed by the semiautomated method WIDER I®. Genotypic characterization of carbapenemase was performed with Hodge test, PCR and specific enzymes (A, B, D) and subsequent sequencing. Clonal relationship conducted with XbaI PFGE and dendrogram was generated using the Dice coefficient and by MLST scheme with the Institut Pasteur. Plasmid analysis was performed by extraction (Kieser method), electroporation into DH10 E.coli, Inc/rep group analysis by PCR and subtyping of Inc-F by RBT.
Results: A total of 295 samples (59 patients) with KPC-3-Kpn were detected (71.2% male, mean age 61.8 years, 13-93). Of the isolates, 94.9% were inpatient: 81.4% General Hospital and 18.6% Provincial Hospital. Distribution of KPC-3-Kpn isolates was: ICU (31.5%), Internal Medicine (23.4%) and Infectious Diseases (11.9%). Surgery areas together accounted for 15.9% of the isolates. In the first 15 weeks, 213 KPC-3-Kpn were isolated (72.2% from 43 patients): 28.6% in respiratory specimens, 26.7% exudates and 15.5% blood. The 90% of ICU isolates were detected in the first 15 weeks (81 isolates from 23 patients), and there were two separated peaks of KPC-3-Kpn isolation, preceding confinement in locations outside of the ICU. All isolates were only susceptible to Tygecicline and gentamicin. Hodge test was positive, detecting SHV-11, TEM-1 and KPC-3. All KPC-3-Kpn showed 99.5% similarity by PFGE with the index case and were assigned to clone ST512. An only Inc-F 140kb plasmid was detected, which K2-FAB formula was: A-/B-.
Conclusions: We describe a highly epidemic outbreak by a clon ST512 of a KPC-3-Kpn. from a case imported from Italy. The spread from the ICU and the surgery´s block was clear, reflecting the extreme importance of isolation measures to reduce nosocomial transmission.

Keywords: Nosocomial infection, Klebsiella pneumoniae, KPC carbapenemase

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14 THERAPEUTIC APPROACHES BASED ON NITRIC OXIDE FOR THE TREATMENT OF COLORECTAL CARCINOMA

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IMIBIC Group: A08. Nuevas terapias en cáncer.
Poster

5-fluorouracil (5-FU) and oxaliplatine are standard therapy for mestastatic CRC, but the development of chemoresistance is inevitable. Although the underlying causative factors are not fully understood, development of drug-resistance has been associated with induction of cancer stem cells (CSCs), which constitute a small sub-population of tumor cells known to be highly resistant to chemotherapy, to be responsible for the initiation, maintenance, dissemination and tumor recurrence. Based on the evidences about the important role of different mechanisms related to nitric oxide (NO) in the biology and treatment of cancer, this study aimed to analyze the impact of NO in colon CSC compared with the effects of 5-FU and oxaliplatine. Here, we show that 5-FU and oxaliplatine have an antiproliferative effect in Caco-2 and HCT116 cells. However, these chemotherapeutic agents abolished the formation of colonospheres in both cell lines, with the exception of Caco-2, which was fully resistant to treatment with 5-FU. On the other hand, the NO donors DETA-NONOate and S-nitroso-cysteine (CSNO) had an antiproliferative effect in both cell lines, and CSNO exerted a dual modulation of cell proliferation in Caco-2 cell. Both DETA-NONOate and CSNO have a great effect in cell viability in both lines, and reduced the formation of colonospheres. Although both NO donors failed to completely eliminate the formation of colonospheres, they caused a significant decrease in their size. Furthermore, analysis by Western blot showed that DETA-NONOate decreased the level of Akt phosphorylation and both donors increased the Erk1/2 phosphorylation in Caco-2. Finally, the expression of cyclin D1 was strongly inhibited by DETA-NONOate and CSNO. These data show that the disturbance of nitrosothiol homeostasis and the ensuing nitrosative stress have an antiproliferative effect in CRC cells and reduce the formation of colonospheres. Therefore, therapeutic approaches based on NO are a promising alternative in the treatment of CRC.

Keywords: Nitric oxide, cancer stem cell, colorectal cancer, 5-fluorouracil, oxaliplatine, DETA-NONOate, CSNO.

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15 INTERACTION BETWEEN TRIB1 RS2954029 POLYMORPHYSM AND DIETARY PATTERNS ON FASTING TRIGLYCERIDES IN PATIENTS WITH CORONARY ARTERY DISEASE AND METABOLIC SYNDROME.

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IMIBIC Group: B02.Nutrigenómica. Síndrome metabólico.
Poster

Background and Aims. TRIB1-rs2954029 polymorphism has been associated with fasting plasma triglycerides and risk of coronary artery disease. Our aim was to investigate whether chronic consumption of a Mediterranean or low fat diet for a year may modulate the effect of TRIB-rs2954029 polymorphism on fasting plasma lipids concentrations in patients with coronary artery disease and metabolic syndrome (MetS).
Methods. 581 metabolic syndrome patients participating in CORDIOPREV study (NCT00924937) were randomized to one of two dietary patterns: 302 patients were assigned to Mediterranean diet group, rich in fat from olive oil (34% total fat energy intake, monounsaturated fat (MUFA) 22%, polyunsaturated fat (PUFA) 6% and saturated fat (SAT) 7%), and 279 patients were assigned to Low-fat diet group (28% total fat calories, MUFA 12%, PUFA, 8% and SFA 8%). TRIB1-rs2954029 polymorphism was genotyped in all patients and fasting plasma lipids levels were measured at baseline and after one year of intervention.
Results. Homozygotes for A allele (AA) had higher fasting triglycerides and ApoB levels (p=0.001 and p=0.02) than carriers of T allele (AT and TT) at baseline. After one year of dietary intervention, significant reductions were achieved in both intervention groups on fasting triglyceride concentrations (p <0.001), without no differences between genotypes.
Conclusion. TRIB1-rs2954029 polymorphism interacts with dietary pattern and modulates fasting plasma triglycerides in patients with coronary artery disease and metabolic syndrome.

Keywords: TRIB1, polymorphism, triglycerides, diet, metabolic syndrome, coronary artery disease

NOTES:

16 FUNCTIONAL ASSESSMENT IN CHILDREN FED WITH TWO DIFFERENT FORMULAS WITH ISOLATED HUMAN MILK PROBIOTICS

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IMIBIC Group : BE07. Metabolismo infantil.
Poster

According to recent studies, breast milk is a source of probiotic bacteria that could play a key role in the initial colonization of the newborn. Currently, the manipulation of the intestinal microbiota by probiotic bacteria is reaching interest in modifying infant formulas with different components that may have a beneficial effect for the infant. There is evidence that administration of these probiotics is beneficial in the prevention and treatment of gastrointestinal infections, and studies suggest that could stimulate an immune response against pathogenic microorganisms. However, the inclusion of probiotics in infant formulas is more controversial, although is known that breast milk contains this type of bacteria.
The aim of this study is to evaluate the safety and functionality of two infant formulas with L. fermentum CECT5716 or strain Bifidobacterium breve CECT7263. A randomized, double-blind, controlled without crossover was done with 3 groups of 63 healthy newborns of both sexes: a group with a standard formula without probiotics, another with the same formula added to L. fermentum CECT5716 and another with the same formula added to B. CECT7263 brief. Nutritional intervention and faeces sampling were taken during 12 months. Anthropometric measurements, the presence of adverse events, the incidence of gastrointestinal and respiratory infections and stool studies (characteristics, fecal microbiota, IgA faecal short chain fatty acids fecal cytotoxicity) and other data such as crying time, average sleep time per day or the presence of regurgitation or vomiting were taken.
The consumption of probiotics L. fermentum CECT5716 and B. CECT7263 from 106ufc/g to 107ufc/g is safe in infants under one year of age. These formulas have been well tolerated demonstrating no significant differences in growth or fecal IgA quantification between the different study groups. The effects in incidence of respiratory and gastrointestinal infections are being evaluated actually.

Keywords: Probiotic, Bifidobacterium, infant formula.

NOTES:

17 EPIGENETIC MECHANISMS MAY MEDIATE THE EFFECT OF TESTOSTERONE IN THE NERVOUS SYSTEM OF C. ELEGANS. APPLICATIONS FOR UNDERSTANDING THE ETIOLOGY OF AUTISM SPECTRUM DISORDERS.

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IMIBIC Group: D03. Genética y enfermedades del comportamiento.
Poster

Current research indicates that the causes of autism spectrum disorders (ASD) are multifactorial and include both genetic and environmental factors. To date, several works have associated ASD with mutations in many genes that encode proteins involved in neuronal synapses; however the environmental factors and the way they can interact with the development of the nervous system remain largely unknown. Recent studies have established a direct relationship between risk for ASD and the exposure of the fetus to high testosterone levels during the prenatal stage. In order to explain the possible mechanisms by which this androgenic hormone may interact with the nervous system we use C. elegans as an experimental model. This nematode has a very well-defined and genetically tractable nervous system that allows the study of basic pathways, and it is estimated that over 83% of the nematode proteome has human orthologous proteins. We observed that testosterone was able to alter the behavioral pattern of the worm, including the mechanosensory response to gentle touch and the pumping rate of the pharynx. This impairment of the behaviour was abolished using specific RNAi against genes orthologs of human genes which code androgen and estrogen receptors. On the other hand the testosterone effect remained through several generations in the absence of the hormone, indicating that some epigenetic mechanisms could be involved. This was confirmed using sodium butyrate, a histone deacetylase inhibitor, which was able to eliminate the effect of testosterone. Our results suggest that testosterone may impair the nervous system functionality through specific androgen receptors and generating epigenetic marks in the genome. This work may provide new paradigms for understanding biological mechanisms involved in ASD.

Keywords: Autism spectrum disorders (ASD); Caenorhabditis elegans; Testosterone; Epigenetics.

NOTES:

18 THE EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELDS REVERSES SCORE PROGRESSION IN A MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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IMIBIC Group: BE05. Estrés oxidativo y nutrición.
Poster

Transcranial magnetic stimulation (TMS) has shown positive effects on spasticity in patients with multiple sclerosis. Meanwhile, the extremely low-frequency electromagnetic fields (ELFEF) application on different models of neurodegeneration is characterized by reduction of oxidative damage, neurogenesis and remyelination in corpus callosum. The objective of this study was to analyze the effect of ELFEF on immobility score shows in an experimental autoimmune encephalomyelitis (EAE) model. For this purpose, we used Dark Agouti rats that were inoculated with myelin oligodendrocyte glycoprotein (MOG), inducing EAE. After 14 days of MOG administration, some of the animals were treated with ELFEF (60 Hz and 0.7 mT for 2 hours a day, every day for 21 days). The rats inoculated with MOG suffered a progressive paralysis of their limbs. These symptoms were reversed by treatment with ELFEF.

In conclusion, the ELFEF blocks the development of clinical symptoms in our experimental model of multiple sclerosis.

NOTES:

19 ANTI-CYCLIC CITRULLINATED PROTEIN ANTIBODIES ACT AS DIRECT INDUCTORS OF THE INFLAMMATION AND THE OXIDATIVE STRESS OBSERVED IN RHEUMATOID ARTHRITIS, WITH DIFFERENTIAL EFFECTS IN EACH WHITE BLOOD CELL TYPE.

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IMIBIC Group: A07. Enfermedades autoinmunes sistémicas e inflamatorias crónicas del aparato locomotor y tejido conectivo.
Poster

Background: No study has evaluated the direct effect of the anti-cyclic citrullinated protein antibodies (anti-CCPs) in the leukocytes and their relationship with the atherogenesis and cardiovascular disease (CVD) observed in Rheumatoid Arthritis (RA).
Objectives: To evaluate the effect of the in vitro treatment with anti-CCPs antibodies in the induction of the pro-oxidative and inflammatory state observed in RA leukocytes, and its relationship with the chronic inflammation and early atherogenesis that commonly characterize this disease.
Methods: 53 RA patients and 31 healthy donors were included. Carotid intima-media thickness (CIMT) was evaluated as atherosclerosis marker; other markers of CVD were also studied. Several pro-coagulant factors, leukocyte activation markers, stress oxidative markers and mitochondrial membrane potential (MMP) were analysed in leukocytes by flow cytometry. Oxidative stress and inflammation plasma markers were also analyzed. Inflammatory molecules were quantified by RT-PCR. Finally, anti-CCPs antibodies were isolated from plasma of RA patients and in vitro treatment of healthy leukocytes was conducted.
Results: Inflammatory factors and nitric oxide levels were found elevated in RA plasma versus controls. The antioxidant capacity and protein tyrosine nitration were lower in RA plasma compared to controls. Additionally, high titles of anti-CCPs were associated to an increased expression of the pro-thrombotic and inflammatory markers, high oxidative stress and pathological CIMT. RT-PCR of the proinflammatory molecules showed that each cell subtype presented a specific expression pattern. The in vitro treatment with anti-CCPs antibodies from RA patients supported the results observed in vivo in RA patients.
Conclusions: The anti-CCPs antibodies directly induce inflammation and oxidative stress in the leukocytes from RA patients. The anti-CCPs effects are different depending on the cell type. Understanding the specific effects induced by anti-CCPs antibodies would help to develop a cellular targeted therapy preventing the atherosclerosis and CVD in RA patients.
Supported by: P08-CVI-04234, PI12/01511, Spanish Rheumatology Society

Keywords: Anti-cyclic citrullinated protein antibodies (anti-CCPs), Atherogenesis, Cardiovascular disease (CVD), Rheumatoid Arthritis.

NOTES:

20 PREVALENCE OF RENAL STONES IN ANDALUCIA. CLINICAL AND SOCIO-DEMOGRAPHIC ASSOCIATED FACTORS.

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IMIBIC Group: Cas04. Urología y Medicina sexual.
Poster

Introduction: Urolithiasis is a prevalent disease with a high rate of recurrence and hight morbility associated, which gives it a great clinical, social and economic importance. From the limited and unrepresentative studies done so far in Spain, the estimated prevalence is 5%. An unquantified increase is being seen in recent years in which various factors could be related. The main objective of our study is to estimate the prevalence of renal lithiasis and their characteristics in Andalusian population ranging from 40 to 65 years, determining which socio-demographic and clinical factors are associated. MATERIAL AND METHODS: We have done an epidemiological observational and transversal study, taking a stratified random sample of the Andalusian population of both sexes from 40 to 65 years old. Data collection was conducted through phone interviews, questioning the chosen subjects about their history of kidney stones, comorbidity and socio-demographic characteristics. We conducted a descriptive, bivariate and multivariate analysis with logistic regression. RESULTS: Surveyed 2465 subjects with a mean age of 51 years (male: 48.7% female: 51.3%), we obtained a prevalence of kidney stones of 16.3% (95% CI: 14.79-17.74%). As variables significantly associated with the presence of urolithiasis in the multivariate study found: The presence of a family history of kidney stones (OR 1.95, 95% CI 1.55-2.44, p <0.001), hypertension (OR 1.58, 95% 1.24-2.02, p <0.001) and BMI (OR 1.60, 95% CI 1.19-2,17 p = 0.008). CONCLUSIONS: The prevalence of urolithiasis obtained shows a significant increase in the environment with respect to the figures previously available to us, which confirms what was suspected beforehand. Hypertension, the presence of a family history of urolithiasis as well as having a high BMI was significantly associated with the presence of kidney stones.

Keywords: Urolithiasis. Prevalence. Associated Factors.

NOTES:

21 EFFECT OF DIET ON BIOMARKERS INVOLVED ON ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CARDIOVASCULAR DISEASE

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IMIBIC Group: B02.Nutrigenómica. Síndrome metabólico.
Poster

Introduction: Cardiovascular disease continues to be a top priority, since work on traditional risk factors has not shown the desired impact on morbidity and mortality, and despite intensive treatment, one in three patients who suffers a coronary event has a recurrence. Therefore, the search for new biomarkers or mediators for the disease will allow us to examine the prevention of these events. Cardiovascular disease depend the interaction between genetics and environmental factors, such as diet.
Objective: To evaluate the influence of two models of healthy diet (Mediterranean diet rich in MUFA or low- fat diet) on the miRNAs involved in endothelial dysfunction in patients with high risk of coronary disease as a diet based therapeutic strategy associated a reduction of cardiovascular risk.
Methodology: We selected 40 patients, age between 20 and 75 years old, with high cardiovascular risk included in the intervention study CORDIOPREV. These patients consumed two models of healthy diets: The Mediterranean diet (34% fat, 22% MUFA, 6% PUFA and 7% SAT) and low-fat diet (28% fat, with 12 % MUFA, 8% and 8% PUFA SAT). Blood samples will be taken at the starting point and one year after the start of dietary intervention. Endothelial progenitor cells (EPC) were isolated from PBMCs (peripheral blood mononuclear cell) by ficoll gradient centrifugation and were cultured in medium EGM. Finally, we analyzed the expression of miRNAs: miR-34a, miR-126, miR130a, miR-221, miR-222 and miR-92a in EPC from each of the patients by RT-qPCR. Statistical analysis of the data was performed using SPSS 15.0.
Results: The patients, who increased circulating levels of EPC after one year of dietary intervention, showed an increase expression of miRNA 126 after consumption of Mediterranean diet, compared with the low fat diet. No significant differences were observed in others miRNAs of study.
Conclusion: Mediterranean diet may improve endothelial damage in these patients, and it could be used as a dietary therapeutic strategy to reduce cardiovascular risk.

Keywords: endothelial cells, mediterranean diet, cardiovascular disease, EPC, miRNAs

NOTES:

22 NOS-3 REGULATION BY OXIDATIVE STRESS IN A CELLULAR MODEL OF CHOLESTATIC DAMAGE

Author/es: Sandra González, Gustavo Ferrín, Clara I. Linares, Patricia Aguilar, Ana Fernández-Álvarez, Marta Casado, Javier Briceño, Luís Casáis, José Luis Montero, Jordi Muntané, Manuel de la Mata

IMIBIC Group: A02. Estrés oxidativo y nitrosativo en hepatopatías agudas y crónicas.

Poster

Background: During cholestatic liver disease, excessive accumulation of hydrophobic bile acids exerts a cytotoxic effect leading to cell death and tissue damage. Oxidative stress plays a key role in this process by promoting the development of fibrosis, cirrhosis, portal hypertension and chronic liver failure. Furthermore, in cellular models of cholestatic damage it has been established a cytoprotective role for nitric oxide (NO).

The aim of the study was to evaluate the regulation of endothelial nitric oxide synthase (NOS-3) in a cellular model of cytotoxicity by glycochenodeoxycholic acid (GCDCA) and its relationship with the oxidative stress and cell death.

Materials and Methods: A kinetic study was performed (0-24 hours) for induction of cell death by GCDCA (0.5 mM) in the human hepatocarcinoma cell line HepG2. The compound Mn (III) tetrakis (4-benzoic acid) porphyrinchloride (MnTBAP, 1mg/mL) was tested as an antioxidant molecule. The detection of reactive oxygen species and assessment of cell death was performed spectrophotometrically by using the probes 2,7-dichlorofluorescein diacetate and dihydroetidium, and by measuring caspase-3 activation and lactate dehydrogenase cellular release, respectively. NOS activity was determined by analyzing nitrite and nitrate accumulation in the extracellular medium. NOS-3 expression was measured by RT-qPCR and western-blot. The promoter activity of Nos-3 gene (1601 bp) was assessed using the luciferase activity assay. The identification of transcription factors (TFs) that could be involved in the NOS-3 regulation was performed using prediction programs. Chromatin immunoprecipitation assay and western-blot were used for further analysis and for the identification of the TFs binding sites in the Nos-3 promoter..

Results: GCDCA administration was associated to oxidative stress increase and Nos-3 promoter activity decrease, with a reduction in NOS-3 expression and cellular NO production. The expression and the binding of TFs cJun, cFos and SP1 to the Nos-3 promoter (identified positions), as well as the phosphorylation of protein kinases JNK and ERK1/2, were related to GCDCA-induced hepatocellular damage. MnTBAP treatment prevented the cellular effects of GCDCA.

Conclusions: GCDCA-induced cell death was associated to NOS-3 expression/activity decrease by oxidative-stress. This fact was related to JNK and ERK1/2 phosphorilation, and Nos-3 promoter binding increase of TFs cJun, cFos and SP1.

Keywords: Nitric Oxide, NOS-3, oxidatives stress, cell death, biliar acid, HepG2

NOTES:

23 PHENOLIC COMPOUNDS IN FRYING OILS, WHETHER NATURAL OR ADDED, REDUCE OXIDATIVE DNA DAMAGE IN OBESE PEOPLE

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Author/es: B02. Nutrigenómica. Síndrome metabólico.
Poster

Introduction: The intake of fried food may alter the cellular redox balance, accumulating reactive oxygen species (ROS) which cause damage in DNA. The p53 protein acts in repair mechanisms against DNA damage by reducing ROS levels. Thus activation of p53 represents an important component of DNA repair, cell cycle control and apoptosis.
Objective: To study the effect of phenolic compounds present in heated oils, in the oxidative damage to DNA during the postprandial state in peripheral blood mononuclear cells of obese people.
Methodology: After 12 hours fasting, 12 obese volunteers received 4 breakfasts prepared with different oils, [virgin olive oil (VOO), sunflower oil (SFO) and two mixtures of seeds oil (sunflower oil / canola oil), one enriched with dimethylpolysiloxane as an antioxidant (SOX) and other phenolic compounds extracted with alperujo (SOP)]. To heat the oils were previously subjected to 20 cycles of 5 minutes. mRNA was isolated from monoucleares cells in the fasting state and 4 hours after breakfast. Then, using OpenArray platform (Applied Biosystems), genica expression was analyzed of p53, APE1, mdm2, and GADD45B GADD45A.
Results: The mRNA levels of p53 increased at 4 hours after the consumption of abreakfast prepared with SFO (p = 0.009) and SOD oils (p = 0.022) as compared with the fasting state, the mRNA levels of APE1 and GADD45A increased at 4 hours after the consumption of the breakfast prepared with SFO(p = 0.009 and p=0.04, respectively) as compared with the fasting state.
Conclusion: Phenolic compounds in heated oils, seem decrease the DNA damage during the postprandial state, in obese people.

Keywords: Frying oils. Obesity. Oxidative DNA Damage.

NOTES:

24 NOVEL REGULATORY ELEMENTS OF PUBERTY: EXPLORING THE ROLES OF MICRORNAS IN THE CONTROL OF PUBERTY AND ITS MODULATION BY METABOLIC CUES

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Poster

Puberty is a key maturational event that is controlled by central neurotransmitters, peripheral signals and environmental cues, which cooperate to ensure its proper timing. Among its various regulators, metabolic factors and energy homeostasis influence puberty onset and growing concerns have mounted on the possibility that the trends of altered timing of puberty in different populations could be linked to the escalating prevalence of metabolic disorders, such as obesity.
In an attempt to decipher the physiological basis of puberty and the potential mechanisms for its deviations, we have undertaken studies to address the putative roles of microRNAs (miRNAs) in this phenomenon. MiRNAs are small, non-coding RNAs with ability to modulate (mainly repress) gene/protein expression. Because of their capacity to regulate multiple gene targets, miRNAs seem to be well suited for the control of complex biological phenomena, such as puberty. In addition, genome-wide association studies (GWAS) suggested the involvement of the RNA-binding factor, LIN28B, in the timing of puberty of humans; the major known role of LIN28 being the inhibition of let-7 miRNA maturation.
Departing from these observations, we have conducted expression analyses of Lin28B, let-7 and related miRNAs, such as mir-9, mir-132 and mir-145, in the hypothalamus, during male and female postnatal/pubertal maturation, and in (rat) models of altered puberty. Our data are suggestive of a role of hypothalamic Lin28/let-7 in the control of puberty, and document that manipulations that disrupt normal pubertal timing do have a discernible impact on the expression patterns of this regulatory pathway.
In order to gain further insight into the physiological role of miRNAs in the regulation of puberty, we have undertaken the development of mouse models of selective elimination of Dicer, the enzyme responsible for generation of mature miRNAs, in key neuronal populations in puberty onset, namely Kiss1 and GnRH neurons. Initial analyses have revealed that elimination of miRNA synthesis in GnRH neurons prevent external signs of puberty in male but nor female mice, while Dicer KO in Kiss1 neurons was compatible with preserved phenotypic indices of puberty in both sexes. Nonetheless, both Kiss1- and GnRH-selective Dicer KOs are infertile, thus suggesting the relevance of miRNA pathways in these neuronal populations. Additional analysis, involving the use of our recently generated inducible Kiss1-Cre mouse line (to conditionally ablate Dicer at timed developmental windows in Kiss1 and GnRH neurons), as well as qPCR based neuron-specific analyses of the miRNA signature of Kiss1 and GnRH cells, are currently in progress in our group to further document the physiological role of miRNAs in the control of mammalian puberty.

Keywords: Puberty, metabolism, miRNA, GnRH, Kiss1, Lin28B, Let-7

NOTES:

25 EFFECTS OF CONSUMPTION OF VIRGIN OLIVE OIL RICH IN PHENOLIC COMPOUNDS ON VASCULAR ENDOTHELIUM

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Poster

Introduction: Previous studies have shown the anti-inflammatory and antioxidant properties of phenolic compounds of virgin olive oil (VOO). However, the effect of bioavailable phenolic compounds on the vascular endothelium is unknown.
Objective: To evaluate the effect of bioavailable phenolic compounds of VOO present in human serum on vascular endothelium.
Methodology: Two virgin olive oil-based breakfasts with high (398 ppm) and low (70 ppm) content of phenolic compounds were administered to 20 metabolic syndrome patients (MetS) following a double blinded, randomized, crossover design. Human umbilical endothelial cell line(HUVEC) were treated with serum pools prepared with serum of the patients obtained at 0, 2 and 4 hours after the consumption of each breakfast. Gene expression analysis was performed by using Openarray system (Applied Biosystem), and mRNA levels are expressed as Fold Change (FC).
Results: Treatment of HUVEC with the serum collected 2 hours after the intake of the breakfast prepared with high phenolic VOO content decreased p65 and MCP-1 gene expression ($p<0.001$ and $p=0.002$ respectively) and increased the MT- CYB, SDHA and SOD1 gene expression ($p=0.004$, $p=0.012$ and $p=0.001$ respectively) as compared with the treatment of HUVEC with the serum collected 2 hours after the intake of the breakfast prepared with low phenolic VOO content and treatment with the serum obtained 4 hours after the intake of the breakfast prepared with high phenolic VOO content decreased the of MCP-1 and CAT gene expression ($p <0.001$ and $p = 0.003$ respectively) and increased MT-CYB gene expression ($p<0.001$) as compared to the treatment with serum obtained 4 hours after the intake of the breakfast prepared with low phenolic VOO content.
Conclusion: Our results suggest that the consumption of a breakfast based on VOO rich in phenolic compounds decreases inflammation and improves antioxidant profile in the vascular endothelium.

NOTES:

26 GUT MICROBIOTA SUFFERS DEEPER CHANGES IN METABOLIC SYNDROME THAT IN OBESITY

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Poster

Introduction: Metabolic Syndrome (MetS) is a multi-component disorder, characterized by different criteria, including abdominal obesity, low HDL levels, high TGL levels, hypertension and impaired insulin sensitivity. Nowadays, the gut microbiota has gained special interest in this field, because it may be involved in the development of the complications associated to the obesity.
Objective: To determine differences in the bacterial community structure of the intestine in obese patients with 3, 4 and 5 MetS criteria (group 3c, 4c and 5c respectively) compared to obese people without MetS (O) and control subjects (C).
Metodology: We extracted the bacterial DNA from feces of 200 patients (40 patients of each group) and we analyzed the intestinal microbiota by qPCR with specific primers for 16S rRNAgene for several relevant phyla, genera and species.
Results: We found a significant decrease in the Bacteriodetes phylum as the number of MetS criteria in the patients increase ($p=0.004$), while we did not observed any significant differences in Firmicutes, Actinobacteria, and Proteobacteria phyla.
We also observed significant differences between groups at the genus Prevotella ($p=0.028$), Eubacterium ($p=0.003$), Lactobacillus ($p=0.010$), and Staphylococcus ($p=0.001$). While we did not observed any significant differences in the genera Bacteroides, Clostridium, Ruminococcus, Peptococcus, Bifidobacterium, Fusobacterium, Desulfovibrio, and Escherichia.
To bacterial species level, Bacteroides fragilis, B. distasonis, and B. thetaiotaomicron decreased as the number of MetS criteria increase ($p=0.003$, $p<0.001$, and $p=0.001$, respectively). In addition, we have found significant differences between groups in the following bacterial species: Ruminococcus flavefaciens subgroup ($p<0.001$), Lactobacillus casei ($p<0.001$), Eubacterium rectale ($p=0.001$), Bifidobacterium adolescentis ($p=0.009$), Bifidobacteium longum ($p<0.001$). Moreover, we also observed differences in the F. prausnitzii and F. nucleatum ($p=0.007$ and $p<0.001$, respectively).
Conclusion: Our data suggest that the presence of MetS affects the composition of gut microbial community but its causal-effect relationship will have to be investigated.

Keywords: gut microbiota, obesity, metabolic syndrome, insulin resistance, cardiovascular disease.

NOTES:

27 DIFFERENTIAL ROLE OF GHRELIN SYSTEM COMPONENTS IN HUMAN PROSTATE CANCER.

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IMIBIC Group: B01. Hormonas y cáncer.
Poster

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths in the Western male population. In all cancers, aberrant and alternative splicing events generate proteins that could influence the tumor cell physiology and survival of patients. In1-ghrelin, a spliced ghrelin gene variant, has been shown to be overexpressed in breast tumors, where it increases breast cancer cell lines proliferation. Recently, ghrelin gene expression has been found in normal and prostate carcinomas. The aim of this study was to investigate the presence of the ghrelin system [(native-ghrelin, In1-ghrelin, ghrelin-O-acyl transferase (GOAT) and ghrelin receptors (GHSRs)] and the potential role of this system in prostate cancer using both human primary prostate samples and prostate cancer cell lines as models. In1-ghrelin and GOAT (the enzyme responsible for ghrelin acylation) mRNA levels were significantly increased in prostate tumors compared to normal prostate tissue while ghrelin mRNA levels were not altered. Comparably, In1-ghrelin and GOAT mRNA expression was found at detectable levels in all the prostate tumor-derived cell lines studied (22Rv1, DU145, PC3, VCaP), while ghrelin expression was not present. Interestingly, In1-ghrelin treatment evoked a significant increase in the proliferation rate of DU145 and VCaP cells as compared with untreated-controls, while native-ghrelin peptide did not induce any significant response. The differential role of alternative spliced In1-ghrelin vs. native-ghrelin in prostate cancer highlights the importance of this recently identified variant in tumor development and point out new clinical pathways in human prostate pathologies, both in early detection of the disease and possible therapeutic targets.

Keywords: Prostate cancer, Ghrelin, alternative splicing.

NOTES:

28 ROLE OF GHRELIN SYSTEM COMPONENTS IN CUSHING'S DISEASE

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Poster

Cushing's disease is a result of hypercortisolism caused by a pituitary adenoma, in which corticotrope tumoral cells over-secrete adrenocorticotropin (ACTH). It has been described that ghrelin stimulates proopiomelanocortin (POMC) expression, the ACTH precursor, and also ACTH release from normal and tumoral corticotrope cells. Ghrelin is modified by the ghrelin O-acyltransferase (GOAT) enzyme that is necessary to bind its only known receptor (GHS-R1a) and to exert the majority of its biological functions. There is also a truncated orphan variant of the receptor (GHS-R1b), which is expressed in a wide variety of tissues. We have recently discovered a novel human ghrelin variant (In1-ghrelin) that is over-expressed in some types of tumors. The aim of this study was to characterize the ghrelin system in human corticotropinomas (n=36) compared to normal pituitary (n=10), and to determine the functional role of In1-ghrelin in cultured cells derived from corticotropinomas. The use of quantitative real time PCR demonstrated a significant over-expression in corticotropinomas of In1-ghrelin and GHS-R1b, and a clear increase in GOAT expression. Additionally, In1-ghrelin expression but not native-ghrelin expression was positively correlated with GOAT, GHS-R1a and GHS-R1b expression. Furthermore, the expression of both receptors is positively correlated with the expression of POMC and CRH-R1. In vitro, native- and In1-ghrelin similarly stimulated free cytosolic calcium levels, which acts as a second messenger involved in hormone release. A significant over-secretion of ACTH was also observed after 24h treatment with native- and In1-ghrelin peptides. Surprisingly, only the treatment with In1-ghrelin evoked a, slight but significant, stimulation in proliferative rate in corticotropinoma cells. These results were also supported by the fact that In1-ghrelin transfection in cultured corticotropinoma cells increased cell proliferation compared to mock-transfected cells. Altogether, our study provides novel findings regarding the role and potential clinical implications of ghrelin system, especially In1-ghrelin, in the pathophysiology of human corticotropinomas.

Keywords: Cushing's disease, Ghrelin, In1-Ghrelin

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29 CHARACTERIZATION OF SOMATOSTATIN SYSTEM IN PROSTATE CANCER: PATHOPHYSIOLOGICAL RELEVANCE

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Poster

Somatostatin (SST) and cortistatin (CORT) bind to a family of five G-protein coupled receptors with seven transmembrane domains (TMD) called somatostatin receptors (sst1-5) to exert their biological actions such as regulation of endocrine secretions, gastrointestinal function, neurotransmission, immune system and inflammation, and proliferation and apoptosis of tumor cells. Our research group has recently identified and characterized novel sst5 variants originated from alternative splicing in human, pig and rodents that lack one or more TMDs. Particularly, in human, we found two new functional variants of sst5 that generate two truncated proteins of five and four TMDs, named sst5TMD5 and sstTMD4, respectively. Both receptors are scarcely and differentially expressed in normal tissues, while sst5TMD4 was especially abundant in subsets of pituitary tumors and breast cancer samples, wherein it could exert a relevant role, probably by blocking sst2 actions. In this study, we analysed the putative role of SST/CST system and particularly the truncated receptors in one of the most relevant endocrine-related cancers, the prostate cancer (PC). Our data reveal a clear overexpression of sst5TMD4 (and also sst1) in PC compared to normal prostate tissues, with no changes in sst2 or sst5 and an almost undetectable expression of sst5TMD5. We also analysed the expression of SST/CST system in androgen sensitive (DU145, PC3) and androgen independent (VCaP and 22Rv1) PC cell lines and found that, the particular expression profile of each cell line could be determining their response to SST/CST in terms of proliferation rate and calcium kinetic. Interestingly, results demonstrated that overexpression of sst5TMD4 stimulated proliferation of VCAP and DU145 cells. Altogether, our results suggest a relevant role of the truncated sst5 variants, particularly sst5TMD4, in PC, where could be associated with a more aggressive behaviour.

Keywords: Prostate cancer; Somatostatin system; Somatostatin truncated receptors; Pathophysiological relevance; sst5TMD4 overexpression; Cell lines; Prostate cancer tissues

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