VI JORNADAS DE JÓVENES INVESTIGADORES

ABSTRACT BOOK

Edificio IMIBIC • Salón de actos
Córdoba, 4 y 5 de mayo de 2015

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Coordinators
Dr. Javier Delgado Lista
Dr. Antonio Romero Ruiz
Dr. Marco Antonio Calzado Canale
Dra. Rosario López Pedrera

Scientific Committee
Dr. Manuel Tena Sempere
Dra. Rosario López Pedrera
Dr. Antonio Rodríguez Ariza
Dr. Javier Delgado Lista
Dr. Antonio Romero Ruiz
Dr. Antonio Camargo
Dr. Marco Calzado Canale

Technical Secretariat
Dª Cristina Horcajada Magarín
Dª Marisa Escabias Parejo

Thank-you notes
The coordinators of the IMIBIC's VI Jornada de Jóvenes Investigadores would like to express their sincere gratitude to all the researchers that have become part of the scientific committee this edition. With no doubt, their effort and advice raise the standards of this meeting year by year. Moreover, they would like to thank the Colegio de Médicos de Córdoba for its commitment on the promotion of the research activity among the medical residents.

VI JORNADA DE JÓVENES INVESTIGADORES
PROGRAMME

Day 1 (4th May)	
9:00-9:30	**Opening ceremony. Inscriptions and Posters display**
9:30-10:45	**SESSION I. Cancer (Oncology and Oncohematology)**
I.a 9:30-9:45	Postoperative time course and utility of inflammatory markers in patients with ovarian peritoneal carcinomatosis treated with neoadjuvant chemotherapy, cytoreductive surgery and HIPEC. **Dimas Javier, Garcilazo Arismendi**
I.b 9:45-10:00	Identification of new substrates for DYRK2 and its implication in carcinogenesis: Cdc25A regulation in the context of lung cancer. **Maribel Lara Chica**
I.c 10:00-10:15	The role of nitric oxide in generation and maintenance of cancer stem cells: new therapeutic opportunities in cáncer. **Jon Peñarando Sáez**
I.d 10:15-10:30	Visfatin expression is tightly regulated by metformin and could serve as a non-invasive biomarker for prostate cancer. **Sebastiano Messineo**
I.e 10:30-10:45	Metformin exerts antitumoral actions in in vitro and in vivo models of prostate cáncer. **André Morais Sarmento**
10:45-11:15	**Coffee Break. Poster Showcase**
11:15-13:00	**SESSION II. Nutrition, Endocrine and metabolic diseases**
II.a 11:15-11:30	Novel cannabidiol derivatives are dual pparγ/cb2 agonists that induce polarization of M2 macrophages and modulate diet-induced obesity. **Inmaculada Velasco Aguayo**
II.b 11:30-11:45	Gonadotropin-inhibitory hormone signaling displays sexually dimorphic roles in the control of energy homeostasis: Studies in the NPFF1 receptor null mouse. **Silvia León Téllez**
II.c 11:45-12:00	Renal damage induced by diets rich in fat and phosphate. **Rafael Ríos Varo**

II.d 12:00-12:15	Bone marrow mesenchymal stem cells are morphological, functional and genetically different between patients with type 2 diabetes and healthy donors. **Gustavo Díez López**
II.e 12:15-12:30	Clinical-histological and molecular characteristics of patients with gastrointestinal and pancreatic neuroendocrine tumors. **Aura Dulcinea Herrera Marínez**
II.f 12:30-12:45	Metabolic phenotypes of obesity influence glucose homeostasis in coronary artery disease patients. **Juan Francisco Alcalá-Díaz**
II.g 12:45-13:00	The gut microbial community in metabolic syndrome patients is modified by diet. **Carmen María Haro Mariscal**
13:00-14:00	**Plenary conference. Rafael F. Duarte** **Hospital Universitario Puerta de Hierro**
14:00-16:00	**Lunch**
16:00-17:00	**SESSION II. Nutrition, Endocrine and metabolic diseases.**
II.h 16:00-16:15	Central ceramide signaling as novel mediator for the metabolic regulation of puberty: interplay with leptin and kisspeptin. **Violeta Heras**
II.i 16:15-16:30	Contribution of lipid droplet-associated rab proteins to the development of insulin resistance in obesity. **Yoana Rabanal Ruiz**
II.j 16:30-16:45	Phosphate restriction preserves bone volume in early and late stages of CKD in rats. **Juan Miguel Díaz Tocados**
II.k 16:45-17:00	Contributions to the analysis of vitamin D for the study of metabolic diseases. **Antonio Mena Bravo**
17:00-17:45	**Conference. Alejandro Lomniczi** **"**Epigenetics of Puberty: New Answers to Old Questions" Division of Neuroscience. Oregon National Primate Research Center (ONPRC)-Oregon Health Science University (OHSU)
17:45-18:15	**Poster Showcase**

18:15-19:00	**SESSION II. Nutrition, Endocrine and metabolic diseases.**
II.l 18:15-18:30	Endocrine and metabolic characterization of double somatostatin and cortistatin knockout mice. **Sergio Pedraza Arévalo**
II.m 18:30-18:45	Telomere length and its relation to dietary fat intake in an elderly population with cardiovascular disease: cordioprev study. **Andreea Corina Baba**
II.n 18:45-19:00	Mediterranean diet improves cellular damage and modulates expression of circulating mir210 in patients with severe endothelial dysfunction and cardiovascular disease. **Rosa Jiménez Lucena**

Day 2 (5th May)

9:00-10:30	**SESSION III. Chronic and Inflammatory diseases. Infectious and Immunological diseases. Organ transplantation.**
III.a 9:00-9:15	Acute kidney injury in the meld era of liver transplantation. Are calcineurin inhibitors so problematic for renal function? **Irene Gómez Luque**
III.b 9:15-9:30	Prevalence of urolithiasis in spanish population aged 40 to 65: prelirene study. **Roque Cano-Castiñeira**
III.c 9:30-9:45	FGF23 increases phosphate-induced smooth muscle cells calcification. **Noemi Vergara Segura**
III.d 9:45-10:00	Prognosis of patients with ulcerative colitis in sustained remission after thiopurines withdrawal. **Estefanía Moreno Rincón**
III.e 10:00-10:15	Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFα. **Carlos Pérez Sánchez**
III.f 10:15-10:30	Aging and chronic kidney disease oxidized albumin promotes cellular senescence and endothelial damage. **Carlos Luna Ruiz**
10:30-11:00	**Coffee Break. Poster Showcase**

11:00-12:30	**SESSION III. Chronic and Inflammatory diseases. Infectious and Immunological diseases. Organ transplantation**
III.g 11:00-11:15	Myogenic differentation in skeletal muscle satellite cells and interstitial mononuclear cells in the absence of injury. **Fernando Leiva-Cepas**
III.h 11:15-11:30	Real-time measurements of tissue oxygen microtension as a marker of bile duct viability in liver transplantation. **Elena Navarro Rodríguez**
III.i 11:30-11:45	Knowledge and practices of primary care professionals about the approach of alcohol: results of alco-ap study. **Esperanza Mª Romero Rodríguez**
III.j 11:45-12:00	Caenorhabditis elegans as an animal model in preclinical assays for pharmacogenetic studies of the antipsychotic drugs risperidone and aripiprazole. **Jaime Osuna Luque**
III.k 12:00-12:15	VCE-003.2 is a novel cannabigerol derivative that enhances neuronal stem cell prosurvival and alleviates symtomatology in murine models of Huntington disease. **Carmen del Río Mercado**
III.l 12:15-12:30	Role of monocytes subsets in the atherothrombosis and endothelial dysfunction associated with rheumatoid arthritis: beneficial effects of tocilizumab. **Patricia Ruiz Limón**
12:30-13:30	**Conference. Alvaro Roldán López** "El profesional con Formación Sanitaria Especializada en los Subprogramas de Formación e Incorporación de la AES" Instituto de Salud Carlos III
13:30-14:00	**Closing ceremony** **Award-giving** **Honors recognition. Dr. Francisco Pérez Jiménez**

SESSION I

Cancer
(Oncology and Oncohematology)

I.a– Postoperative time course and utility of inflammatory markers in patients with ovarian peritoneal carcinomatosis treated with neoadjuvant chemotherapy, cytoreductive surgery and HIPEC

Authors: *F. J. Medina Fernández MD, F. C. Muñoz-Casares MD, PhD, A. Arjona-Sánchez MD, PhD, A. Casado-Adam MD, I. Gómez-Luque MD, D. J. Garcilazo Arismendi MD, H. Thoelecke MD, S. Rufián Peña MD, PhD, J. Briceño Delgado MD, PhD*

Group: GC18 *Translational research in surgery of solid organ transplantation*

Background: Inflammatory markers may help monitor postoperative evolution of surgical patients and detect complications. However, to date, the effect that neoadjuvant chemotherapy and hyperthermic intraperitoneal chemotherapy (HIPEC) may have in the postoperative kinetics of these parameters remains unknown.
Methods: Between July 2011 and June 2014, all patients who underwent neoadjuvant chemotherapy, cytoreductive surgery, and HIPEC for ovarian peritoneal carcinomatosis were studied. Patients were divided into four groups: no complications, noninfective complication, and infective complications during the first and second postoperative weeks. Retrospectively, C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), white blood cell count, platelet-to-lymphocyte ratio, and prothrombin ratio were collected from postoperative days 1–14. Postoperative behavior of each parameter was carefully evaluated across groups.
Results: The study included 122 patients. Only CRP and NLR showed promising results. CRP presented a mean peak value at 48 h (186.1 mg/L), while NLR peaked at 24 h (10.21 mg/L). Both parameters rose with infective complications. Statistically significant differences were found at several time points compared with uncomplicated patients. A simple test comparing the peak value of CRP with the value when an infective complication was suspected accurately diagnosed these complications with sensitivity of 81"%, specificity of 91"%, and negative and positive predictive value of 93.1 and 76" %, respectively. This comparison presented lower diagnostic performance when NLR was used.
Conclusions: Both CRP and NLR are useful in monitoring postoperative evolution in these patients; however, only CRP is useful for detecting infective complications.

I.b– Identification of new substrates for DYRK2 and its implication in carcinogenesis: Cdc25A regulation in the context of lung cancer

Authors: *Maribel Lara-Chica, Carla Jiménez Jiménez, Paula Moreno, Moisés Pérez, Eduardo Muñoz, Marco A. Calzado*

Group: GC4. *Inflammation and cancer*

Dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) is a serine/threonine protein kinase belonging to the evolutionary conserved CMGC family of protein kinases. Recent studies have shown that this protein plays a very important role in carcinogenesis, since DYRK2 has the ability to phosphorylate and regulate relevant proteins implicated in this process such as Stat3, p53, c-Jun/c-Myc or SIAH2. In this sense, the identification of new substrates for DYRK2 is highly relevant to clarify and understand its function in cancer development and progression.

To assess this point, initially we performed a kinase array through which we identified new potential and relevant substrates for DYRK2 such as B-Raf, β-Catenin, ETS1 and Cdc25A. Given the absolutely critical role of this phosphatase as a key regulator of the cell-cycle progression, we focused on studying the effect of DYRK2 on Cdc25A in the context of lung cancer. In this report, we demonstrate that DYRK2 overexpression down-regulates Cdc25A at both endogenous and exogenous levels. This degradation depends on DYRK2 kinase activity and is performed by ubiquitination and mediated by the proteasome. Compared with the rest of the DYRK family, DYRK2 is the most effective in degrading Cdc25A. We also described that DYRK2 knockdown increases Cdc25A stability, whereas DYRK2 expression facilitates Cdc25A polyubiquitination and degradation. Cdc25A protein half-life is decreased under DYRK2 overexpression, whereas this does not occur when a DYRK2 kinase dead mutant is overexpressed. The study of DYRK2 and Cdc25A endogenous protein expression in lung cancer cell lines reveals an important inverse correlation between these two proteins among them. Significantly, we described how this correlation occurs specially in a cellular model system of human bronchial epithelial cell squamous differentiation. Taken together, our findings show DYRK2 as a new regulator of Cdc25A, a target protein in cancer basic research, since it governs different key pathways in cancer processes. These results certainly help to improve our knowledge of the tumorigenic process and could open a road to the development of new therapeutical strategies against cancer based on the cell-cycle progression.

I.c– The role of nitric oxide in generation and maintenance of cancer stem cells: new therapeutic opportunities in cancer

Authors: *Peñarando J, López-Sánchez LM, Cañas A, Valverde A, Hernández V, Lopez-Pedrera C, De la Haba-Rodriguez JR, Aranda E, Rodríguez-Ariza A.*

Group: GC6. *New therapies in cancer*

Various studies have revealed a nitric oxide (NO)-induced poor outcome phenotype in cancer. However, the mechanisms responsible for the effects of NO in tumor biology and disease progression remain poorly understood. Many cancers, including breast and colon cancer, are driven by a subpopulation of cells that display stem cell properties, known as cancer stem cells (CSCs), which mediate metastasis and contribute to tumor relapse. It is therefore essential to investigate the role of NO in the generation and/or maintenance of these cell subpopulations. Here, we show that the pre-treatment of colon (HCT-116, Caco-2, DLD1) or breast (MCF-7, BT-474) cancer cells with the NO scavenger carboxy-PTIO (c-PTIO) markedly inhibited their subsequent ability to form tumorospheres in suspension. Also, NO depletion altered their capacity to growth as tumor spheroids when cultured in matrigel. This indicates that the selective removal of NO produced by tumor cells can abolish the ability of self-renewal in colon and breast cancer CSCs. Furthermore the effect was NO-specific, since the use of NO donors (DETANONOate and CSNO) eradicated the anti-CSC activity of c-PTIO. The NO scavenging pre-treatment down-regulated Notch, Bmi-1 and wnt/beta-catenin signaling pathways, which are known to be involved in the generation and expansion of breast and colon CSCs. The depletion of endogenous NO in breast cancer cells by the heterologous expression of the bacterial NO-consuming enzyme flavohemoglobin also impaired tumorosphere formation and the growth of tumor spheroids. Furthermore, tumor cell xenograft experiments showed that c-PTIO pre-treatment of colon cancer cells markedly reduced the size tumors formed compared to control cells. These data show that NO exerts autocrine and/or paracrine effects on stem cell signaling pathways which are essential for the generation and maintenance of CSCs subpopulations in tumors and may constitute a therapeutic target which can be efficiently blocked by selective elimination of this molecule.

Funded by: Funded by MINECO-ISCIII: PI13/00553.

I.d– Visfatin expression is tightly regulated by metformin and could serve as a non-invasive biomarker for prostate cancer

Authors: *Messineo S, Brunetti A, Foti DP, Castaño JP, Luque RM*

Group: GC8 *Hormones and cancer*

Prostate cancer (PC) is a highly prevalent pathology in the male population, and is strongly associated with obesity. Indeed, several adipokines preferentially secreted by visceral fat depots have been suggested to be associated with the development and progression of PC. Visfatin/NAMPT (nicotinamide phosphoribosyl transferase), an adipokine that harbours strong insulin-mimetic activities and is associated with obesity, has been found to overexpressed in PC-cells and to increase the malignancy of PC-derived cell lines. However, the potential value of visfatin as an aggressiveness marker in PC patients and its regulation in PC cells is still to be fully elucidated. Interestingly, some indications from our group (unpublished observations) and others indicate that hypoxia-inducible factor 1 Alpha (HIF1α) and the high-mobility group AT-hook 1(HMGA1) might act as key regulators of visfatin production, and also, that metformin (a widely used antidiabetic drug whose role in PC is still unknown) could inhibit visfatin production. Therefore, this study was designed to ascertain whether visfatin could serve as a molecular biomarker in the diagnosis/prognosis of PC patients, and to determine whether metformin can regulate visfatin expression in PC cells and, if so, what are the underlying molecular mechanisms. Our results revealed that visfatin plasma levels are more elevated in PC patients with higher Gleason-scores (GC10-9>GC8>GC7). Interestingly, PC-cell lines transfected with a visfatin gene promoter-driven luciferase reporter vector treated with metformin under normoxia or hypoxia conditions (induced with CoCl2 administration) showed that metformin treatment induces a remarkable decrease of visfatin gene expression in PC cells in a dose-dependent manner, under both normoxia and hypoxia conditions. Of note, those changes occurred in parallel with a decrease in the levels of HIF1α and HMGA1, two putative regulators of visfatin production. Altogether, our data strongly suggest that anti-cancer activity of metformin could be, in part, mediated through a down-regulation of local visfatin/HMGA1/HIF1α-axis in PC cells under normoxia/hypoxia conditions, and suggest that visfatin could serve as a non-invasive biomarker of aggressiveness in PC patients.

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I.e– Metformin exerts antitumoral actions in in vitro and in vivo models of prostate cancer

Authors: *André Sarmento-Cabral, Fernando López-López, Manuel D. Gahete, Justo P. Castaño, Raúl M. Luque*

Group: GC8 *Hormones and cancer*

Obesity is a chronic endocrine-metabolic disease and is one of the most serious and complex threats for the human health. Obesity is associated with an increased incidence of some types of cancers such as prostate cancer (PC), the most common cancer in the Spanish male population. Interestingly, metformin, a widely used antidiabetic drug, might represents a very promising opportunity to treat both pathologies (obesity and PCa) as some retrospective clinical studies have shown that the incidence of PC is lower in patients treated with metformin. However, the endocrine-metabolic, cellular and molecular mechanisms underlying the association between obesity and higher incidence/aggressiveness of PC and the putative pharmacological effectiveness of metformin in PC are still unknown. In this study, we have implemented a triple strategy by using: 1) primary normal prostate (NP) cell cultures from mice treated with metformin (10mM); 2) Human PC cell lines (PC3, 22Rv1 and LNCaP) treated with different doses of metformin (10μM- 5mM) and; 3) Immuno-suppressed mice inoculated with PC3 and LNCaP cells, fed a high-fat (HF) or low-fat (LF) diet, and treated with vehicle or 250mg/Kg of metformin (n=5-6 mice/group). Our results indicate that metformin modulates key metabolic, endocrine and pathologic components (e.g. IGF-I, insulin/IGF-I/somatostatin receptors and In2-ghrelin variant expression) in NP cell cultures. Interestingly, metformin had no evident effect on the proliferation of 22Rv1 cells but significantly diminished proliferation in PC3 and LNCaP cells (at 24-, 48-, and/or 72-h). Remarkably, we found that metformin also have a significant in vivo effect as it reduces PC3 derived xenograft tumor growth in mice fed both LF- and HF-diets compared to vehicle-treated mice (after 6-7 weeks of treatment). Altogether, our data suggest that metformin modulates NP cell function and exerts beneficial effects in the inhibition of PC cells growth in vitro and in vivo, specially, under HF-conditions.

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SESSION II

Nutrition, Endocrine and metabolic diseases

II.a– Novel cannabidiol derivatives are dual PPARγ**/CB2 agonists that induce polarization of M2 macrophages and modulate diet-induced obesity**

Authors: *Inmaculada Velasco, Carmen Navarrete, Miguel Ángel Sánchez Garrido, Irene Cantarero, María Jesús Vazquez, Silvia León, Juan M. Castellano, Marco A Calzado, Manuel Tena-Sempere and Eduardo Muñoz*

Group: GC10. *Hormonal regulation of energy balance, puberty and reproduction*

Since its postulation more than 20 years ago, the concept of "immunometabolism" has gradually evolved and different types of immune cells are now known to contribute to adipose tissue inflammation and insulin resistance. Macrophages (M1 type) have been shown to accumulate in the adipose tissue of obese animals, inducing a state of low-grade inflammation that could trigger insulin resistance. In contrast, M2 macrophages expressing high levels of arginase 1 (M2a phenotype) are anti-inflammatory and prevent adipose tissue inflammation. Recent evidence indicates that, besides the IL-4/STAT6 axis, peroxisome proliferator-activated receptors and co-activators (PPARγ and PGC-1α) and HIF-1α/2α transcription factors participate in M2 differentiation.

The use of cannabinoids in medicine is severely limited by their psychoactive effects, which are largely or entirely mediated by the CB1 receptor. Thus, a conceivable possibility would be to use cannabinoids that do not target that receptor, such as cannabidiol (CBD) and its quinone derivatives. As part of our study on the structure-activity relationship of CBD, we have generated a library of novel non-electrophilic CBD quinol derivatives, from which the compounds 3, 8 and 10 were selected for further in vitro characterization and efficacy validation in a murine model of diet-induced obesity.

Using binding affinity and transcriptional assays, we found that those compounds are dual PPARγ and CB2 agonists, as well as CB1 antagonists. Compound 8 blunts IL-17-induced M1 polarization and enhances basal and IL-4-induced M2 polarization, especially of Arg1+ macrophages. However, neither PPARγ nor STAT6 were involved in compound-8-induced M2 polarization that depends on KLF-4 expression. We also showed that this compound stabilizes HIF-1α, induces the transcriptional activity of the erythropoietin promoter in different cell types and inhibits the expression of COX-2 and PGE2 secretion in primary macrophages.

In addition, adult male mice, fed for >8-wks with either high fat diet (HFD) or the corresponding low-fat, control diet (CD), were used for in vivo validation. Daily administration of # 8 for 3-wks induced a significant reduction in food intake and body weight gain, in both HFD and CD mice, with a ~10% drop of BW at the end of the treatment, irrespective of the diet. Compound 8 significantly ameliorated also glucose tolerance following i.p. administration of a glucose bolus, in both HFD and CD animals. Of note, these effects were dose-dependent, as they were observed for a daily dose of 20 mg/kg, but not of 10 mg/kg of VCE004-8. The actions of # 8 on glucose handling were mimicked by the # 3, which reversed HFD-induced glucose intolerance.

In conclusion, our study documents the potent biological actions of cannabinoid quinones on macrophage differentiation, and highlights their potential for the treatment of obesity and its co-morbidities.

II.b– Gonadotropin-inhibitory hormone signaling displays sexually dimorphic roles in the control of energy homeostasis: Studies in the NPFF1 receptor null mouse

Authors: *S. León, M.J. Vázquez, A. Barroso, D. García-Galiano, F. Ruiz-Pino, M. Manfredi-Lozano, A. Romero-Ruiz, C. Dieguez, R. Nogueiras1, J. Roa, L. Pinilla, M. Tena-Sempere*

Group: GC10. *Hormonal regulation of energy balance, puberty and reproduction*

RF-amide-related peptide-3 (RFRP-3), the mammalian ortholog of the avian gonadotropin-inhibiting hormone (GnIH), has been proposed as major inhibitory signal for reproductive axis, acting via the NPFF1 receptor (NPFF1R). In addition, RFRP-3 has been recently suggested to modulate feeding, with reported orexigenic actions; a function that might contribute to the integrative control of energy homeostasis and reproduction. However, characterization of the metabolic effects of RFRP-3/GnIH signaling remains superficial and largely restricted to few pharmacological studies. As a means to address the putative physiological roles of GnIH/RFRP-3 signaling in the control of metabolic homeostasis, we report here the metabolic phenotyping of the first mouse line with constitutive inactivation of NPFF1R. Congenital elimination of NPFF1R did not consistently alter adult body weight (BW) neither did it affect BW responses to high fat diet (HFD) in males. In good agreement, indirect calorimetry failed to detect any alteration in total energy expenditure or RQ ratio, in spite of decreased spontaneous locomotor activity in the light phase. In contrast, NPFF1R null female mice tended to be slightly heavier and displayed exaggerated BW increases in response to obesogenic insults, such as HFD or ovariectomy. These were associated to an increased percentage of fat mass, as revealed by body composition analyses, with no difference in total energy expenditure or spontaneous locomotor activity. NPFF1R KO males, but not females, fed on HFD showed perturbed glycemic responses in glucose tolerance tests, even though basal glucose and insulin concentrations were not significantly different from WT levels. In addition, the patterns of food intake were affected in NPFF1R KOs of both sexes, with modest decreases in acute food intake and altered responses to leptin and ghrelin; feeding suppression following leptin administration was exaggerated in NPFF1R null mice, while the orexigenic responses to ghrelin were partially blunted in the absence of NPFF1R signaling. In sum, we provide herein the metabolic characterization of a mouse model of congenital elimination of the canonical receptor for GnIH/RFRP-3. Our data are compatible with a (modest) role of GnRH/RFRP-3 as orexigenic factor that mediates part of the effects of ghrelin on food intake. In addition, our study is the first to disclose the deleterious impact of the lack of NPFF1R signaling on body weight and fat composition, locomotor activity and glucose homeostasis, which exaggerates some of the metabolic consequences of concurrent obesogenic insults, such as HFD, in a sexually dimorphic manner.

II.c– Renal damage induced by diets rich in fat and phosphate

Authors: *Ana Isabel Raya, Rafael Ríos, Carmen Pineda, Ignacio López, Mariano Rodríguez, Escolástico Aguilera-Tejero*

Group: GC13. *Calcium metabolism. Vascular calcification*

"Fast-foods" play a major role in the pathogenesis of Type II diabetes (TIIDM). In addition to their high fat content, these foods are usually rich in phosphate (P), which is widely used as a food additive. Chronic kidney disease is a common complication of TIIDM. In this work we evaluated the effect on kidney histology of feeding diets with high fat and/or P content to rats with normal renal function.

Twenty-two female Wistar rats were divided in 4 groups: A control group (n=5) was fed a standard diet with 0.6% P and 4% fat (NPNF); the second group (n=5) was fed a 0.6% P and 35% fat (NPHF); the third group (n=6) was fed a 1.2% P and 4% fat (HPNF); and the fourth group (n=6) was fed a 1.2% P and 35% fat (HPHF). Diets were fed for 30 days and then rats were sacrificed to obtain renal tissue. Tissue samples were processed for staining with hematoxylin and eosin, periodic acid-Schiff, Masson's Trichrome and Von Kossa stains.

All rats fed HPHC diet showed moderate to severe tubular hyperplasia, tubular dilation, interstitial infiltrate and nephrocalcinosis. Tubular hyperplasia with focal crowding and increased nuclear to cytoplasmatic ratio was accompanied by a loss of brush border in proximal tubules and marked thickening of the tubule basement membrane. Dilation of tubular lumens, which were lined by a flattened epithelium, was also noted. Interstitial infiltrate was composed by mononuclear cells which were surrounding hyperplastic tubules. Calcifications were mainly located in the juxtamedullary cortex, with some scattered small areas in outer and inner medulla. Minor lesions were observed in rats fed NPHF and HPNF.

In conclusion, feeding high fat or high P diets has the potential of inducing minor lesions in the kidneys but the combination of both factors causes a significant renal damage.

II.d– Bone marrow mesenchymal stem cells are morphological, functional and genetically different between patients with type 2 diabetes and healthy donors

Authors: *G.Díez, V. Martín, M. Muñoz, S. Noguera, M.L. Castilla, T. Muñoz, P.Marí, M.Carmona, L.López, S. Cañadillas, R. Jiménez, R. Gutiérrez, M.Luque, I. Herrera*

Group: GC14 *Cell Therapy*

Introduction: Diabetes impairs in new blood vessel formation and wound healing. Mesenchymal stem cells derived from bone marrow (MSCs) are being used to promote healing, and improve blood flow in peripheral vascular disease of the lower extremities; although it remains unclear if diabetes impairs their functional and therapeutic capacity. The main objective of our study is to demonstrate wether bone marrow MSCs from diabetic patients and healthy donors are different in morphology, differentiation, in vitro pro-angiogenic capacity and gene expression profile.
Methods: we performed in vitro cultures of human MSCs (7 samples of diabetic type II patients and 4 of healthy donors) to analize differences in morphology, capacity of differentiation, in vitro pro-angiogenic capacity and gene profile expression. Results were validated with real-time quantitative PCR for genes MMP13 and SMOC 2, both involved in blood vessel growth.

Results: Morphology cultures showed a lower growing speed and aged appearance, in addition, electron microscopy revealed differences between diabetic and healthy donors in cell organelles. Moreover, differentiation studies , showed heterogeneity in both type of cells: diabetic MSCs did not differentiate to osteocytes, but no differences were found in adipogenic differentiation. MSCs of healthy donors formed more vessel structures than diabetic MSCs. Gene expression profile demonstrated a gene expression pattern generally decreased. Diabetic MSCs were characterized by low expresion of MMP13 and SMOC2.
Conclusions: Diabetes mellitus induces changes in the homeostasis of bone marrow MSCs showing a limited proliferative capacity, inability to differentiate to osteocyte lineage and reduced functional capacity. MSCs of diabetic patients have morphological, functional and genetic differences compared to healthy donors.

II.e– Clinical-histological and molecular characteristics of patients with gastrointestinal and pancreatic neuroendocrine tumors

Authors: *Aura D. Herrera-Martínez D, Manuel D. Gahete, Rafael Sánchez-Sánchez, Teresa Caro-Cuenca, Raquel Serrano-Blanch, Raúl M. Luque, Maria Ángeles Gálvez-Moreno, Justo P. Castaño*

Group: GC8 *Hormones and cancer*

Gastrointestinal and pancreatic neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms, whose incidence is recently rising. Unfortunately, an advanced stage is often found at diagnosis, suggesting the necessity to identify new diagnostic, prognostic, and therapeutic molecular markers. Therefore, we aimed to determine key clinical, histological and molecular characteristics of patients with NETs and the putative relationships among them. Hence, an observational retrospective study with 107 NETs patients was carried out by collecting clinical/histological characteristics and by measuring the expression levels of ghrelin and somatostatin/cortistatin systems components (by quantitative-PCR) in formalin-fixed paraffin-embedded NET samples (n=25; 52.2% G2, 43.5% G1 and 4.3% G3) and in control-adjacent non-tumoral tissues. Mean age was 55±17 years (55.1% males); 39.5% of cases were diagnosed incidentally, 31.6% were functioning tumors and, 48% had metastasis at diagnosis. Tumor size was associated with invasive characteristics ($p<0.001$), vascular invasion ($p<0.001$) and metastasis ($p<0.001$). Mortality was associated to metastasis ($p<0.007$), relapsed disease ($p<0.01$), persistent disease ($p<0.04$), vascular invasion ($p<0.04$) and nerve infiltration ($p<0.05$). Functionality was not associated to mortality. Somatostatin receptor (sst) subtypes-1 and -2, cortistatin and ghrelin O-acyl-transferase enzyme were overexpressed in pancreatic samples compared to control-tissues ($p<0.01$). Presence of skin lesions at diagnosis was correlated to sst3 expression ($p<0.05$). sst1 expression was negatively associated with vascular invasion ($p<0.05$). Ghrelin-receptor was negatively associated with nerve invasion ($p<0.01$). Interestingly, mortality tended to associate with somatostatin ($p=0,083$) and sst3 ($p=0.08$). Altogether, our results indicate that there are significant relationships between clinical-pathological characteristics and the evolution/prognosis of NETs patients. Moreover, the molecular analysis of the tumors demonstrated the presence of several components of the ghrelin and somatostatin systems in tumoral tissues, where they display relevant associations with clinical-histological parameters that might help to better understand NETs pathophysiology and, to identify novel molecular targets with potential prognostic and/or therapeutic value for NETs patients.

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II.f –Metabolic phenotypes of obesity influence glucose homeostasis in coronary artery disease patients

Authors: *Alcalá Díaz JF, Gómez Delgado F, Haro C, Marín C, Pérez Caballero AI, Delgado-Lista J, Pérez Martínez P, López Miranda J.*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Background: Recent evidences suggest that not all obese subjects display a clustering of metabolic and cardiovascular risk factors, and, likewise, not all lean subjects present a healthy metabolic and disease free profile. Our aim was to compare the glucose and insulin responses in subjects with normal weight, overweight and obese patients, according to their metabolically healthy or abnormal status.
Material and methods: 1002 coronary patients from the CORDIOPREV clinical trial (NCT00924937) were submitted to a 75-g oral glucose tolerance test (OGTT). We examined the phenotypic flexibility, measured with the OGTT among normal weight (BMI<25), overweight (BMI 25.0-29.9) and obese (BMI ≥30) patients, according to their metabolically normal or abnormal status. Abnormal metabolically status was defined by the presence of ≥2 cardiometabolic abnormalities, included elevated blood pressure, elevated triglyceride level, decreased HDL-C level, elevated glucose level, insulin resistance (HOMA-IR) and systemic inflammation (hsCRP).
Results: Metabolically healthy patients displayed lower response of plasma glucose compared with those metabolically abnormal, independently whether or not they were obese (P<0.001 and P<0.001, respectively). Interestingly, between metabolically abnormal subjects, the insulin response was directly related to BMI groups (P<0.05).
Conclusion: Our findings showed that certain types of the metabolic phenotypes of obesity are more favourable modulating phenotypic flexibility after a 75g-OGTT. To identify these phenotypes may be the best strategy for personalized treatment of obesity.

II.g– The gut microbial community in metabolic syndrome patients is modified by diet

Authors: *Haro C, Rangel-Zuñiga OA, Alcala-Diaz JF, Sonia García-Carpintero, García-Rios A, López-Miranda J, Camargo A, Pérez-Jiménez F.*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Introduction: The gut microbiota acts collectively as an organ which is fully integrated in the host metabolism. While a balanced microbiota confers benefits to the host, microbial imbalances have been associated with metabolic disorders such as dyslipidemia, insulin resistance, and type 2 diabetes. In fact, some studies have suggested that gut microbiota changes may be involved in the development of metabolic syndrome (MetS), a multi-component disorder frequently associated to obesity. In this sense, the shaping of the gut microbiome is currently considered a therapeutic target since specific changes in this microbial community might counteract the development of obesity and MetS.
Objective: We aimed to test the effect of consuming two healthy diets, a Mediterranean diet and a low-fat high-carbohydrate diet, for two years, in the gut microbiota of MetS patients and a control group of patients without MetS.
Methodology: We analyzed the differences in the bacterial community structure between the groups, after 2 years of dietary intervention (Mediterranean or low-fat diet) through quantitative PCR using primers targeting specific bacterial taxa.
Results: We observed, at basal time, that the abundance of Bacteriodes, Eubacterium and Lactobacillus genera is lower in the control group than in MetS patients, while B. fragilis group, P. distasonis, B. thetaiotaomicron, F. prausnitzii, F. nucleatum, B. longum, B. adolescentis, R. flavefaciens subgroup, and E. rectale are depleted in MetS patients (all P-values <0.05).
Additionally, we find that long-term consumption of Mediterranen diet partially restores the population of P. distasonis, B. thetaiotaomicron, F. prausnitzii, B. adolescentis, and B. longum in MetS patients (all P-values <0.05).
Conclusion: Our results suggest that the Mediterranean diet could be a useful tool to restore potentially beneficial members of the gut microbiota, although the stability of these changes over time remains to be assessed.

II.h– Central ceramide signaling as novel mediator for the metabolic regulation of puberty: interplay with leptin and kisspeptin

Authors: *Violeta Heras, Inmaculada Velasco, María Jesús Vazquez, F. Ruiz-Pino, Silvia Leon, María Manfredi-Lozano, Alexia Barroso, Juan M. Castellano, F. Gaytan, Leonor Pinilla and Manuel Tena-Sempere*

Group: GC10. *Hormonal regulation of energy balance, puberty and reproduction*

Puberty is a key developmental event, under the precise control of metabolic signals and different hormones, whose timing in humans appears to be changing in recent years. Ceramides, a family of sphingolipids of ubiquitous nature and pleiotropic function, have been recently proposed as hypothalamic signaling mediators in the neuronal networks governing energy homeostasis. In fact, ceramides are (i) oppositely regulated by the metabolic hormones, leptin and ghrelin; (ii) partially mediate the orexigenic effects of ghrelin; and (iii) seem to be involved in central lipotoxicity and ER stress, thereby modulating thermogenesis and causing body weight gain. While the metabolic state, leptin and ghrelin are known to modulate puberty onset, the putative role of central ceramide signaling in the central control of puberty remains unexplored. Similarly, the potential interplay of ceramides and the puberty-inducing neuropeptide, kisspeptin, has not been addressed so far.

We report herein a series of functional studies in immature female rats, either fed ad libitum or subjected to chronic sub-nutrition, in which pubertal onset was studied following manipulation of central ceramide signaling by treatment with either a specific precursor (C6:0) or inhibitor (Myriocin: Myr) of the novo synthesis of ceramides. In addition, the roles of ceramides in mediating leptin or kisspeptin effects on puberty onset and/or GnRH secretion were explored by a combination of in vivo and ex vivo studies.

Chronic intracerebral administration of C6:0 to enhance ceramide synthesis centrally resulted in precocious puberty onset, as evidenced by earlier vaginal opening and first ovulation, without affecting basal gonadotropin levels. In contrast, persistent blockade of central ceramide synthesis by Myr had opposite effects, with significantly delayed vaginal opening and first ovulation, but preserved gonadotropin levels, in Myr-treated rats. Immature female rats subjected to chronic sub-nutrition displayed delayed puberty, which was rescued by treatment with either leptin or kisspeptin. These permissive/stimulatory effects of kisspeptins were largely prevented, while those of leptin were partially delayed, by co-administration of the ceramide inhibitor, Myr. However, Myr did not attenuate kisspeptin-induced GnRH release ex vivo.

In sum, our data are the first to document a potential stimulatory role of central ceramide signaling in the control of female puberty, with a putative function as mediator of at least part of kisspeptin, and eventually leptin, effects on the timing of puberty. Notably, ceramide signaling did not apparently influence GnRH/gonadotropin secretion, suggesting the involvement of alternative effector pathways for the pubertal actions of ceramides, whose nature is presently under investigation in our laboratory.

II.i– Contribution of lipid droplet-associated rab proteins to the development of insulin resistance in obesity

Authors: *Rabanal Y., Guzmán-Ruiz R., Fernández-Vega A., Trávez A., Moreno N. R., Garcia-Navarro S., López-Miranda J., Tinahones F., Vázquez-Martínez R., Malagón M. M.*

Group: GC11 *Metabolism and adipocyte differentiation. Metabolic Syndrome*

Lipid droplets (LDs) are complex subcellular organelles responsible for lipid storage and mobilization in adipocytes. These organelles are surrounded by a phospholipid monolayer that harbors a wide variety of proteins, including several members of the Rab family of GTPases such as Rab18, which regulates lipid traffic to and from LDs. Investigating how LD-associated Rabs are regulated is pivotal for better understanding the lipid management in adipocytes and their contribution to lipotoxicity and metabolic disease. To identify potential regulators of Rab proteins, we carried out a proteomic analysis of isolated LDs from control vs. insulin stimulated 3T3-L1 cells. Among all the proteins differentially expressed under these conditions, we focused our attention on GDI2, which is known to bind GDP-bound Rab GTPases, inhibiting the exchange of GDP by GTP and, therefore, blocking Rab action. Accordingly, overexpression of GDI2 in 3T3-L1 cells inhibited the association of Rab18 to the LD surface, which led to a reduction in both lipogenesis and lipolysis. Notably, GDI2 expression and/or Rab18 distribution were significantly altered in in vitro models of insulin resistance in 3T3-L1 cells (i.e. high insulin-high glucose and TNF-a treatment). Finally, in a comparative proteomic screening we found that GDI2 protein expression was up-regulated in visceral adipose tissue from metabolically unhealthy obese individuals as compared to metabolically healthy obese and lean subjects. In sum, our data suggest that up-regulation of GDI2 and the subsequent impaired recruitment of Rab proteins to the LD surface contribute to the dysregulation of lipid metabolism observed in obese subjects and highlight the relevance of Rab proteins and their regulatory proteins in the development of insulin resistance.

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II.j– Phosphate restriction preserves bone volume in early and late stages of CKD in rats

Authors: *JM. Díaz-Tocados, ME. Rodríguez-Ortiz, C. Herencia, JM. Martínez-Moreno, A. Montes de Oca, N. Vergara, CG. Carvalho, M. Rodríguez, JM. Frazão, Y. Almadén, JR. Muñoz-Castañeda*

Group: GC13 *Calcium Metabolism Vascular Calcification*

Dietary phosphate (P) restriction may be beneficial in early Chronic Kidney disease (CKD) even when serum P is not yet elevated. One question to be addressed is whether in early CKD are detected bone abnormalities which could be prevented by dietary P restriction. In this study, we investigated the differential effects of high vs low P diet (HPD vs LPD) on bone remodeling in sham, uninephrectomized (1/2Nx, CKD stage 2) and 5/6Nx rats (CKD stage 4-5).

Sham and 1/2Nx male wistar rats were fed a HPD (1,2%) or LPD (0,2%). After 3 weeks, rats were euthanized, blood was collected and femurs were processed for histomorphometry. In order to examine progression of bone disease in parallel with worsening of renal function, some of the 1/2Nx rats underwent surgical removal of 2/3 of the remnant kidney maintaining the same P diet. Subsequently, 5/6Nx rats were euthanized one week later. Serum creatinine was slightly higher in 1/2Nx rats as compared with sham, albeit serum P was similar in HPD and LPD. Serum PTH decreased in sham and 1/2Nx rats fed on LPD and FGF23 increased significantly in HPD rats. After additional renal ablation (5/6Nx), plasma P, PTH and FGF23 increased in HPD vs LPD. In sham rats, histomorphometric analysis showed higher osteoid surface and trabecular separation in HPD than in LPD. In 1/2Nx rats, osteoid thickness, number of osteoclasts and osteoblasts and trabecular separation were increased in HPD vs LPD rats; noteworthy, bone volume was reduced. 5/6Nx-HPD rats showed remarkable loss of mineralized bone and peritrabecular fibrosis, whereas in Nx5/6-LPD rats bone volume was maintained and fibrosis was almost nonexistent. In conclusion, phosphate restriction prevents bone abnormalities in early CKD, even when phosphate levels are within the normal range and deterioration of renal function is marginal.

II.k– Contributions to the analysis of vitamin D for the study of metabolic diseases

Authors: *A. Mena-Bravo, F. Priego-Capote, MD. Luque de Castro*

Group: GC21 *Metabolomics. Identification of bioactive components*

The analysis of vitamin D status, with special emphasis on 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, is gaining interest in clinical studies due to the classical and non-classical effects attributed to this prohormone. In this research, two studies were developed: optimization of the method for quantitative analysis of vitamin D (both D2 and D3) and its main metabolites —monohydroxylated vitamin D (25-hydroxyvitamin D2 and 25-hydroxyvitamin D3) and dihydroxylated metabolites (1,25-dihydroxyvitamin D2, 1,25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3) in human serum, and the influence of the two steps preceding determination (viz. sample collection and preparation) on the quantitative analysis of these analytes. The quantitative method is based on direct analysis of serum by an automated platform involving on-line coupling of a solid-phase extraction (SPE) workstation to a liquid chromatograph–tandem mass spectrometer. Detection of the seven analytes was carried out by the selected reaction monitoring (SRM) mode, and quantitative analysis was supported on the use of stable isotopic labeled internal standards (SILISs). The method was externally validated according to the vitamin D External Quality Assurance Scheme (DEQAS) through the analysis of ten serum samples provided by this organism. The analytical features of the method support its applicability in nutritional and clinical studies targeted at elucidating the role of vitamin D metabolism. Two preparation approaches, deproteination and SPE, have been additionally evaluated in terms of sensitivity to delimit their application, thus establishing that detection of 1,25-dihydroxyvitamin D cannot be addressed by protein precipitation. Statistical analysis revealed that serum and plasma provided similar physiological levels for vitamin D3, 24,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3, while significantly different levels were obtained for 1,25-dihydroxyvitamin D3, always higher in plasma than in serum. Sample collection and treatment have proved to be significant in the analysis of vitamin D and its relevant metabolites.

II.I– Endocrine and metabolic characterization of double somatostatin and cortistatin knockout mice

Authors: *Pedraza-Arévalo S, Córdoba-Chacón J, Pozo-Salas AI, L-López F, de Lecea L Gahete MD, Castaño JP, Luque RM.*

Group: GC8 *Hormones and Cancer*

Somatostatin (SST) and cortistatin (CORT) are two highly related neuropeptides involved in the regulation of various endocrine secretions. In particular, SST and CORT are two primary negative regulators of growth hormone (GH) secretion. Consequently, single SST- or CORT-knockout (KO) mice exhibit elevated GH levels; however, this does not lead to increased IGF-I levels or somatic growth. This apparent lack of correspondence has been suggested to result from compensatory mechanisms between both peptides. To test this hypothesis, in this study we explored, for the first time, the consequences of simultaneously deleting endogenous SST and CORT by generating a double SST/CORT-KO mouse model and exploring its endocrine and metabolic phenotype. Our results demonstrate that simultaneous deletion of SST and CORT induced a drastic elevation of endogenous GH levels, which, surprisingly, did not lead to changes in growth rate or IGF-I levels, suggesting the existence of additional factors/systems that, in the absence of endogenous SST and CORT, could counteract GH actions. Notably, elevation in circulating GH levels were not accompanied by changes in pituitary GH expression or by alterations in the expression of its main regulators (GHRH and ghrelin) or their receptors (GHRH-R, GHS-R or SST/CORT receptors) at the hypothalamic or pituitary level. However, the expression of hepatic GH-sensitive genes, except for IGF-I, was augmented (GH-R and PRL-R) or reduced (EGF-R) in double-KOs compared to controls suggesting that liver of double-KO mice had partial resistance to GH actions. Moreover, although double-KO male mice exhibited normal glucose and insulin levels as well as normal pancreatic expression of glucagon and insulin, they had improved insulin sensitivity and a slightly improved glucose clearance compared to control mice. Therefore, these results suggest the existence of an intricate interplay among the known (SST/CORT), and likely unknown, inhibitory components of the GH/IGF-I axis to regulate somatic growth and glucose/insulin homeostasis.

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II.m– Telomere length and its relation to dietary fat intake in an elderly population with cardiovascular disease: cordioprev study

Authors: *Baba, A C., Rangel-Zúñiga, OA., López-Moreno, J., León-Acuña, A., Quintana-Navarro, GM., Cruz-Teno, C., López-Miranda, J., Pérez-Martínez, P.*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Objective: Accelerated telomere shortening is associated with aging and aging-related diseases. The lifestyles, and fundamentally the diet, are factors that may reduce the rate of telomere shortening, which leads to a decreased cellular senescence and an increased regenerative capacity of endothelium. Our aim was to study whether the quality and the quantity of the fat intake influences the telomeres length in elderly patients with established cardiovascular disease.
Materials and Methods: A total of 310 patients from the CORDIOPREV clinical trial (NCT00924937), 65 year or older, where included in our study. Information about dietary habits was obtained using a semi-quantitative 146-items food frequency questionnaire. DNA was isolated from peripheral blood samples using "Salting Out" method. Relative telomeres length (RTL) was measured by real time PCR. We estimated the relative ratio of telomere repeat copy number (T) normalized against a single copy gene(S) for each sample and for the reference DNA sample.
Results and Conclusions: Our findings showed that there is a significant difference between the RTL and the percentage of saturated fatty acids intake. Subjects with <8.09" % Kcal / day saturated fatty acids intake (Tertile 1) showed longer RTL compared to the other two groups (Tertile 2 = 8.10 to 9.64 Kcal/day and Tertile 3 > 9.65% Kcal/day) (p <0.05). No significant differences between the RTL and the percentage of total fat intake, the percentage of monounsaturated fatty acids intake and the percentage of polyunsaturated fatty acids intake were observed. No significant differences were observed between gender and RTL. Our results suggested that the RTL could be associated with the quality of fat intake, as we showed that the quantity of fatty acids intake is directly related to the cellular senescence. These findings suggest that dietary habits are important factors and powerful tools that regulate the cellular senescence which lead to a healthy aging process.
This project is cofounded by Ministerio de Economia y Competitividad (ISCIII- PI13 / 00185) and FEDER funds.

II.n Mediterranean diet improves cellular damage and modulates expression of circulating mir210 in patients with severe endothelial dysfunction and cardiovascular disease

Authors: *Jiménez R, Rangel O, Alcalá-Díaz, JF, Camargo A, Haro C, Delgado-Lista J, López-Miranda J, Marín C.*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Introduction: Coronary artery disease is the most important cause of death in developed countries. An early manifestation of cardiovascular disease (CVD) is endothelial dysfunction. Circulating microRNAs (miRNAs) are emerging as sensitive biomarkers of CVD, and diet is known to modulate endothelial damage and influence the expression of miRNAs.
Objective: To study the effect of two healthy diets (Mediterranean diet rich in virgin olive oil, and a low-fat diet) on cellular damage and expression levels of the circulating miR210, in patients with severe endothelial dysfunction and high cardiovascular risk.
Methodology: 40 patients with severe endothelial dysfunction and high cardiovascular risk, between 20 and 75 years old, were selected from the CORDIOPREV intervention study. These patients were randomized to consume a Mediterranean diet (MedDiet) and one low-fat diet high in complex carbohydrates. Blood samples were extracted at baseline and after the first year of dietary intervention. We quantified the cellular apoptosis levels in HUVEC (Human umbilical vein endothelial cells) incubated with serum from each patient, in presence and absence of TNF-α , and the expression of plasma circulating miR-210 at baseline and after one year of dietary intervention.
Results: We observed that cellular apoptosis ($p<0.05$) in HUVEC decreased after consumption of the MedDiet compared with the low-fat diet. No significant differences were observed in HUVEC incubated with TNF-α. Furthermore, the expression of circulating miR-210 increased after consumption of the MedDiet ($p<0.05$), compared with the low-fat diet, in patients with CVD and metabolic syndrome (MetS). In addition, we observed a negative correlation between miR210 expression and cellular apoptosis in HUVEC.
Conclusion: The MedDiet induces lower cellular apoptosis levels in HUVEC than the low-fat diet in patients with severe endothelial dysfunction and CVD. This finding could be modulated by the effect of MedDiet on miR-210 expression in patients with CVD in the presence of MetS.

SESSION III

Chronic and Inflammatory diseases. Infectious and Immunological diseases. Organ transplantation

III.a– Acute kidney injury in the meld era of liver transplantation. Are calcineurin inhibitors so problematic for renal function?

Authors: *Irene Gómez, Rubén Ciria, Miriam Cortés, Manuela Torres, Shirin Khorsandi, Anny Sofía Cadenas, Pedro López Cillero, John O'Grady, Manuel de la Mata, Nigel Heaton, Javier Briceño*

Group: GC18. *Translational research in surgery of solid organ transplantation*

Background and aims. Acute kidney injury (AKI) after liver transplantation (LT) is a common problem with complex management. Calcineurin inhibitors (CNI) have to be well-balanced due to their important immunosuppresive effect but also supposed deleterious effect on kidney function. The aims were: Primary, analyze the specific effect of CNI on AKI in a large cohort of patients. Secondary, to analyze the profile of AKI-RIFLE categories in the post-transplant setting and its impact on post-transplant-survival.

Methods. A retrospective analysis of 400 (2007-2012) consecutive patients transplanted at Reina Sofia-Cordoba and King's College Hospital-London was performed. Exclusion criteria were paediatric transplants, death<14 days and CNI-free regimes. Several peri-transplant variables were collected. Endpoints were: Development of AKI (RIFLE-Risk-Injury-Failure) and 1-year survival. Univariate, multivariate and general linear models were performed.

Results. A total of 330 patients were included. Incidence of AKI-Risk-Injury-Failure in the first 2 weeks after LT was 59,8%, 34,3% and 8,4%, respectively. The development of any AKI-type had no impact on overall 1-year survival. In the multivariate analysis, intraoperative transfusions (OR=1,15[1,04-1,3]), peak-post-transplant-transaminases (OR=2,9[1,01-8,77], pretransplant eGFR (OR=10,54[1,88-59,1]) and normal-CNI-dose strategies (OR=0,29[0,14-0,61]) were independent predictors of AKI_Risk. Similarly, peak post-transplant-transaminases (OR=3,3[1,39-7,8]) and normal-CNI-dose strategies (OR=0,31[0,15-0,62]) were predictors of AKI_Injury. Only peak post-transplant-transaminases (OR=6,8[2,6-16,8]) was a predictor of AKI_Failure. Renal function after the transplant suffers a drop in almost all cases in the first 2-4 days after the transplant independent of the immunosuppression strategy. In the general linear models, renal-protective strategies with low-CNI doses were only useful to prevent severe impairment in patients with extremely-low pre-transplant eGFR(<30ml/min/1.73m2), being useless in the rest (P<0,05).

Conclusions. AKI is frequent after LT. Transfusion, immediate liver function, pretx status and IS are factors that predict mild AKI. However, only immediate liver function predicts severe AKI. AKI happens similarly in all pre-tx eGFR status groups. Low CNI regimes are mildly useful in severely impaired eGFR pretx status patients being useless when pretransplant normal kidney function is preserved.

III.b– Prevalence of urolithiasis in spanish population aged 40 to 65: prelirene study

Authors: *Cano Castiñeira, Roque; Arias Vega, Raquel; Carrasco Valiente, Julia; Pérula de Torres, Luis Angel; Requena Tapia, Maria José; Jiménez García, Celia; Criado Larumbe, Margarita*

Group: GC12 *Epidemiological Research in Primary Care*

Objective: To estimate prevalence of renal lithiasis in Spanish population aged from 40 to 65 and determine socio-demographic and co-morbidity factors associated.
Material and Methods: Observational and transversal study, population base, selecting a random and stratified sample by CC.AA, sex and age groups, through a personal telephone survey including socio-demographic and clinical variables. A descriptive statistical analysis for each variable, as well as a bivariate analysis (Chi-squared test, Student-t test, Mann-Withney U test p<0.05), calculating the prevalence ratio (PR) and the confidence interval (CI95%), and a multiple logistic regression analysis were carried out.
Results: A total of 2445 people were surveyed, 51.2% females, 55.9% aged 40-52. The prevalence of renal lithiasis was 14.6% (CI95%:13.1-15.9) and the annual incidence 2.9% (CI95%: 2.2-3.6). No significant differences were found in lithiasis prevalence with sex and level of education but with age, higher in aged group 45-50 (PR=1.58;CI95%:1.12-2.21; p<0.001) and 60-65 (PR=1.47;CI95%:1.02-2.13;p=0.040), with higher social class (PR=1.75;CI 95%:1.07-2.86;p=0.024), with renal lithiasis family history (PR=2.01;CI95%:1.73-2.33;p<0.001), high blood pressure (HBP) (PR=1.61;CI95%:1.35-1.93;p<0.001), with diabetes (PR=1.68;CI 95%: 1.19-2.38;p=0.002), hypercholesterolaemia (PR=1.20;CI95%:1.02-1.41;p=0.032) and overweight (PR=1.31;CI95%:1.01-1.69;p=0.037) or obesity (PR=1.45;CI95%:1.03-2.03;p=0,033).
The multivariate analisys confirmed the association between renal lithiasis and aged 46-50 (OR: 1.51), higher social class (OR:2.54), renal lithiasis family history (OR:2.81) and HBP (OR:1.68).
Conclusions: The results confirm the high auto-declared renal lithiasis prevalence that was observed in others previous studies around. There is a relationship between prevalence of renal lithiasis and to pertain to a high social class, having a family history, be middle aged and presence of co-morbidity (HBP). It is important to make an effort aimed to prevention, through the promotion of healthy dietary habits in the population, over all in those patients who have risk factors associated.

III.c– FGF23 increases phosphate-induced smooth muscle cells calcification

Authors: *Vergara N, Herencia C, Martínez-Moreno JM, Rodriguez-Ortiz ME, Diaz-Tocados JM, Almaden Y, Muñoz-Castañeda JR, Rodríguez M.*

Group: GC13. *Calcium metabolism. Vascular calcification*

Elevated fibroblast growth factor 23 (FGF23) is associated with cardiovascular disease in patients with chronic kidney disease (CKD) and it is responsible to the phosphate homeostasis. Vascular smooth muscle cells (VSMC) are located around endothelial cells and they contribute to the maintenance of blood vessels structure. These cells may be differentiated under several stimuli into functional (contractile and synthetic) or dysfunctional (osteogenic) phenotypes. Recent studies had also proposed that the transition from contractile to synthetic phenotype could be involved in pathological processes of cardiovascular abnormalities.

The present study was aimed to evaluate whether FGF23 alone or in combination with high phosphate is involved with the ability of these VSMC to change their phenotype.

To determine the effects of high phosphate in the development of VSMC phenotypes, we treated aortic human smooth muscle cells (AoSMC) with normal or high levels of phosphate. In addition, high concentrations of FGF23 were also added alone in combination with high phosphate to examine the contribution of FGF23 to these changes. After 9 days, we analyzed expression of contractile, synthetic and osteogenic genes, calcium content and matrix mineralization deposition involved in phenotypic changes.

We found that high phosphate addition into AoSMC culture increased calcium content and matrix mineralization as compared with normal phosphate while there was not an increase in the expression of synthetic VSMC genes. On the contrary an increase in osteogenic genes expression was observed. Remarkably, high FGF23 concentration greatly enhanced the procalcificant effects of phosphate. There were not significant changes between contractile and synthetic phenotypes although whether there was a significant increase in osteogenic genes expression.

In conclusion, high FGF23 increases phosphate-induced smooth muscle cells calcification and enhances osteogenic transdifferentiation.

III.d– Prognosis of patients with ulcerative colitis in sustained remission after thiopurines withdrawal

Authors: *Moreno-Rincón, Estefanía; Benítez, José Manuel; Serrano-Ruiz, Francisco Javier; Vázquez-Morón, Juan María; Pallarés-Manrique, Héctor; Herrera-Justiniano, José Manuel; Leo-Carnerero, Eduardo; Gómez-García, María Rosario; Cabello-Tapia, María José; Castro-Fernández, Manuel; Rojas-Feria, María; Castro-Laria, Luisa; Argüelles-Arias, Federico; Camargo-Camero, Raquel; Alcaín-Martínez, Guillermo; Iglesias-Flores, Eva; García-Sánchez, Valle*

Group: *other*

Background. The ideal length of treatment with thiopurines in patients with ulcerative colitis (UC) in sustained remission remains unknown. It is widely accepted that the drug withdrawal is associated with a worse outcome. The aim of this study was to analyze the outcome after this withdrawal and to identify predictors of relapse.
Methods. A multicenter and retrospective study was designed. A total of 102 patients with UC who discontinued thiopurines in a situation of sustained remission were included. All the patients were followed up until last revision or until relapse (understood as the occurrence of signs and symptoms of UC that required a rescue treatment).
Results. After thiopurines withdrawal, overall relapse was recorded in 32.35% of the patients: 18.88% in the first year, 36.48% in the third and 43.04% in the fifth year after withdrawal. On multivariate analysis, predictors of relapse were the time from diagnosis of UC until the starting of thiopurines (hazard ratio [HR], 1.01, 95% confidence interval [CI] 1.01-1.02, p=0.039), the number of relapses before the withdrawal (HR 1.3, 95% CI 1.01-1.66, p=0.029), pancolitis (HR 5.01, 95% CI 1.95-26.43, p=0.028), the duration of treatment with thiopurines (HR 0.15, 95% CI 0.03-0.66, p=0.013) and the situation of biological remission at withdrawal (HR 0.004, 95% CI 0.0001-0.14, p=0.002).
Conclusions. The withdrawal of thiopurines in patients with UC, although in sustained remission, is related to a high relapse rate. Clinical variables such as the extent of the disease, the duration of treatment or time from diagnosis to the start of thiopurines should be considered before stopping these drugs.

III.e– Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFα

Authors: Carlos Pérez-Sánchez, Patricia Ruiz-Limón, Carmen Castro-Villegas, Ma Angeles Aguirre, Yolanda Jiménez-Gomez, Pilar Font, Antonio Rodriguez-Ariza, Juan Ramon Peinado, Eduardo Collantes-Estévez, Nuria Barbarroja, and Chary López-Pedrera

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Introduction: The advent of anti-tumor necrosis factor alpha (anti TNFα) drugs has considerably improved medical management in rheumatoid arthritis (RA) patients, although it has been reported to be ineffective in a fraction of them. MicroRNAs (miRNAs) are small, non-coding RNAs that act as fine-tuning regulators of gene expression. Targeting miRNAs by gain or loss of function approaches have brought therapeutic effects in various disease models. The aim of this study was to investigate serum miRNA levels as predictive biomarkers of response to anti-TNFα therapy in RA patients.

Methods: In total, 95 RA patients undergoing anti-TNFα/disease modifying antirheumatic drugs (anti-TNFα/DMARDs) combined treatments were enrolled. Serum samples were obtained at 0 and 6 months and therapeutic efficacy was assessed. miRNAs were isolated from the serum of 10 patients before and after anti TNFα/DMARDs combination therapy, cDNA transcribed and pooled, and human serum miRNA polymerase chain reaction (PCR) arrays were performed. Subsequently, selected miRNAs were analyzed in a validation cohort consisting of 85 RA patients. Correlation studies with clinical and serological variables were also performed.

Results: Ninety percent of RA patients responded to anti TNFα/DMARDs combination therapy according to European League Against Rheumatism (EULAR) criteria. Array analysis showed that 91% of miRNAS were overexpressed and 9% downregulated after therapy. Functional classification revealed a preponderance of target mRNAs involved in reduction of cells maturation - especially on chondrocytes - as well as in immune and inflammatory response, cardiovascular disease, connective tissue and musculoskeletal system. Six out of ten miRNAs selected for validation were found significantly upregulated by anti TNFα/DMARDs combination therapy (miR-16-5p, miR-23-3p, miR125b-5p, miR-126-3p, miRN-146a-5p, miR-223-3p). Only responder patients showed an increase in those miRNAs after therapy, and paralleled the reduction of TNFα, interleukin (IL)-6, IL 17, rheumatoid factor (RF), and C-reactive protein (CRP). Correlation studies demonstrated associations between validated miRNAs and clinical and inflammatory parameters. Further, we identified a specific serum miRNA signature (miR-23 and miR-223) that may serve both as predictor and biomarker of response to anti-TNFα/DMARDs combination therapy.

Conclusions: miRNA levels in the serum of RA patients before and after anti-TNFα/DMARDs combination therapy are potential novel biomarkers for predicting and monitoring therapy outcome.

III.f– Aging and chronic kidney disease oxidized albumin promotes cellular senescence and endothelial damage

Authors: *Carlos Luna, Matilde Alique, Estefanía Navalmoral, Sagrario Soriano, Julia Carracedo, Rafael Ramírez*

Group: GC7 *Nephrology. Cell damage in chronic inflammation*

The increase levels of oxidized proteins with aging and chronic kidney disease have been considered a cardiovascular risk factor. However, it is unclear that whether oxidized albumin, the most abundant serum protein may induce endothelial damage. The results in this study indicated that with aging process and chronic kidney disease situations, levels of oxidized proteins increased. Among these, oxidized albumin seems to play a principal role. In vitro studies, endothelial cells cultured with oxidized albumin exhibited an increment of oxidative stress and underwent senescence. In addition, endothelial cells cultured with oxidized albumin shown a reduction in endothelial cell migration measured by wound healing. Taken together, we provide the first evidence that oxidized albumin induces endothelial injury which then contributes to the increase cardiovascular disease in elderly and chronic inflammation.

III.g– Myogenic differentation in skeletal muscle satellite cells and interstitial mononuclear cells in the absence of injury

Authors: *Fernando Leiva-Cepas, Ignacio Ruz-Caracuel, Eduardo Agüera, Ignacio Jimena, Evelio Luque & José Peña*

Group: GE1 *Oxidative stress and nutrition*

Introduction. It has been noted that trophic factors released during denervation are implicated in the activation of satellite cells and that pericytes associated with muscle capillaries could be a stem cell subpopulation with myogenic commitment. The aim of this study was to test whether satellite cells and pericytes from normal muscle could be activated by treatment with an extract of denervated muscle.
Methods. Normal Wistar rats were treated with denervated muscle extract during 5 consecutive days; normal rats without treatment were used as control. Soleus muscles were then dissected and processed for histological, histochemical and immunohistochemical analysis. Myogenic differentiation was evaluated by desmin immunohistochemistry. A qualitative and quantitative analysis of satelite cells and pericytes was performed on transmission electron microscopy.
Results. We observed isolated and grouped mononucleated cells desmin positive in the interstitial space and satellite cells desmin positive located in the muscle fibers surface; the quantitative analysis showed significant differences between control and experimental group. The transmission electron microscopy analysis confirmed the inmunohistochemical observations and showed a significative increase in the number of pericytes in the experimental group.
Discussion. Our results suggest that trophic factors contained in denervated muscle extract can activate myogenic differentiation of both satellite cells and interstitial cells in the absence of injury. In conclusion, the microscopic evidence of this experimental study, suggest that perycites could play a role in the proplasic response of skeletal muscle in relation with satellite cells.

III.h– Real-time measurements of tissue oxygen microtension as a marker of bile duct viability in liver transplantation

Authors: *Elena Navarro Rodríguez, Ruben Ciria Bru, Ana Belen Gallardo Herrera, Marina Sanchez Frías, Javier Medina Fernández, María Dolores Ayllón Terán, Sebastián Rufián Peña, Pedro López Cillero, Javier Briceño Delgado*

Group: GC18 *Translational research in surgery of solid organ transplantation*

Aims: The main aim was to evaluate bile duct viability by assessing its microvascular quality using an innovative real-time oxygen tension device by testing different areas in both donor and recipient's side. Findings were subsequently correlated with histopathological results.
Methods: Observational prospective cohort study with 18 patients included from November 2013 to September 2014. Tissue oxygen microtension measurements were made using Oxylite® device in different areas of recipient and donor's bile duct intraoperatively after biliary anastomosis was made.
Results: Mean oxygen microtension value in the graft bile duct at anastomosis level was 106 (92,5-118) mmHg, being 125 (108,5-134,5) mmHg 1.5 cm proximal to the hilar plate. Mean micro-oxygenation value in the bile duct recipient was 117,5 (100,5-150) mmHg, whilst a value of 138 (119-183) mmHg was observed 1.5 cms distal to the anastomosis. Tissue oxygen microtension was statistically higher in distal areas to section border of the biliary anastomosis, with an overall pO2 increase distal to the anastomosis of 17,94 mmHg (p<0,001) and 21,61 mmHg (p<0,001) in the graft and recipient, respectively. Biliary anastomosis was performed above the cystic duct insertion in the donor bile duct in 10 patients, with significant higher values of pO2 microtension (p=0.017). Histological injury grade 2-3 in biliary mural stroma and grade 1-3 in peribiliary vascular plexus of graft's bile duct graft were associated with lower tissue oxygen pressure, as well as injury grade 2 in biliary epithelium and grade 1-3 in peribiliary vascular plexus of recipient's bile duct were associated with lower micro-oxygenation (p<0,05).
Conclusion: Our results demonstrates that terminal border of donor and recipient bile duct are low-vascularized areas. Tissue microoxygenation improves significantly in areas close to the hilar plate and to the duodenum in the donor and recipient's sides, respectively. Histopathological findings of bile duct injury are associated to worst tissue microoxigenation.

III.i– Knowledge and practices of primary care professionals about the approach of alcohol: results of ALCO-AP study

Authors: *Esperanza María Romero Rodríguez, Fernando Leiva-Cepas, José Ángel Fernández García, Luis Ángel Pérula de Torres y Grupo colaborativo ALCO-AP*

Group: GC12 *Epidemiological Research in Primary Care*

-Objectives: assess knowledge, attitudes and practices of doctors and nurses in primary care (PC) to the implementation of interventions postulated by the Program of Preventive Activities and Health Promotion -PAPPS- aimed at patients alcohol consumption.
-Material and Methods: a descriptive, cross-sectional study. Scope: health centers of the National Health System. Were invited to participate doctors and nurses PC. For an alpha error of 5% and an accuracy of 3% and a rate of 50%, would be included in the study 1068 Professional. They were invited to engage all partners and professionals semFYC a random sample of health centers in the country. Once established their intention to participate, they completed an online survey. Descriptive and inferential statistics bivariate and multivariate analysis was performed ($p<0.05$).
-Results: 1116 subjects completed the survey (86% doctors and 14% nurses), 65% were women. 46% do not know how many grams of a standard drink. 48% have a fundamental misunderstanding of what is meant by "consumer risk" concept. 33% performed systematic exploration of alcohol consumption by more than 65% of the time in his usual consultation and 47% give medical advice. 65% (screening test) and 81% (council) knows PAPHP recommendations in relation to alcohol consumption.
-Conclusions: degree of knowledge of PC professionals around the approach to patients with excessive alcohol consumption has to be increased as well as the assumption that interventions targeting these patients should be performed more commonly in clinical practice.

III.j– Caenorhabditis elegans as an animal model in preclinical assays for pharmacogenetic studies of the antipsychotic drugs risperidone and aripiprazole

Authors: *Jaime Osuna-Luque, Nuria Cascales-Picó, M. Mar Gámez-del-Estal and Manuel Ruiz-Rubio*

Group: GC20 *Genetic and Behavior Diseases*

Aripiprazole and risperidone, both classified as atypical antipsychotic drugs, are the only Food and Drug Administration approved medications for treating irritability-aggression in autism spectrum disorder (ASD). Aripiprazole differs of risperidone in the mechanism of action. Risperidone is a dopamine D2 and serotonin 5-HT2A receptors antagonist. Aripiprazole function as a partial agonist of dopamine D2 receptor, and as partial agonist of 5-HT1A and antagonist of 5-HT2A serotonin receptors, respectively. Although both medicaments appear to present similar efficacy, aripiprazole seems to be potentially better tolerated than risperidone presenting fewer side effects. The causes of these differences are unknown. In this scenario, the establishment of animal models to study the basis of differential biological mechanisms of drug targets might be essential. C. elegans presents several advantages as animal model, especially with respect to their nervous system composed of 302 neurons and 56 glial cells. This nematode has dopamine and serotonin receptors orthologs to human D2 and 5-HT human receptors, which are coded by genes belonging to a super-family of seven-transmembrane G-protein-coupled receptors. Furthermore, dopamine and serotonin has been found to participate in a wide array of nematode behaviors, including gentle touch response and pharyngeal pumping rate. We have obtained results showing that risperidone and aripiprazole were able to alter both behaviors and the response differ in mutants deficient in different dopamine and serotonin receptors. On the other hand their effect on behavior remained to some extent in successive generations, indicating that epigenetic mechanisms could be involved. This presumably epigenetic effect was stronger with risperidone than aripiprazol that may explain the differences in the side effects of treatments in humans. The present study provides novel insights on the molecular neurobiological mechanism of these drugs.

III.k– VCE-003.2 is a novel cannabigerol derivative that enhances neuronal stem cell prosurvival and alleviates symtomatology in murine models of huntington disease

Authors: *Carmen del Rio, Javier Diaz-Alonso, Irene Cantarero, Carmen Navarrete, Juan Paraíso Luna, Marco A. Calzado, Ismael Galve-Roperh, and Eduardo Muñoz*

Group: GC4. *Inflammation and cancer*

Huntington's disease (HD) is a rare neurodegenerative disorder characterized by motor abnormalities, cognitive dysfunction, and psychiatric symptoms. The primary cause of the disease is a mutation in the huntingtin gene, which results in an abnormal polyglutamine protein that becomes toxic for striatal and cortical neuronal subpopulations. Different cannabinoids have shown to be neuroprotective in experimental animal models of neurodegeneration and neuroinflammation through cannabinoid receptors (CBs)-dependent and -independent mechanisms. We have previously found that resorcinyl-to-paraquinol oxidation of CBG (VCE-003) increases its PPARg agonistic activities and acts as immunosuppressor. However VCE-003 is an unstable electrophilic compound, with limited prospects for further development. We have generated novel non-electrophilic VCE-003 derivatives that activate PPARg, from which we have selected the VCE-003.2 for further characterization.

VCE-003.2 is a PPARg agonist with neuroprotective activity in vitro and in vivo. This compound did not affect the proliferative activity of neural progenitor cells in proliferative conditions but showed a prosurvival activity in differentiating conditions. This activity was mediated by activation of the ERK 1+2 pathway and was prevented by a PPARg antagonist. In addition, VCE-003.2 prevented quinolinic acid (QA)-induced inhibition of mitochondrial activity and caspase-3 activation that paralleled with an increase in the percentage of PCNA+ cells. VCE-003.2 protection from QA excitotoxicity was dependent on PPARg. This compound also protected from glutamate-, H2O2-, and mut-Huntinting-induced cytotoxicity in N2A, NSC34 and ST cells respectively and reduced huntingtin aggregates in striatal cells.

VCE-003.2 was extremely neuroprotective in mice intoxicated with 3-nitropropionate (3NP) or QA improving motor deficits and reactive gliosis. In the 3NP model VCE-003.2 preserved the loss of striatal neurons, inhibited the upregulation of proinflammatory markers and improved the levels of antioxidant defenses. In conclusion, this study highlights the therapeutic potential of non-electrophilic CBG-quinone derivatives for the treatment of HD and other neurodegenerative and neuroinflammatory diseases

III.I –Role of monocytes subsets in the atherothrombosis and endothelial dysfunction associated with rheumatoid arthritis: beneficial effects of tocilizumab

Authors: *P. Ruiz-Limon, N. Barbarroja , C. Perez-Sanchez , Y. Jimenez-Gomez , M. Abalos-Aguilera , P. Font , M. Aguirre , P. Segui , J. Calvo , R. Ortega , E. Collantes-Estevez , A. Escudero , R. Lopez-Pedrera*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Background: Monocytes play a key role in the pathogenesis of the atherothrombosis. It has been described three subsets of monocytes with different actions in the vascular pathology: MON1 (CD14brightCD16-), MON2 (CD14dimCD16bright) and MON3 (CD14brightCD16bright). The distribution of these monocytes subsets and its profile associated with cardiovascular disease (CVD) in rheumatoid arthritis (RA) remain unravelled. Tocilizumab (TCZ) is a new therapeutic drug, very effective in RA, but its effects on CVD are still unknown

Objectives: 1) To functionally characterize the different monocytes subsets in RA patients and analyze their role in the endothelial dysfunction, altered oxidative status and proinflammatory/prothrombotic profile associated to RA.

2) To evaluate the effect of TCZ in the proatherotrombotic profile of these cells and its association with the clinical outcome.

Methods: Thirty RA patients and 15 healthy donors were included. Endothelial function was measured through post occlusive hyperaemia using Laser-Doppler. Carotid intima media thickness (CIMT) was used as atherosclerosis marker. MON1, MON2 and MON3 were characterized by flow cytometry and isolated using immuno-magnetic selection. Different proinflammatory cytokines, peroxides levels and cellular activation markers were analyzed in the three different subsets. Selected RA patients (10) received 162 mg/week subcutaneous TCZ. Blood samples were collected before and after 6 months of treatment.

Results: CD16+ (MON2-MON3) monocytes were significantly extended in RA patients. These subsets had increased protein expression of proinflammatory cytokines, markers of cellular activation and peroxide levels. RA patients had impaired endothelial function, with a reduced perfusion value after ischemia. Increased MON2 and MON3 and reduced MON1 percentage were associated with a pathologic CIMT thickness. Clinical parameters such as evolution time, C reactive protein, anti-CCPs antibodies and rheumatoid factor levels strongly correlated with endothelial dysfunction, decreased percentage of MON1 monocytes and increased number of MON2 and MON3 subsets. Furthermore, higher expression of proinflammatory/prothrombotic molecules and endothelial adhesion markers in these CD16+ cells correlated with the alteration in endothelial function and the clinical parameters. After 6 months of treatment, TCZ reduced clinical parameters of inflammation, autoinmunity and joint damage. Endothelial function was significantly restored. TCZ reduced the expression of inflammatory and prothrombotic molecules, peroxide and peroxinitrite levels. The reduction of proinflammatory cytokines and oxidative stress in RA monocytes significantly correlated with the improvement of the joint damage and endothelial function.

Conclusions: 1) RA patients display an increased number of MON2 and MON3 monocytes directly associated to the autoimmune and inflammatory profile, the progression of the disease and the altered microvascular function, indicating that CD16+ subpopulation might play a key role in the CVD pathogenesis associated with RA. 2) TCZ induces an improvement of the proatherothrombotic profile in RA patients.

POSTER SESSION

1. Characterization of a novel protein associated to lipid droplets in adipocytes and hepatocytes, Rab34

Authors: *Trávez A., Rabanal-Ruiz Y., Guzman-Ruiz R., Garcia-Navarro S., Gracia-Navarro F., Vázquez-Martínez R, and Malagón M.M*

Group: GC11 *Metabolism and adipocyte differentiation. Metabolic Syndrome*

The adipose tissue (AT) plays a central role in managing the energy stores and as an endocrine organ by producing adipokines that regulate food intake and energy homeostasis, immunity and cardiovascular function. The major cellular component of the AT, the adipocytes, store energy in form of triglycerides (TAGs) within the large lipid droplet (LD) characteristic of this cell type. In contrast to their physiological role in adipocytes, accumulation of LDs in other cell types, as occurs in hepatocytes in obese individuals, is commonly associated with pathological processes. This abnormal retention of lipids, known as steatosis, is characteristic in fatty liver disease.
In order to identify novel regulators of LD function, we carried out a comparative proteomic study of lipid droplets (LDs) isolated from 3T3-L1 adipocytes, exposed or not to insulin (10 μM). This approach enabled the identification of a novel LD-associated protein, the small GTPase, Rab34, which had been previously characterized in other cell types as a component of the Golgi apparatus that mediates intracellular transport. Here, we demonstrate that while Rab34 does localize to the Golgi at early stages of adipogenesis in 3T3-L1 cells and human adipocytes (day 3), it is linked to LDs in differentiated adipocytes. These observations suggest a novel pathway for the incorporation of proteins to the LD surface involving the participation of the Golgi apparatus. Moreover, quantification of Rab34 in human adipose tissue samples revealed a significant increase in the protein content of this GTPase in obese individuals, as compared to lean subjects. Notably, as observed in adipocytes, a redistribution of Rab34 from the Golgi to nascent LDs occurred in HepG2 cells in response to fatty acid exposure (oleic acid; 200 μM, 16 h). Together, these data suggest a significant role of Rab34 in LD dynamics, both under physiological and pathological conditions.

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2. A role for the cytoskeletal protein, Septin11, in the regulation of caveolae dynamics and lipid transport in adipocytes

Authors: *Moreno N.R., Rodríguez A.,Vázquez-Martínez R., Frühbeck G., and Malagón M.M.*

Group: GC11 *Metabolism and adipocyte differentiation. Metabolic Syndrome*

In adipocytes, the cytoskeleton undergoes important changes in relation to adipogenesis and obesity. Recently, septins have been considered as new components of the cytoskeletal network that interact with other cytoskeletal elements (actin and tubulin) profoundly modifying their dynamics. In this work, we have focused on characterizing septin 11 (SEPT11). Specifically, we evaluated the localization of SEPT11 in adipocytes by immunocytochemistry and subcellular fractionation techniques. Finally, GST-pulldown assays and a Yeast-Two Hybrid (Y2H) screening were used to identify the SEPT11 interactome.

In both the adipocyte cell line, 3T3-L1 cells, and in human adipocytes, SEPT11 distributes as bundles resembling actin filaments and is mainly cytosolic at early adipogenic stages while it is membrane-bound and forms ring-like structures at the cell surface that are reminiscent of caveolae in differentiated cells. In this line, the Rho GTPase activating protein known to regulate actin polymerization, ARHGAP21, was found to interact with SEPT11 by Y2H. In addition, GST-pulldown experiments revealed that SEPT11 also interacts with a major component of caveolae, caveolin-1, as well as with a member of the fatty acid-binding protein family, FABP5, which has been reported to localize to caveolae. Together, these data suggest a role of SEPT11 in caveolae function and, in particular, in lipid transport. Notably, exposure of adipocytes to fatty acids (oleate, palmitate) significantly increased the protein content of SEPT11. Moreover, oleate also induced the translocation of SEPT11 from the plasma membrane to the surface of the organelles responsible of lipid storage and mobilization in adipocytes, the lipid droplets. Altogether, these results suggest that SEPT11, through its relation with actin filaments and caveolae components, may play a role in the transport of fatty acids in adipocytes and can thus represent a potential biomarker of insulin resistance in obesity.

3. Effect of diet on cellular senescence and cellular damage in patients with severe endothelial dysfunction and cardiovascular disease

Authors: *Fernández C., Rangel O., Jiménez R., Gómez Delgado F., Carpintero S., Pérez-Martínez P., López Miranda J., Marín C.*

Group: GC9 *Nutrigenomics. Metabolic Syndrome*

Introduction: The endothelial dysfunction, an early manifestation of cardiovascular disease (CVD), is related to increase oxidative stress, caused by increased production of reactive oxygen species (ROS) and inflammatory environment. These alterations promote senescence and apoptosis of endothelial progenitor cells (EPCs) and induces a decrease in the ability of vascular repair. Previous studies show that diet can modulate these cellular mechanisms.
Objective: Our aim was to study the effect of two healthy diets (a low fat diet and a Mediterranean diet –MedDiet) on molecular alterations involved in CVD, in patients with severe endothelial dysfunction and high cardiovascular risk, in order to use the diet as a therapeutic strategy.
Methodology: We selected 40 patients from the CORDIOPREV clinical trial, with severe endothelial dysfunction and CVD, 20 patients with circulating EPC increased and 20 patients with circulating EPCs decreased after one year of dietary intervention. All patients were performed blood extractions at baseline and after one year of dietary intervention. We quantified the circulating EPCs in peripheral blood. We studied cellular senescence and intracellular ROS levels in Human Umbilical Vein Endothelial Cells, incubated with serum from each patient. Also, we analyzed the TLR4 gene expressionin peripheral blood mononuclear cells from each patient.
Results: Our results show a cellular senescence decreased after one year dietary intervention, independently of the diets group. In addition, we observed a lower intracellular ROS levels after consumption of MedDiet versus low fat diet. Furthermore, in patients with circulating EPC levels decreased, we observed that the TLR4 levels were not differentially expressed after the MedDiet, compared with the low fat diet.
Conclusion: The Mediterranean diet could regulate oxidative stress and pro-inflammatory factor inducing less cellular damage and decreasing the rate of cellular senescence to endothelial cells involved in the vascular repair. This could decrease the cardiovascular risk in patients with severe endothelial dysfunction.

4. Is there a relationship between renal lithiasis, cardiovascular risk factors and erectile dysfunction? An epidemiologic study in Spanish population aged from 40 to 65

Authors: *Roque Cano Castiñeira, Isabel López Macías, Isabel Conesa Pedrosa, Raquel Arias Vega R , Julia Carrasco Valiente, Aurora Blanco Mora, Enrique Rodríguez Guerrero, José Antonio Luque Domínguez, Macarena Lara Doblas, Luís Ángel Pérula de Torres, Maria José Requena Tapia, Rafael Prieto, Celia Jiménez García, Margarita Criado Larumbe*

Group: GC12 *Epidemiological Research in Primary Care*

Objectives: The erectile dysfunction (ED) is more prevalent in older men and there is a relationship with some risk factors like cardiovascular diseases, diabetes or metabolic syndrome, whose common denominator is the vascular insufficiency promoted by arteriosclerosis and the endothelial dysfunction. The objective was to estimate the prevalence of erectile dysfunction (ED) in the Spanish population 40-65 years old and study the association between it, kidney stones and other cardiovascular risk factors.

-Material and Methods: Observational and transversal study, population base. A sample of 1193 male aged 40 to 65 living in Spain was selected by a random and multistage sampling. Sociodemographic (age, sex, physical activity and social class), and clinic (arterial hypertension, diabetes, hypercholesterolemia, gout, weigh and size) variables were taken, as well as variables of renal lithiasis and ED. It was estimated the prevalence ratio (RP) and it was applied the Chi squared test.

-Results: The ED prevalence is 3.4% (CI95%:2.29-4.4), the higher in subjects aged 60-65 (RP=12.43; CI95%:3.65-42.30; p<0.001). We found association between ED and HTA (p=0.008), DM (p=0.042) and hypercholesterolemia (p=0.023). Although the 15.0% of them had obesity and 47.5% were overweight, however no association was found between those factors and ED and nor with the uric acid levels, physical activity and social class. The 27.5% of patients suffering ED had also renal lithiasis (p=0.016).

-Conclutions: The association between ED and cardiovascular risk factors that we observed in our study indicates the importance of assessing the cardiovascular risk in patients who have ED and the necessity of promoting some measures to reduce it in the population. Likewise, it should be assessed the presence of ED in patients with renal lithiasis.

5. The type and amount of dietary fat determine the postprandial increase in LPS levels and inflammatory response in patients with Metabolic Syndrome

Authors: *Javier López-Moreno, Antonio Camargo-García, Sonia García-Carpintero, Francisco Gómez-Delgado, Ana León-Acuña, Rosa Jiménez-Lucena, Pablo Pérez-Martínez, José López-Miranda*

Group: GC9 *Nutrigenomics. Metabolic Syndrome*

Introduction. Metabolic syndrome is a postprandial metabolic alteration which predisposes to a state of chronic low-grade inflammation and an increased oxidative stress. We hipothezed that the long-term consumption of diets differing in the type and amount of dietary fat may influence the postprandial intestinal absorption of bacterial endotoxin, which may be responsible, at least partially, of the postprandial inflammatory response after meals intake.
Objective: To determine the effect of the amount and type of dietary fat on postprandial endotoxemia.
Methodology: A subgroup of 75 patients from the LIPGENE study were randomized to receive 1 of 4 diet (HSFA, HMUFA, LHFCC and LHFCC n-3) for 12 week. We determined the plasma lipoproteins and glucose concentrations, and gene expression in peripheral blood mononuclear cells and adipose tissue. Lipopolysaccaride (LPS) and LPS binding protein (LBP) plasma levels were determined by ELISA, at fasting and postprandial state (4 hours after a fat challenge reflecting the fatty acid composition of the dietary intervention), 12 weeks post- dietary intervention.
Results: We did not observe any statistically significant differences between diets in the LPS fasting levels. By contrast, we observed a postprandial increase in the LPS levels after the intake of the HSFA meal (p=0.009), whereas we did not find any postprandial changes after the intake of the other diets. In addition, we did not find any statistically significant differences in the LBP plasma levels at fasting nor postprandial states. Moreover, we observed a positive relationship between the LPS plasma levels and the expression of IkB-a and MIF1 genes in mononuclear cells (R=0.326; p=0.006; and R=0.339; p=0.004, respectively).
Conclusions: Our results suggest that the consumption of a saturated-rich diet increase the intestinal permeability and absorption of LPS, which increase the postprandial endotoxemia levels and the postprandial activation in the inflammatory response.

6. Correlation between both kidneys biopsy in expanded criteria donors and transplant survival

Authors: *García-Rubio, JH; Ruiz García, J; Campos Hernández, JP*

Group: GA7 *Urology and Sexual Medicine*

Objective: The objective was to study the relation between same-donor renal biopsies and analyze whether the score influences graft survival.
Methods: We retrospectively reviewed histological results of expanded criteria donors and the graft survival in patients followed-up at Reina Sofia hospital (Cordoba, Spain) between January 2004 and October 2012. We analyzed clinical and demographic variables from the donors, as well the association between the scores of same-donor biopsies who had different scores for each kidney and the graft survival with a T-test for paired data. A Kaplan-Meier with Log Rank test was performed between the higher score and the lower score groups. We excluded retransplantation and those who received a combined transplantation (liver or pancreas).
Results: We analyzed 168 kidneys that had been biopsied, from 84 donors. Of the whole sample, 35.7% (n=30) had the same score for each kidney, while 64.3% (n=54) had discrepancies. In this second group, 81.8% (n=44) had a difference of one point, whereas the remaining 18.2% (n=10) had a larger difference. Both kidneys were suitable for trasplant in 72.7% of cases (n=40), only one in 14.5% (n=8), and none in 12.7% (n=7). To analyze the survival of the paired kidneys there remained 48 kidneys from 24 donors with a different score for each kidney. We observed a difference in favor of the better scores, with a difference of 11 months up to the time of the analysis (P=.045). We found no significant differences in the Log Rank test between the survival rate for the group with a less favorable score (95% CI, 61.26-95.67) versus those with a more favorable score (95% CI, 66.76-93.03).
Conclusions: A high percentage of biopsies had a different score for the two kidneys from the same donor. This difference was important for graft survival. We therefore recommend doing a biopsy of both kidneys.

7. Effect of hypoxia on human mesenchymal stem cells phenotype and functionality

Authors: *Paco-Meza LM, López L, Holgado A, Carmona MD, Nogueras S, Martin V, Blanco A, Jiménez R and Cañadillas S-Herrera C.*

Group: GC14 *Cell Therapy*

Background: Human mesenchymal stem cells (hMSCs) have been investigated for cell therapy in a wide range of diseases. MSCs are a potent source of trophic factors and actively remodel their immediate microenvironment in response to external stimuli such as oxygen tension. hMSCs in the laboratory are usually cultured under normoxic conditions corresponding to atmospheric oxygen (21%). Nevertheless, physiological oxygen tension in the bone marrow is much lower (<7%). Hypoxia is considered an important factor in many aspects of stem-cell biology, including: proliferation, potentiality, migration and paracrine activity. In this study, we tested the hypothesis that hypoxia could influence on hMSC properties. We evaluated whether low oxygen level affected h-MSCs phenotype and functionality.

Methods: h-MSCs from five human healthy donors were cultured in growth medium under either normoxic (21%O2) or hypoxic (3%O2) conditions for 48 and 72 hours and then were transferred to normoxic conditions to study: 1) hMSCs phenotype by flow cytometry; 2) hMSCs proliferation; 3) Adipogenic and Osteogenic differentiation after 21 days in adipogenic and osteogenic differentiation medium respectively; 4) Their ability to migrate towards VEGF and SDF-1 growth factors; and 5) VEGF secretion capacity (VEGF was measured by ELISA in supernatants).

Results: Hypoxic pre-conditionig did not change neither phenotype nor differentiation potential of hMSCs. However, hMSCs cultured under hypoxic conditions (72h) showed an increase in VEGF secretion, which was significantly higher compared to hMSCs incubated in normoxia. Moreover, hypoxic pre-conditioned hMSCs (48h and 72h) exhibited a greater capacity of migration in presence of VEGF in comparison to hMSCs incubated under normoxic conditions. On the contrary, SDF-1-induced migration showed no significant changes between hypoxic and normoxic cultures.

Conclusion: Our results suggest that a Hypoxic pre-conditioning enhances hMSCs migration ability and VEGF secretion capacity. This fact could be an advantage for their use in cell therapy.

8. Analysis of a cohort of recent onset rheumatoid arthritis: determinants of early remission

Authors: *Jiménez Gasco Rocío, Escudero Contreras Alejandro, Font Ugalde Pilar, Calvo Gutiérrez Jerusalem, Ortega Castro Rafaela, López Medina Clementina, y Collantes Estevéz Eduardo*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Objectives: to determine the factors that may influence the time at which early remission is achieved in patients with recent-onset rheumatoid arthritis.
Methods: A logistic regression analysis was performed on a sample of 50 patients diagnosed with rheumatoid arthritis of recent onset, in order to determinate the association among the disease remission 1 year after diagnosis(determined by DAS-28 <2.6) and a sociodemographic variable, seven clinics variables and the basal dose methotrexate measured at diagnosis.
Results: The sex of the patient, the 'health assessment questionaire' (HAQ) and the dose of methotrexate were significantly ($p<0.05$) related to disease remission 1 year after diagnosis. Women were less likely to achieve remission year (OR=0.009; p=0.044) compared with men. Patients who had higher HAQ scores at diagnosis also were less likely to achieve remission (OR = 0.028; p = 0.044). For every milligram of additional weekly methotrexate administered at diagnosis there is four times more likely to achieve remission year (OR = 4.208; p = 0.026).
Conclusions: Women appear to suffer more aggressive forms of the disease compared with men. High values of HAQ at diagnosis are less likely to achieve remission1year after diagnosis. However, the dose of methotrexate with which treatment is initiated is the most relevant factor for the control of rheumatoid arthritis on the short term and could help to raise new therapeutic strategies such as the beginning with an intense treatment.

9. Analysis of the characteristics of patients with chronic back pain referred from primary care to a rheumatology service

Authors: *López Medina, Clementina; Calvo Gutiérrez, Jerusalen; Jiménez Gasco, Rocío; Castro Villegas, Maria Carmen; Ortega Castro, Rafaela; Font Ugalde, Pilar; Escudero Contreras, Alejandro; Collantes Estévez, Eduardo*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Objectives: To analyse the clinical, analytical and radiographical characteristics of patients with chronic back pain (CBP) and younger than 45 years old referred from Primary Care (PC) to a Rheumatology service.
Methods:A descriptive analysis which included 95 patients referred from Primary Care with chronic back pain and younger than 45 years old who underwent a full clinical and analytical examination which aimed to determine Spondyloarthritis criteria (sacroiliac joints X-ray, HLAB-27) in each patient. The patients also answered an anamnesis undertaken by a specialist clinician in Rheumatology. The aim of which was to determine in each patient the presence of items which define the inflammatory back pain (IBP) by ASAS. In this way, patients were classified in two different groups: a) those that meet IBP criteria, and b) those that do not meet IBP criteria (non-IBP). An analysis of the connection between the two groups of patients with different variables took place.

Results: Out of the 95 patients, 35 of them (38.9%) were classified as IBP and 55 (61.1%) as non-IBP. Table 1 represents the most important clinical and analytical characteristics from DLI patients, compared with non-DLI patients. Also, the table shows the results of the bivariate and multivariate logistic regression.
The multivariate logistic regression model showed that patients with pathologic SI joints X-ray have a 78.78 higher risk of having IBP as well as a 4.19 increased probability in patients with a positive result in HLA-B27.
Out of the 35 IBP patients, 27 of them (77.14%) met the ASAS criteria for axial SpA, with a 19.82 higher risk of having IBP.
Conclusions: This research suggests that IBP is the most distinctive clinical characteristic of axial SpA and it is defined as an accurate criterion for referring patients with chronic back pain to a Rheumatology service.

10. Changes in cellularity in the striatum in an experimental model of multiple sclerosis: Effects of N-acetyl-cisteyne

Authors: *Aguilar-Luque M, Leiva-Cepas F, Luque E, LaTorre-Luque M, Giraldo AI, Conde C y Bahamonde C, Galván-Jurado A, Escribano BM, Túnez I*

Group: GE1 *Oxidative stress and nutrition*

Aim: The main aim of this study was to analyze the changes in cellularity and astrocytes in the striatum nucleus in a experimental autoinmune encephalomyelitis (EAE), similar to multiple sclerosis in human, as well as the effects of N-acetyl-cisteyne (NAC).
Material and Methods: The study was performed on 15 male rats, they were divided into 3 group as following: i) control; ii) EAE; iii) EAE+NAC. The EAE inductor agent was myelin oligodendrocyte glucoprotein (MOG). NAC was administered of gastric catheter. The treatment was applied during 21 days starting 14 days after inoculation with MOG. To determine the cellularity and astrocytes we applied Nissl and immunohistochemistry (with GFAP and DAPI) techniques.
Image analysis was performed using the Image-Pro Plus 6.0 Image Analysis Program. Statistical analysis was carried out using the Sigma Stat 3.1 software package, a one-way ANOVA was used followed by the Tukey´s test
Results: EAE group show a decrease in cellularity and a high increase in astocytes population. On the other had, the treatment with NAC reversed towards normality the changes.
Conclusions: In brief,
- EAE group presents a decrease in cellularity accompanied by an increase in the number of astrocytes.
- The application of NAC shows an imporvement towads normality values, indicating a positive effect of antioxidants.
However, more experimental and clinical studies in this line are required

11. Duodenal Iron after ferric citrate administration in uremic rats

Authors: *Vergara N, Diaz-Tocados JM, Rodriguez-Ortiz ME, Almaden Y, Herencia C, Martínez-Moreno JM, Muñoz-Castañeda JR, Rodríguez M.*

Group: GC13 *Calcium Metabolism Vascular Calcification*

In CKD gastrointestinal (GI) iron absorption is impaired; therefore in these patients intravenous iron is given to assure effective iron supplementation. Control of phosphate is another difficult task for CKD patients. Available phosphate binders are not totally effective and there is a continuous search for therapies directed to phosphate control.

Ferric citrate (FC) is a new iron-based phosphate binder that has been shown to reduce phosphate absorption and it also increases serum iron levels.

The present study evaluated intestinal iron absorption in uremic rats, 2 and 24 h after administration of FC orally (350 mg/kg body). Rats underwent 5/6 nephrectomies(5/6Nx) and were maintained in either normal or low iron diet for one week; then, a single oral dose of FC or water was administered and animals were sacrificed at 2 and 24 hours and plasma and duodenal samples were collected.

Our results showed that in 5/6 Nx rats fed with a low iron diet for 7 days induced a decrease in iron related parameters such as hemoglobin, hematocrit, erythrocytes number, plasma and intestinal iron. Two hours after a single oral dose of FC (150 mg/kg body) there was an increased duodenal content of iron (measured by atomic absorption) and by Perl's staining together with an increase in serum iron levels.

In conclusion, iron in the form of FC is rapidly and absorbed at intestinal level in uremic animals and therefore it seems to be good option as phosphate binder plus Iron therapy in moderate CKD.

12. Efficacy of opportunistic detection of atrial fibrillation in people aged 65 or older in primary care: a cluster-randomised clinical trial

Authors: *Virginia González Blanco y Grupo Colaborativo estudio DOFA-AP*

Group: GC12 *Epidemiological Research in Primary Care*

-Objective: To prove whether early opportunistic screening for Atrial Fibrillation (AF) by measuring blood pulse is more effective than regular practice in subjects aged older than 65 attending Primary Care centers.
-Methods: A cluster-randomized controlled trial conducted in Primary Care centers of the Spanish National Health Service. A total of 345 physicians and nurses will be allocated to one of the two arms of the trial by stratified randomization, 284 of them recruited patients aged 65 or older that met eligibility criteria. The Experimental Group researchers (EG) made an opportunistic search of AF, while those belonged to the Control Group (CG) followed the regular guidelines. It was made an ECG to those patients who had an irregular pulse to confirm the AF diagnostic. Test Chi-square was applied and the Relative Risk (RR) was estimated.
-Results: A total of 6971 people were evaluated (average age 74.9; confidence interval 95% -CI 95% -, 74.7 to 75.0; range: 65-104); 58.6% women. The blood pulse was irregular in 4.3% of patients from EG and in 14.9% from CG (p<0.001). 163 new cases of AF were detected (2.3%). The AF incidence was 1.1% in the EG and 6.7% in CG (RR: 6.04; CI95%: 4.38 a 8.33; p<0.001).
-Conclutions: The opportunistic detection of AF by measuring blood pulse is not a recommended strategy as a screening method in patients aged 65 years or older attending Primary Care centers, due to his low diagnostic efficiency.

13. Endothelial microparticles induced by uremia modulate physiological endothelial repair

Authors: *Carmona A, Buendía P, Luna C, Guerrero F, Jiménez MJ, Moyano R, Soriano S, Martín Malo A, Ramírez R, Aljama P, Carracedo J.*

Group: GC7 *Nephrology. Cell damage in chronic inflammation*

Introduction: Patients with chronic kidney disease (CKD) have chronic microinflammation caused by uremia and it has been proposed as one of the mechanisms that cause endothelial dysfunction. To maintain vascular homeostasis, endothelial cells damaged are replaced by endothelial progenitor cells (EPCs). In this process of activation / damage / repair, the endothelium produces endothelial microparticles (EMP), which are small vesicles involved in processes signaling and communication intercellular.
Objective: The aim of this study was determine the effect of EMP induced by uremia (MPus) on EPCs regenerating process.
Material and Methods: MPus were obtained from mature endothelial cells (HUVECs) treated with uremic serum (US 10%, 24h). MPus was purified using a serial centrifugation protocol. The MPus were quantified by flow cytometry. EPCs were purified from healthy donors with a conditioned medium. Different concentrations of MPus was added to EPCs. After 21 days in culture, colony forming units (UFCs) were quantified by optical microscopy and EPCs ability to create new vessels were analyzed by angiogenesis in matrigel.
Results: Results are expressed as mean ± SD. UFCs reduction was observed with the highest concentration of MPus compared with control. The capacity of create new vessels in vitro was decreased as increased the concentration MPus (quantified by area and Nb Meshed segments). (See table)
Conclusion: EMP obtained from the activation of endothelial cells may cause a decrease in the angiogenic and proliferative activity of EPCs. CKD patients have elevated levels of EMP that could alter vascular endothelial regenerative processes.

14. Neuroligin and neurexin deficient mutants of Caenorhabditis elegans. A model to explain neurobiological mechanisms in some cases of Autims Spectrum Disorders

Authors: *Saúl Adán Ruiz, Angel Rodríguez Ramos, María del Mar Gámez del Estal and Manuel Ruiz Rubio*

Group: GC20 *Genetic and Behavior Diseases*

Neurexins and neuroligins are synapse adhesion molecules that have an essential role in synaptic transmission. The dysfunctions of these molecules do not abolish completely the synaptic transmission, but disrupts the normal neural networks causing impairments in the neurodevelopment process. Mutations in the genes encoding neurexins and neuroligins have been implicated in cases of autism spectrum disorders (ASD). In C. elegans nrx-1 and nlg-1 genes are orthologues to human NRXN1 and NLGN1 genes, which encode alpha-neurexin-1 and neuroligin-1 proteins respectively. Previously we have shown that human NLGN-1 and NRXN1 were functional in the worm. Here we show that strains of C. elegans deficient in nrx-1 or nlg-1 and double mutants nrx-1;nlg-1 are similarly impaired in gentle touch response, pharyngeal pumping rate and osmotic avoidance response. However, these mutants differ respecting of the roaming behavior. Thus, the exploratory behavioral phenotype observed in nrx-1 mutants was markedly different to nlg-1-deficient mutants. The nrx-1 had a "hyper-reversal" phenotype increasing the number of changes of direction with respect to the wild type strain, the nlg-1 mutants presented a "hypo-reversal" phenotype. The significance of these differences in the behavior might be important for understanding the functional role of these molecules in neurodevelopmental disorders. Furthermore, using RNA-seq tools we generate a gene expression profile of nrx-1 or nlg-1 and double mutants nrx-1;nlg-1. The significance of the differentially expressed genes and their possible role in ASD is discussed.

15. Optimization of Biological Therapy in a cohort of patients with Rheumatoid Arthritis in HURS, Córdoba

Authors: *Ortega Castro R, Calvo Gutiérrez J, Font Ugalde P, Castro Villegas MC, Jiménez Gasco R, Ruíz Vilchez D, Romero Gómez M, Escudero Contreras A, Cardenas Aranzana M, De la Fuente Ruiz S y Collantes Estévez E.*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Optimization of Biological Therapy (BT) in patients with Rheumatoid Arthritis (RA) in remission, is a strategy employed in rheumatology practice in recent years consisting in dose reduction or enlargement dose interval (1,2)

Some published studies (3-6) and recommendations of RA (7) management guidelines suggest that patients in sustained remission, could get the same benefit with a lower dose (8)

Recently, there has been a consensus document from the Spanish Society of Rheumatology and hospital pharmacy about the unification of criteria for dose optimization with BT. The objectives of this paper are based on minimizing the variability among professionals in optimizing these drugs, striving for the minimum effective dose for each patient, limiting the occurrence of adverse effects and promoting economic savings.

Purpose: To compare the clinical and laboratory characteristics that define the activity of RA patients in remission treated with BT at baseline and at one year after its optimization.

Method: Observational prospective study of 40 patients followed during 12 months, from a cohort of patients with RA (ACR 1987 criteria) in BT treatment (Anti TNF, Abatacept or Tocilizumab) after reaching sustained clinical remission according DAS28 (ESR) (Table 1: Optimization according to drug). We compared the clinical and laboratory features at the beginning of optimization and at 12 month of follow-up.

Results: Of the 40 patients with RA who were optimized, 80% were women with a mean age of 55.25 ± 14.05 years, a DAS28 at baseline optimization 2.1 ± 0.91 and a mean duration of disease of 16.3 ± 15.3 years. The most widely used drug with spacing pattern was etanercept (27.5%), followed by tocilizumab (25%), both infliximab and adalimumab with 15%, abatacept 12.5% and golimumab 5%.

No statistically significant differences were found when contrasting clinical and laboratory parameters of activity at baseline and at 12 months, maintaining remission rates close to 100% at one year of follow-up (DAS28 medium at 12 months 2.30 ± 0.77)

Conclusions: The optimization of BT is a routine clinical practice in our hospital, employing more frequently enlargement dose interval rather than reducing it, managing to maintain remission status one year after the optimization. The adequacy to the published consensus recommendations regarding therapeutic target compliance and the rate of dose reduction was excellent, so we can conclude that optimization can be a useful performance in patients who are in sustained remission.

16. STAT3 31313A>G (rs744166) polymorphism influences the metabolic syndrome phenotype and anti-TNFs response in women with moderate-severe plaque psoriasis

Authors: *Gómez FJ, Carmona PJ, Rodriguez-Martin A, Gonzalez-Padilla M, Lorente A, Isla-Tejera B, Jiménez-Puya R1, Velez A, J. Ruano*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Considering that STAT3, a JAK-STAT pathway transcription controller, is involved in the development of the plaque of psoriasis and plays a key role in weight regulation and glucose homeostasis, we conducted this study to investigate the relationship between STAT3 31313A>G (rs744166) polymorphism, the clinical manifestations of metabolic syndrome and the response to anti-TNF in a cohort of 300 patients with moderate-severe plaque psoriasis treated with anti-TNFs. Following an observational, prospective, single centre study design, STAT3 31313A>G (rs744166) polymorphism was genotyped in by qRT-PCR in all patients of a cohort. Clinical, anthropometric and demographic data related to psoriasis evolution and cardiovascular risk factors were obtained. In addition, glucose, insulin, lipid profile, lipoprotein (a) and PCR fasting plasma concentrations were determined. Physical activity and food intake habits were assessed using the scale Godin Leisure-Time Exercise Questionnaire (GLTEQ) and the semi-quantitative food frequency PREDIMED questionnaire. Resting blood pressure at rest was measured and body mass index, the HOMA-IR index of insulin resistance and the Mediterranean Diet score were calculated. The therapeutic efficacy was measured by the PASI75 value and time-to-relapse after the anti-TNF was temporary withdrawn (NCT01753245). Women with AA/AG genotypes presented lower weight, waist, waist-to-height index, hypertension and metabolic syndrome, as well as higher values of PASI75 and time to relapse as compared with carriers of the GG genotype. No significant differences were found in the men group on the basis of this polymorphism. Our data suggests STAT3 31313A>G (rs744166) as a potential genetic factor that could explain differences in cardiovascular risk and response rates to antiTNFs drugs in the psoriatic population.

17. The Metabolic Syndrome influences treatment outcomes in a Spanish cohort of patients with moderate-to-severe plaque psoriasis treated with anti-TNF

Authors: *Carmona PJ, Gómez F, Lorente A, Rodriguez-Martin A, Gonzalez-Padilla M, Isla-Tejera B, Jiménez-Puya R, Velez A, J. Ruano*

Group: GE3 *Inflammatory immune-mediated cutaneous diseases*

Background: Psoriasis is a chronic inflammatory skin disease. In recent years substantial epidemiological evidence indicates that psoriasis is associated with a predisposition to develop metabolic dysregulation leading to obesity, insulin resistance and the Metabolic Syndrome. However, the nature of this association and the potential underlying mechanisms remain unclear.
Objective: This study aimed to explore whether the metabolic status of patients with moderate to severe psoriasis could determine differences in disease characteristics and treatment outcomes with anti-TNF drugs.
Methods: Single-centre prospective study from a cohort of 200 Caucasian patients without cardiovascular disease and moderate-to-severe psoriasis treated with etanercept or adalimumab as first biologic was analyzed (ClinicalTrials.gov Identifier: NCT01753245). The population was divided into 4 groups: (1) normal weight, (2) overweight or obese subjects but without metabolic syndrome, (3) overweight patients with metabolic syndrome and (4) obese patients with metabolic syndrome. Characteristics of psoriasis, metabolic profile and response to anti-TNF treatment (PASI75/PGA and 'time to relapse' after anti-TNF drug discontinuation) were compared.
Results: No differences in PASI75 were found between the metabolic groups. Only 'time to relapse' values were significantly higher in patients with either normal weight, overweight or obesity, but without metabolic syndrome, as compared with those ones who fulfilled criteria for metabolic syndrome criteria (p=0.045). An inverse correlation was observed between 'time to relapse' and homeostasis model assessment-estimated insulin resistance index (r=0.288, p=0.021).
Conclusion: Our results suggest that the status of insulin resistance associated with the metabolic syndrome, and does not simply overweight or obesity, determines a shorter duration of the effect achieved during psoriasis treatment with anti-TNF once the drug is temporary withdrawn.

18. Transgenerational epigenetic inheritance of the testosterone-induced alterations in the behavioral pattern of C. elegans. An approach towards the understanding of the etiology of autism spectrum disorders

Authors: *Gámez-Del-Estal MM, Porta-de-la-Riva M, Cerón J and Ruiz-Rubio M.*

Group: GC20 *Genetic and Behavior Diseases*

Introduction: In the pathogenesis of metabolic syndrome, increased oxidative stress plays a key role in close relationship with endothelial dysfunction, insulin resistance and chronic inflammation. The aim of this study was to analyze the expression levels of genes involved in oxidative stress in patients with metabolic syndrome after two hypocaloric diets intervention with or without the addition of exercise.
Methods: 40 subjects with metabolic syndrome were selected and randomized into 4 groups: 1 group with hypocaloric Mediterranean diet (MED), another with similar diet but performing physical exercise (MEDE); a third group with hypocaloric diet rich in carbohydrates (CHO) and another with similar diet but also same exercise that MEDE (CHOE). The mRNA expression levels of genes gp91phox, Nrf2 and SOD2 were determined at baseline and after the time period of 3-month intervention, using the platform OpenArrayTM multiarray.
Results: The consumption of CHO diet increased the mRNA levels of gp91phox gene as compared to MED diet, independently of the physical exercise ($p = 0.004$, $p = 0.018$, respectively); the consumption of CHO diet increased the mRNA levels of Nrf2 ($p = 0.022$), and it remained unchanged when patients consumed this diet but they also performed moderate to high endurance training; by contrast, the antioxidant SOD2 gene increased ($p = 0.022$) after the performing of moderate to high endurance training when patients consumed CHO diet, but the expression of this gene remained unchanged when patients performed the same endurance training but they consumed the Med diet.
Conclusion: Our results suggest that physical exercise is an additional factor to the diet in the modulation of oxidative stress process in patients with metabolic syndrome.

19. Use of methotrexate in patients with recent onset rheumatoid arthritis alter serum cholesterol and triglyceride levels

Authors: *Calvo Gutiérrez, Jerusalem; Ortega Castro, Rafaela; Jimenez Gascon, Rocío; Font Ugalde, Pilar; Castro Villegas, Maria Carmen; Escudero Contreras, Alejandro; Collantes Estévez, Eduardo*

Group: GC5 *Systemic Autoimmune and Chronic Inflammatory Diseases of the Musculoskeletal System and Connective Tissue*

Introduction: There is an inverse relationship between lipid measurements and the presence of clinical cardiovascular events (CVE) in rheumatoid arthritis (RA). Studies in RA show that a high rate of inflammation correlates with low serum lipids and after intensive treatment to reduce the proinflammatory state, lipids tend to increase (1), being only increased triglyceride levels which were significantly associated with the presence of CVD (2)

In patients with early-stage RA, inflammatory markers are elevated and lipids affect both quantitatively (usually decreased) and qualitatively (subfractions are alteradas (3). Navarro-Millán (4) showed that the use of methotrexate (mtx) for 24 weeks increased lipid levels, although its use is associated with a trend towards reduction of CVD and that is why it has been suggested that presents cardioprotective properties and producing no effect on the characteristics of lipids (5)

Objectives: To assess the influence of mtx use in cholesterol (COL), triglycerides (TG) and hemoglobin (Hb) levels, in a cohort of patients with recent-onset RA for 5 year follow-up and record the presence or absence of CVD.

Patients and methods: Longitudinal observational study that included 50 patients diagnosed with RA (ACR criteria / EULAR 2010) of less than 6 months from the onset of symptoms and who had started treatment with mtx or hydroxychloroquine (HCQ). The presence or absence of CVD baseline was recorded and performed analytical control (no treatment), at one and five years of follow up. Was compared in both groups (mtx or hydroxychloroquine), clinical activity as DAS28 (ESR) and Hb levels, Total COL and TG after initiation of treatment.

Results: 72% were women with a mean age of 50.39 ± 16.13 years and an average duration of disease less than six months. 64% started treatment with mtx with an average dose of 9.08 ± 1.89 mg / week. The proportion of smoking, hypertension and diabetes was low, although the inflammatory burden was high with an average DAS28> 4. The mean total and basal Tg and col was 199.18 ± 37.48 mg / dl and 95.60 ± 46,65mg / dl respectively, both being within normal limits, like the mean Hb levels (12.54 ± 1.81 g / dl). (Table 1 Baseline clinical characteristics analytical-collected).

During follow any CVD was not described in any of the groups. When comparing the change in the levels of Col and Hb at one and five years compared to baseline in both groups, no significant differences were found, maintaining stable serum levels before and after the introduction of treatment; we found reduced levels of TG in mtx- group at one year follow-up ($p < 0.05$). We also observed a significant reduction in clinical activity as DAS28 when compared with baseline, happening the same in both groups. ($p < 0.01$). (Figures 1 and 2)

Conclusions: We could conclude that the introduction of mtx in patients with recent-onset RA improves clinical activity and the underlying inflammation without an increase in the levels of total Col or EVC development. There is a decrease in Tg levels significantly when compared with the use of hcq, which corroborate its cardioprotective profile.

20. A clinical, radiological and molecular study of surgically treated patients harboring non-functional pituitary adenomas

Authors: *Álvaro Toledano-Delgado, Alejandro Ibáñez-Costa, Esther Rivero-Cortés, Rosa Ortega-Salas, Jesús Adolfo Lozano-Sánchez, Mª Ángeles Galvez, Andrés de la Riva, Justo P. Castaño, Raúl M. Luque*

Group: GC8 *Hormones and cancer*

Non-functioning pituitary adenomas (NFPAs) are the most common tumors in the sellar region in adults. Typically, NFPAs are clinically characterized by headache and visual defects caused by mass effect and, by the absence of hormone hypersecretion. Moreover, NFPAs frequently invade adjacent intracranial structures, preventing from total surgical resection, which may lead to tumoral re-growth after surgery; however, there is no a reliable marker to predict tumor relapse after surgery. For that reason, an observational retrospective study with 20 patients harboring NFPAs was carried out by collecting clinical/radiological characteristics and, by measuring expression level of key genes involved in pituitary function in NFPA-tissues collected after surgical resection. Mean age was 60.1; mean time of follow-up was 26.43 months; 61.2"% were men; 52% did not show hormonal deficit and, 9.5% were diagnosed of pan-hypopituitarism. Total resection of the tumor was achieved in 5% of the cases, and 28% of the cases needed a second surgery due to recurrence, with a mean follow–up of 11.3 months.

In 31% of cases less than 50% of the tumor was resected, showing a III-IV grade according to KNOPS classification (an indicator of cavernous sinus invasion). As expected, a high expression of the alpha-chain of glycoproteins was frequently observed in NFPA-tissues. Dopamine and somatostatin receptors (currently used as pharmacological biomarkers for the treatment of pituitary adenomas) were also expressed, especially dopamine receptor-2 and somatostatin receptor-3. Interestingly, we found a remarkable expression of ghrelin (a well-known activator of pituitary function) and GnRH-R (the receptor of gonadotropin releasing hormone) in NFPA-tissues where their expression was directly correlated with KNOPS classification (i.e. a higher expression of ghrelin and GnRH-R was detected in invasive tumors). Our results indicate that the evaluation of the molecular phenotype of NFPAs could contribute to better understanding their pathophysiology and to identify novel targets with potential prognosis and/or therapeutic value for patients with NFPAs.

21. Advances in the search for biomarkers to develop a screening tool for the diagnosis of lung cancer

Authors: *M.M. Delgado-Povedano, M. Calderón-Santiago, B. Jurado-Gámez, F. Priego-Capote, M.D. Luque de Castro*

Group: GC21 *Metabolomics. Identification of bioactive components*

Lung cancer is the carcinogenic disease with the highest mortality rate owing to the advanced stage at which it is usually diagnosed. For this reason, different biofluids have been studied to find new screening tools to detect lung cancer at earlier stages. In this research, a non-invasive biofluid as human sweat was used as clinical sample to develop a screening tool for lung cancer. With this aim, a method based on analysis of the metabolites in sweat to discriminate between patients with lung cancer versus smokers as control individuals was developed. In a first step, a cohort of 23 patients diagnosed with lung cancer and 18 control individuals was studied. The capability of the metabolites identified in sweat to discriminate between both groups of individuals was studied and, among them, a trisaccharide phosphate presented the best independent performance in terms of the specificity/sensitivity pair (80 and 72.7%, respectively). Additionally, two panels of metabolites were configured using the PanelomiX tool. The first panel (80% specificity and 69% sensitivity) was composed by suberic acid, a tetrahexose and a trihexose, while the second panel (69% specificity and 80% sensitivity) included nonanedioic acid, a trihexose and the monoglyceride MG(22:2). Thus, the combination of the five metabolites led to a single panel providing 80% specificity and 79% sensitivity. The study was expanded with a second cohort including 34 lung cancer patients and 14 control individuals. The new panel providing 85% specificity and 87% sensitivity was composed by urocanic acid, a tetrahexose, two dipeptides (GluVal and γ-GluLeu), and a monoglyceride MG(22:2). Finally, a first approach to find metabolomics differences in sweat to discriminate between early and advanced stages of lung cancer is being carried out.

22. Circulating microRNAs as biomarkers of response to bevacizumab in colon cancer patients

Authors: *Marta Toledano, Carlos Pérez-Sánchez, Vanesa Hernández, Juan de la Haba, Antonio Rodriguez-Ariza, Enrique Aranda*

Group: GC6 *New Cancer Therapies*

Bevacizumab, a humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor (VEGF), is currently a major anti-angiogenic drug in cancer. Although bevacizumab activity has been associated with the development of hypertension secondary to treatment, currently there are no predictive biomarkers of response to this anti-angiogenic drug. MicroRNAs (miRNAs) are short non-coding RNAs with important gene-regulatory roles. Stable miRNAs derived from tissues and organs are present in blood and circulating miRNAs are promising biomarkers for predicting response to treatments. This study aimed to determine if there are circulating miRNAs, including those associated with hypertension, which may be predictive biomarkers of response to bevacizumab.

In a retrospective study, plasma samples from 37 colon cancer patients who received bevacizumab were analyzed using a human plasma miRNA PCR array, and the circulating levels of those miRNA of interest were validated using RT-PCR.

Our results show that circulating levels of three specific miRNAs (miR-10b-5p, miR-143-3p and miR-17-3p) were significantly increased in non-responders patients. Furthermore, no significant differences were observed when patients were compared for tumour TNM stage or K-RAS mutation status. No significant correlation was found between any of the analyzed miRNAs and plasma VEGF. However, levels of miR-223-3p, miR-374a-5p and miR-145-5p positively correlated with angiotensin converting enzyme (ACE) plasma levels. In addition, a negative correlation was found between duration of treatment and circulating miR-143-3p and miR-17-3p levels. Finally, to determine those miRNAs with potential predictive value of response to bevacizumab a ROC curve analysis was performed and three miRNAs (miR-10b-5p, miR-143-3p and miR-17-3p) showed an area under the curve of 0.8 with a significant p value.

In conclusion, circulating levels of miR-10b-5p, miR-143-3p and miR-17-3p, which have previously been associated with colon cancer and hypertension, may have predictive value of response to bevacizumab in colorectal cancer patients.

23. Clinical association between metabolic syndrome, levels of C-reactive protein and testosterone and aggressive prostate cancer

Authors: *Gomez Gomez E, Carrasco Valiente J, Valero Rosa J, Garcia Rubio JH, Campos Hernandez P, Ruiz Garcia J, Carazo Carazo JL, Luque R.M, Blanca Pedregosa A.M, Requena Tapia MJ.*

Group: GA7 *Urology and Sexual Medicine*

Introduction & Objectives: Prostate cancer (PCa) is the most commonly diagnosed malignancy among men worldwide. Epidemiologic studies suggest that obesity, metabolic syndrome (MetS) and inflammation might be associated with increased risk and death of PCa; however, the existing epidemiologic data are somewhat conflicting, and therefore, further studies are necessary to clarify whether obesity/MetS might be a predictive factor of aggressive PCa. Thus, the aim of the study was to determine the possible clinical association between obesity/MetS, C-reactive protein (CRP) and testosterone levels and increased risk of aggressive PCa.

Material & Methods: 639 patients with no active cancer or infectious or autoimmune disease were scheduled for prostatic biopsy. Demographic/clinical variables and treatments/laboratory parameters were evaluated.

MetS was evaluated according to the criteria of the Adult Treatment Panel III. It was evaluated as a dichotomous and quantitative variable depending on the cumulative number of MetS components.

Biopsies were evaluated through ISUP 2005 criteria, defining aggressive PCa when Gleason grade was ≥7.

Results: 39.1% of patients met criteria for MetS. The % of PCa patients with or without MetS was 37.8 and 36.1% respectively, being the Gleason grade ≥7 in 22 and 17.4% of these patients, respectively (p>0.05). For each cumulative individual MetS component that was met in the cohort, the relative risk of diagnosis a PCa or an aggressive PCa was 1.12; 95% CI (0.99-1.26) or 1.16; 95% CI (1.01-1.34), respectively.

A high CRP and low testosterone levels were associated with an increased risk of aggressive PCa. There was a correlation between CRP levels and Gleason grade on the biopsy (p <0.05).

Patients with MetS had higher CRP and lower testosterone levels vs. patients without MetS (4.8 vs. 3.7mg/L, p=0.02; and 4.5vs. 5.4ng/mL, p<0.001, respectively).

In multivariate analysis, CRP levels >2.5ng/ml (adjusted by age, PSA levels, digital rectal examination, number of biopsies and 5α-reductase intake) was found to be an independent risk factor of aggressive PCa diagnosis. The best predictive model of positive biopsy of aggressive PCa was found when both the cumulative number of MetS components and a level of CRP>2.5ng/ml were combined [ROC curve of 0.78 (0.74-0.83); p<0.001].

Conclusions: Overall, presence of MetS was not directly associated with an increased PCa diagnosis; however, the prevalence of aggressive PCa was higher in patients that met a higher number of MetS components. CRP/testosterone levels were associated with aggressive PCa. Levels of CRP>2.5ng/ml might be used as an independent risk factor in aggressive PCa. These results reinforce the notion that PCa aggressiveness is influenced by certain specific MetS components, which deserve to be identified and mechanistically characterized.

24. Extracellular domain fragments of truncated sst5TMD4 receptor increase malignancy features in the breast cancer derived cell line MDA-MB-231

Authors: *Maria Eugenia Prados-González, David Rincón-Fernández, Antonio J. Martínez-Fuentes, Raúl M Luque, Manuel D. Gahete, Justo P. Castaño*

Group: GC8 *Hormones and Cancer*

Growing evidence suggest a functional relevant role of tumor-derived extracellular domain fragments of a variety of receptors in the development and/or progression of tumoral pathologies, which could represent novel and useful diagnostic, prognostic and/or therapeutic tools for the management of tumoral pathologies. Of particular interest is the case of the truncated somatostatin receptor sst5TMD4 since this receptor is expressed in a number of endocrine-related tumors such as breast, prostate, pituitary, and thyroid. Thus, in contraposition to other seven transmembrane G-coupled protein receptors, the C-terminal tail of the sst5TMD4 presents an extracellular location suggesting that this domain could be processed by extracellular proteases to release receptor-derived fragments with potential pathophysiological significance. In order to test this hypothesis, the present study implemented an in silico approach to identify putative cleavage sites in the sst5TMD4 extracellular domain. We found two putative metalloproteinase cleavage sites, whose processing by metalloproteinases (MMP)-2 and/or MMP-16 could generate three soluble fragments with 7, 10 or 17-aminoacids.

To explore their putative biological effects, these sst5TMD4-derived extracellular fragments were chemically synthesized and originally tested using a battery of functional and molecular assays on the breast cancer-derived cell line MDA-MB-231. In particular, the three extracellular fragments of the sst5TMD4 augmented the malignant features of the MDA-MB-231 cells in that, treatment with these peptides increased the capacity of the cells to proliferate and migrate and, their capacity to form mammospheres which was associated with changes in key specific molecular markers. Although the processing of the sst5TMD4 extracellular domain by MMPs to release soluble fragments with pathophysiological consequences is still to be fully demonstrated, these data support such contention, and reveal a plausible role of these sst5TMD4 extracellular fragments in breast cancer aggressiveness.

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25. Molecular characterization of the Engrailed-1 and -2 variants of the family of the HOX genes in human prostate cancer: potential value as biomarkers

Authors: *Enrique Gómez-Gómez, Daniel Hormaechea-Agulla, Julia Carrasco-Valiente, José Valero Rosa, Alejandro Ibáñez-Costa, María M. Moreno, Manuel D. Gahete Justo P. Castaño, María José Requena, Raúl M. Luque*

Group: GA7 *Urology and Sexual Medicine*

Prostate cancer (PC) is the most common malignancy in the male population however; molecular diagnostic/prognostic markers that better define this pathology are very limited and frequently found to be unspecific (i.e. PSA). Therefore, it is necessary to provide new clues for novel diagnostic/prognostic/therapeutic targets in this pathology. HOX genes belong to a family of homeodomain-containing transcription factors that determine cell/tissue identity during normal embryonic development which, have been shown to be re-expressed by different tumoral cell-types. Interestingly, some studies have indicated that the engrailed variants (EN1 and EN2; members of HOX-family) might be used as a potential diagnostic markers of early PC however; these few studies are conflicting and incomplete and, to date, limited information is available concerning the presence of these variants in PC-cells. Therefore, the main goals of this study were to analyze the expression levels of EN1- and EN2-variants in PC-tissues and cell lines and, to determine urinary levels of EN2-variant in patients with and without PC. To that end, we implemented a triple strategy by using: 1) paraffin-embedded PC-tissues obtained from radical prostatectomies and its adjacent normal-control tissues; 2) normal and androgen-dependent (LnCaP/VCaP/22Rv1) and androgen-independent (DU145/PC3) PC-cell lines and; 3) Urinary fluids from patients with PC and control-patients. Expression of EN2-variant, but not EN1-variant, was up-regulated in PC-tissues compared to normal-adjacent tissues. Moreover, EN1/EN2-variants were not expressed in a normal-prostate cell-line while, EN2-variant was over-expressed in all PC cells-lines analyzed (LnCaP>>DU145>VCaP>PC3>22Rv1). Interestingly, only DU145 cells expressed EN1-variant. Median urinary levels of EN2-variant collected from PC and controls-patients without prostate massage were similar (~0.8ng/ml). Altogether, our ongoing studies suggest a potential role of EN-variants, especially EN2, in PC cells. Therefore, additional experiments are planned to determine the functional role of these EN-variants in PC-cells as wells as, additional measures of urinary and plasma EN2 levels in PC and control patients following a prostate massage.

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26. Role of In1-ghrelin derived peptides in the malignant features of the breast cancer derived cell line MDA-MB-231

Authors: *Virginia Ruiz-Murillo, Manuel D. Gahete, David Rincón-Fernández, Francisco Gracia-Navarro, Raúl M Luque, Justo P. Castaño*

Group: GC8 *Hormones and Cancer*

Breast cancer is the most frequently malignant tumor type and the leading cause of cancer-related death in the female global population. However, despite the enormous efforts invested in the study of this pathology, novel and more reliable diagnostic, prognostic and/or therapeutic targets are still required for the appropriate management of the patients. In this regard, the ghrelin system is gaining importance as putative tool for the identification of novel tumoral-molecular markers. Specifically, our group has recently identified a splicing variant of the ghrelin gene named In1-ghrelin originated by the retention of the intron 1. Interestingly, this splicing variant has been found to be overexpressed in a number of endocrine-related tumors, including breast cancer and pituitary tumors.

However, its putative role in the pathogenesis of breast cancer is yet to be fully elucidated. Of note, the In1-ghrelin protein precursor is predicted to be proteolytically processed to generate two putative peptides (In1-19 and In1-40), which could be secreted from tumoral cells and display pathological effects. Therefore, the aim of this project was to explore the putative role of the In1-ghrelin derived peptides in the malignant features of the breast cancer derived cell line MDA-MB-231. The results generated herein indicate that the treatment with both In1-ghrelin derived peptides similarly increases the proliferation and migration rate, as well as, the capacity to form mammospheres of this cell line, which was associated with the activation of relevant signaling pathways and with the expression of key specific molecular markers. Altogether, these results demonstrate a significant functional role of the In1-ghrelin derived peptides in the regulation of the malignant behavior of breast cancer cells.

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27. 24-hour pattern of falls in hospitalized elderly patients in a general hospital of North-Eastern Italy

Authors: *Pablo Jesús López-Soto, Juan Manuel Carmona Torres, Cinzia Canella, Paola Caponcelli, Roberto Manfredini, María Aurora Rodríguez-Borrego, Fabio Fabbian*

Group: GA2 *Comprehesive Nursing Care. Multidisciplinary Perspective*

Introduction: Many efforts in identifying the circumstances of the fall and risk factors of falling in the elderly have been carried out. However, little attention has been paid to time of fall. The incidences in many acute events do not occur randomly, but there are temporal patterns (Smolensky et al., 2014). Recent evidence shows the importance of the time of fall (Manfredini et al., 2011; López-Soto et al., 2014). Therefore, we were aimed to verify whether in-hospital falls exhibit a rhythmic pattern.
Methods: In-hospital falls in elderly people in a general hospital of North-Eastern Italy between 1 January 2013 and 31 December 2013 were recorded. In addition to the descriptive analysis, we conducted a chronobiological analysis to verify the presence of rhythmicity. This analysis generate harmonic(s) which best explain the variance of the set of data through a nonlinear reiteration least squares fitting method (Refinetti et al., 2007). The percentage of overall variance (PR) serves as the representative parameter of the goodness of fit. The parameters calculated for the period were: Midline Estimated Statistic of Rhythm (rhythm-estimated mean; MESOR), amplitude (one-half the difference between the peak and trough of the fitted approximation), peak (acrophase) and trough (bathyphase) with reference to local midnight (00:00h).
Results: A total of 228 in-hospital falls were registered. The average age of patients who fell was 80.28 (±7.57) years. Falls were more common in men (65.64%) than in women (34.36%). The majority of falls took place in medical services (76.21%), being lower in emergency (17.18%) and surgical (6.61%) services. Descriptively, a high incidence of falls was observed during the early morning decreasing progressively along the day. According to this data, the chronobiological analysis showed a presence of a 24-hours temporal pattern ($p = 0.01$) with a peak incidence at the beginning of the morning (~ 05: 30am) and a batiphase during the late afternoon (~ 17.30%), with a percentage of 31.78% rhythmic. The amplitude was 2.35 (± 0.75) and MESOR at 9:21 (± 0.53) hours.
Conclusion: Evidence shows that a 24-hour temporal pattern fits the incidence of in-hospital falls. The chronobiological pattern in the in-hospital falls is of great importance for the proper development of specific preventive measures.

28. Abuse by intimate partner violence in men's health profession

Authors: *Carmona Torres, Juan Manuel; López Soto, Pablo Jesús; Muñoz Villanueva, Mª del Carmen; Rodríguez Borrego, Mª Aurora*

Group: GA2 *Comprehesive Nursing Care. Multidisciplinary Perspective*

To date, most studies on Intimate Partner Violence (IPV) in health professionals have been focused on the group of nurses (women). In recent years, it has been shown that men suffer from this problem (Ocampo, 2011).
The objective of this study was to determine the prevalence of violence perpetrated by an intimate partner in male health professionals working in the Public Health System of the Spanish State (PHSSS).
Methods: Cross-sectional multicenter study. The subjects studied were male health professionals who carried out their professional activity in the PHSSS, Primary Care or Specialty Care. The instrument used for data collection was a self-administered online questionnaire, which was composed at the same time by the questionnaire of Sherin et al. (1998) [adapted from Chen et al. (2005) and which comprises 4 questions to measure abuse. Data collection was conducted from October 2014 to April 2015.
Results: 273 men with a mean age of 46.5 (SD ± 9.625) years participated in the study. 64.8% were physicians, 32.6% nurses and 2.6% nursing assistants. 83.2% of the study subjects were married. 68.1% of the study subjects had a mean of 1.47 children (SD ± 1.636). The main financial support of the study subjects was the salary of both partners (69.2%). 2.9% of male health professionals suffered some form of abuse. Of the total number of abused men, 50% were nurses and 50% physicians.
Conclusions: The data presented show the presence of IPV in male health professionals. For this reason, we propose to study this phenomenon in depth and to develop tools to prevent it.

29. Effect of age and CMV latent infection on CD8+CD56+ T cells (NKT-like) frequency and functionality

Authors: *Fakhri Hassouneh, Carmen Campos, Nelson López-Sejas, Corona Alonso, Raquel Tarazona, Rafael Solana and Alejandra Pera*

Group: GC1 *T and NK cell Immunosenescence Antiviral Immune Response*

Changes in the T cell pool caused by CMV infection have been proposed to contribute to immunosenescence. However, the detrimental role of CMV over the immune system has been questioned and, although it is clear that CMV contributes to T cell senescence, it also has some beneficial effects in young individuals improving the immune response to other pathogens. It has been shown that T cells expressing CD56 (also called NKT-like cells) expand with age and CMV infection and play an important role in the immune response against cancer, yet little information about their contribution to the response to infections is known. Thus, here we propose an analysis of NKT-like cells (CD8+CD56+ T cells) responses to a bacterial toxin (Staphylococcal Enterotoxin B, SEB), in the context of CMV latent infection and aging.

Our results show that NKT-like cell percentage increases with a combination of both CMV latent infection and age. The response to SEB and the polyfunctional index of NKT-like cells also increase with age in CMV-seropositive individuals. However, NKT-like cells have lower SEB-responses and polyfunctional index, and a different polyfunctional profile than overall CD8+ T cells. CMV infection alters the polyfunctional profile of overall CD8+ T cells but not the NKT-like's. NKT-like CD57+ cells, as overall CD8+CD57+ T cells, are expanded in CMV-seropositive individuals. These CD57+ cells are more polyfunctional than their CD57− counterparts. NKT-like cell polyfunctionality of both CD57− and CD57+ subsets increases with a combined effect of age and CMV latent infection. In contrast overall CD8+CD57+ T cells polyfunctional index is independent of age and CMV infection, whereas CD8+CD57− T cell subset acquires polyfunctionality with age in CMV-seropositive individuals.

30. Expression of NKp30, NKp46 and DNAM-1activating receptors on resting and IL-2 activated NK cells from healthy donors according to CMV-serostatus and age

Authors: *Nelson López, Carmen Campos, Alejandra Pera, Juan J. Gordillo, Fakhri Hassouneh, Raquel Tarazona y Rafael Solana*

Group: GC1 *T and NK cell Immunosenescence Antiviral Immune Response*

Human Natural Killer (NK) cells are innate lymphoid cells with capacity to kill tumor cells and virus-infected cells. According to the expression of CD56 and CD16 several NK cell subsets have been identified, a major CD56dimCD16+ subpopulation characterized by higher cytotoxic capacity, two CD56bright subsets (CD16- and CD16+) that represent different maturation stages and the fourth CD56-CD16+ subset that correspond to activated dysfunctional NK cells. Previous studies have shown quantitative changes in the frequency, phenotype and distribution of NK cell subsets depending on CMV serostatus and age. Here, we present new data on the expression of NKp30, NKp46 and DNAM-1 activating receptors on resting and IL-2 activated NK cells NK cells from CMV-seronegative and seropositive healthy young donors and in CMV-seropositive elderly individuals. Our results showed that CMV serostatus of healthy young donors is associated to phenotypic differences on both CD56bright and CD56dim NK cells with an increase of NKp46 and a decrease of NKp30 expression respectively.

The reduced expression of DNAM-1 was related to ageing whereas lower

NKp30 expression was associated to CMV seropositivity. In vitro NK cell activation by IL-2 increased the expression of NKp46 and NKp30. In summary, both age and CMV serostatus influence the expression of these cytotoxicity activating receptors that will have functional consequences. In elderly donors is difficult to isolate age from the effect of chronic CMV infection since in our study all elderly donors were CMVseropositive. The possibility of modulating the expression of these activating receptors by cytokines as IL-2 may open new opportunities for improving age-associated deterioration of NK cell function.

31. Mediterranean diet improves cellular damage and modulates expression of circulating mir210 in patients with severe endothelial dysfunction and cardiovascular disease

Authors: *Jiménez R, Rangel O, Alcalá-Díaz, JF, Camargo A, Haro C, Delgado-Lista J, López-Miranda J, Marín C*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Introduction: Coronary artery disease is the most important cause of death in developed countries. An early manifestation of cardiovascular disease (CVD) is endothelial dysfunction. Circulating microRNAs (miRNAs) are emerging as sensitive biomarkers of CVD, and diet is known to modulate endothelial damage and influence the expression of miRNAs.
Objective: To study the effect of two healthy diets (Mediterranean diet rich in virgin olive oil, and a low-fat diet) on cellular damage and expression levels of the circulating miR210, in patients with severe endothelial dysfunction and high cardiovascular risk.
Methodology: 40 patients with severe endothelial dysfunction and high cardiovascular risk, between 20 and 75 years old, were selected from the CORDIOPREV intervention study. These patients were randomized to consume a Mediterranean diet (MedDiet) and one low-fat diet high in complex carbohydrates. Blood samples were extracted at baseline and after the first year of dietary intervention. We quantified the cellular apoptosis levels in HUVEC (Human umbilical vein endothelial cells) incubated with serum from each patient, in presence and absence of TNF-α , and the expression of plasma circulating miR-210 at baseline and after one year of dietary intervention.
Results: We observed that cellular apoptosis ($p<0.05$) in HUVEC decreased after consumption of the MedDiet compared with the low-fat diet. No significant differences were observed in HUVEC incubated with TNF-α. Furthermore, the expression of circulating miR-210 increased after consumption of the MedDiet ($p<0.05$), compared with the low-fat diet, in patients with CVD and metabolic syndrome (MetS). In addition, we observed a negative correlation between miR210 expression and cellular apoptosis in HUVEC.
Conclusion: The MedDiet induces lower cellular apoptosis levels in HUVEC than the low-fat diet in patients with severe endothelial dysfunction and CVD. This finding could be modulated by the effect of MedDiet on miR-210 expression in patients with CVD in the presence of MetS.

32. Interaction between diet and endotoxemia on the development of metabolic syndrome

Authors: *García-Carpintero Sonia; Camargo Antonio; López-Moreno Javier; Haro-Mariscal Carmen; Gómez-Delgado Francisco; Marín-Hinojosa Carmen; López-Miranda José; Pérez-Jiménez Francisco*

Group: GC9. *Nutrigenomics. Metabolic syndrome*

Introduction: Metabolic syndrome (MetS) is characterized by a chronic low-grade inflammation that favors the development of atherosclerosis, the pathogenic substrate responsible of cardiovascular diseases in this syndrome. The intestinal absorption of lypopolysaccharide (LPS) may be related with the inflammatory status. Both, LPS and lypopolysaccharide binding protein (LBP) act as mediators in the inflammatory response, and may therefore be involved in the insulin resistance phenomenon. We hypothesized that some of the benefits induced by the Mediterranean diet may be mediated by changes in the endotoxemia and therefore, in the inflammatory status.
Objective: We aimed to evaluate the grade of endotoxemia and inflammation in patients that developed or lost the metabolic syndrome.
Methodology: A subgroup of 177 patients within the CORDIOPREV study was divided into 4 groups depending on the evolution of MetS: group 1 developed MetS; group 2 lost MetS, group 3 maintained MetS and group 4 no MetS, half of each group received a type of diet (Mediterranean diet or low fat diet). Fasting blood samples were taken at baseline and after 2 years of dietary intervention and plasma levels of LPS, LBP, adiponectin, resistin, TNF-α, IL-6, MCP-1 and leptin were determined by ELISA. Statistical analysis by ANOVA for repeated measures was conducted using the SPSS version 20.0 program.
Results: Our study showed that the long term consumption of the Mediterranean diet increase the levels in plasma the TNF- α (p=0.001) in patients that developed MetS. In patients that lose MetS decrease the levels of IL-6 (p=0.033), adiponectin (p=0.024) and LBP (p=0.020) and increase MCP-1 (p=0.000). The consumption long term low fat diet increase the levels of MCP-1 (P=0.001) in patients that maintained MetS and decrease the levels of IL-6 (P=0.030). The levels of MCP-1 (p=0.002) have decreased in control group with MetS.
Conclusion: Our results suggest that diet may play an important role altering the endotoxemia, and inflammation in the development of MetS.

33. High phosphate diet induces renal damage and accelerates progression of chronic kidney disease

Authors: *JM. Díaz-Tocados, ME. Rodríguez-Ortiz, C. Herencia, JM. Martínez-Moreno, A. Montes de Oca, N. Vergara, J. Dorado-Toro, R. Ortega, M. Rodríguez, Y. Almadén, JR. Muñoz-Castañeda*

Group: GC13. *Calcium metabolism. Vascular calcification*

High Phosphate (P) is harmful in the context of Chronic Kidney Disease (CKD). As CKD progresses, serum P levels increases and causes skeletal and cardiovascular disorders. However, the moment in which P triggers its deleterious effects is unclear. Therefore, the beneficial effects of P restriction in early CKD stages must be clinically useful. In our study, we examine the effects of High P diet (HPD, 1.2%P) or Low P diet (LPD, 0.2%P) in sham and uninephrectomized (1/2Nx) rats. After three weeks, rats were euthanized and renal oxidative stress, kidney histology and pro-inflammatory profile were studied. In 1/2Nx rats, serum creatinine increased slight but significantly, however, phosphate levels were similar to Sham levels in both HPD and LPD rats. Through MALDI-Imaging, we detected that P loading induced significant changes in the distribution and composition of the peptide profile in the kidney of sham rats fed with HPD or LPD. Renal glutathione peroxidase (GPx) activity increased in HPD as compared with LPD in sham and 1/2Nx rats suggesting an adaptive response to oxidative stress. We also found histological changes indicating renal fibrosis, renal calcification, brush border membrane deterioration and high proliferation of tubular cells in respond to cellular damage in kidneys from HPD rats. Renal damage in LPD rats was not appreciated.

In order to investigate phosphate-induced renal damage concomitant to the loss of renal function, some of the 1/2Nx rats underwent surgical removal of 2/3 of the remnant kidney keeping the same P diet and euthanized one week later. In 5/6 Nx-HPD rats, renal GPx activity declined after additional kidney ablation. Interestingly, rats on HPD developed vascular media calcification that was not observed in 5/6 Nx-LPD rats.

Thus, HPD promotes renal damage in early CKD stages and phosphate restriction prevents vascular calcification and loss of kidney function.

34. Molecular and functional studies indicate that somatostatin, dopamine and ghrelin neuroendocrine systems are differentially expressed in human tumoral brain tissues

Authors: *Cristóbal Blanco-Acevedo, Alejandro Ibáñez-Costa, David Rincón-Fernández, Rafael Sánchez-Sánchez, Rosa Ortega-Salas, Juan Solivera-Vela, Cristóbal Blanco-Pinto, Manuel D. Gahete, Jesús Lozano-Sánchez, Andrés De La Riva, Justo P. Castaño, Raúl M. Luque*

Group: GC8. *Hormones and cancer*

Affiliations: 1Service of Neurosurgery, Hospital Universitario Reina Sofia, 14004 Córdoba, Spain.
2. Hormones and Cancer" group, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Universitario Reina Sofia (HURS); Department of Cell Biology, Physiology and Immunology, University of Córdoba; CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn) and; Campus de Excelencia Internacional Agroalimentario (ceiA3), 14004 Córdoba.
3. Anatomical Pathology Service, Reina Sofia University Hospital, Cordoba, Spain.
4. Department of Neuroanatomy of University Carabobo-Venezuela.

The molecular signals that regulate the functional morphological processes of brain tumoral pathologies are rather unknown. The presence of key neuroendocrine systems, such as somatostatin, dopamine and ghrelin families, have been found to be dysregulated in pituitary and multiple extra-cerebral tumors; nevertheless, the presence and role of these systems in human brain tumors has not been fully elucidate. Hence, we evaluated the clinical and histological data of 14 patients with brain tumors and analyzed the molecular profile and functional response of these brain tumoral tissues/cells. Mean age was 53 years (range 13-83); 72% male; location in brain lobe: 35.7% right-frontal, 14.2 left-frontal, 14.2% right-temporal, 7.1% left-temporal, 7.1% right-temporo-occipital, 7.1% right-occipital, 7.1% left-occipito-parietal and 7.1% right-brainstem. Histopathological classification in degrees and types: 14% Grade-I, 28% Grade-II, 14% Grade-III and 43% Grade-IV. According to histological type: 64% gliomas, 22% metastasis, and 14% meningiomas. Gene expression was assessed by quantitative-PCR and found that somatostatin and dopamine receptors (sst and DRD, respectively) were differentially expressed in brain tumors, being sst2>>>sst1>>sst5 and DRD4>>DRD1>>DRD2=DRD5. Some components of the ghrelin system were also expressed in brain tumors. Specifically, although the expression of ghrelin receptors was almost absent in brain tumors, GOAT enzyme and native-ghrelin were expressed at relevant levels, being the expression of native-ghrelin significantly over-expressed compared with In1-ghrelin variant expression. Functional assays using primary brain tumoral cell cultures revealed that native-ghrelin treatment did not influence cell viability which might be explained by the absence of its receptor. However, similar to that found in other tumoral cell types, In1-ghrelin treatment was able to increase cell viability in brain tumoral cells. Altogether, our results provide the first quantitative evidence showing that somatostatin/dopamine/ghrelin systems are expressed in tumoral brain tissues, thereby suggesting that these systems could be involved, at least in part, in some of the pathological features observed in these pathologies.

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35. Breast cancer is associated with a deterioration of glucose/insulin homeostasis in obese premenopausal women but not in normoweight women

Authors: *Alicia Villa-Osaba, Laura López-Sánchez, Antonio J. Martínez-Fuentes, Isabel M. Luque, Marina Álvarez de Benito, Francisco Gracia-Navarro, José Lopez-Miranda, Manuel D. Gahete, Raúl M. Luque, Justo P. Castaño*

Group: GC8. *Hormones and cancer*

Breast cancer (BC) is the leading cause of cancer-related death in female population and its development and progression is strongly conditioned by endocrine factors as obesity, type-2 diabetes or metabolic syndrome, which share common features as insulin resistance (IR) and altered glucose homeostasis. Although IR could be the main cause of this relationship, this association has not been yet proved in humans, probably due to an inadequate determination of IR and/or patient stratification. For that reason, in this prospective study we aimed to analyze the putative relationship between IR and BC, by appropriately determining the IR of patients with or without BC with different BMI and menopausal status. Specifically, anthropometric, clinical and biochemical data were collected and/or determined in 148 volunteer women (71 control patients without BC and 77 patients with BC) who were classified by their BMI [normo-weight (<25), overweight (25-30), or obese (>30)] and subjected to an oral glucose tolerance test (OGTT). Results showed that BC presence was associated with high plasmatic levels of glucose, triglycerides, glycosylated hemoglobin and VEGF, with higher levels of glucose and insulin during the OGTT and high IR (determined by calculating several IR indexes), especially in premenopausal patients. When the population was stratified by BMI, we found that these associations were due to a worsening of glucose and insulin homeostasis in BC overweight patients (BMIe25) but not in normo-weight patients. Moreover, these associations were especially marked in obese patients (BMI≥30) compared to overweight patients. Altogether, our results indicate, for the first time, that an exacerbated dysregulation of metabolic homeostasis is directly linked to BC since we found a clear association between BC and a severe impairment of glucose/insulin homeostasis in obesity or overweight BC patients, but not in normo-weight patients, and this association was especially marked in premenopausal women.

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36. Functional implications of somatostatin receptor type 3 in non-functioning pituitary adenomas

Authors: *Mª Carmen Vázquez-Borrego, Alejandro Ibáñez-Costa, Miguel A. Japón, Mª Ángeles Gálvez, Eva Venegas-Moreno, Luis Jiménez-Reina, Alfonso Soto-Moreno, Manuel D. Gahete, Justo P. Castaño, Raúl M. Luque*

Group: GC8. *Hormones and cancer*

Non-functioning pituitary adenomas (NFPAs) constitute one third of all human pituitary tumors and are usually late diagnosed due to the absence of clinical manifestations associated to hormone hypersecretion. Most of these tumors are considered macroadenomas (size >1cm) which are associated with multiple comorbidities (e.g. headache and visual defects) related to the tumor mass effects. The first-line therapy in the management of NFPAs is the surgery; however, tumoral relapses are frequent because the tumoral mass is, in many cases, surgically inaccessible and extend to neighboring intracranial structures. Therefore, identification of novel therapies deem necessary to manage this pathology. Of note, somatostatin analogs (SSA) with high affinity for somatostatin receptors 2 or 5 (sst2, sst5) are successfully used to treat pituitary secreting adenomas; however, these drugs are not usually effective on NFPAs, which might be explained by the predominant expression of sst3, and low levels of sst2/sst5, in NFPAs. Hence, the aim of this study was to determine the functional role of sst3 in primary human NPFA cell cultures by analyzing the effect of an sst3-specific non-peptidic ligand (L-796,778; Merck) on key functional parameters including cell viability, apoptosis, hormone secretion, gene expression and second messenger signaling. Firstly, we analyzed by quantitative real-time PCR a series of 36 NFPAs and 10 normal pituitaries (NP) which demonstrated a clear alteration in the expression pattern of key hormones (e.g. FSH) and receptors (e.g. sst3, truncated sst5TMD4 or dopamine receptor subtype-2) in NFPAs compared to NP-samples. Remarkably, our results indicate that in vitro treatment of primary NPFA cell cultures with L-796,778 reduced cell viability and increased apoptosis by a Ca2+-independent mechanism. Altogether, our study provides novel evidences regarding the role and potential clinical implications of sst3 in the pathophysiology of NFPAs, suggesting that pharmacological treatment targeting this receptor could be a novel therapeutic alternative for these tumors.

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37. Effects of Classic vs. Novel Somatostatin Analogs in Pituitary Adenomas: a Head-to-Head in vitro Study

Authors: *Alejandro Ibáñez-Costa, Esther Rivero-Cortés, Mª Carmen Vázquez-Borrego, Manuel D. Gahete, Luis Jiménez-Reina, Miguel A. Japón, Andrés de la Riva, Eva Venegas-Moreno, Miguel Ángel Arráez, Silvia Maraver-Selfa, Inmaculada González-Molero, Inmaculada Gavilán, Juan Antonio García-Arnés, Herbert A. Schmid, Alfonso Soto-Moreno, María A. Gálvez, Raúl M. Luque, Justo P. Castaño*

Group: GC8. *Hormones and cancer*

Somatostatin analogs (SSA) represent the first-line treatment for pituitary adenomas (PAs) to control hormone secretion and/or tumor growth. However, some patients are unresponsive or escape from octreotide, a classic somatostatin receptor 2 (sst2)-preferring SSA, but pasireotide, a novel multi-sst-preferring SSA, may help to overcome this problem. It has been proposed that the expression pattern of sst1-sst5 in PAs and its correspondence with sst binding profile of the SSA could define and predict responsiveness to SSA therapy. To test this notion and better define the cellular/molecular features associated to octreotide and pasireotide responsiveness, we performed a systematic, parallel comparison of in vitro effects on primary PA cell-cultures, evaluating sst1-5 expression, intracellular Ca2+ signaling ([Ca2+]i), hormone secretion and cell viability, in a series of 97 samples [36 somatotropinomas, 16 corticotropinomas, 34 non-functioning PAs (NFPAs), 5 prolactinomas, and 6 normal pituitaries]. Somatotropinomas showed sst5>sst2 expression, yet octreotide appeared to reduce [Ca2+]i more efficiently than pasireotide, while both SSA similarly decreased GH release/expression and cell viability. Corticotropinomas expressed high sst5 levels, but displayed a limited response to pasireotide, while octreotide moderately reduced [Ca2+]i levels, ACTH release and cell viability. NFPAs preferentially expressed sst3 and were poorly responsive to SSA, and surprisingly, both SSA increased cell viability in a relevant proportion of cases. Prolactinomas mainly expressed sst1 and were mostly unresponsive to SSA. Finally, both SSA decreased [Ca2+]i in distinct proportions of normal pituitary samples. Our results indicate that both SSA act on the main PA-subtypes by exerting similar and distinct effects on [Ca2+]i, hormone release and cell viability. Since no evident correspondence was found between the observed effects and the specific sst1-sst5 profile of the tumors, it is plausible that additional factors, besides the simple abundance of a given sst, such as receptor dynamics, interaction and signaling would substantially influence the response of PA cells to SSA.

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38. A novel ghrelin splicing variant, In1-ghrelin, is overexpressed in prostate cancer where increases aggressive features

Authors: *Daniel Hormaechea-Agulla, Alejandro Ibañez-Costa, Enrique Gómez-Gómez, José Valero Rosa, Julia Carrasco-Valiente, Jose A. Ramos-Fernández, María M. Moreno, Michael D. Culler, Manuel D. Gahete, María José Requena, Justo P. Castaño, Raúl M. Luque*

Group: GC8. *Hormones and cancer*

Prostate cancer (PC) is the most commonly diagnosed malignancy and the second leading cause of cancer-related deaths among men. An emergent cancer hallmark with diagnostic/prognostic/therapeutic potential is aberrant/alternative splicing. The ghrelin system comprises a complex molecular family that regulates multiple key pathophysiological processes and comprises several peptides and splice variants, modifying enzymes [ghrelin-O-acyltransferase (GOAT)], and receptors (GHSR1a/1b). In1-ghrelin variant has been shown to be overexpressed in breast and pituitary tumors where it increases proliferation. Recently, ghrelin gene expression has been found in normal and PC. Therefore, we aim to determine the presence of ghrelin system components (native-ghrelin, In1-ghrelin, GOAT and GHSR1a/1b) and to explore the functional role of this system in PC cells. Samples from human PC, normal prostate (NP), and androgen-dependent and -independent PC cell lines were used. Expression profile was determined by quantitative-PCR. Functional parameters (i.e. proliferation, PSA secretion, migration, signaling) were analyzed in response to treatments with native-ghrelin and In1-ghrelin peptides. Moreover, functional consequences of over-expression or inhibition of In1-ghrelin variant (using stable transfected cells and siRNAs, respectively) was determined in PC cells. Our data revealed that expression pattern of this system is dysregulated in PC, where In1-ghrelin and GOAT are markedly over-expressed. Interestingly, In1-ghrelin treatment did not affect cell viability but increased PSA expression/secretion, androgen-receptor expression and Ca2+ signaling in cultured NP cells. Moreover, In1-ghrelin treatment and/or over-expression increased proliferation, migration and p-ERK and p-AKT levels in PC cells, whereas In1-ghrelin silencing reduced cell proliferation and PSA secretion. Altogether, our results indicate that In1-ghrelin is over-expressed in PC, where it can regulate cell proliferation, migration and PSA secretion, thus suggesting a possible pathophysiological role for this splice variant in human prostate and supporting the idea that ghrelin system could contribute to the PC oncogenesis and may provide novel tools to explore diagnostic/therapeutic targets in PC.

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