27th November
Universitat de Barcelona. Campus de Bellvitge. 4a Planta
ORAL COMMUNICATIONS

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ORAL COMMUNICATIONS 1
Livestock-associated methicillin-resistant Staphylococcus aureus in humans, Barcelona, Spain, 2012-2013

Mariana Camoez, Jordi Càmara, Fé Tubau, Ana Hornero, Miquel Pujol, Josefina Liñares, Maria Angeles Domínguez.

Research Group: Epidemiology of bacterial infections. Investigation of the pathogenesis and molecular basis of antimicrobial resistance

Abstract:

Livestock-associated methicillin-resistant Staphylococcus aureus sequence type 398 (MRSA-ST398) has been described as a new clonal lineage able to colonize and infect humans in several countries around the world. The increased rate of this clone from 2010 to 2011 in our setting, lead us to investigate the prevalence and the molecular features of MRSA-ST398 isolates, identified from 2012 to 2013. All MRSA strains were screened by Smal-PFGE. Nontypable MRSA strains by Smal (NT-Smal) were re-analyzed by Apal-PFGE. Molecular typing including SCCmec, agr, MLST and DNA microarray hybridization were performed for all NT-Smal isolates. Molecular characterization revealed that 16 of 1,127 (1.4%) MRSA strains isolated during this 2-year period belonged to the MRSA-ST398 clone. The majority of the MRSA-ST398 isolates belonged to PFGE type A (56%), carried SCCmec V (94%) and was spa type t011 (75%). Most of the strains revealed resistance to ciprofloxacin (81%) and low virulence genes content was observed. All MRSA-ST398 isolates in this study had a variety of genes encoding proteins associated with biofilm formation. Colonization or infection by this clone occurred most often in debilitated patients. Three cases were hospital-acquired. Our study shows that the MRSA-ST398 clone was present in low numbers in the Southern Barcelona metropolitan area. Colonization was mainly of community origin and in some patients, persisted for a long period. Of note, hospital transmission was hypothesized in a few cases. We report two MRSA-ST398 isolates showing the genetic markers suggesting being associated with a human adapted ST398 phenotype.
ORAL SESSION 1

Orexin and sleep quality in anorexia nervosa: clinical relevance and influence in treatment outcome

Sarah Sauchelli Toran, Susana Jiménez-Murcia, Isabel Sánchez, Nadine Riesco, Nuria Custal, Jose C. Fernández-García; Lourdes Garrido-Sánchez; Francisco J Tinahones; Howard Steiger; Mimi Israel; Rosa M. Baños; Cristina Botella; Rafael de la Torre; Jose M Fernández-Real; Francisco J. Ortega; Gema Frühbeck; Roser Granero; Salome Tárrega; Ana B Crujeiras; Amaia Rodríguez; Xavier Estivill; Jacques S Beckmann; Felipe F Casanueva, Jose M Menchón; Fernando Fernández-Aranda

Research Group: Eating Disorders Unit, Pshychiatric Service

Abstract:

BACKGROUND AND AIMS: Orexins/hypocretins are orexigenic peptides implicated in the regulation of feeding behavior and the sleep/wake cycle. Little is known about the functioning of these peptides in anorexia nervosa (AN). The aims of the current study were to evaluate the extent to which orexin-A might be linked to sleep and treatment outcome in AN.

METHOD: Fasting plasma orexin-A concentrations were measured in 48 females with AN at the start of a day hospital treatment and in 98 normal-eater/healthy-weight controls. The Pittsburgh Sleep Quality Index was used to examine sleep quality. Other psychopathological variables were evaluated with the Symptom Checklist-Revised (SCL90R) and the Eating Disorder Inventory-2 (EDI). Patients were assessed at the start and end of treatment by means of commonly used diagnostic criteria and clinical questionnaires.

RESULTS: The AN patients presented more sleep disturbances and poorer overall sleep quality than did the healthy controls (p=0.026) but there were no global differences between groups in plasma orexin-A concentrations (p=.071). In the AN sample, orexin-A concentrations were associated with greater sleep disturbances (|r|=.30), sleep inefficiency (|r|=.22) and poorer overall sleep (|r|=.22). Structural Equation Modeling (SEM) showed that both elevated orexin-A concentrations and inadequate sleep predicted poorer treatment outcome.

CONCLUSION: Plasma orexin-A concentrations contribute to poor sleep quality in AN, and both of these variables are associated with therapy response
Mechanisms governing the assembly, trafficking and function of the RetGC/GCAPs protein complex responsible for cGMP synthesis in retinal photoreceptor cells

**Santiago López Begines**, Sergi Tosal, Anna Plana-Bonamaisó, Maria José Fidalgo, Josep Maria Estanyol, Isidre Casal, **Ana Méndez**

Research Group: *Molecular and cellular basis of sensory disorders*

Abstract:

Visual perception is initiated at retinal photoreceptor cells, where light activates an enzymatic cascade that reduces free cGMP. As cGMP drops, cGMP-channels close, the photoreceptors hyperpolarize and emit a signal. As the light extinguishes, cGMP levels are restored by new synthesis to reestablish sensitivity. cGMP synthesis relies on guanylate cyclase/guanylate cyclase activating protein (RetGC/GCAP) complexes, in which GCAPs confer Ca2+-sensitivity to the cyclase important for signal termination. Despite extensive biochemical and structure-function analyses of guanylate cyclase and GCAP proteins, little is known about the mechanisms governing protein complex assembly, trafficking and anchoring to the rod outer segment in photoreceptors *in vivo*. We here have studied the molecular determinants in the GCAP proteins of their subcellular distribution *in vivo*. By transiently expressing mutants of GCAP1 at key residues in the retinas of GCAP1/GCAP2 double knockout mice and studying the effect of the mutations in protein localization, we have observed that GCAP1 requires binding to retGC in order to be transported to the sensory cilium. Therefore these proteins are transported as a pre-formed complex. We have applied proteomic approaches to identify new protein partners of the retGC/GCAP complex that may mediate complex assembly, trafficking, anchoring or regulation *in vivo*. Pull-down assays were performed with the Ca2+-bound and Ca2+-free forms of GCAP1 using sensory cilia preparations from bovine retinas as starting material. A number of selected interaction candidates were identified –among them, genes linked to blindness- that are now being confirmed by co-localization assays by confocal microscopy and size exclusion chromatography.
Impact of immunomodulatory treatment on disability progression in relapsing remitting multiple sclerosis

Laura Bau Vila, Matas E, Cobo-Calvo A, Mañé-Martínez MA, Hernández-Regadera JJ, Romero-Pinel L, Martinez-Yélamos S.

Research Group: Neurologic Diseases and Neurogenetics

Abstract:

BACKGROUND: Increasing evidence suggests that immunomodulatory drugs reduce short-term disability progression in relapsing-remitting multiple sclerosis (RRMS). However, treatment effectiveness and long-term effects remain unclear.

OBJECTIVE: To assess the effectiveness of immunomodulatory drugs in RRMS.

METHODS: We selected 525 RRMS patients seen from disease onset and followed up in our MS unit. Patients were divided in two subsets according to the date of the first assessment in our clinic: 158 untreated patients were assessed from 1980 to 1995 and 367 patients were assessed from 1995 to 2010, when disease modifying drugs were available. Time to Expanded Disability Status Scale (EDSS) 3, 4 and 6 in both groups was studied by Kaplan Meier survival analysis.

RESULTS: We found significant differences in survival curves for EDSS 3 (log rank p=0,001), 4 and 6 (log rank p<0,001). Time to irreversible EDSS 3 showed a significant increase in the contemporary cohort when compared to the historical untreated cohort (p75= 13,69 vs 9,06 years).

CONCLUSIONS: Our results showed a delay on disability progression in the contemporary cohort. Apart from immunomodulatory treatment, other changes in MS management may have contributed to our findings.
Phenotypic virulence factors among hypermucoviscous Klebsiella pneumoniae isolates causing bacteraemia

Meritxell Cubero, Sara Martí, Maria Angeles Domínguez, Josefina Liñares, Carmen Ardanuy

Research Group: Epidemiology of bacterial infections. Investigation of the pathogenesis and molecular basis of antimicrobial resistance

Abstract:

BACKGROUND: Despite the importance of classical non-hypermucoviscous Klebsiella pneumoniae (non-HvKp) in pathogenicity, a new hypervirulent hypermucoviscous clinical K. pneumoniae phenotype (HvKp) emerged in Asia in 1986 and its prevalence is increasing all over the world.

OBJECTIVES: We aimed to study the association between the HvKp phenotype and the presence of magA and rmpA genes, together with an evaluation of the virulence factors in a collection of K. pneumoniae clinical isolates obtained between 2007 and 2013.

METHODOLOGY: HvKP phenotype was identified by “string” and hypermucoviscosity tests. Adhesion was studied with the Biofilm Ring Test®. Air-Liquid-biofilm formation was identified by visual examination of the air-liquid interface (thick pellicle was covering all liquid surface). Resistance to pooled human serum was determined.

RESULTS: Among the HvKp phenotype, we selected 15 isolates (K1) with both genes, 12 isolates (9 K2; 3 non-K1/K2) with only the rmpA gene, and 11 isolates (non-K1/K2) without genes. Seven non-HvKP isolates were selected as control samples.

Hypermucoviscosity and Air-Liquid-biofilm formation increased with the number of genes present in the K. pneumonia strain, with the highest values observed for strains with both genes. In addition, the serum resistance rate was higher in isolates with magA and/or rmpA genes. In contrast, adhesion to a solid surface was inversely proportional to the presence of these genes.

CONCLUSION: The hypervirulent clones K1/K2 presented the highest levels of hypermucoviscosity, Air-Liquid-biofilm formation and serum resistance. By contrast, the slower adhesion rates suggest that hypermucoviscosity could interfere with initial adhesion to a solid surface.
When learning is its own reward: intrinsic reward-related activation of dopaminergic midbrain enhances word-learning

Pablo Ripollés, Josep Marco-Pallarés, Helena Alicart, Anna Mestres-Missé, Claus Tempelmann, Antoni Rodriguez-Fornells & Toemme Noesselt

Research Group: Cognition and Brain Plasticity Unit

Abstract:

Human beings are constantly engaged in learning processes. But, what can influence learning so that certain information sticks to our memory? Recent evidence suggests that memory formation can be modulated by brain activity within the ventral striatum (VS, a core region of reward processing), the substantia nigra/ventral tegmental area complex (SN/VTA, a source of dopaminergic projections) and the hippocampus (HP). However, in these studies, reward-related activations were associated to motivational factors, rather than to the process of learning itself. Thus, while it is clear that reward can be associated to the motivational aspects of learning, can learning be its own reward? And, more importantly, can this reward enhance memory performance? Confirming this hypothesis, we showed that participants—who were engaged in a word-learning paradigm in which no explicit reward was provided—exhibited enhanced fMRI-signals within the VS, HP and SN/VTA when successfully learning the meaning of new-words. This activity was not related to novelty, attention or exertion of effort, but rather to intrinsic reward-related effects. Moreover, greater activity and functional connectivity among the SN/VTA, HP and VS was related to better memory performance. In a second experiment, new-words which were remembered after a 24-hour retention delay were associated to higher pleasantness ratings and increased electrodermal activity during encoding. Together, our results suggest that learning, under certain circumstances, could be fuelling itself through reward-related mechanisms, possibly via dopaminergic modulation of the midbrain.
Loss of function of a regulator of energy metabolism halts axonal degeneration in X-linked adrenoleukodystrophy

*Pablo Ranea Robles*, Ruiz Montserrat, Galino Jorge, Schlüter Agatha, Fourcade Stéphane*, and Pujol Aurora*

Research Group: *Neurometabolic Diseases Lab*

Abstract:

X-linked adrenoleukodystrophy (X-ALD) is a rare disease, with fatal prognosis and currently lacking satisfactory treatment. X-ALD is characterized by central demyelination and/or axonal degeneration in the spinal cord, and is caused by loss-of-function of the peroxisomal transporter ABCD1. As a result, very long-chain fatty acids (VLCFA) such as C26:0 accumulate in tissues and plasma, being considered the pathognomonic sign of the disease. The murine model of X-ALD (*Abcd1*) exhibits a late-onset axonal degeneration with mainly spinal cord (corticospinal tracts) involvement. Our recent functional genomics analysis revealed that excess of C26:0 produced oxidative stress of mitochondrial origin, which compromises energy metabolism and suppresses the mitochondrial biogenesis pathway driven by the SIRT1/PGC-1α/PPARγ network, very early in the pathogenetic cascade.

In this study, we have identified TF40, a novel transcriptional regulator of energy metabolism in the brain, which shows increased expression levels in the X-ALD mouse model’s central nervous system. Interestingly, TF40 is regulated by C26:0 via an oxidative stress-dependent mechanism, *ex vivo*, in an organotypic spinal cord slice culture model. Further, its ubiquitous deletion by homologous recombination in the X-ALD mouse, normalizes mitochondrial respiration and bioenergetic failure, as well as the downstream consequences of axonal degeneration and associated locomotor disabilities.

Altogether, these results highlight TF40 as a novel therapeutic target, and suggest that its pharmacological inhibition may be a valuable strategy to treat X-ALD, as well as other axonopathies in which energetic homeostasis is impaired.
Baseline MxA mRNA expression predicts interferon beta response in multiple sclerosis patients

Elisabet Matas Martín Laura Bau, María Martínez-Iniesta, Lucía Romero-Pinel, M.Alba Mañé-Martínez, Álvaro Cobo-Calvo, Sergio Martínez-Yélamos

Research Group: Neurologic Diseases and Neurogenetics

Abstract:

BACKGROUND: Myxovirus resistance protein A (MxA) is a molecule induced after interferon-beta injection, mostly used to evaluate its bioactivity. There is little available data on clinical utility of baseline MxA mRNA status. The objective of the study is to investigate whether baseline MxA mRNA expression can predict relapse and disease progression in multiple sclerosis patients treated with interferon-beta.

METHODS: Baseline blood samples were obtained before the first interferon-beta dose was administered to evaluate MxA mRNA expression using real-time polymerase chain reaction (PCR). Demographic and clinical variables were prospectively recorded to define treatment responder and non responder groups.

RESULTS: 104 patients were included in the study. Baseline MxA mRNA expression was significantly lower in the group of patients who met the definition of responders (1.07 vs 1.95, Student t test, p<0.001). A threshold of 1.096 was established using Receiver Operating Characteristic analysis to differentiate between responders and non responders (sensitivity 73.9%, specificity 69.0%). Survival analysis using this threshold showed that time to next relapse (p< 0.001) and to EDSS progression (p=0.01) were significantly higher in patients with lower MxA titers.

CONCLUSION: The results suggest that baseline MxA mRNA levels may be useful for predicting whether multiple sclerosis patients will respond or not to interferon-beta treatment.
ORAL SESSION 2

Glucose deprivation induces apoptosis through ATF4-dependent TRAIL-R2/DR5 up-regulation

Raffaella Iurlaro, Estefanía Lucendo-Gutierrez, Daniel Palou-Gramón, Cristina Muñoz-Pinedo

Research Group: Cell death regulation

Abstract:

Glucose deprivation can induce different types of cell death, such as apoptosis, generally through the mitochondrial pathway, or necrosis. Previously, we described that Bax/Bak deficient MEFs subjected to glucose withdrawal die in a caspase-8 dependent manner and independently of the mitochondrial pathway and of death receptor/ligand interactions. Knockdown of caspase-8 prevented cell death due to glucose starvation also in HeLa cells. Here we described that HeLa cells subjected to glucose deprivation die by caspase-dependent apoptosis because co-treatment with the pan-caspase inhibitor Q-VD but not the inflammatory caspase inhibitor Y-VAD protects from death. We also showed that Bax/Bak deficient HCT116 cells are protected from death due to glucose withdrawal by Q-VD and caspase-8 silencing, although in this cell line the protection is partial. We show by immunoprecipitation and immunohistochemistry experiments that after glucose removal caspase-8 interacts with p62 and LC3-II, proteins that associate with the autophagosome. However, we could not demonstrate significant translocation of caspase-8 to these organelles or an essential role of p62 to activate caspase-8 and induce apoptosis after glucose removal. However, we demonstrated that FADD is essential for cell death execution under glucose deprivation. We observed that glucose deprivation induces endoplasmic reticulum stress in different cell lines, as shown by ATF4 and CHOP induction, as well as death receptors. We describe that ATF4 but not CHOP is responsible for TRAIL-R2 (DR5) induction after glucose withdrawal. Furthermore, we show by immunoprecipitation that DR5 interacts with caspase-8 and localizes mostly at Golgi apparatus before and after the treatment, where maybe it could be accumulating and recruiting caspase-8. Moreover, the knockdown of DR5 in HeLa cells protects from apoptosis due to glucose deprivation, an effect that is more significant when Bcl-XL is stably expressed in these cells, suggesting that a component of death upon glucose deprivation is dependent on the mitochondrial pathway. However, we demonstrated that apoptosis induced by glucose removal is not dependent on DR5 ligand, as knockdown of TRAIL does not prevent cell death.
ORAL SESSION 2

SirT2-dependent epigenetic control of the cell cycle

*Núria Sima Teruel*, Paloma Martínez-Redondo*, Berta N. Vazquez, Marcus Krüger³, Anne K. Voss⁴, Lourdes Serrano², Alejandro Vaquero

Research Group: Chromatin Biology Lab

Abstract:

The members of the family of NAD⁺-dependent HDACs Sirtuins are major players in stress response, genome stability and cell cycle control. Among the seven mammalian Sirtuins, SIRT1 and SIRT2 are the main H4K16Ac deacetylases in mammals. Despite its cytoplasmic localization, SIRT2 shows a high specificity for H4K16Ac *in vitro*, and its loss correlates with hyperacetylation of H4K16Ac during mitosis. Moreover, SIRT2 regulates H4K20me1 deposition through deacetylation of H4K16Ac and determines the level of H4K20me2,3 throughout the cell cycle. In addition, SIRT2 deacetylates the H4K20me1 methyltransferase, PR-SET7, in K90 regulating its chromatin levels during G₂/M. In recent studies SIRT2 and PR-SET7 have been proposed as regulators of a mitotic checkpoint. Here we show that the main H4K16 HAT, MOF, plays an antagonistic role to SIRT2 in cell cycle control and genome stability through regulation of H4K20me1 during G₂/M. SIRT2 deacetylates MOF in K113, K116 and K175 and promotes its degradation during G₂ and mitosis. Interestingly, MOF, SIRT2 and PRSET7 seem to form a ternary complex during G₂ suggesting a tight control of H4K20me1 and cell cycle. In order to determine the functional implications of this antagonism *in vivo*, we have generated a double KO MOF/SIRT2 mouse model and studied the impact of this interplay in genome stability, cancer and aging.
Epigenetic inactivation of SLFN11 confers resistance to platinum agents in ovarian and lung cancer.

Vanesa Nogales Trallero, Anna Martinez, Catia Moutinho, Sebastian Moran, Manel Esteller

Research Group: Cancer Epigenetics

Abstract:

Currently, one of the major issues in cancer fight is that, although existing a large number of antitumor drugs, in many cases the tumor does not respond or stops responding to the therapy. Epigenetic marks, as DNA methylation, are features heritable through mitosis that determine genes expression. These marks are usually altered in cancer, conferring an advantage to the tumor; therefore, they can be used as cancer biomarkers of easy detection due to the high stability of DNA molecule. Given these facts, we decided to determine the methylome of the well characterized cancer cell line panel NCI-60, to find epigenetic predictive biomarkers of chemotherapy response. By performing correlational analysis between our methylation data and the available data of NCI-60 sensitivity to antitumor drugs from the Developmental Therapeutic Program, we found that promoter CpG island methylation of the SLFN11 was associated with increased resistance to platinum compounds. We then validated these findings in vitro by performing epigenetic analysis of SLFN11 as well as sh-silencing and over-expression of SLFN11 in several cancer cell lines. We also identified the BRCA1-interacting partner DHX9, as a protein partner of SLFN11, suggesting a mechanistic pathway for the observed chemoresistance effect. Most importantly, we have been able to extend these findings clinically, following the observation that those patients with ovarian and non-small cell lung cancer carrying SLFN11 promoter methylation had a poor response to both cisplatin and carboplatin treatments. Overall, these results identify SLFN11 epigenetic inactivation as a predictor of resistance to platinum drugs in human cancer.
Lineage tracing of cardiac myocytes during zebrafish heart regeneration

Isil Tekeli Mario Notari, Anna Garcia-Puig, Cristina García-Pastor, Isabelle Aujard, Ludovic Jullien, Angel Raya

Research Group: CMRB

Abstract:

Adult zebrafish can regenerate their heart when 20% of the ventricle is amputated through the dedifferentiation and proliferation of existing cardiomyocytes. However, it is still unclear whether all cardiomyocytes have similar regenerative potential, or only cardiomyocytes located in particular regions of the heart are able to carry out the regeneration process.

Here, in order to address this, we developed a cardiomyocyte-specific labeling method based on a photo-inducible Cre/lox system. This technique is based on creating transgenic zebrafish that are able to perform site-specific recombination reactions only in the cardiomyocytes upon photo-activation. As a result of this recombination, activated cardiac muscle cells express green fluorescent protein. This fluorescent labeling is irreversible, and the descendants of these labeled cells are permanently labeled as well, as long as they remain as cardiomyocytes.

First, we showed that cardiomyocytes labeled in embryonic hearts by this method are able to survive and contribute to different heart muscle lineages of the adult zebrafish heart. Next, we used these adult zebrafish with labeled cardiomyocytes to investigate the fates of cardiomyocytes from different regions of the myocardium during regeneration. For this, we performed cardiac amputation in zebrafish with different labeled regions throughout their hearts. Our results showed that only cardiomyocytes immediately adjacent to the injury site contributed to the regenerated tissue.

Overall, our studies indicate that heart regeneration in the adult zebrafish is a rather static process, in contrast to the highly dynamic plasticity of cardiomyocyte fates that takes place during embryonic heart regeneration.
ORAL SESSION 2

Targeting GMP synthesis, ribosomal biogenesis and p53-cell cycle control as a therapeutic approach in colorectal cancer

Ferran Riaño-Canalias, Joffrey Pelletier, Antonio Gentilella, Oscar Yanes, Sara C. Kozma, Ramon Salazar and George Thomas

Research Group: Cancer Metabolism

Abstract:

Sporadic colorectal Cancer (sCRC) is the most frequent cause of cancer in men and women in Europe. It represents ~ 80% of CRCs. Almost all sCRCs display deregulated c-Myc signaling, which drives hyperactivation of ribosome biogenesis, a key determinant in c-Myc driven tumorigenesis. Critical for the studies described here, inhibition of high rates of ribosome biogenesis appears to serve as an ‘Achilles heel’ in c-Myc driven sCRCs. Here we have set out to elucidate the underlying mechanisms of the crosstalk between ribosome biogenesis, impaired by Mycophenolic acid (MPA), and c-Myc, in view to exploit this connection therapeutically, in collaboration with Novartis Oncology. MPA impairs ribosome biogenesis and induces p53 through the inhibition of GMP synthesis. Consistent with earlier studies, we show that MPA treatment inhibits rRNA synthesis and causes p53 upregulation by triggering a checkpoint that involves the redirection of a preribosomal complex made-up of two ribosomal proteins, RPL5, RPL11, and noncoding 5S rRNA, to the binding and inhibition of E3-ubiquitin ligase human double minute 2 (HDM2). In the process of elucidating the role of MPA in inducing the RPL5/RPL11/5S rRNA-Hdm2 inhibitory complex, we have found that alternative mechanisms are involved in p53 stabilization, likely due to nucleotide imbalances induced by MPA. Elucidation of the basic mechanisms by which MPA affects sCRCs progression will provide insights into the role of inhibition of GMP synthesis in suppressing c-Myc driven tumor progression.
ORAL SESSION 2

TBC1D16 hypomethylation: A novel epigenetic biomarker for melanoma


**Research Group:** *Cancer Epigenetics*

**Abstract:**

Metastasis is responsible for most cancer-related deaths, and, among common tumor types, melanoma is one with great potential to metastasize. Here we study the contribution of epigenetic changes to the dissemination process by analyzing the changes that occur at the DNA methylation level between primary cancer cells and metastases. We found a hypomethylation event that reactivates a cryptic transcript of the Rab GTPase activating protein TBC1D16 to be a characteristic feature of the metastatic cascade. This short isoform of TBC1D16 exacerbates melanoma growth and metastasis both *in vitro* and *in vivo*. By combining immunoprecipitation and mass spectrometry, we identified RAB5C as a new TBC1D16 target and showed that it regulates EGFR in melanoma cells. We also found that epigenetic reactivation of TBC1D16-47KD is associated with poor clinical outcome in melanoma, while conferring greater sensitivity to BRAF and MEK inhibitors. Importantly, this study informs about the molecular pathways affected by the epigenetic reactivation of TBC1D16 gene and the consequences on chemotherapy responses. Thus, our results contribute to unveil the complex mechanisms driving melanomagenesis and melanoma progression, and defend TBC1D16 as a crucial modulator of the melanoma progression and the clinical response to chemotherapy.
ORAL SESSION 2

Role of p110α/PI3K in the sprouting angiogenic process and its mechanism of action

Ana Angulo Urarte, Pedro Maria Casado-Izquierdo, Pedro Cutillas, Jaime Millán and Mariona Graupera

Research Group: Vascular Signaling

Abstract:

Class I PI3K signalling is required in a cell-autonomous manner in endothelial cells (ECs) for blood vessel growth. ECs express all class I PI3K isoforms, but only the catalytic subunit p110α is required for vessel morphogenesis (Graupera et al. Nature 2008). However, it is still unclear how p110α/PI3K signalling regulates sprouting angiogenesis. By using a tamoxifen-inducible endothelial Cre line and retinas as model of study, we have found that p110α signalling is required to guarantee vessel function. Reduced p110α activity in ECs resulted in complete inhibition of cell migration and intercalation; two processes required for sprout elongation. Consequently, p110α loss of function (LOF) vessels resulted in tubular structures composed of single ECs with aberrant shape, multiple protrusions and no lumen. These vessels failed to stabilize upon anastomosis and showed an increase in cortical actin cables and hyper-phosphorylation of myosin light chain (MLC). Surprisingly, p110α inactivation in ECs did not correlate with an increase in RhoA-GTP, and inhibition of ROCK activity did not rescue the p110α LOF phenotype. To further understand how p110α regulates pMLC activity, we next performed a phospho-proteomic screening. Interestingly, we found that inactivation of p110α in ECs resulted in increased phosphorylation of Mprip and Ppp1r12 (key proteins involved in the dephosphorylation of pMLC) that reduce their activity. Our data not only have shed light into the physiological role of p110α/PI3K, but have also identified novel downstream effectors of this signalling hub. It would be relevant to underscore whether this interaction between p110α, Mprip and Ppp1r12 is extend beyond ECs.
c-Myc and ribosome biogenesis: Role of the RPL5/RPL11/5S rRNA-Hdm2 inhibitory complex in stabilizing p53

_Carmen Morcelle Magaña_, Francisco Moron, Sandra Menoyo, Albert Tauler, Sara C. Kozma, and _George Thomas_

**Research Group:** _Cancer Metabolism_

**Abstract:**

In most human cancers c-Myc expression is de-regulated and/or elevated. This leads to hyperactivation of ribosome biogenesis, as the three RNA polymerases are c-Myc target genes. Based on the role of the RPL5/RPL11/5S rRNA complex in the regulation of p53 and in the importance of this complex in c-Myc-tumorigenesis, we queried whether c-Myc induction, by increasing ribosome biogenesis, leads to the availability of more RPL5/RPL11/5S rRNA complex, the inhibition of Hdm2 and p53 stabilization, or instead selectively drives ribosome biogenesis, consuming the RPL5/RPL11/5S rRNA complex, releasing Hdm2 and inducing p53 degradation. Our long-term goal would be to determine the underlying molecular mechanisms by which the cell senses c-Myc function as it raises, the barrier against the development of tumors in an early stage of oncogenesis. We have focused on sporadic colorectal cancer (CRC), which shows upregulation c-Myc target genes in nearly 100% tumors analyzed. Two distinct strategies have been employed: A) c-Myc depletion to evaluate if loss of c-Myc would reduce or increase the availability RPL5/RPL11/5S rRNA complex, which in turn would either relief or induce the inhibition of Hdm2, causing a decrease or increase in p53 levels, respectively; and B) increasing c-Myc expression in a dose dependent manner in a non-tumorigenic system, to determine how the different c-Myc levels would affect the formation of the complex. Our preliminary results show that reduction on c-Myc levels leads to a p53 decrease, which would supports the model of deregulated c-Myc triggering p53 stabilization.
ORAL COMMUNICATIONS 3
Cancer, warts or asymptomatic infections: clinical presentation matches codon usage preferences in human papillomaviruses

Marta Félez-Sánchez, J.H. Trösemeier, S. Bedhomme, I. González-Bravo, C. Kamp, I.G. Bravo

Research Group: Virus and Cancer

Abstract:

OBJECTIVES: Viruses rely completely on the hosts’ machinery for translation of viral transcripts. However, for most viruses infecting humans codon usage preferences (CUPrefs) do not match those of the host. Human papillomaviruses (HPVs) are a showcase to tackle this paradox: they present a large genotypic diversity and a broad range of phenotypic presentations, from asymptomatic infections, to productive lesions and cancer. We examined here the CUPrefs for human PVs to determine whether variations in CUPrefs could be explained by differences in tissue tropism, association with disease and/or timing of gene expression.

METHODS: We examined the codon usage bias of the six major PV genes across 156 HPVs from five distinct phylogenetic groups. Codon Adaptation Index was used to evaluate intergenomic adaptation between viral and host genomes. Correspondence and multidimensional scaling analysis in combination with cluster analysis were used as complementing methods in order to elucidate common patterns on codon usage among HPVs.

RESULTS: We demonstrated that phylogenetic relationships between HPVs explained only a small proportion of CUPrefs variation. Instead, the most important explanatory factor for viral CUPrefs was infection phenotype: orthologous genes in viruses with similar clinical presentation displayed similar CUPrefs.

CONCLUSIONS: Our results suggest that CUPrefs in HPVs reflect either variations in the mutation bias or differential selection pressures depending on the clinical presentation and expression timing. We propose that poor viral CUPrefs may be central to a trade-off between strong viral gene expression and the potential for eliciting protective immune response.
Identification of a microRNA signature with a prognostic value in stage II colon cancer


Research Group: Biomarkers and susceptibility unit

Abstract:

Colorectal cancer is the third most common cancer worldwide. About a quarter of patients are diagnosed with stage II disease, but still disease recurrence occurs after surgery in 20-25% of them. Prognostic biomarkers are needed to select which patients require adjuvant chemotherapy. Micro-RNAs (miRNAs) are small non-coding RNAs that are key post-transcriptional regulators of gene expression.

We aim to identify a signature of miRNA with prognostic value in stage II colon cancer patients.

A series of 100 patients diagnosed with stage II colon cancer were included in the study (http://www.colonomics.org). Total RNA was isolated from tissue samples of tumor and normal adjacent mucosa. Has performed a small RNA sequencing using the Applied Biosystems SOLID platform. Quantification of specific miRNAs was done mapping the reads to reference of mature miRNA sequences annotated in miRBase v20. A total of 22 events were recorded, with a minimum follow-up of 3 yeas. Cox models and data mining techniques for survival analysis were used to elaborate an univariate and multivariate profile. A functional annotation of the selected miRNAs has been performed using the MirTarBase database, that provides validated target genes for miRNAs.

A final list of 22 miRNA were selected for further validation. An independent validation series of 256 patients diagnosed with stage II/III CRC has been collected for the validation. Finally has obtain a prognostic stage II classifier with 4 miRNAs. The functional analysis of the selected miRNAs showed an enrichment on cancer pathways.
Dietary intake and biomarkers of acrylamide exposure and risk of endometrial and ovarian cancer in the EPIC study

*Mireia Obón-Santacana, Eric J Duell* and the EPIC Group

Research Group: *Unit of Nutrition and Cancer. Cancer Epidemiology Research Program (PREC).*

Abstract:

Endometrial and ovarian (EC, OC) are the 4th and 5th most common cancers diagnosed in European women. IARC classified acrylamide (AA) as ‘probably carcinogenic to humans (group-2A)’. AA is formed in carbohydrates rich foods during baking, frying or roasting. Recent prospective cohort studies found that women with high AA intake had elevated risk of developing EC and OC. Using prospectively collected dietary and lifestyle information in 368,010 women from the EPIC cohort, we assessed the association between estimated AA intake and EC and OC risk. We also identified which food groups and lifestyle variables were determinants of hemoglobin adduct concentrations of AA and glycidamide (HbAA, HbGA) in non-smoking postmenopausal control women. Further, we studied the relation between these biomarkers and the risk of EC and OC in two nested case-control studies. Dietary intake of AA was not associated with overall/type-1 EC risk; however, positive associations with type-I were observed in women who were both non-users of OCs and never smokers. We did not observe any evidence that AA intake was associated with risk for overall OC or OC subtypes. The main food determinants of HbAA and HbGA in non-smoking women were biscuits, crackers and dry cakes. Alcohol and BMI were the principal determinants of the ratio HbGA/HbAA. None of the biomarkers variables had an effect on EC risk (overall/type-1); however, positive associations were observed between some quintiles of HbGA and OC, but no linear dose-response trend was found. The results of these studies may lead to public health prevention strategies.
Adenoviruses BiTE at cancer: an immunotherapy approach to improve oncolytic virotherapy

Carlos Alberto Fajardo, Luis Rojas, Rafael Moreno, Sonia Guedan, Marcel Arias, Ramon Alemany

Research Group: Cancer virotherapy

Abstract:

Oncolytic adenoviruses that selectively infect and kill cancer cells without harming normal cells have gained considerable attention as anticancer drugs. Despite their potential, clinical trials with oncolytic adenoviruses have shown that, even though being successfully delivered to the tumor, adenovirus-infected cells are efficiently cleared from the tumor microenvironment by infiltrating virus-specific lymphocytes. We hypothesize that arming oncolytic adenoviruses with bispecific T-cell engagers (BiTEs), a new class of immunotherapeutic antibodies that re-direct T-cells to cancer cells, might favor antitumor rather than anti-viral immune responses. We have engineered the oncolytic adenovirus ICOVIR15K to express an EGFR-targeting BiTE (cBiTE) antibody. The virus ICOVIR15K-cBiTE was successfully rescued and it retained the oncolytic properties of its parental counterpart in vitro. cBiTE expression could be detected in supernatants from ICOVIR15K-cBiTE infected cells and its specific binding to CD3+ and EGFR+ cells was demonstrated by flow cytometry. Interestingly, co-cultures of unstimulated human PBMCs and EGFR+ cells infected with the BiTE-expressing virus led to augmented T-cell activation when compared to the parental virus, as evidenced by an increase in activation markers, cytokine release and T-cell proliferation. Furthermore, supernatants from ICOVIR15K-cBiTE infected cells induced a significant increase in cell death of EGFR+ cells in the presence of PBMCs. In vivo studies in A549 xenograft mouse models have shown that the intratumoral administration of the cBiTE-expressing virus in combination with human PBMCs improves the antitumor efficacy of ICOVIR15K. Thus, arming oncolytic adenoviruses with BiTEs represents a promising strategy to overcome key limitations in oncolytic virotherapy.
Physical activity (PA), cardiorespiratory fitness (CRF), nutrition and weight control in breast cancer (BC) patients and survivors

Noémie Travier, Nutrition and Cancer Unit ICO, Physiology Unit UB-Bellvitge, Breast Cancer Functional Unit ICO-HUB, Julius Center (University Medical Centre of Utrecht), Josep Maria Borràs, Antonio Agudo


Abstract:

Improvement in screening and treatment has increased the number of BC survivors. Because numerous patients and survivors experience side-effects likely to affect quality of life (QoL) and disease prognosis, reducing them has become a health priority.

This thesis investigates whether PA, CRF, nutrition and weight control might be modifiable determinants related to QoL and BC prognosis using the PREDICOP-F\(^1\) and PACT\(^2\) trials' data.

The single-arm pre–post PREDICOP-F\(^1\) trial, offered diet and PA sessions to overweight and obese BC survivors recruited shortly after treatment. This trial involved 42 BC survivors from the Catalan Institute of Oncology who showed a significant 8% weight loss and significant decreases in BMI, fat mass and waist circumference at the end of the 12-week intervention. Significant decreases in total energy, saturated fat and carbohydrate intakes were observed while QoL and CRF significantly increased.

In the PACT\(^2\) trial, 204 BC patients undergoing chemotherapy from 7 Dutch hospitals were randomly assigned to usual care or an 18-week exercise intervention. At 18 weeks, the increase in physical fatigue was significantly lower in the intervention group compared to control while submaximal CRF and muscle strength were significantly higher in the intervention group. QoL outcomes favored the exercise group without reaching statistical significance.

This thesis indicates that efforts should be put on the promotion of exercise during and after BC treatment. Offering exercise sessions as part of routine care might help trigger this lifestyle change. More research is however still needed to make recommendations arguing this will improve BC prognosis.

\(^1\)Prevención de las Recaídas mediante Ejercicio, Dleta y COntrol de Peso en pacientes con cáncer de mama - Estudio de Factibilidad

\(^2\)Physical Activity during Cancer Treatment
Epigenetic profiling uncovers the suppressive role of caveolae in Ewing Sarcoma

Juan Huertas martínez, Frank Court, Santiago Rello-Varona, David Herrero Martin, Olga Almacellas, Miguel Sáinz-Jaspeado, Silvia Garcia-Monclús, Laura Lagares-Tena, Raquel Buj, Lourdes Hontecillas-Prieto, Silvia Mateo-Lozano, Ana Sastre, Daniel Azorin, Jaume Mora, Josep Roma, Sebastian Moran, Soledad Gallego, Miquel Angel Peinado, Xavier Garcia del Muro, Javier Alonso, Enrique de Alava, Dave Monk, Manel Esteller and Oscar M Tirado

Research Group: Sarcoma Research

Abstract:

Ewing Sarcoma (ES) is the second most common bone tumor in childhood. ES harbors a characteristic gene translocation that gives rise to a fusion protein, most commonly EWS/FLI1 (EF). DNA methylation is common in cancer. Inactivation of some tumors suppressors by hypermethylation is a hallmark in cancer. In the present study, epigenomic profiling identifies differential hypermethylation in the N-shore island of the PTRF promoter of ES tumor samples and cell lines. Accordingly, ES cells do not express PTRF. Epigenetic silencing of PTRF was confirmed by bisulfite genomic sequencing on ES cells and human samples. A673 treated with 5-AZA (demethylating agent) resulted in re-expression of PTRF and an increase in caveolae formation as shown by electron microscopy. Likewise, gain of function experiments also showed an increase in caveolae formation but paralleled by a significant increase in cell death in vitro, together with a lower capacity to form tumors in vivo. The tumor suppressive effect derived from CAV1-PTRF interaction was verified also in EW7 cells. These results suggest that CAV1-PTRF interaction and caveolae formation promote cell death in ES cells. Co-expression of CAV1-PTRF in p53 mutant ES cells had no cell death inducing effect despite increased caveolae formation. Knockdown of p53 in TC252/PTRF-transfected cells resulted in a reduction of cell death. In summary, our results suggest that PTRF acts as a tumor suppressor in ES. PTRF re-expression enhances caveolae formation in ES cells, thus modulating CAV1 localization that results in p53-mediated cell death.
ORAL SESSION 3

Generation of tumorgraft models to characterize novel tumor suppressor genes implicated in lung carcinogenesis

**Carolina Pereira**, Pol Gimenez-Xavier, Gonzalo Gomez, Enric Condom, Alberto Villanueva, David Pisano, Montse Sanchez Cespedes

**Research Group:** *Genes and Cancer*

**Abstract:**

The poor prognosis and lack of effective therapies throw lung cancer (LC) to the deadliest cancer worldwide. The mutational spectrum determines tumor onset, and thus, the discovery of LC-related genes has been crucial to the development of targeted drugs, which are now available for patients bearing specific gene mutations.

Here, aiming to discover novel tumor suppressor genes (TSG), 8 tumorgrafts were generated to perform exome and transcriptome sequencing. Tumorgrafts constitute valuable systems to overcome tumor heterogeneity: once implanted in mice, human stromal components and normal cells are drifted. Plus, they are able to maintain the original histological features, genetic background and gene expression profiles, facilitating the detection of mutated genes. Overall, from the 4428 mutated genes found, we focused specifically on the subset that would presumably lead to biallelic gene inactivation, a common feature exhibited by tumor suppressors. Given that homozygous deletions are also strongly indicators of the TSG presence, we scanned the exomes to find regions where there were sequencing reads in the healthy tissue and no reads in the corresponding tumor. These stringent filters allowed us to reduce the amount of candidate genes from thousands to several dozens. Currently, we are characterizing *B2M* gene, which was found to be mutated in 5.1% in 79 tested LC cell lines and in 4.0% in a panel of 174 lung primary tumors. *In vitro* experiments are ongoing to fully characterize this gene and hopefully disentangle its role in lung tumor formation.
The multiple roles of carcinoma associated fibroblast-soluble factors in chemoresistance in colorectal cancer

Samuel Gonçalves Ribeiro, Natalia Guillén Díaz-Maroto, Mireia Berdiel, Antonio Soriano, Jordi Guardiola, Mercedes Martínez-Villacampa, Gabriel Capellà, Alberto Villanueva, Eva Martínez-Balibrea, David G. Mollevi

Research Group: Chemoresistance and predictive factors to tumor response and stromal microenvironment

Abstract:

The importance of the tumor microenvironment (TME) as an important contributor to cancer progression, and its role in the development of de novo resistance to treatments has become increasingly apparent. The effect of TME on resistance to targeted therapies is conceptually easier to understand, since various soluble factors may activate signaling events converging in the same pathway downstream of the targeted molecule/receptor. However, the mechanisms of microenvironment-mediated drug resistance for nonspecific conventional chemotherapeutic agents, such as platinum compounds or antimetabolites, are still unclear.

Here we describe a mechanism induced by soluble factors released by carcinoma-associated fibroblasts (CAFs) that induce the translocation of AKT, Survivin and P38 to the nucleus of the tumor cells. These changes are guided to ensure DNA repair and the correct entrance and exit from mitosis in the presence of oxaliplatin or 5-fluorouracil. We used conditioned media (CM) from normal colonic fibroblasts and paired CAFs to assess dose response curves of oxaliplatin and 5-fluorouracil, separately or combined, compared with standard culture medium. We also evaluated a colony-forming assay and cell death to demonstrate the protective role of CAF-CM. We further characterized the signaling events induced by CAF-soluble factors in several CRC cell lines. Immunofluorescence confirmed the translocation of AKT, P38 and Survivin to the nucleus induced by CAF-soluble factors. The inhibition of STAT3 or P38 provides a promising strategy for overcoming microenvironment-mediated resistance. Conversely, AKT inhibition induces an antagonistic effect that relieves STAT3-mediated compensatory feedback.
POSTERS SESSION A
A.1 Absence of maternal methylation in biparental hydatidiform moles from women with NLRP7 maternal-effect mutations reveals widespread placenta-specific imprinting.


Research Group: Genomic Imprinting and Cancer Group

Abstract: Familial recurrent hydatidiform mole (RHM) is a maternal-effect autosomal recessive disorder usually associated with mutations of the NLRP7 gene. It is characterized by HM with excessive trophoblastic proliferation, which mimics the appearance of androgenetic molar conceptuses despite their diploid biparental constitution. It has been proposed that the phenotypes of both types of mole are associated with aberrant genomic imprinting. However, no systematic analyses for imprinting defects have been reported. Here, we present the genome-wide methylation profiles of both spontaneous androgenetic and biparental NLRP7 defective molar tissues. We observe total paternalization of all ubiquitous and placenta-specific differentially methylated regions (DMRs) in four androgenetic moles; namely gain of methylation at paternally methylated loci and absence of methylation at maternally methylated regions. The methylation defects observed in five RHM biopsies from NLRP7 defective patients are restricted to lack-of-methylation at maternal DMRs. Surprisingly RHMs from two sisters with the same missense mutations, as well as consecutive RHMs from one affected female show subtle allelic methylation differences, suggesting inter-RHM variation. These epigenotypes are consistent with NLRP7 being a maternal-effect gene and involved in imprint acquisition in the oocyte. In addition, bioinformatic screening of the resulting methylation datasets identified over sixty loci with methylation profiles consistent with imprinting in the placenta, of which we confirm 22 as novel maternally methylated loci. These observations strongly suggest that the molar phenotypes are due to defective placenta-specific imprinting and over-expression of paternally expressed transcripts, highlighting that maternal-effect mutations of NLRP7 are associated with the most severe form of multi-locus imprinting defects in humans.
POSTER SESSION A

A.2 DYRK1A, A NOVEL REGULATOR OF GluN1/GluN2A RECEPTORS: IMPLICATIONS FOR DOWN SYNDROME AND ALZHEIMER’S DISEASE.

Macarena Gómez de Salazar, Cristina Grau, Krisztina Arató, José M. Fernández-Fernández, Paula Sanchís, David Soto, Aitana Valderrama, Carlos Sindreu, Cristina Fillat, Isidre Ferrer, Susana de la Luna, Xavier Altafaj

Research Group: iGluRs

Abstract: N-Methyl-D-Aspartate (NMDA) glutamate receptors play a pivotal role in synaptic plasticity processes, but under certain conditions their activation can induce neuronal dysfunction and excitotoxicity, which are associated to synaptic dysfunctions present in Alzheimer's disease (AD) and Down syndrome (DS). Regarding the latter condition, we previously shown that DYRK1A kinase (a DS candidate gene product) overexpression promoted an increased surface expression of GluN1/GluN2A receptors, together with a prolonged decay of NMDA-induced calcium currents. Furthermore, we identified the DYRK1A phosphorylation site at GluN2A Ser (1048) intracellular domain. Functionally, DYRK1A-mediated phosphorylation of GluN2A reduces its internalization rate, increases the surface expression and potentiates NMDA-evoked current density. Considering the regulatory effects of phosphorylation on NMDAR surface density, subcellular distribution (synaptic, vs. extrasynaptic) and biophysical properties, these data support a role of DYRK1A as a direct and specific regulator of GluN1/GluN2A subtype of NMDARs.

Interestingly, DYRK1A upregulation has been described in DS and AD murine models. In agreement with this, we have detected an increase of the DYRK1A-mediated phosphorylation of GluN2A(Ser1048) in adult brain of DS murine models and we are currently exploring whether these changes are also present in AD mouse models. In both models, our efforts are currently focused to determine, by a proteomics-based approach, the subcellular phosphorylation pattern of NMDARs. These data will contribute to understand the role of NMDAR phosphorylation in the initial stages of these related synaptopathies, with potential therapeutic applications.
A.3 Emotion regulation and excess weight: impaired affective processing characterized by dysfunctional insula activation

Trevor Steward, Maria Picó-Pérez, Fernanda Mata, Ignacio Martínez-Zalacaín, Marta Cano, Fernando Fernández-Aranda, Murat Yucel, Carles Soriano-Masa, Antonio Verdejo-García

Research Group: Eating Disorders Unit

Abstract: Maladaptive coping strategies such as overeating in response to negative affect are thought to accompany obesity onset. This study sought to examine the neural correlates of emotion regulation in overweight and healthy-weight individuals using functional magnetic resonance imaging (fMRI). We hypothesized that overweight participants would display heightened emotional reactivity and deficient down-regulation capability. 14 overweight subjects and 14 healthy-weight controls performed a reappraisal task during fMRI scanning: they were exposed to 24 blocks of negative affective or neutral pictures that they were instructed to Observe (neutral pictures), Maintain (sustain the emotion elicited by the negative pictures) or Regulate (downregulate the emotion provoked by the negative pictures through previously trained reappraisal techniques). Task-related activations during two conditions of interest (Maintain>Observe and Regulate>Maintain) were analyzed using the general linear model in SPM8 software. Our results found that overweight subjects had decreased insula activation as well as reduced activity in the orbitofrontal cortex and cerebellum when experiencing negative emotions (Maintain>Observe). However, when instructed to regulate these negative emotions by means of cognitive reappraisal (Regulate>Maintain), overweight subjects exhibited increased insula activation. Analysis of neuroimaging and behavioral results using path analysis revealed a complex relationship between body mass, insula activation, focal attention and negative emotion reappraisal capacity. Jointly, these findings indicate that excess weight is linked to dysfunctional insula activation during negative emotion experience and cognitive reappraisal.
A.4 The Role of Systemic Chemotherapy in Mycosis Fungoides and Sezary Syndrome: a Retrospective Review of 94 Cases

Viviana Paredes, Eva Gonzalez-Barca, Eva Domingo, Santiago Mercadal, Anna Sureda, Alberto Fernandez de Sevilla, Teresa Estrach, Octavio Servitje

Research Group: Hematopoietic and lymphoid tumours

Abstract:
BACKGROUND: Mycosis fungoides (MF) and Sezary Syndrome (SS) are the most common forms of cutaneous T-cell lymphomas (CTCL). Advanced MF forms and SS have poor prognosis. Due to their low frequency there is no standard systemic treatment for MF/SS.
OBJECTIVE: evaluate response rates and survival of patients treated with systemic chemotherapy.
MATERIALS AND METHODS: Medical records from MF and SS patients treated with systemic chemotherapy from 1980 to 2011 in two University hospitals in Barcelona, Spain, were reviewed. The EORTC/ISCL staging system and response criteria were applied.
RESULTS: Ninety-four patients were included, 51 (54.3%) with MF and 43 (45.7%) with SS. Systemic chemotherapy was administered at a median time of 23.9 months from diagnosis. In MF, CHOP and CHOP-like were the most frequently used regimens (73.5%), while in SS group, patients were mostly treated with Chlorambucil (72%). Overall response rate (ORR) was 51.85%; median duration of response was 11 months; with a median follow-up of 26 months, 66 patients (70.2%), relapsed or progressed. Median progression free survival (PFS) was 18.9 months; stage IIIIB or higher was statistically related to worse PFS in multivariate analysis (36.6 vs. 14.2 months; p=0.001). The median overall survival (OS) was 35.6 months
CONCLUSIONS: This is the larger published study focusing the response and outcome in advanced MF/SS patients treated with systemic chemotherapy and provides reliable information on what can be expected in this scenario. These results could be used as the basis for further studies comparing classical systemic chemotherapy with new targeted treatments.
POSTER SESSION A

A.5 Downregulation of PP2A-Cdc55 at anaphase onset by Zds1 and separase

Soraya Játiva, Inés Calabria and Ethel Queralt

Research Group: Cell cycle

Abstract: Exit from mitosis and completion of cytokinesis require the inactivation of mitotic cyclin-dependent kinase (Cdk) activity. In budding yeast, Cdc14 phosphatase is a key mitotic regulator that is activated in anaphase to counteract Cdk activity. In metaphase, Cdc14 is kept inactive in the nucleolus sequestered by its inhibitor Net1. At anaphase onset, downregulation of PP2A-Cdc55 phosphatase by separase and Zds1 protein promotes Net1 phosphorylation and consequently, Cdc14 release from the nucleolus. The mechanism by which Zds1 and separase impinge on PP2A-Cdc55 activity remains to be elucidated. Previous results show that Zds1 exert its biological function as PP2A-Cdc55 regulator, by controlling the subcellular localisation of the PP2A regulatory subunit Cdc55. Our previous results suggest that the activity of PP2A-Cdc55 cannot be modulated solely through regulation of its localization, and that an additional regulatory step may be required to control PP2A-Cdc55 activity during mitotic exit. Here we show that Cdc55 regulatory subunit is phosphorylated during anaphase upon PP2A-Cdc55 downregulation. Our results suggest that PP2A-Cdc55 activity is modulated throughout Cdc55 posttranslational modifications in a separase and Zds1-dependent manner.
A.6 Breath methane in functional constipation: response to treatment with Ispaghula husk

Ana Belen Vega López, Antònia Perelló, Lourdes Martos, Inmaculada García, Montserrat Garcia, Victoria Andreu, Aqueda Abad, Mercè Barenys

Research Group: Digestive tract pathology (Hospital de Viladecans)

Abstract:
Methane production has been associated with chronic constipation (CC) and with changes in gut motility. To determine CH4 production in CC compared to controls, and to assess whether the therapeutic response to Ispaghula husk in CC differs between CH4-producers (M+) and non-producers (M-).

METHODS: Forty-eight patients with chronic constipation and 19 healthy age- and-sex-matched volunteers (HV) filled in a 1-week symptom diary and a dietary questionnaire. They then underwent a lactulose breath test (LBT) to measure H2 and CH4 production (peak and area under the time-concentration curve, AUC-) and a colonic transit time (CTT) assessment. In patients, measurements were repeated after a 4-week treatment with Ispaghula husk.

RESULTS: Prevalence of M+ patients was 60.5% vs 52.6% in HV (p = 0.37). Patients had longer CTT and greater production of both H2 and CH4 during LBT. There was a significant correlation between CH4 production and CTT (r = 0.51; p = 0.07). Treatment response rate was similar for M+ and M- patients (58.3% vs 52.9%; p = 0.76) as were the increases in bowel movements and consistency, changes in abdominal discomfort and bloating. In M+, treatment reduced CTT (-10 ± 35 h; p = 0.029 vs baseline) and CH4 levels: peak CH4 (-13± 24 ppm;p = 0.014) and CH4-AUC (-817± 3100 ppm/min;p = 0.04).

CONCLUSIONS: Although CH4 production has been associated with CC pathophysiology, we found that CH4 status did not negatively affect the response to Ispaghula husk. Measurement of CH4 levels as a biomarker tool for CC requires further appraisal.
A.7 Multiband analysis of the amplitude of spontaneous low frequency fluctuations in obsessive compulsive disorder

Mónica Giménez, Andrés Guinea Izquierdo, Ignacio Martínez Zalacaín, Cinto Segalàs, Marta Subirà Coromina, Eva Real, Narcís Cardoner, Pino Alonso, J.Menchón, Carles Soriano Mas

Research Group: Psychiatry and Mental Health

Abstract:
Obsessive–compulsive disorder (OCD) is a common neuropsychiatric disorder characterized by alterations in fronto-subcortical brain circuits, although the extent of these functional abnormalities is still unclear. Neuroimaging studies, in general, and resting-state functional Magnetic Resonance Imaging (rs-fMRI) assessments, in particular, have provided relevant information for integrating alterations of fronto-subcortical circuitry in neurobiological models of OCD. Nevertheless, rs-fMRI studies have been mainly focused on examining between-region functional connectivity alterations at low frequency signal fluctuations (i.e., <0.08 Hz), but other resting-state related measurements, such as the amplitude of low frequency fluctuations (ALFF), may also provide valuable information, especially if assessed at different frequency bands. Here we used a voxel-wise approach to examine resting-state fractional ALFF (fALFF) values at four different frequency bands (from 0.01 Hz to 0.25 Hz) in a large sample of OCD patients (n=65) compared to a healthy control group (n= 50). OCD patients showed decreased fALFF values in medial orbitofrontal regions and increased fALFF values in the dorsal medial prefrontal cortex (DMPFC) at low frequency bands (<0.08 Hz). Interestingly, this pattern was reversed at higher frequency bands, where increased fALFF values also appeared in medial temporal lobe structures and medial thalamus. Moreover, clinical variables such as overall and symptom-specific severities were associated with fALFF values across the different frequency bands. Our findings provide novel data about the extent and regional distribution of functional brain alterations in OCD, which will help to further develop and refine neurobiological models of the disorder.
POSTER SESSION A

A.8 Blockade of proangiogenic Semaphorin 4D unravels a novel pro-invasive mechanism that involves both vascular pericytes and macrophages.

Iratxe Zuazo, Patricia Carrasco, Laura Martín, Marta Páez-Ribes, Julia Sallaberry, Oriol Casanovas.

Research Group: Tumoral Angiogenesis

Abstract:

One of the main consequences of vessel pruning and inhibition of neovessel growth by anti-angiogenic therapy is an increase in intra-tumoral hypoxia levels. Growing evidence indicates that tumor cells escape from this hypoxic environment to better nourished locations, thus presenting hypoxia as a positive stimulus for malignant invasion. Our group has previously described vascular targeting and antitumor effects of the blockade of Semaphorin 4D (Sema4D) pro-angiogenic molecule in the RIP1-Tag2 spontaneous mouse model of pancreatic neuroendocrine cancer. Anti-Sema4D therapy effectively impairs tumor growth and prolongs survival. Surprisingly, although there is no induction of intratumor hypoxia by anti-Sema4D therapy, the increase in local invasion and distant metastases are comparable with the ones produced by VEGFR inhibition. Using the RIP1-Tag2 mouse model we sought to describe the molecular foundations that regulate the hypoxia-independent mechanism triggering invasiveness and dissemination after anti-Sema4D therapy. In the present study, we characterize the expression of the receptors for Sema4D, PlexinB1 and CD72, and their contribution to the crosstalk mechanisms between endothelial cells and pericytes, suggesting a role for vascular cells in establishing the malignant phenotype. We also describe a mechanism that involves the recruitment of Sema4D-positive macrophages to the tumor invasive fronts and their release of a pro-invasive molecule that stimulates tumor cell aggressiveness and invasion. Our results unravel a novel mechanism of tumor aggressiveness that implicate Sema4D positive macrophages and Plexin B1 positive vascular cells that mediate local tumor cell invasion and distant metastasis.
A.9 Emotion Regulation Strategies and Frontolimbic Functional Connectivity

Maria Picó-Pérez, Narcís Cardoner, Pino Alonso, Marta Subirà, Clara López-Solà, Cinto Segalàs, Ignacio Martínez-Zalacaín, Eva Real, Ximena Goldberg, Rosa Hernández-Ribas, José M. Menchón, Carles Soriano-Mas

Research Group: Psychiatry and Mental Health

Abstract:

Emotion regulation may be thought of as the capacity of prefrontal cortex regions to regulate activity in limbic regions, but this frontolimbic connectivity may differ as a function of the emotion regulation strategy at use. In this study we aimed at differentiate the corticolimbic functional connectivity correlates of two emotion regulation strategies: cognitive reappraisal and suppression.

30 healthy controls completed the Emotion Regulation Questionnaire (ERQ) and underwent a resting-state functional magnetic resonance imaging acquisition. Functional connectivity (FC) of basolateral and centromedial-superficial amygdala (BLA/CMS) with the prefrontal cortex was estimated with a seed-based approach. Scores in the suppression and reappraisal factors of the ERQ were correlated with FC maps.

Overall, what we found was that FC between dorsomedial prefrontal regions and BLA, and between left dorsolateral prefrontal cortex and left CMS amygdala, correlated directly with suppression scores. Conversely, FC between the right ventrolateral prefrontal cortex and CMS amygdala correlated directly with reappraisal scores, while FC between ventromedial prefrontal regions and CMS amygdala correlated inversely with reappraisal capacity.

In conclusion, in a sample of healthy controls, suppression strategies appear to be more associated with dorsomedial prefrontal-BLA connectivity, while reappraisal strategies are positively associated with right ventrolateral prefrontal-CMS amygdala connectivity, and negatively with ventromedial prefrontal-CMS amygdala connectivity.
A.10 Fluorinated thiazolines induce apoptosis in human acute myeloid leukemia cells

Helena Pomares, Ana M Cosialls, Claudia M Palmeri, Daniel Iglesias-Serret, Cristina Moncunill-Massaguer, José Saura-Esteller, Sonia Núñez-Vázquez, Alba Pérez-Perarnau, Enric Gamundi, Montserrat Arnan, Gabriel Pons, Eva M González-Barca and Joan Gil

Research Group: Apoptosis and Cancer

Abstract:

Fluorinated thiazolines are a novel family of small molecules containing a thiazole scaffold and at least one fluor in the structure of the active compounds. The fluorinated thiazoline with the highest apoptosis-inducing activity is fluorizoline, previously termed compound 1a, which has been proven to selectively target prohibitins (PHBs). In this study, the proapoptotic effect of fluorizoline was assessed in two cell lines of acute myeloid leukemia (AML) with altered p53 status: U-937 and HL-60. Fluorizoline induced apoptosis in both cell lines with an EC50 in the low micromolar range (<10 µM) at 24 and 48 hours. Furthermore, the sensitivity to fluorinated thiazolines was also tested in 22 primary samples from newly diagnosed patients with AML. Most samples analyzed displayed substantial sensitivity to fluorizoline with an EC50<20 µM at 24 and 48 hours. In comparison, the structurally similar inactive analog called compound 2a, which does not contain any fluorine atom, was unable to induce apoptosis. Time-course experiments were performed in order to determine the expression of apoptosis-related genes through the transcriptional analysis by RT-MLPA and Western blot. Induction of NOXA mRNA and protein levels as well as the decrease of MCL1 protein levels were found as the apoptotic events induced by fluorizoline. Therapeutic interventions leading to remission and avoiding relapse are not available for many hematologic cancers, being particularly evident AML. These results suggest that targeting PHBs could be a new and promising therapy for leukemia and other hematologic neoplasias.
A.11 Macrophage cell therapy for renal regeneration

R. Guiteras, M Flaquer, G. Hotter, A. Sola, JM Cruzado

Research Group: Experimental Nephrology

Abstract:

BACKGROUND. Alternatively activated macrophages (M2) have regenerative and anti-inflammatory characteristics. The aim of this study was to evaluate whether bone marrow-derived M2 macrophages (BM-M2) cell therapy induced tissue repair in the UUO mice model of chronic kidney damage.

METHODS. UUO surgery was performed on 8 week-old C57BL6J male mice. At day 7 after surgery, 1x10^6 cells were injected via the tail vein. Transfused macrophages were examined by immunofluorescence staining and in vivo tracking. Mice were killed and evaluated on day 9 and day 15 after UUO surgery. Tubular injury, interstitial fibrosis and pro-inflammatory cytokines were evaluated. A group of n=2 mice were used for in vivo BM-M2 macrophage cell therapy tracking and cell sorting.

RESULTS. In vivo tracking demonstrated that infused macrophages got into the obstructed kidney. However, UUO mice treated with BM-M2 macrophage cell therapy, showed similar structural renal damage and markers of fibrosis (IFTA, fibronectin, α-SMA, TGF-β1, MMP-9) as well as similar pro-inflammatory cytokine gene expression (MCP-1, IL-6, iNOS, CD40) and tubular markers (aquaporin-1, megalin, NGAL) at day 9 and 15 than controls. We further assessed the phenotype of infused M2 in the UUO kidney by confocal microscopy and cell-sorting. Interestingly, we found a high percentage (75%) of switching to M1. In order to overcome the plasticity of M2 performed cell therapy of genetically modified M2 stabilized cell line (RAW264.7) in the UUO model. We found that kidney pro-inflammatory cytokine levels such as MCP-1, MMP9 and iNOS as well as kidney fibrosis were significantly reduced. Moreover, by confocal microscopy and isolation of the exogenous M2-RAW from the whole kidney we demonstrated that M2 phenotype was more preserved.

CONCLUSIONS. Our findings demonstrate that BM-M2 cell therapy does not provide therapeutic effect in the UUO model of renal fibrosis because infused BM-M2 cells switch their phenotype to M1. Stabilized M2 cell therapy is able to overcome this limitation and reduce renal inflammation and fibrosis.
POSTER SESSION A

A.12 Uncovering the role of lysyl oxidase-like 2 in rhabdomyosarcoma.

**Olga Almacellas-Rabaiget**, Juan Huertas-Martínez, Silvia García-Monclús, Santiago Rello-Varona, David Herrero-Martin, Roser López-Aleman and Oscar M. Tirado

**Research Group: Sarcoma Research**

**Abstract:**

Rhabdomyosarcoma (RMS) is the most common soft tissue malignancy in childhood and adolescence that originates as a consequence of regulatory disruption in the growth and differentiation of muscle precursor cells. Previous results of our group deciphered the epigenetic profiling of RMS, compared to healthy tissue, by Infinium 450k Illumina methylation array. This analysis revealed the hipomethylation of a potential enhancer of lysyl oxidase-like 2 (LOXL2). LOXL2 is an amine oxidase that catalyzes the covalent crosslinking of collagen and elastin in the extracellular matrix. Apart from its traditional role, novel functions have been attributed to both intra- and extracellular LOXL2 related to tumor progression. In this study we first confirmed the overexpression of LOXL2 in RMS cell lines compared to other sarcoma cells by qRT-PCR and Western blot. We also functionally characterized LOXL2 in RMS cell lines and in myogenic cells, used as control cells. The functional analysis of LOXL2 included: subcellular localization by immunofluorescence and subcellular fractionation; post-translational modifications: glycosylation and proteolytic processing by tunicamycin treatment and secretion by analysis of the conditioned media by Western blot. Moreover, we characterized the neoplastic phenotype after silencing LOXL2 expression in RH4 RMS cell line. LOXL2 silencing resulted in a decrease in the clonogenic capacity and cell migration. On the other hand, gain of function experiments in LOXL2 under-expressed cells (RH28) using wild type or mutated (catalytically inactive) constructs of LOXL2 will be crucial to further explore its role in these tumors. Our preliminary results suggest an oncogenic role of LOXL2 in RMS.
POSTER SESSION A

A.13 Deciphering the role of peripheral and central nervous system metabotropic glutamate receptors in pain: An optopharmacological approach

Marc López-Cano, Joan Font, Jesús Giraldo, Amadeu Llebaria, Francisco Ciruela

Research Group: Neuropharmacology and Pain

Abstract:

Metabotropic glutamate (mGlu) receptors are G protein-coupled receptors (GPCRs) which are activated by glutamate, thus modulating synaptic transmission both in the central and peripheral nervous system. Interestingly, mGlu receptors have been related to pain transmission, thus constituting an attractive drug target for pain treatment. Indeed, the blockade of mGlu5 receptor with selective negative allosteric modulators (NAMs) has been recently proposed for the treatment of pain symptomatology.

In collaboration with Amadeu Llebaria (CSIC, Barcelona) we developed several photoactivable mGlu5 receptor compounds to control pain transmission at the peripheral and central nervous system level. Interestingly, these molecules might serve not only to understand how these receptors participate in pain transmission but also might be refined with some pharmacotherapeutic purposes. Accordingly, we synthesized some mGlu5 receptor NAM-caged compounds using the rasaglurant molecule as a template.

Overall, our optopharmacological approach using light-activable compounds allowed us to manipulate pain transmission with high spatio-temporal resolution both at the peripheral and central level of behaving animals.
The etiology, incidence, and impact of preservation fluid contamination during liver transplantation

Isabel Oriol, Laura Lladó, Marina Vila, Carme Baliellas, Fe Tubau, Núria Sabé, Joan Fabregat and Jordi Carratalà

Research Group: Infections of the respiratory tract and in the immunocompromised patients

Abstract:

BACKGROUND: The role of contaminated preservation fluid in the development of infection after liver transplantation has not been fully elucidated. We reviewed the incidence and etiology of contaminated preservation fluid to determine its impact on the subsequent development of infection after liver transplantation.

METHODS: We prospectively studied 50 consecutive liver transplants, and cultured the following samples in each instance: preservation fluid (immediately before and at the end of the back-table procedure, and just before implantation), blood, and bile from the donor, and ascitic fluid from the recipient. When any culture was positive, blood cultures were obtained and targeted antimicrobial therapy was started.

RESULTS: The incidence of contaminated preservation fluid was 92% (46 of 50 cases of liver transplantation per year), but only 26% (13/50) were contaminated by recognized pathogens. Blood and bile cultures from the donor were positive in 32% and 6% respectively, whereas ascitic fluid was positive in 22%. The most frequently isolated microorganisms were coagulase-negative staphylococci. In eight cases, the microorganisms isolated from the preservation fluid concurred with those grown from the donor blood cultures, and in one case, the isolate matched with the one obtained from bile culture. No liver transplant recipient developed an infection due to the transmission of an organism isolated from the preservation fluid.

CONCLUSION: Contamination of the preservation fluid is frequent in liver transplantation, and it is mainly caused by saprophytic skin flora. Transmission of infection is low, particularly among those recipients given targeted antimicrobial treatment for organisms isolated in the preservation fluid.
POSTER SESSION A

A.15 Proteomic and imaging studies to study the mechanism underlying trafficking of the RD3/retGC/GCAPs complex in photoreceptor cells

Plana Anna, López-Begines S., Andilla J., Loza-Alvarez P., Méndez A.

Research Group: Molecular and cellular basis of sensory disorders

Abstract:

In photoreceptor cells of the retina, cGMP acts as the second messenger in the light response. Retinal guanylyl cyclase (RetGC) is the enzyme responsible for cGMP synthesis, and its correct transport, activity and regulation is absolutely required for rod and cone photoreceptor survival and proper function. Mutations in RetGC lead to Leber’s Congenital Amaurosis (LCA) which represents the earliest and most severe form of retinal dystrophy, accounting for at least 5% of all inherited retinopathies. Recently the group of Dr. Robert Molday in Canada has identified RD3, the protein encoded by a novel gene that has been linked to LCA, as a RetGC interacting protein that appears to assists its stability and trafficking to the sensory cilium. Little is known about the overall trafficking mechanism of the RetGC complex to the rod outer segment or sensory antenna of the cell, a central question in photoreceptor cell biology. We here report the generation and characterization of specific antibodies to RetGC and RD3, and their use in immunolocalization assays on bovine retinal sections, with co-markers of different cellular compartments and landmark cytoskeletal structures. Our results indicate that the RetGC/RD3/GCAP1 complexes move along actin filaments from their site of synthesis to the base of the connecting cilium. This is the first indication of the involvement of actin-based transport in this process. We are carrying parallel proteomic analysis, by performing RD3 pull-down assays from bovine retinal extracts, in order to identify new protein interactors of RD3 that may reveal its role in the process.
A.16 Set up of an in vitro mismatch repair assay in a diagnostic laboratory

González-Acosta Maribel; Pineda, Marta; Hinrichsen, Inga; Fernández, Anna; Rueda, Daniel; Balmaña, Judith; Lázaro, Conxi; Plotz, Guido; Capellá, Gabriel

Research Group: Hereditary cancer

Abstract:
A significant proportion of DNA mismatch repair (MMR) variants identified in suspected Lynch syndrome patients are missense. They are classified as variants of unknown significance (VUS) precluding diagnosis. One key step to sort out uncertainty is to determine whether the variants result in non-functional proteins. The in vitro MMR assay is used to assess the mismatch repair, likely the most important function of a MMR protein. However, robustness of the assay, critical for its routine use in the clinical setting, requires technical specialization and accurate reagent preparation. Also, standardized protocols are lacking.

The aim of the present work was to set up the in vitro MMR assay for the functional characterization of VUS in MLH1 and PMS2 genes meeting quality control standards.

Reference materials and standardized operative procedures (SOP) for HEK293T cells transfection, whole cell protein extraction, nuclear extraction, mismatched plasmid substrate generation, repair buffer, and MMR assay were provided by Dr. Plotz and optimized in our laboratory. Monitoring of cell lysis for nuclear extraction was assessed by trypan blue staining and enrichment for nuclear extract proteins by western blot. Average protein concentration in nuclear extracts was 4.7 µg/µl. Transfection efficiency was up to 60% and protein concentration of whole cell extracts was about 10 µg/µl. Use of HPLC-purified oligomers and verification of complete digestion improved the quality of the mismatched plasmid. Control plasmids were used in each experiment. Assay performance was preliminarily validated with MLH1 D41H VUS which showed a decreased activity (23%±6 of the wildtype level) with minimal interexperimental variability, supporting its pathogenicity.

High quality reagents and optimized protocols are critical to standardization of the in vitro MMR repair assay allowing to achieve robust and clinically interpretable results.
Defining the inheritance pattern of MLH1 epimutations helps in the genetic counseling of families

Estela Dámaso, Marta Pineda, Mar Arias, Pilar Mur, Sira Moreno, Edurne Urrutia, Matilde Navarro, Joan Brunet, Conxi Lázaro, Ángel Alonso, Capellá, Gabriel

Research Group: Hereditary cancer

Abstract: Constitutional epimutations in MLH1 have been identified in a subset of Lynch syndrome patients (0-2%). Two types of constitutional MLH1 epimutations have been defined: primary epimutations, which arise de novo and are reversible between generations, and secondary epimutations, linked to in cis genetic alterations and dominantly transmitted.

PURPOSE: The aim of this study was the analysis of the inheritance pattern of 3 constitutional MLH1 epimutations identified in a Spanish series of Lynch syndrome patients.

METHODOLOGY: Mutational analysis of promoter and intron 1 was performed by Sanger sequencing. MLH1 methylation in blood DNA was assessed by MS-MLPA. Clonal bisulfite sequencing of the promoter was used to determine the methylated allele. Inheritance pattern was determined by haplotype analysis in probands' first-degree relatives.

RESULTS: The MLH1 epimutant carriers included in this study developed multiple Lynch syndrome tumors at early age. Case 1 is a 49-year-old male who was diagnosed with two colorectal cancers at ages 32 and 34. The patient has no history of cancer in his-first degree relatives. Case 2 is a 57-year-old female affected by two colorectal cancers at ages 29 and 44 and endometrial cancer at age 49. Her mother was affected by breast cancer at age of 77 years. Case 3 is a 60-years-old female diagnosed with colorectal cancer at ages 37 and 59, endometrial cancer at 43 and kidney cancer at 55. Patient’s mother was diagnosed with endometrial cancer at age 50. Reported alterations associated with secondary epimutations were not detected in the MLH1 promoter of the probands. Only frequent SNPs were identified: cases 1 and 3 were heterozygous for rs1800734 and case 2 was heterozygous for rs34566456. No evidence of MLH1 methylation was found in available probands' relatives: the father and two daughters of case 1, four sisters of case 2, and two sisters and two children of case 3. In PBL DNA of cases 1 and 3, clonal bisulfite sequencing confined methylation to allele A of rs1800734. Subsequent haplotype analysis in these cases revealed that MLH1 methylation was reversed in children who inherited the proband epimutated allele. The observed intergenerational erasure allowed classifying these epimutations as primary. In case 2, although haplotype analysis was not performed, the absence of methylation in proband sisters also suggested the primary type of the epimutation. In addition, methylated allele was maternally transmitted in case 1. The lack of availability of samples precluded the parental origin analysis of the remaining two cases.

CONCLUSION: The inheritance pattern analysis of MLH1 epimutations is essential for determining the type of epimutation. This classification is relevant for assessing the risk of epimutation transmission and genetic counseling of these families.
A.18 Electrophysiological correlates of semantic anticipation during speech comprehension

Patricia León Cabrera, Joaquín Morís Fernández, Antoni Rodríguez Fornells

Research Group: Brain Plasticity and Cognition

Abstract:

Words that are more predictable given a previous context show facilitated processing over low predictable ones. Such facilitation has been traditionally viewed as associated with reduced amplitudes in the N400 component. However, this facilitation effect could be due to words being predicted or because they are easier to integrate with their context after they appear. A way to understand the contribution of each of these processes is to test the possible existence of neural correlates of anticipation prior to target words. We did so by inserting a 1000 ms delay between the penultimate and the final word of sentences of different semantic constraining levels, and measuring the event related potentials generated during that period. In addition, half of the final words were congruent with the context while the other half were incongruent. A slow potential with a frontal distribution developed during the delay interval, beginning 200 ms after the start of the delay and increasing progressively until the presentation of the final word. The amplitude of the slow potential varied as a function of contextual constraint, larger for low than high contextual constraint, suggesting that it indexed anticipatory processes. On the other hand, amplitude variations elicited by final words were not consistent with an interpretation of the N400 based exclusively on predictive processes. Our results provide strong support to theories of semantic anticipation although they require of additional processes to be adequately explained.
POSTER SESSION A

A.19 Validation of Prohibitin as the target for novel apoptosis-inducing compound fluorizoline

José Saura-Esteller, Cristina Moncunill-Massaguer, Alba Pérez-Perarnau, Claudia Mariela Palmeri, Sonia Núñez-Vázquez, Ana M. Cosialls, Diana M. González-Gironès, Helena Pomares, Anne Korwitz, Sara Preciado, Fernando Albericio, Rodolfo Lavilla, Gabriel Pons, Thomas Langer, Daniel Iglesias-Serret, and Joan Gil

Research Group: Apoptosis and Cancer

Abstract:

Fluorinated thiazolines are new synthetic compounds that induce p53-independent apoptosis in a wide range of tumor cell lines. Among all the thiazolines analyzed, compound 1a (fluorizoline) was selected due to its strong pro-apoptotic activity. High-performance affinity purification with magnetic nanobeads proved that fluorizoline binds directly to prohibitin 1 and 2 (PHBs), two proteins involved in the regulation of cell survival, apoptosis, metabolism and inflammation. Treatment with fluorizoline induces mitochondrial fragmentation, cristae disorganization, and cytochrome c release from the mitochondria. Depletion of PHBs in MEF cells provided strong resistance to fluorizoline treatment, strongly pointing Prohibitin as a molecular target for fluorizoline. OPA1 is an IMM protein which is cleaved upon apoptotic stresses by OMA1 protease and is normally protected by Prohibitin presence in the mitochondria. However overexpression of a non-cleavable form of OPA1 and lack of OMA1 did not render cells more resistant to fluorizoline-induced apoptosis. Fluorizoline produced increases in ROS production, which were found to be independent of PHB presence in the cell. Prevention of ROS production by fluorizoline with MTBAP did not modify cell viability at any extent, suggesting that fluorizoline-induced increases in ROS are not necessary for its activity. Our work demonstrates that fluorizoline requires PHBs to induce apoptosis.
A.20 Motor rehabilitation induced by Music Supported Therapy in sub-acute stroke patients: a single-case study

Neus Ramos Escobar, Jennifer Grau Sánchez, Antoni Rodríguez Fornells and Esther Duarte

Research Group: Cognition and Brain Plasticity

Abstract:

Stroke is the condition most strongly associated with long-term disability in developed countries. The most frequent impairment in stroke patients are motor disabilities. Music Supported Therapy (MST) has been developed (Schenider et al., 2007) as a complementary therapy to restore the upper limb function in stroke patients. Several studies have demonstrated the effectiveness of the therapy, however, there are no previous studies that investigated how the improvement progress along the treatment. The purpose of this study is to see the progression of the motor improvements due to the treatment over the time occurring. We performed a single-subject experimental procedure with an ABAB design with two chronic stroke patients. The design consisted of 4 different phases of four weeks each one. The first phase (Baseline) consisted in evaluating the patients in the motor domain, once a week, during 4 weeks. During the second phase (MST1) the treatment was applied during the 4 weeks and the patients were evaluated again in the motor domain, once a week. In phase 3 (Withdrawal) and 4 (MST2), we replicated the Baseline and MST1 respectively. Our results indicate improvements during the treatment phases but not during the Baseline and Withdrawal phases. Moreover we have found that the ameliorament of the motor function follow a progressive increment along the weeks in some parameters but not in others. In conclusion, the results support previous findings demonstrating the effectiveness of MST and highlight the fact that not all the elements of the recovery process proceed evenly.
A.21 MYC-amplification predicts sensitivity to in vivo GC/RA-based treatments

Octavio A Romero, Sara Verdura, Manuel Torres-Diz, Antonio Gomez, Sebastian Moran, Enric Condom, Manel Esteller, Alberto Villanueva, Montse Sanchez-Cespedes

Research Group: Genes and Cancer

Abstract:

INTRODUCTION: BRG1 inactivation and MYC amplification are common alterations and mutually exclusive events in lung cancer. BRG1-containing SWI/SNF complexes work to activate or repress transcription of certain genes by altering the chromatin structure and orchestrate the response to retinoid acid (RA) and glucocorticoids (GCs) among other functions. In vitro GC/RA treatment reduces growth, triggers pro-differentiation gene expression signatures and down-regulates MYC in MYC-amplified lung cancer cell lines. In contrast, most lung cancer cells lacking BRG1 do not respond to RA and GC treatment.

OBJECTIVES: To test the in vivo effectiveness of GC/RA treatment in MYC-amplified lung cancers and to determine whether the combination of this treatment (GC/RA) with epigenetic drugs azacitidine and SAHA (A/S) is an effective in vivo therapy in lung cancers carrying BRG1 inactivation.

RESULTS: In vivo GC/RA treatment reduced tumor-cell viability, cell proliferation and improved overall survival of mice implanted with the MYC-amplified cells, but not of mice implanted with the BRG1-mutant cells. The combination with A/S did not improve overall survival or have an effect in necrosis or cell proliferation.

CONCLUSIONS: RA/GC-based treatments, used as a single combination or in combination with epigenetic drugs or with other therapies, may have a therapeutic window in lung cancer patients bearing amplification at any of the MYC-family genes.
POSTER SESSION A

A.22 A novel prohibitin-binding compound induces the mitochondrial apoptotic pathway mainly through NOXA upregulation.

_Sonia Núñez-Vázquez_, José Saura-Esteller, Cristina Moncunill-Massaguer, Alba Pérez-Perarnau, Claudia Mariela Palmeri, Ana M. Cosials, Diana M. González-Gironès, Helena Pomares, Anne Korwitz, Gabriel Pons, Thomas Langer, Daniel Iglesias-Serret, and Joan Gil.

Research Group: Apoptosis and Cancer

Abstract:

We previously described diaryl trifluorothiazoline compound 1a (hereafter referred to as fluorizoline) as a first-in-class small molecule that induces p53-independent apoptosis in a wide range of tumor cell lines. Prohibitin 1 and 2 (PHBs), two proteins involved in the regulation of several cellular processes, including apoptosis, are the targets of fluorizoline, as cells depleted of these proteins are highly resistant to fluorizoline treatment.

Here we demonstrate that BAX and BAK are necessary for fluorizoline-induced cytotoxic effects, thereby proving that apoptosis occurs through the intrinsic pathway. The fact that fluorizoline was able to induce apoptosis in WT Jurkat cells, while overexpression of BCL-XL conferred resistance to the pro-apoptotic effects of fluorizoline, confirm the importance of the activation of the intrinsic apoptotic pathway.

In addition, expression analysis revealed that PHBs mediate the modulations of the expression of various BCL-2 family members upon fluorizoline treatment, which was not present in PHB-depleted MEFs. We also observed a time-dependent upregulation of NOXA and BIM in HeLa cells. Furthermore, Noxa-/Bim-/- MEFs and NOXA-downregulated HeLa cells were resistant to the pro-apoptotic effects of fluorizoline.

All together, these data demonstrate that fluorizoline induces the mitochondrial apoptotic pathway predominantly through the upregulation of NOXA and, depending on the cell type, also of BIM.
A.23 TIGAR modulation in wild type and mutated TP53 cancer cell lines: the crosstalk with PFKFBs

**Helga Simon-Molas**, Ana Rodríguez-García, Àurea Navarro-Sabaté, Esther Castaño, María Nieves Calvo-Vidal, **Ramon Bartrons** and Anna Manzano

**Research Group**: Nutrition, Metabolism and Gene Therapy

**Abstract:**

Metabolic reprogramming is a key step in tumor initiation and proliferation. One of the key enzymes involved in this phenotype is 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2), which can be codified by four different genes (PFKFB 1-4). Among them, the most studied one in cancer is PFKFB3, which has been found overexpressed in human tumors. The product of PFK-2 is fructose-2,6-bisphosphate (Fru-2,6-P2), the main positive allosteric regulator of the glycolytic enzyme phosphofructokinase-1 (PFK-1).

TP53-Induced Glycolysis and Apoptosis Regulator (TIGAR) is a bisphosphatase enzyme that converts Fru-2,6-P2 into fructose-6-phosphate, thus inhibiting glycolysis and triggering glucose-6-phosphate to the pentose phosphate pathway. TIGAR is expressed in a wide range of tumors lacking TP53. Therefore, it is interesting to study TIGAR modulation in both TP53-wild type and mutated cancer cell lines.

Previous work demonstrated that some oxidant molecules can trigger TIGAR induction, but there are no previous references about TIGAR modulation in response to oxidative stress caused by metabolic alterations. Here we report that PFKFB3 silencing triggers Akt signaling pathway and TIGAR induction in HeLa, which results in a senescent phenotype. On the other hand, the same stimulus triggers AMPK phosphorylation and autophagy in the HCT 116 cell line. Whether TP53 status explains these differences has not been clarified yet. However, we describe here an Akt-dependent TIGAR induction in the absence of TP53 function, supporting the idea that other transcription factors are involved in TIGAR regulation.
c-Myc is a heterodimeric transcription factor which binds to E-box motifs, regulating the expression of genes involved in anabolic cell proliferation. Over 90% of sporadic colorectal carcinomas (sCRC) analyzed by TCGA show mutations in the Wnt/APC/β-catenin pathway leading to persistent c-Myc activation. The underlying intrinsic tumor mechanisms that discriminate c-Myc’s role in normal cell proliferation versus its oncogenic role when persistently activated are not well understood. Recently, our laboratory has identified a preribosomal complex, made-up of ribosomal protein (RP)L5, RPL11 and non-coding 5S rRNA, which in response to impaired or hyperactivated ribosome biogenesis binds to Hdm2, independent of the ARF tumor suppressor, to stabilize p53 and induce apoptosis. Consistent with these findings, hypomorphic mutations in RPs limit c-Myc’s ability to drive oncogenesis and inhibition of ribosome biogenesis in c-Myc driven tumors leads to their selective apoptosis. However, it has been shown that oncogenic c-Myc can selectively mediate mTORC1 phosphorylation of initiation factor eIF4E binding protein (4E-BP), allowing upregulation of mRNA translation, principally the RP mRNA family. This suggests that oncogenic c-Myc not only drives transcriptional activation of ribosome biogenesis to facilitate high rates of protein synthesis, but also steers the translational machinery. Here, we will discern the ability of oncogenic c-Myc to selectively signal to 4E-BP versus its effects on global translation, and determine the effects of deregulated c-Myc on transcription versus translation employing ribosome profiling in cell lines that express different levels of c-Myc fused to the Estrogen Receptor (ER), whose activation is achieved by treatment with 4-hydroxy-tamoxifen.
INTRODUCTION: Lymphangioleiomyomatosis (LAM) is a rare lung disease primarily affecting women of childbearing age and characterized by cystic lung destruction. The disease is caused by loss-of-function mutations in tuberous sclerosis complex 1 and 2 (TSC1/2) genes. The origin of LAM cells remains unclear, but diverse data indicate that it might be a different organ to the lung. In breast cancer, lung metastasis events have been associated with low expression of TSC1/2. Moreover, the expression of these genes correlates with canonical mediators of lung metastasis.

HYPOTHESIS: The transcriptional programs of breast cancer that metastasize to lung could be similar to those of LAM cells.

RESULTS: First, we have shown that lung metastasis mediators and breast stem cell markers are relatively overexpressed in LAM tissues and cell models. Then, following this evidence, in silico analysis of gene expression correlations was performed to predict novel drug targets and surface markers for LAM. The assessment of the predictions has shown the presence of some of these markers in LAM patient tissues and cellular models.

CONCLUSION AND FUTURE WORK: Collectively, this study reveals novel LAM biomarkers linked to breast cancer that metastasize to lung, which in turn could provide a better characterization, the possibility to use them as a tool to isolate LAM cells and/or therapeutic opportunities for LAM.
A.26 Music-supported Therapy in the neurorehabilitation of motor deficits after a stroke

Jennifer Grau Sánchez, Joanna Sierpowska, Neus Ramos Escobar, Nohora Rueda Moreno, Susana Redón Bolós, Misericordia Veciana de las Heras, Esther Duarte Oller and Antoni Rodríguez Fornells

Research Group: Cognition and Brain Plasticity

Abstract:

Motor deficits are the most common outcome after a stroke, affecting the ability of patients to accomplish everyday life activities. Because of the impact in the life of patients and the associated financial costs, it is a health care priority to develop effective and efficient treatments to restore motor deficits.

Music-supported therapy (MST) has been recently developed to enhance the use of the affected upper extremity after a stroke. In MST patients are trained to play a piano and electronic drum pads in order to improve fine and gross movements, respectively. Based on basic evidence from neuroscience regarding the neuroplastic changes after learning to play an instrument, MST is aimed to promote brain plasticity in motor and pre-motor brain regions affected after a stroke. Previous studies have shown motor gains associated to MST in acute and chronic stroke patients. However, the effects of MST have not been appropriately contrasted with conventional therapy and no previous study has evaluated the impact in the mood and quality of life of patients that could be associated to motor gains.

In the present project, a randomized controlled trial is being conducted to compare for the first time the effectiveness of MST (n=20) compared to conventional treatment (n=20) in subacute stroke patients. In our design, motor functions are evaluated using 3-D movement analysis and behavioural motor tests as well as cognitive functions and quality of life are assessed before and after the treatment. Moreover, functional and structural changes in the brain of patients are evaluated using Magnetic Resonance Imaging. At this stage of the project, we have included 10 participants in each group of treatment and can present behavioural data regarding motor, cognitive and emotional gains.
A.27 Generation of a novel cell culture system to derive human midbrain specific neuroepithelial stem cells

Giulia Carola, Neus Bayò Puxan, Angelique Di Domenico, Armida Faella, Irene Fernàndez Carasa, Angel Raya, Antonella Consiglio

Research Group: Stem Cell and Neuronal Plasticity - IBUB

Abstract:

Parkinson’s Disease (PD) is clinically characterized by motor impairments, such as tremor, rigidity and bradykinesia, although non-motor features are also important in later stages of the disease. Neuropathologically, it is characterized by progressive preferential loss of striatal projecting neurons of the substantia nigra pars compacta, a specific subtype of dopaminergic neurons (DA n) patterned as ventral midbrain (vmDan).

Induced pluripotent stem cells (iPSC) offer an unprecedented opportunity to model human disease, since they can be generated from patients and differentiated into disease-relevant cell types, including neurons, which would capture the patients’ genetic complexity. In our laboratory it has been developed a protocol for the stable long-term cultivation of Da neurons derived from human induced pluripotent stem cells.

However, currently differentiation always results in mixed cultures consisting of several cell types. Particularly, differentiation into dopaminergic neurons, which are of essential importance for phenotypic screening in Parkinson’s disease, results also in the generation of serotonergic and GABAergic neurons. Consequently, future screening campaigns with such cultures will suffer from these limitations. In this project we aim on the generation of neuroepithelial stem cell cultures that are specific for the developing midbrain (midbrain NESC). This will be achieved by identifying the optimal combination of growth factors and signaling molecules mimicking the developing human midbrain. As preliminary results we found that greater than 45% of all neurons generated by using this modified protocol, were ventral midbrain DA neurons of the A9 subtype, as judged by specific marker expression such as Girk2 and FoxA2. Functional studies are being performed. These cultures will be the ideal starting point for phenotypic screening as well as for transplantation based cell replacement approaches.
A.28 Human oscillatory activity in near-miss events

Helena Alicart, David Cucurell, Ernest Mas-Herrero, Josep Marco-Pallarés

Research Group: Cognition and Brain Plasticity

Abstract:

Near-miss events are situations in which an action yields a negative outcome but very close to the goal. They are known to influence behavior, especially in gambling scenarios. Previous fMRI studies have described an “anomalous” activity of brain reward areas following near-misses. Nevertheless, the participation of these areas in the expectation and in the outcome phase cannot be easily disambiguated with the fMRI technique. The goal of the present research was to study electrophysiological correlates in the expectation and outcome phases of near-miss events.

EEG was recorded while participants were playing in a simplified version of a slot machine. Four possible outcomes were presented in a balanced pseudorandom order to ensure fixed proportions: gain (p=1/7), near-miss (p=2/7), loss (p=3/7) and no-information (designed as a control condition, p=1/7).

In the outcome phase, time-frequency analysis for the theta (4-8 Hz), alpha (9-13 Hz), low beta (15-22 Hz) and beta-gamma (25-35 Hz) frequency bands showed larger power increases for wins and near-misses compared to losses. In the anticipation phase, power changes for these frequency-bands were lower than in the resolution phase. P300 ERP analysis also showed larger amplitude in near-miss responses compared to losses.

The current pattern of results is in agreement with previous neuroimaging studies showing that near-miss events recruit brain areas of the reward network. Likewise, these results show that near-misses are not processed as losses, but their oscillatory responses are very similar to the ones elicited in the gain condition in the outcome phase.
POSTERS SESSION
B
POSTER SESSION B

B.1 Modulation of limbic and prefrontal connectivity by electroconvulsive therapy in treatment-resistant depression: a preliminary study

Marta Cano, Narcís Cardoner, Mikel Urretavizcaya, Ignacio Martínez-Zalacaín, Ximena Goldberg, Esther Via, Oren Contreras-Rodríguez, Joan Camprodon, Aida de Arriba-Arnaú, Rosa Hernández-Ribas, Jesús Pujol, Carles Soriano-Mas, José M. Menchón

Research Group: Psychiatry and Mental Health

Abstract:

BACKGROUND: Although current models of depression suggest that a sequential modulation of limbic and prefrontal connectivity is needed for illness recovery, neuroimaging studies of electroconvulsive therapy (ECT) have focused on assessing functional connectivity (FC) before and after an ECT course, without characterizing functional changes occurring at early treatment phases.

OBJECTIVE: To assess sequential changes in limbic and prefrontal FC during the course of ECT and their impact on clinical response.

METHODS: Longitudinal intralimbic and limbic-prefrontal networks connectivity study. We assessed 15 patients with treatment-resistant depression at four different time-points throughout the entire course of an ECT protocol and 10 healthy participants at two functional neuroimaging examinations. Furthermore, a path analysis to test direct and indirect predictive effects of limbic and prefrontal FC changes on clinical response measured with the Hamilton Rating Scale for Depression was also performed.

RESULTS: An early significant intralimbic FC decrease significantly predicted a later increase in limbic-prefrontal FC, which in turn significantly predicted clinical improvement at the end of an ECT course.

CONCLUSIONS: Our data support that treatment response involves sequential changes in FC within regions of the intralimbic and limbic-prefrontal networks. This approach may help in identifying potential early biomarkers of treatment response.
B.2 Personality patterns to predict food addiction in patients with eating disorders

Ines Wolz*, Ines Hilker*, Roser Granero; Susana Jiménez-Murcia, Ashley N Gearhardt; Carlos Dieguez; Felipe F. Casanueva; Ana B. Crujeiras; José M Menchón & Fernando Fernández-Aranda

* Shared first authorship

Research Group: Eating Disorders Unit

Abstract:

OBJECTIVES: The present study aimed to investigate if ED patients differ in specific personality traits depending on a positive screening of food addiction and to find a model to predict food addiction in eating disorder patients using measures of personality and impulsivity.

METHODS: 278 patients having an eating disorder self-reported on food addiction, impulsivity, personality, eating and general psychopathology. Patients were then split into two groups, depending on a positive or negative result on the food addiction screening. Analysis of variance was used to compare means between the two groups. Stepwise binary logistic regression was used to obtain a predictive model for the presence of food addiction.

RESULTS: Patients with food addiction had lower self-directedness, more negative urgency and a higher lack of perseverance than patients without this “diagnosis”. The probability of food addiction can be predicted by high negative urgency, high reward dependence, and low lack of preméditation.

CONCLUSIONS: Eating disorder patients who have more problems to pursue tasks to the end and to focus on long-term goals seem to be more likely to develop addictive eating patterns.
B.3 Declining Mortality among Hospitalized Patients with Community-Acquired Pneumonia

Antonella F. Simonetti, Carolina Garcia-Vidal, Diego Viasus, Dolors García-Somoza, Jordi Dorca, Francesc Gudiol, Jordi Carratalà

Research Group: Infections of the Respiratory Tract and in Immunocompromised Patients

Abstract:

Little information is available on the changes over time in community-acquired pneumonia (CAP) management and their impact on 30-day mortality in hospitalized patients. We performed a prospective, observational study of non-severely immunosuppressed hospitalized adults with CAP from 1995 to 2014. 4558 patients were included. Thirty-day mortality decreased from 9.6% in the first study period (1995-1999) to 4.1% in the last period (2010-2014); with a progressive downward trend (-0.2% death/year; P for trend=.003]). Over time, patients were older (P=.02), had more comorbidities (P=.037), more frequently presented severe illness according to the PSI (P<.001), and septic shock (P<.001), and more often required intensive care unit admission (P<.001). Combination antibiotic therapy (P<.001) and fluoroquinolone use (P<.001) increased. Factors independently associated with 30-day mortality were increasing age (OR 1.04; 95% CI, 1.03 – 1.05), comorbidities (OR, 1.48; 95% CI, 1.04 - 2.11), shock at admission (OR, 4.95; 95% CI, 3.49 – 7.00), respiratory failure (OR, 1.89; 95% CI, 1.42 – 2.52), bacteremia (OR, 2.16; 95% CI, 1.58 – 2.96) and Gram-negative bacilli etiology (OR, 4.79; 95% CI, 2.52 – 9.10). Fluoroquinolone use as empiric treatment either in monotherapy or in combination (OR 0.45; CI 0.29 – 0.71) was independently associated with a decrease in mortality. In conclusion, Thirty-day mortality decreased significantly over time in hospitalized patients with CAP in spite of an upward trend in patient age and other factors associated with poor outcomes. The use of fluoroquinolones as empiric treatment was independently associated with better survival over time.
POSTER SESSION B

B.4 Unraveling the oncogenic role of EphA2 receptor in Ewing Sarcoma

Silvia García-Monclús, Juan Huertas-Martinez, Laura Lagares-Tena, Santiago Rello-Varona, David Herrero-Martin, Roser López-Alemany and Oscar M. Tirado

Research Group: Sarcoma Research

Abstract:

Ewing sarcoma (ES) is the second most common bone malignancy, affecting mainly children and young adults. This highly aggressive tumor of mesenchymal origin harbors a characteristic chromosomal translocation (EWS/FLI1 mainly). EphA2 is a tyrosine kinase receptor that has been found overexpressed in a wide variety of tumors and correlated with malignant phenotype. Accordingly, our group previously described the implication of EphA2 receptor in promoting angiogenesis in ES cells. In this study we report that EphA2 receptor is phosphorylated at S897 in a panel of ES cell lines, which determines the ligand-independent function of the receptor and is related to its oncogenic properties. Thus, stable silencing of EphA2 in two different ES cell lines resulted in a lower clonogenic capacity in vitro and in a decrease of tumoral growth in immunodepressed mice in vivo. Furthermore, silencing of EphA2 resulted in an abolishment of the migration and invasion capacities of ES cells in vitro. We performed an experimental metastasis assay, injecting through the tail vain of mice both wild-type or EphA2 silenced ES cells and observed a reduction in the lung metastasis incidence. To further study the effect of silencing EphA2 in ES cells, we performed an Affymetrix GeneChip Microarray. Ingenuity pathway analysis revealed an implication of this receptor in cell signaling, cellular movement and survival. Globally, our results suggest that EphA2 acts as an oncogene in ES. The use of drugs or genetic tools that block EphA2 function or expression may be of therapeutic use for the treatment of ES.
POSTER SESSION B

B.5 Distinct roles of class IA PI3K isoforms in pericytes during sprouting angiogenesis

Ana M Figueiredo, Ralf H Adams, Mariona Graupera

Research Group: Signaling Pathways in Angiogenesis

Abstract:

Pericyte (PC) depletion inhibits tumour growth but facilitates tumour cell dissemination and metastasis. It is therefore critical to understand which signalling pathways regulate PC biology. Data from our laboratory have identified the p110α/PI3K isoform as the major driver of PIP3 production in endothelial cell during angiogenesis. Similarly, p110α signalling in smooth muscle cells has been shown to regulate vascular remodelling. Using pharmacological and genetic tools together with cultured PC and retinal systems, we next sought to validate whether p110α is also critical to mediate PC functions in vessel morphogenesis. As expected, pharmacological inhibition of p110α, but not inhibition of p110β, in wild-type cultured PC led to reduced Akt phosphorylation and reduced cell motility. Surprisingly, however, inhibition of p110β, but not inhibition of p110α resulted in PC arrest. Next, we used the tamoxifen-inducible-pericyte-Cre (PDGFRβ(BAC)-CreERT2), in combination with loxP-flanked and constitute kinase dead (D933A and D931A) alleles for p110α and p110β respectively, to genetically inactivate p110α (p110αD933A/iΔPC) and p110β (p110β D931A/iΔPC) in retinal pericytes. Analysis of both retinas at postnatal day 6 revealed that inactivation of p110β, but not p110α led to reduced PC coverage and proliferation. Interestingly, inhibiting both isoforms resulted in changes in retinal vasculature. However, while inactivation of p110α principally led to reduced radial expansion, lack of p110β activity resulted in decreased vascular density. Taken together, our data indicate that isoform-specific PI3K signalling in PC regulates retinal angiogenesis in a different manner.
POSTER SESSION B

B.6 Epithelial to Mesenchymal Transition in Pancreatic Human Beta, Alpha, Delta and PP Cells Expanded in-vitro

José Luis Moreno; Montserrat Nacher, Cristofer García, Noelia Tellez, Eduard Montanya

Research Group: Diabetes, Nutrition and endocrinological diseases

Abstract:

The ability of epithelial cells to transition into mesenchymal cells illustrates an inherent plasticity of the epithelial phenotype. A recent study using a genetic lineage tracing method has confirmed the presence of epithelial-to-mesenchymal transition (EMT) in pancreatic human β-cells during in-vitro expansion. These beta cells, that initially underwent EMT, can be now induced to reddifferentiate in vitro. Until the moment, it is unknown whether alpha, delta and PP cells undergo also EMT which in turn could be an additional source of insulin-producing cells.
POSTER SESSION B

B.7 Structural basis for dominance or recessivity pathogenesis of GLIALCAM mutations causing MLC2 Leukodistrophy

Xabier Elorza Vida, Tanit Arnedo, Tania López-Hernández, Juan Fernández-Recio, Raúl Estévez

Research Group: Physiology of Brain Transport Diseases

Abstract:

GlialCAM is a cell adhesion molecule that functions as an auxiliary subunit of both MLC1 and CIC-2 proteins. Mutations in this gene cause Megalencephalic leukoencephalopathy with subcortical cysts (MLC), a heterogeneous neurodegenerative leukodystrophy. While recessive GLIALCAM mutations cause the classical clinical phenotype (MLC2A), dominant mutations in GLIALCAM cause a different remitting phenotype (MLC2B).

Previous work shows that GlialCAM is localized at cell-cell contacts in the astrocytic network, and is required for the proper localization of MLC1 and CLC2 in these astrocyte junctions. Most mutations in GLIALCAM generate a defect in trafficking. Recessive mutations can be rescued biochemically in astrocytes by coexpressing with the WT protein, while dominant mutations cannot be rescued. Thus, GlialCAM can interact homophilically in the same cell (cis), as well as interacts with GlialCAM molecules present in the contacting cell (trans). These interactions are mediated by the extracellular domain, mainly by the first immunoglobulin-like domain IgV.

The objective of this project is to understand the molecular basis of the dominance or recessivity of GLIALCAM mutations. Using homology modeling and computer-based docking experiments, we generated a 3D structural model of the IgV domain of GlialCAM, and modelled the interaction of two IgV domains in contact of two GlialCAM molecules. We have tested this model using site directed mutagenesis and biochemical assays. Our results give a structural explanation of the biochemical basis of dominance and recessivity of the mutations found in the IgV domain.
**POSTER SESSION B**

**B.8 Zebrasfish models for the study of CIC channels**

**Carla Pérez Rius**, Héctor Gaitán Peñas, Raúl Estévez, Alejandro Barallo

**Research Group:** Physiology of Brain Transport Diseases

**Abstract:**

The CIC gene family comprises a group of chloride channels and transporters expressed in different tissues and fulfill diverse physiological roles. The chloride channel group is formed by CIC-1 which is relevant for the repolarization of the skeletal muscle fibers, CIC-2 is widely distributed, being present in almost every cell, and CIC-K is important for salt reabsorption in the renal tubules and for the potassium enrichment of the inner ear endolymph. Functional defects in all these channels lead to diseases: myotonia congenita, leukoencephalopathy with ataxia and Bartter’s syndrome, respectively.

We have identified and characterized the zebrafish orthologs for these CLC channels. Many zebrafish genes are present as duplicates due to the teleosts’ genome duplication. Such is the case for CLCN1, for which the zebrafish genome shows two paralogs: clcn1a and clcn1b. Both are expressed in skeletal muscle and show similar electrophysiological properties to mammalian CIC-1. CLCN2, however, has suffered an additional single gene duplication after the genome duplication, and three paralogs exist: clcn2a, clcn2b and clcn2c. clc-2a and clc-2b could be considered the homologs to mammalian CIC-2 in terms of expression pattern (although they have experienced sub-functionalization) and electrophysiological characteristics, including their interaction with the CIC-2 subunit, GlialCAM. clc-2c is expressed exclusively in a teleost-specific cell type: the ionocytes. Lastly, in the zebrafish there is a single clcnk gene, expressed in the distal segment of the embryonic pronephros, similarly to its obligatory subunit, barttin.

We will present the characterization of these zebrafish CIC channels and our progress in obtaining loss of function alleles as zebrafish disease models.
POSTER SESSION B

B.9 Caveolin-1 is required for TGF-β-induced activation of the metalloprotease TACE/ADAM17 through the regulation of Src and Nox1 activities.

J. Moreno-Càceres, J. Mainez, R. Mayoral, P. Martín-Sanz, G. Egea and I. Fabregat

Research Group: Biological clues of the invasive and metastatic phenotype

Abstract:

Transforming growth factor-beta (TGF-β) plays a dual role in hepatocytes, inducing both pro- and anti-apoptotic responses, the balance among them decides cell fate. Survival signals are mediated by the epidermal growth factor receptor (EGFR) pathway, which is activated by TGF-β. We have previously described that caveolin-1 (Cav1) is required for the activation of the metalloprotease tumor necrosis factor (TNF)-α-converting enzyme/a disintegrin and metalloproteinase 17 (TACE/ADAM17) and hence the transactivation of the EGFR pathway. The specific mechanism by which TACE/ADAM17 is activated has not been uncovered yet. Here we show that TGF-β induces sarcoma kinase (Src) phosphorylation in hepatocytes, a process that is impaired in Cav1-/- hepatocytes, coincident with a decrease in p-Src in detergent resistant membrane (DRM) fractions. TGF-β-induced activation of TACE/ADAM17 and EGFR phosphorylation were blocked using the Src inhibitor PP2. Cav1+/+ hepatocytes showed early reactive oxygen species (ROS) production induced by TGF-β, a fact that was not seen in Cav1-/- cells. These ROS were inhibited by the NADPH-oxidase (NOX)-1 inhibitor STK301831, which also impaired TACE/ADAM17 activation and, consequently, EGFR phosphorylation. Finally, STK301831 did not impair Src phosphorylation, but PP2 blocked early ROS production, evidencing that Src is involved in NOX1 activation. As expected, inhibition of both Src and NOX1 increased TGF-β-induced cell death in Cav1+/+ cells. In conclusion, Cav1 is required for TGF-β-mediated activation of TACE/ADAM17 through a mechanism that involves phosphorylation of Src and NOX1-mediated ROS production.
B.10 Therapeutic effects of novel anticancer tambjamine analogs in human lung cancer

P Manuel-Manresa, V Soto-Cerrato, L Korrodi-Gregório, AM Rodilla, R Quesada, R Ramos, A Villanueva and R Pérez-Tomás

Research Group: Cancer Cell Biology Research Group

Abstract:

BACKGROUND: Lung cancer ranks among the leading cause of cancer-related deaths worldwide. Nowadays the success in increasing the 5-year patient survival rate is less than 15%. For this reason, our challenge is to offer a new therapeutic strategy against cancer focused on the modulation of the intracellular pH (pHi) through the anion transport ability of our tambjamine analogs (tmbjs). The aim is to identify effective tmbjs with anticancer properties against different lung cancer cell lines, characterize the cell death that they induce and corroborate these results in in vivo models and also in human lung primary cultures.

MATERIALS AND METHODS: Four lung cancer cell lines were selected to perform MTT assay to evaluate cell viability after tmbjs treatment. In order to characterize the type of cell death induced, different apoptotic, autophagic and stress-kinases markers were assessed by Western-blot and immunofluorescence techniques. An orthotopic lung mouse model was established and tmbj were administered in alternate days during 3 weeks. Furthermore, the establishment of several primary cultures from human lung tumour samples was performed and were characterize for specific epithelial markers. Also viability assays were conducted.

RESULTS: After tmbjs treatment, cell viability was significantly reduced. Processes as cellular stress, apoptosis and autophagy was observed after 24 hours treatment. In vivo assays confirmed the efficacy of our tmbjs, by reducing the size of lung tumours after 3 weeks of treatment. Finally, the tmbjs effect on patient-derived lung primary cultures has shown to being even more cytotoxic than cis-platinum, the first line chemotherapeutic agent.
B.11 Mitochondrial swelling and cell death processes induced by anion transporters in lung cancer

Ananda M Rodilla, V Soto-Cerrato, P Manuel-Manresa, L Korrodi-Gregório, AM Rodilla, R Quesada and R Pérez-Tomás

Research Group: Cancer Cell Biology Research Group

Abstract:

Lung cancer is the leading cause of cancer death worldwide. Despite of new advances in diagnosis and clinical care, the success of standard treatments is still limited, especially in chemotherapy. Therefore, novel anticancer compounds with different mechanisms of action are eagerly needed. In this view, we propose a new therapeutic strategy against cancer that involves intracellular pH (pHi) modulation. Cancer cells have a reversed pH gradient compared to normal cells, which allows cancer progression by promoting proliferation and evasion of apoptosis. Hence, anion transporter compounds, such as tambjamine analogs, have been selected in this study for their potential role as anticancer agents through the modulation of the pHi.

First, the effect of our compounds on cell viability was evaluated in several lung cancer and in cancer stem cells, by the MTT assay. A significant decrease was observed in most of them, and compounds 3 and 9 were chosen for further studies. To characterize the type of cell death, we analyzed different molecular markers related to apoptosis and autophagy by Western blot and we observed some evidence of caspases activation and LC3II accumulation. Also, anion transporter treatment resulted in the activation of MAPK and JNK in A549 cells. At the same time, it was observed at phase contrast and electron microscope, that compounds also induced massive cytoplasmic vacuolization. We tested several markers to distinguish among different potential organelles, which could have undergone this phenomenon. We used LAMP1, LC3II and EEA1 by immunofluorescence and we could exclude lysosomes, autophagosomes and early endosomes, respectively. Finally TOMM20, mitochondrial marker, proved to be positive. That was subsequently corroborated by transfecting A549 cells with the subunit VIII of human cytochrome C oxidase mCherry-MITO7 plasmid.

Altogether, these results show that these anion transporters have potent cytotoxic effects in lung cancer cells, inducing an imbalance in anion homeostasis, which triggers mitochondrial swelling, MAPK and JNK kinases activation and cell death which involves several features of different cell death processes such as apoptosis and autophagy.
**POSTER SESSION B**

**B.12 The role of parkinson’s disease-associated receptor GPR37 in the hippocampus: functional interplay with the adenosinergic system.**

*Xavier Morató*, Joao Pedro Lopes, Carolina Souza, Pinhal C, Nuno Machado, Paula Canas, Henrique Silva, Igor Stagljar, Jorge Gandía, Víctor Fernández-Dueñas, Rafael Luján, Rodrigo Cunha, *Francisco Ciruela*

**Research Group:** *Neuropharmacology and Pain Research*

**Abstract:**

GPR37 is an orphan G protein-coupled receptor mostly enriched in brain areas such as the cerebellum, striatum, and hippocampus. Identified as a substrate of parkin, an E3 ubiquitin ligase involved in the ubiquitination and proteasome-mediated degradation/clearance of misfolded proteins, GPR37 has been suggested to play a role in Parkinson’s disease. Also, GPR37 has been found to be down-regulated in the amygdala and hippocampus of major depressive disorder patients. Distributed throughout the brain, the function of GPR37, however, remains unknown. We now provide the first mapping of GPR37 within the hippocampus, where GPR37 is widely expressed and localized at the level of the extrasynaptic plasma membrane of dendritic spines, dendritic shafts, and axon terminals. GPR37 per se does not appear to play a role in learning and memory, since knocking out GPR37 (GPR37-KO) did not alter the performance in different hippocampal-related memory tasks. This is in agreement with slice electrophysiology experiments showing no differences both in short-term plasticity paired-pulse facilitation and long-term potentiation between WT and GPR37-KO mice. However, we report a potential functional interaction between GPR37 and adenosine A2A receptors (A2AR) in the hippocampus, with A2AR modulating the GPR37-associated phenotype. Thus, the absence of GPR37 appeared to sensitize mice to hippocampal A2AR-mediated signaling, as observed by the effect of the A2AR antagonist SCH58261 increasing synaptic depotentiation, reducing novel object recognition memory and reverting the anxiolytic effect of GPR37 deletion. Collectively, these findings afford insight into the localization and role of the orphan GPR37 within the hippocampus with potential involvement in A2AR function (i.e., A2AR sensitization).
B.13 Bioengineering of a myocardial graft from human iPSC-derived cardiomyocytes

Juan Crespo Santiago, Ángel Raya Chamorro

Research Group: Center of regenerative medicine of Barcelona (CMRB)

Abstract:

After a myocardial infarction the death of cardiomyocytes (CMs) specifically results in compromised contractility of the whole heart, for which there is currently no cure, as the heart cannot regenerate by itself. Cardiac tissue engineering is a promising strategy to introduce in-vitro created piece of tissue into the heart, to achieve muscular regeneration. Scaffold-based engineered tissues have been shown to align CMs, lengthen them, promote their electrical coupling and improve contractile function, in summary, to create more mature-like cardiac tissues. The aim of this project is to generate functional tissue grafts of human myocardium to be delivered into an in-vivo mouse model of heart infarction, using human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM). After differentiation of iPSC into CMs, we fabricate collagen-based scaffolds and we subject them to mechanical strain for few days with a custom-made pumping system, to induce cell maturation and therefore a functional piece of cardiac tissue suitable to be grafted in-vivo. Maturation of the CMs is analyzed in terms of cell and tissue morphology and protein organization and expression. Besides some functional studies such as electrical synchronization with current pulses and calcium transients. So far results suggest clear signs of maturation in the stimulated group versus the non-stimulated control group, nevertheless some experiments are still required to fully demonstrate positive effect of mechanical pumping, and successfully achieve in-vivo grafting.
The role of extracellular matrix proteins in zebrafish heart regeneration

Anna García Puig, Senda Jiménez Delgado, Cristina García Pastor, Ángel Raya Chamorro

Research Group: Center of regenerative medicine of Barcelona (CMRB)

Abstract:

Heart disease is one of the principal causes of death and disability in many countries. After myocardial infarction, human heart is unable to regenerate and replaces the lost myocardial cells with a fibrotic scar tissue. In contrast, zebrafish has the ability to regenerate its heart even after a resection of the 20% of the myocardium. In our lab we are interested in elucidating the molecular mechanisms turned on during zebrafish heart regeneration. It is known that after a myocardial injury zebrafish cardiomyocytes are able to dedifferentiate, re-enter cell cycle, and proliferate to fill the injury site with newly formed cardiomyocytes. However, most of the factors involved in this process are largely unknown. Studies have shown that the extracellular matrix (ECM) has an important role in tissue repair. Thus, studying the zebrafish heart ECM will help us to better understand the regenerative ability of these animals. In order to characterize the ECM proteins of the zebrafish heart, we decellularized zebrafish hearts and made a proteomic study of the resulting ECM. We compared non-injured zebrafish heart ECMs to heart ECMs at different time points of the regeneration process, and some proteins, such as different types of collagens, fibrinogen, peristatin and fibronectin, were differentially expressed. Peristatin has been studied in conditions such as neoplasia, healing and cardiac lesions, so we decided to validate its expression by RT-PCR and In situ hybridization, as well as determinate its producing cell type. We saw that peristatin b was only produced at the wound and by fibroblasts. Right now we are generating a zebrafish line that will conditionally knock-down this gene in order to unveil its role during regeneration.
B.15 Disease Model For Familial Hypertrophic Cardiomyopathy Using Patients Specific iPS Cells

Juan Luis Vázquez Rentería, Ángel Raya Chamorro, Yvonne Richaud, Senda Jiménez Delgado, Jonathan de Smedth

Research Group: Center of regenerative medicine of Barcelona (CMRB)

Abstract:

Familial Hypertrophic cardiomyopathy is a cardiac disease that affects 1:500 people world wide, is the most heritable disease of the cardiac disease and is characterized for the thickness of muscle heart, could be an asymptomatic or show a severe symptomatic. Mutations in sarcomeric proteins are the most common related with HCM, mutations in the MYBPC3 one of the most related with FHCM, our case of study involves two patients, one asymptomatic and the other with a severe symptomatology. Our goal is generate a model disease from specific iPS cells derived from the two patients and study the reason of those symptomatic differences. We want to understand how the interaction between cMyBP-C and Actin is affected and how this defect derives in HCM.
B.16 Dissecting the role of the Epidermal Growth Factor Receptor catalytic activity during liver regeneration


Research Group: Biological clues of the invasive and metastatic phenotype

Abstract:

Liver regeneration (LR) is a very complex and well-orchestrated phenomenon, involving a large number of growth factors and cytokines, in order to reestablish liver function in a minimum time to avoid the risk of liver failure. The proliferation of hepatocytes is the main mechanism during LR, in response to mitogenic signals, like Epidermal Growth Factor (EGF), which plays essential roles. To elucidate the specific role of the EGFR pathway during LR after partial hepatectomy (PH), we have generated a transgenic mouse model expressing a hepatocyte-specific truncated form of the human EGFR, which acts as a negative dominant mutant (∆EGFR) and allows defining the EGFR tyrosine kinase-dependent functions.

Results indicate a critical role for EGFR catalytic activity during the early stages of LR. Thus, after 2/3 PH, ∆EGFR livers displayed lower and delayed proliferation and lower activation of proliferative signals, which correlated with overactivation of the Transforming Growth Factor-beta (TGF-β) pathway. Altered regenerative response was associated with amplification of cytostatic effects of TGF-β through induction of cell cycle negative regulators. However, no apparent increase in apoptosis was observed in the livers of ∆EGFR mice after surgery. Interestingly, lipid synthesis was severely inhibited in ∆EGFR livers after PH, revealing a new function for EGFR kinase activity as lipid metabolism regulator in regenerating hepatocytes. In spite of these alterations, ∆EGFR mice were able to fully regenerate the liver, recovering the total liver mass, due to compensatory mechanisms, such as the overactivation of the HGF/c-Met pathway.

In conclusion, our studies demonstrate that EGFR catalytic activity is critical during the initial phases of LR and provide key mechanistic insights into how this kinase acts to regulate liver physiology.
B.17 Dissecting the role of the Epidermal Growth Factor Receptor (EGFR) in Diethyl-nitrosamine-induced hepatocarcinogenesis in mice


**Research Group:** *Biological clues of the invasive and metastatic phenotype*

**Abstract:**

**Hepatocellular Carcinoma (HCC)** is a common cancer with an increasing incidence. During hepatocarcinogenesis there is a disruption of the balance between cell death and survival signals. One of these de-regulated survival signals is the Epidermal Growth Factor Receptor (EGFR) pathway.

To unravel the role of EGFR in hepatocarcinogenesis our group generated a novel transgenic mouse model expressing a hepatocyte specific truncated form of the human EGFR, which acts as negative-dominant mutant (ΔEGFR) and allows defining its tyrosine kinase dependent functions. Mice were treated with Diethyl-nitrosamine to induce liver tumorigenesis.

**RESULTS:** Transgenic mice showed a delay in the appearance and in the growth of tumors, associated to attenuation of the inflammatory process, which correlated with lower expression of IL-6 and TNF-α in tumor-surrounding area. A significant increase in the levels of the NADPH oxidase NOX4 was observed in the transgenic mice. This NOX is down-regulated by EGF and negatively modulates hepatocyte proliferation. However, once tumors appear in transgenic mice, they were able to progress properly tie in with up-regulation of Hepatocyte Growth Factor (HGF), suggesting that this cytokine, among others, might replace mitogenic function of the EGFR ligands.

**CONCLUSION:** Our studies suggest that the catalytic activity of EGFR plays an important role during initial phases of hepatocarcinogenesis, and this model will open new possibilities for exploring the EGFR pathway as a targeted therapeutic strategy in liver pathologies.
POSTER SESSION B

B.18 CD154-CD40 T-cell costimulation pathway is a key mechanism in kidney ischemia-reperfusion injury. CD40 gene silencing modulates downstream renal mediators and distant inflammatory response.

Laura de Ramon, Elia Ripoll, Marc Lucia, Ana Merino, Josep M Aran, Pérez-Rentero S, Nuria Lloberas, Josep M. Cruzado, Josep M. Grinyó, Juan Torras

Research Group: Experimental Nephrology

Abstract:

Ischemia-reperfusion occurs in a great many clinical settings and contributes to organ failure or dysfunction. CD154-CD40 signalling in the leukocyte-endothelial cell interactions or T-cell activation facilitates tissue inflammation and injury. Here, a siRNA anti-CD40 was tested in rodent warm and cold ischemia models to check the therapeutic efficacy and anti-inflammatory outcome of in vivo gene silencing.

In the warm ischemia model different doses were used, resulting in clear renal function improvement and a structural reno-protective effect. Renal ischemia activated the CD40 gene and protein expression, which was inhibited by intravenous siRNA administration. CD40 gene silencing improved renal inflammatory status, as seen by the reduction of CD68 and CD3 T-cell infiltrates, attenuated pro-inflammatory and enhanced anti-inflammatory mediators. Furthermore, siRNA administration decreased a spleen pro-inflammatory monocyte subset and reduced TNFα secretion in spleen T cells.

In cold ischemia model with syngeneic and allogeneic renal transplantation, the most effective dose induced similar functional and structural effects. Our data show the efficacy of our siRNA in modulating both the local and the systemic inflammatory milieu after an ischemic insult. Thus, CD40 silencing could emerge as a novel therapeutic strategy in solid organ transplantation.
POSTER SESSION B

B.19 Target Memory Reactivation during sleep promotes strengthening and weakening of overlapping event episodes as a function of temporal encoding contiguity

Javiera P Oyarzún, Joaquín Morís, David Luque, Ruth de Diego-Balaguer, Lluís Fuentemilla

Research Group: Cognition and Brain Plasticity

Abstract:

We examined whether Target Memory Reactivation (TMR) entails the replay of overlapping episodes. After participants encoded two consecutive sets of different memories sharing a common element, we enhanced the recall of a subset of the first encoded memories by presenting, during a nap, a sound cue associated only with the second set. Interestingly, we observed the opposite effects when the encoding of the two set of overlapping memories was separated by a 3 hours interval. In this case, recall of the first subset of memories encoded was impaired by the targeted sleep reactivation of the linked memories encoded in the second set. Recall performance after the nap and electrophysiological correlates of memory processing during sleep indicated that sleep replay can either strengthen or weaken overlapping memories as a function of the encoding contiguity between events.
POSTER SESSION B

B.20 MAX Inactivation in Small Cell Lung Cancer Disrupts MYC–SWI/SNF Programs and Is Synthetic Lethal with BRG1

Octavio A. Romero*, Manuel Torres-Díz*, Eva Pros, Suvi Savola, Antonio Gomez, Sebastian Moran, Carmen Saez, Reika Iwakawa, Alberto Villanueva, Luis M. Montuenga, Takashi Kohno, Jun Yokota, Montse Sanchez-Cespedes

Research Group: Genes and Cancer

Abstract:

Our knowledge of small cell lung cancer (SCLC) genetics is still very limited, amplification of L-MYC, N-MYC, and C-MYC being some of the well-established gene alterations. Here, we report our discovery of tumor-specific inactivation of the MYC-associated factor X gene, MAX, in SCLC. MAX inactivation is mutually exclusive with alterations of MYC and BRG1, the latter coding for an ATPase of the switch/sucrose nonfermentable (SWI/SNF) complex. We demonstrate that BRG1 regulates the expression of MAX through direct recruitment to the MAX promoter, and that depletion of BRG1 strongly hinders cell growth, specifically in MAX-deficient cells, heralding a synthetic lethal interaction. Furthermore, MAX requires BRG1 to activate neuroendocrine transcriptional programs and to upregulate MYC targets, such as glycolysis-related genes. Finally, inactivation of the MAX dimerization protein, MGA, was also observed in both non–small cell lung cancer and SCLC. Our results provide evidence that an aberrant SWI/SNF–MYC network is essential for lung cancer development.

SIGNIFICANCE: We discovered that the MYC-associated factor X gene, MAX, is inactivated in SCLCs. Furthermore, we revealed a preferential toxicity of the inactivation of the chromatin remodeler BRG1 in MAX-deficient lung cancer cells, which opens novel therapeutic possibilities for the treatment of patients with SCLC with MAX-deficient tumors.
POSTER SESSION B

B.21 Arming oncolytic adenoviruses with transgenes to engage stroma toxicity and immunostimulation against cancer

*Marcel Arias*, Carlos Fajardo, Sônia Guedan, Luis Rojas, Rafael Moreno, Ramon Alemany

**Research Group:** Cancer virotherapy

**Abstract:**

The lysis of tumour cells is the most obvious mechanism of virotherapy. Theorically, in the absence of stromal barriers and antiviral immune responses, the virus would spread until complete tumour lysis. However, the fact that immunity eliminates the virus from the infected tumour cells means that the strong immunosuppressive environment of tumours has been modified by the virus, hence raising the possibility of an antitumour immunotherapy mechanism.

Adressing the first mentioned hurdle, a stroma-targeted aerolysin, a pore-forming toxin, has been cloned into ICOVIR-15K, the oncolytic Ad platform commonly used in the group. Upon secretion of a proaerolysin from infected cells, the inhibitory domain is cleaved due to the presence of an MMP-2/9 sensitive linker peptide. To assess the potency of this therapy, cytotoxicity studies in single culture or bystander contexts have been performed.

Growing evidence suggests that the immune system has a determining role in tumour rejection, since tumours create immunosuppressive microenvironments around them by expressing immunosuppressive proteins that inhibit lymphocyte activity, such as CD200 and CD200R, whose interaction results in activation of a RasGAP-mediated intracellular inhibitory pathway. A truncated version of CD200, CD200tr, has been identified as an antagonist of CD200R. Interestingly, Human herpesvirus 8 encodes K14, a protein which shows sequence similarity to CD200 and binds CD200R with comparable affinity. Viruses encoding soluble full-length and truncated CD200 and K14 proteins have been designed and are being tested through MLRs for their role in PBMC immunomodulation.
POSTER SESSION B

B.22 What infant development and language pathologies can tell us about the role of attention in language learning?

Anna Martinez-Alvarez, Ferran Pons, Spiros Christou, Maria Jose Buj Pereda, Monica Sanz-Torrent, Llorenç Andreu, Ruth de Diego-Balaguer

Research Group: Cognition and Brain Plasticity

Abstract:

Given that speech is a sequence of sounds that unfolds in time, orienting of attention to speech in the temporal domain is necessary to predict forthcoming information. Many language rules require tracking predictive information while ignoring intervening elements (is reading, is playing). This study investigated the involvement of temporal endogenous orienting in language learning in infancy and language pathology. In the first line of research we followed the developmental trajectory of temporal endogenous attention in infants at 12 and 15 months of age by collecting data using an eye tracker system and assessing general cognitive capacities. Results showed that 15-month-olds, but not 12-month-olds could use temporal information to adapt their anticipatory behavior. In the second line of research we tested typically developing (TD) children and children diagnosed with Specific Language Impairment (SLI) to explore (i) the effects of attentional deficits on language learning, and (ii) whether deficits in endogenous orienting in time is involved in language learning. We collected data from reaction time paradigms and the assessment of non-verbal cognitive capacities. Results showed that both Attention Deficit-Hyperactivity Disorder scores and temporal endogenous orienting scores correlated with language performance in SLI. These findings suggest that (i) attention deficits in SLI – but not SLI per se – modulate language rule learning, and (ii) endogenous temporal orienting is implicated during rule learning in SLI. This study has implications for our understanding of cognitive processes underlying language, and for the assessment of childhood disorders, especially since early cognitive deficit identification is crucial for successful intervention.
POSTER SESSION B

B.23 Counterfactual thinking is impaired in non-psychotic first-degree relatives of schizophrenia patients.

Áuria Albacete, Fernando Contreras, Clara Bosque and José M. Menchón

Research Group: Schizophrenia and other psychotic disorders

Abstract:

OBJECTIVES: Counterfactual Thinking (CFT) is a mental process about spontaneous alternatives to past outcomes usually evoked as in a “if only” type of response. In schizophrenia there is a global impairment in CFT, which might be the expression of a general cognitive impairment. In the present study we explored CFT in biological first-degree non-psychotic relatives of schizophrenia patients (SCZ-RELs) and healthy control subjects (HCs) through an experimental paradigm in a bottom-up design.

METHODS: The ability to generate counterfactual thoughts in front of a hypothetical scenario with a negative outcome was assessed in 30 SCZ-RELs and 36 HCs. We further explored whether socio-demographic variables predicted performance in the experiment. Participants were matched by gender, age and educational level. Differences between groups were assessed using the Student’s-t parametrical test and the U-Mann Whitney non-parametrical test for continuous variables, and the $\chi^2$ or p-Fisher tests for categorical variables. In multivariate analyses, binary, ordinal or linear regression models were used depending on whether the dependent variable was considered categorical, ordinal or continuous, respectively. Statistical analysis was performed using the statistical package SPSS Version 18.0.

RESULTS: Results suggested that SCZ-RELs generated less counterfactual thoughts than HCs. As well as schizophrenia patients, relatives seem to have difficulties activating alternatives that could help them face reality transforming a negative outcome into a positive through conditional reasoning. Moreover, only employment status was found to be statistically related, having retired subjects more probabilities of generating less counterfactual alternatives.
B.24 Assessing the role of LRRK2 G2019S mutation and the genomic background to the development of PD-related neurodegeneration.


Research Group: Stem Cell and Neuronal Plasticity - IBUB

Abstract:

Despite the advances in the identification of genes and proteins involved in Parkinson's disease (PD), there are still appreciable gaps in our understanding of the mechanisms underlying the chronic neurodegenerative process in PD. In the lab, it has been demonstrated that iPSC technology can be used to observe phenotypes relevant to neurodegeneration in PD, and also provided first proof-of-principle evidence that neurons with the genome of a sporadic PD patient exhibited similar phenotypes as seen in iPSC derived from patients with monogenic LRRK2 (G2019S) PD. In the present study we generated a complementary set of iPSC lines from asymptomatic patients carrying pathogenic LRRK2 mutations, whose gene pool may have a prevailing protective effect. We then corrected the LRRK2 mutation by using TALEN-mediated genetic engineering in the asymptomatic LRRK2-iPSC lines, as well as in our already established LRRK2-PD iPSC lines. Dopaminergic neurons differentiated in parallel from this subset of iPSC lines have been cultured over a long time span and monitored for the appearance of neurodegeneration phenotypes. We also performed phenotypic analysis to address the contribution of the LRRK2 mutation to disease phenotypes such as alpha-synuclein accumulation or sensitivity towards neurotoxic drugs. The availability of a refined set of PD patient-specific iPSC lines representing symptomatic and asymptomatic cases of familial PD sharing the same pathogenic mutation in LRRK2, as well as isogenic iPSC lines in which the mutation has been edited out, will provide a unique test bed for revealing the specific genetic determinants contributing or preventing to the neurodegeneration in PD.
POSTER SESSION B

B.25 Crosstalk between TGF-β-induced Epithelial-Mesenchymal transition and stemness in hepatocellular carcinoma.

Andrea Malfettone, Jitka Soukupova, Joan Fernando, Petra Koudelkova, Esther Bertran, Àngels Fabra, Markus Grubinger, Bhavna Rani, Gianluigi Giannelli, Wolfgang Mikulits, Isabel Fabregat

Research Group: Biological clues of the invasive and metastatic phenotype

Abstract:

Apoptosis and the epithelial–mesenchymal transition (EMT) process are two physiological events that take place during human hepatic fibrosis and hepatocarcinogenesis. Transforming Growth Factor-beta (TGF-β) acts as a tumor suppressor and apoptosis inducer in these processes. However as disease progresses towards malignancy, liver tumor cells acquire mechanism to overcome TGF-β suppressor effects by undergoing EMT. Recent studies have established a link between the acquisition of mesenchymal traits and the expression of stem-cell markers. Here we aimed to analyze the cross-talk between the TGF-β pathway and the acquisition of a dedifferentiated phenotype with Cancer Stem Cell (CSC) characteristics along with the EMT phenotype in human liver tumor cells.

Long-term treatment of Hep3B cells to TGF-β induces EMT and switches stem-cell gene expression from epithelial (EpCAM, CD133) to mesenchymal (CD44). Interestingly, these cells acquire advantage in colony formation ability and growth in liver spheroids, with higher ability to migrate and invade. Long-term treatment with TGF-β in PLC cells does not induce a full EMT, but induces CD44, even though maintaining EpCAM and CD133 expression. This does not induce any advantage in colony formation ability and growth in liver spheroid but enhances cell migration and invasion. Then, we sorted the CD44+ after chronic treatment with TGF-β in PLC cells and we found that TβT-PLC<sup>CD44+</sup> cells show a mixed epithelial/mesenchymal phenotype and simultaneous expression of EpCAM, CD133 and CD44. However, TβT-PLC<sup>CD44+</sup> cells do not show advantage in colony formation ability and growth in liver spheroid but higher capacity to migrate and invade. The cancellation of TβRI in mesenchymal-like HLE cells produced an incomplete MET, but caused a decreased expression of CD44, associated with the lowest ability to form clones and liver spheroids. Moreover, trying to translate our in vitro results to clinical samples, we analyzed the expression of EMT and CSC marker in 22 human HCC tissues. We found that TGF-β expression correlates with mesenchymal genes (VIMENTIN and CXCR4) and CD44 expression. However, CD44 expression does not significantly correlate with either mesenchymal or epithelial genes.

CONCLUSION: Up-regulation of mesenchymal stem genes, such as CD44 expression, by TGF-β in HCC cells does not require a full EMT. These results reinforce the hypothesis that regulation of CD44 and EMT by TGF-β could be not strictly related.
POSTER SESSION B

B.26 Neural correlates of moral sensitivity as an endophenotype for Obsessive Compulsive Disorder


Research Group: Psychiatry and Mental Health

Abstract:

INTRODUCTION: Patients with Obsessive-Compulsive Disorder (OCD) show a heightened moral sensitivity related with nuclear cognitive distortions of the disorder. Such increased moral sensitivity in OCD patients has been previously associated with an hyperactivation in ventromedial, dorsolateral prefrontal and lateral temporal cortices (VMPFC, DLPFC, LTC).

We compared brain activation maps during a moral dilemma task across OCD patients, first-degree healthy relatives (HR) and a healthy control group (HC), and we analyzed the correlations between such brain activations and clinical and psychometric variables.

METHODS: Eighteen OCD patients, 19 HR and 19 HC were included in a fMRI block-design study in which fMRI activation maps were compared across the three groups. According to previous literature, analyses were focused on VMPFC, DLPFC and LTC regions of interest. Results were reported with a p<0.05 significance level (AlphaSim corrected). Correlations between regional activations and disorder’s severity as well as cognitive rigidity scores were also assessed.

RESULTS: During the moral dilemma condition, OCD patients showed a hyperactivation in the DLPFC region in comparison to HC, which was also observed in the HR group in comparison to HC and correlated positively with cognitive flexibility measures in HR group. The VMPFC region showed a hyperactivation during moral dilemma in the OCD group in comparison to HC, but this difference was not observed in the HR group. Finally, the previously observed LTC hyperactivation in OCD in comparison to HC was not replicated in this study.

CONCLUSIONS: Our results suggest that the DLPFC hyperactivation during conflictive situations with moral content is a candidate endophenotype for OCD.
POSTER SESSION B

B.27 Episodic boundaries trigger rapid memory replay of the encoded event sequence to promote their bound representation in long-term memory

Ignasi Sols, Sarah Dubrow, Lila Davachi, Lluís Fuentemilla

Research Group: Cognition and Brain Plasticity

Abstract:

We show, in humans, that the ability to form bound memory representations within and across episodic events during encoding is supported by their online rapid memory replay at the detection of contextual shift. In finding that the degree of memory replay predicted later participants’ ability to preserve the information of the items in the sequence, we concluded that memory replay during encoding is a critical neural mechanism to the online formation of enduring and bound memory representations for sequential event episodes.
POSTER SESSION B

B.28 Modeling Retinitis Pigmentosa in *Caenorhabditis elegans*: toward the molecular mechanisms behind the disease

*Karinna Rubio-Peña*, Laura Fontrodona*, David Aristizábal-Corrales*, Silvia Torres, Eric Cormes, Francisco J. García-Rodríguez, Xènia Serrat,Montserrat Porta-De-La-Riva, Julián Cerón

**Research Group:** Modelling Human Diseases in *C. elegans*

**Abstract:**

Using the worm as model we have deepened in the study of a subtype of Retinitis Pigmentosa, a hereditary degenerative disease that affects the retina producing gradual blindness due to the loss of photoreceptors by apoptosis. *C. elegans* is a suitable multicellular model to investigate splicing-related genes mutated in the autosomal dominat subtype of RP (s-adRP). These genes are essential and ubiquitously expressed, yet their impairment causes a disease only in the retina, the most transcriptionally active tissue of the human body.

Transcriptomic analyses of worms with s-adRP genes partially inactivated by RNAi revealed a very low intron retention that may not explain a final apoptotic outcome. However, these worms presented an increase in the expression of *atl-1* (homolog of human ATR) which is required for the DNA damage and replicative stress response. Moreover, the upregulation of *atl-1* correlates with the increment of the expression of *egl-1*, which is an activator of apoptosis. Interestingly, we observed that apoptosis, and the upregulation of these genes was taking place mostly in hypodermal cells that present high transcriptional activity. Hence, just as it happens in humans, the impairment of s-adRP genes produces an effect related to cell specific transcriptional requirements.

We propose that a mild impaired function of splicing factors could cause genomic instability driven by the presence of R-loops and replicative stress associated with transcriptionally active tissues in both organisms. Then, taking advantage of the CRISPR/Cas9 genome editing technique, we plan to introduce specific human mutations into *C. elegans*, turning the worms into a model of adRP where to screen for therapies in the context of personalized medicine.
POSTER SESSION B


Angelique Di Domenico, Giulia Carola, Neus Bayò Puxan, Yvonne Richaud, Angel Raya, Antonella Consiglio

Research Group: Stem Cell and Neuronal Plasticity - IBUB

Abstract:

Our understanding of Parkinson’s disease (PD) pathogenesis, the second most common age-related progressive neurodegenerative disease, remains elusive despite decades of thorough investigation, arguably owing to the lack of suitable experimental models recapitulating the disease. Induced pluripotent stem cells (iPSC) derived from somatic cells of patients are an innovative tool for in vitro modeling of complex diseases and may also provide a source for cell replacement therapies. It has been previously demonstrated that iPSC technology can be used to observe disease-associated phenotypes relevant to PD neurodegeneration, in particular impaired axonal outgrowth and deficient autophagic vacuole clearance. iPSC disease modeling has provided first hand proof-of-principle evidence that neurons with a sporadic PD patient genome exhibited similar phenotypes compared to ones derived from patients with familial PD. Our group has successfully derived both patient-specific dopaminergic neurons (DAn) and glial cells from iPSC. PD specific associated phenotypes, such as α-synuclein accumulation and alterations in autophagic machinery, have been observed in our model. In the present project we propose to take advantage of this disease recapitulating approach in PD modeling to investigate whether DAn degeneration in PD is truly a cell-autonomous phenomenon, or whether it is influenced by an altered cross-talk between DAn and glial cells. We are now investigating the effects of co-culturing different iPSC-derived patient-specific DAn/astrocyte combinations from PD and control patients. By recapitulating the accurate neurodegenerative phenotypes seen in PD pathology in a progressive manner through the use of iPSC technology, several unknown mechanisms will be unveiled to aid in the future development of specific PD-targeted therapies.
POSTER SESSION B

B.30 Investigating glia-neuron cross-talk in the pathogenesis of Parkinson´s disease using patient-specific iPSC-derived cells.

**Joanna Sierpowska;** Andreu Gabarrós, Alejandro Fernández-Coello, Ángels Camins, Sara Castañer; Montserrat Juncadella, **Antoni Rodríguez-Fornells**

**Research Group:** Cognition and Brain Plasticity

**Abstract:**

Electrical stimulation mapping (ESM) is currently a gold standard in assessing cognitive and motor functions during the awake brain surgery in patients harboring intrinsic brain lesions (tumors and vascular malformations). The most common intraoperative speech processing assessment task is a naming task - at the same time as the current of low intensity is applied to the exposed brain cortex, patients are instructed to name the objects presented by the neuropsychologist. If the electrical stimulation provokes impairment in naming, the area is considered as crucial for speech processing and thus considered very carefully during tumor resection.

Usually this procedure is carried out only in one language. However, in bilingual populations both languages should be assessed intraoperatively as well as the language switching (LS) mechanism. LS enables effective bilingual communication, but the neural bases underlying this process are not fully clear.

In the present study we aimed to explore the possible involvement of the frontal (middle and inferior areas) cortex in LS using ESM.

Data from 9 Spanish-Catalan bilingual patients were collected and analyzed. Within the frontal lobe, LS related points were mainly distributed across the middle frontal gyri (11/18), in distinction to the regular language production sites which were placed predominantly within the inferior frontal area (36/38), being this difference statistically significant.

Our ESM results highlight the importance of the middle frontal regions in LS. Furthermore, it allowed the neurosurgeons to plan tumor resection avoiding the damage to both areas crucial for language production and for the LS.