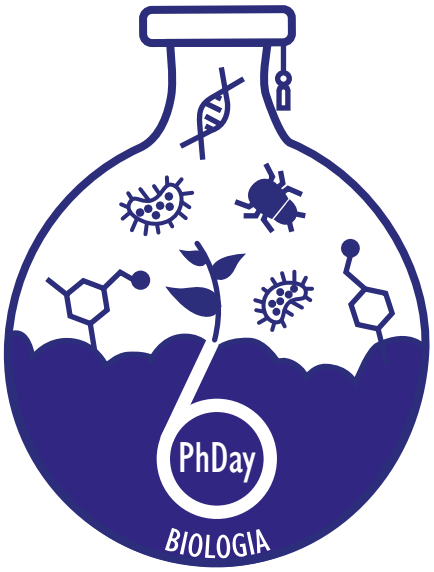
LIBRO DE ABSTRACTS

6º JORNADA PhDay BIOLOGÍA



6 OCTUBRE 2022

FAC. CC. BIOLÓGICAS

UCM

UNIVERSIDAD COMPLUTENSE MADRID

Colegio Oficial de Biólogos de la Comunidad de Madrid

ESCUELA DE DOCTORADO
UNIVERSIDAD COMPLUTENSE DE MADRID

COORDINACIÓN

Paula Losada Oliva
Ainhoa Collada Marugán
Gabriel Munar Delgado
María del Carmen Ureña Lara

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JORNADA
6º PhDay BIOLOGÍA

6 OCTUBRE 2022

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HASTA LAS 18:00

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A novel, rapid molecular method for the diagnosis of avian metapneumovirus by nanoprobe

10:30 — 10:45 **Clara Melguizo Ávila**
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Guillermo Velayos Monge
Checklist of Vascular Plants from Rio Muni, Equatorial Guinea.

Lisbeth Herrera Castillo
Different management conditions affect metabolic rate in goldfish

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RESÚMENES

COMUNICACIONES ORALES

A novel, rapid molecular method for the diagnosis of avian metapneumovirus by nanoprobe.

Pablo Cea[1,4], Sonia Arca-Lafuente[2], Ana Doménech[3,4], Esperanza Gómez-Lucía[3,4], Laura Benítez[1,4], Ricardo Madrid[1,4].

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Keywords: molecular method, virus diagnosis, avian metapneumovirus, RT-LAMP, gold nanoparticles, point-of-care (POC) diagnostic.

Summary

Early diagnosis of active viral infections is crucial to prevent transmission among the population, as we have seen during the present COVID-19 pandemic. Single-stranded RNA viruses, like avian metapneumovirus (aMPV), are currently diagnosed using molecular methods, such as the gold standard qRT-PCR. These techniques require expensive equipment and specialized training, which limits their use. aMPV is the cause of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in chickens, both severe emerging diseases that produce important economic losses in avian industry.

We are developing a point-of-care (POC) diagnostic system for aMPV detection based on a novel, fast and simple method that can be performed from sample collection to final reading on a farm in an integrated manner. In this system, the amplification by LAMP of the viral RNA and subsequent recognition by the DNA probe triggers a change in the aggregation of gold nanoparticles, which leads to a change of color, visible with the naked eye. In this study, a panel of 50 positive samples provided by CESAC reference laboratory has been analysed. Negative samples included avian infectious bronchitis, infectious laryngotracheitis, and Newcastle disease virus. Our results show high levels of specificity and sensitivity after 90 min, which could be reduced to 40 min with further optimization. Upon in vivo validation, a marketable and cheap diagnostic kit will be implemented in the animal health system as a routine method for assaying TRT. Funded by Spanish Ministerio de Ciencia e Innovación (PID2020-114956GB-I00) and Comunidad de Madrid (IND2019/BIO-17124).

Is *Hanseniaspora uvarum U1* an effective biocontrol agent against *Aspergillus flavus*?

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Keywords: microbiology, mycotoxins, biocontrol, *Aspergillus flavus,* aflatoxin B1

Summary

Recent studies have demonstrated that between 60% to 80% of all crops, globally, are contaminated with mycotoxins. Amongst all these mycotoxins, Aflatoxin B1 (AFB1) stands out for being the most potent natural carcinogen described. *Aspergillus flavus* is the main producer of this mycotoxin and due to climate change its incidence is expected to increase. Traditionally, fungicides have been the main choice to avoid fungi contamination, but in the last decade, more sustainable alternatives have been studied: such as the use of biocontrol agents.

In previous studies we have characterized the potential as biocontrol agent against *A. flavus* of the epiphytic yeast of grape *Hanseniaspora uvarum* U1. The aim of the present work is to further characterize this potential. New results have indicated that *H. uvarum* U1 is able to form biofilms, which enables it to successfully colonize different surfaces. Likewise, amongst the mechanisms involved in its interaction with *A. flavus*, results show that this yeast is able to produce volatile compounds that significantly affect the production of AFB1. However, nutrient competition doesn't seem to be a mechanism involved in its biocontrol potential since the niche overlapping index obtained ($NOI_{AF/HU}$=0.33 y $NOI_{HU/AF}$=1) indicates a nutritional dominance of *A. flavus* over *H. uvarum* U1. Finally, *H. uvarum* U1 maintains a high viability, even 4 months after lyophilization, which is a desirable characteristic for its management and industrial application.

These studies were financed by RTI 2018-097593-B-C21R.

Pulmonary surfactant biophysics and surfactant protein SP-B levels in COVID-19-related Acute Respiratory Distress Syndrome.

Paula Losada-Oliva[1], Chiara Autilio[1], David Sanchez-Ortiz[2], Alvaro Mingote[2], Raquel Castejon[2], Gonzalo Hernandez[3], Gema Díaz[2], Mercedes Echaide[1], Clara Salas[2], Juan Antonio Vargas[2], Jesús Pérez-Gil[1].

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Keywords: Pulmonary surfactant, Air-liquid interfaces, Covid-19-related ARDS, Proteins, Adsorption

Summary

Pulmonary surfactant (PS) is a membrane-based lipid-protein complex that forms films at the respiratory air/liquid interface, reducing the surface tension and thus minimizing the work of breathing. Since PS alterations have been described in non-COVID-19 direct ARDS as a result of the disease, PS might also play a major role in the severity of COVID-19 disease, which frequently leads to ARDS. This is the first ex vivo study that evaluates PS adsorption into air-liquid interfaces and the changes in the maturation, amount and/or oligomerization state of PS proteins, as a measure of the surfactant biophysical function, in COVID-19-related ARDS.

Bronchoalveolar lavages (BAL) were collected from 40 patients divided in 3 groups: (1) COVID-19-related ARDS patients, (2) patients without COVID-19 but with affected lungs due to various lung diseases and (3) patients without COVID-19 or any other lung disease. The biophysical properties of PS in BAL were assessed by an in-lab developed Surfactant Adsorption Test. PS protein levels were analyzed by Western Blot. BAL cell count was performed by flow cytometry. Results were normalized by phosphatidylcholine (PC) total amount.

Like in other forms of ARDS, the neutrophil/lymphocyte ratio measured in the BALs was significantly higher in COVID-19-related-ARDS group compared with the control group. Though no significant differences were observed in BAL total PC among the three groups, PS adsorption and accumulation at the air-liquid interface were significantly impaired in COVID-19-related ARDS patients compared to the other two groups, indicating a poor biophysical activity. However, no significant differences were detected between COVID-19-related-ARDS patients in acute or recovery phases, for both cell ratio and PS activity. Furthermore, a variation in the amount of PS proteins was also observed in BAL of COVID-19-related ARDS. These results support the idea that PS impairment has a role in the severity of COVID-19 onset leading to ARDS.

El desajuste de sincronizadores ambientales afecta a la homeostasis energética y la ansiedad en carpín (*Carassius auratus*).

Nuria Saiz[1], Miguel Ángel Marugal[1], Lisbeth Herrera-Castillo[1], Germán Vallejo-Palma[1], Ignacio Ruiz-Jarabo[1], Nuria de Pedro[1], María Jesús Delgado[1], Esther Isorna[1].

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Keywords: ingesta, metabolismo, sistema circadiano, cortisol, ansiedad.

Summary

En los peces se ha descrito una alteración en el funcionamiento del sistema circadiano debida a un desajuste entre señales sincronizadoras como el fotoperiodo y la alimentación. En este trabajo se estudió cuáles son las consecuencias de dicha disrupción temporal sobre el balance energético y la ansiedad en el carpín. Se dividieron 60 peces en tres grupos experimentales: Control, mantenido bajo fotoperiodo de 12h de luz y 12h de oscuridad (12L:12D), alimentado diariamente 1h después del encendido de las luces (ZT1); y dos modelos de cronodisrupción: sin el ciclo ayuno-ingesta (alimentación aleatoria, fotoperiodo 12L:12D); y otro en luz continua y alimentado a ZT1. La tasa metabólica aumentó en los peces en ausencia de uno de los dos sincronizadores. En paralelo, hubo un aumento del cortisol plasmático, así como mayores indicadores de ansiedad en pruebas comportamentales. También se observó una mayor ingesta en ambos grupos, asociada a una modificación en la expresión de reguladores neuroendocrinos de la alimentación (cart, orexina, npy, leptina). Por último, los animales con alimentación aleatoria mostraron un mayor crecimiento e índice hepatosomático. En general, el desajuste de sincronizadores ambientales aumenta la ansiedad, el estrés y la tasa metabólica, generando un mayor gasto energético. Además, en animales que no pueden anticipar la llegada de alimento se produce un balance energético positivo, similar a las alteraciones metabólicas descritas en modelos de mamíferos. Estos resultados sugieren que una homeostasis temporal resulta esencial para mantener una homeostasis energética en los peces, y evitar el estrés y ansiedad inducidos por una cronodisrupción.

The endocannabinoid system in the breast tumor microenvironment: Role in tumor progression and response to immunotherapy.

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Keywords: breast cancer, endocannabinoid system, tumor microenvironment, immunotherapy, medical cannabis.

Summary

Breast cancer (BC), one of the leading causes of female mortality worldwide, is a heterogeneous disease in which cancer cells interact with their surrounding stroma (tumor microenvironment, TME), whose alteration is now considered critical for cancer development and progression, as well as potential diagnostic tools and therapeutic targets.

The medical use of cannabis is increasing among cancer patients due to its palliative and potential anti-tumoral properties. The molecular target of cannabinoids is the endocannabinoid system (ECS), a complex cell communication system which is deregulated in BC. Our group has previously demonstrated that the overexpression of cannabinoid receptor 2 (CB_2R) in BC cells is associated with more aggressive phenotypes, but also that its activation by cannabinoids leads to anti-tumoral responses. However, components of the ECS, and more specifically CB_2R, are also expressed by immune cells, one of the main cell populations in the TME, where its activation has been associated with immunosuppressive actions.

We hypothesize that the ECS plays an essential role in the communication between BC cells and their surrounding stroma. To validate this hypothesis, we conducted *in vivo* experiments in a transgenic model of HER2-positive BC (the MMTVneu mouse). Our results show that lack of CB_2R slows down tumor growth, an effect accompanied by a higher immune infiltration, specifically of T cells. Additional preliminary results suggest that the absence of CB_2R is associated to a less immunosuppressive environment.

Together, our results indicate that the CB_2R expressed by the TME, specifically by the immune compartment, plays a role in BC progression.

The unsolved mystery of the philippine flat-headed frog, *Barbourula busuangensis* (Anura: Bombinatoridae).

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Keywords: *Barbourula*, Philippines, endemism, reproduction, chytrid, natural history.

Summary

The Philippine flat-headed frog, *Barbourula busuangensis* is an endemic anuran occurring in the island of Palawan and some adjacent, minor islands. *Barbourula* constitutes a very old clade, and it remains as one of the less studied anuran groups, despite the many reasons that make it strikingly interesting. Perhaps how these frogs reproduce is the most remarkable gap in our knowledge on them. Based on observations of gravid females carrying few, large, unpigmented eggs, and on the fact that no tadpoles of this species have ever been seen, the species might have a peculiar reproductive mode, involving direct development.

The current project is a multi-scope research to investigate the natural history of this anuran species. Do to so, several field study techniques are being used. Firstly, individuals of *B. busuangensis* belonging to two different populations (population 1 inhabiting the river in Malbato [Bintuan] and population 2 in a river in San Rafael [Busuanga]) have been marked along transects by means of micro-transponders. By the use of mark-recapture analysis we will be able to determine population dynamics. Secondly, skin swabs have been collected to assess the prevalence and impact of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in different localities. Thirdly, the reproductive behavior is being studied by active and passive methods (ethological observations and placing of automatic recording devices to record anuran calls). Finally, climatic data are being registered with data loggers, both at macro- and microhabitat level. In this presentation, an overview of the preliminary results will be discussed.

The role of climate and structural characteristics in spatial patterns of tree mortality and damage in Mediterranean forests.

Pedro Rebollo Orozco[1,2], Daniel Moreno-Fernandez,[1,3], Verónica Cruz-Alonso[1,4], Miguel A. Zavala[1], Antonio Gazol[5], Lorena Gómez-Aparicio[6], Julen Astigarraga[1], Enrique Andivia[1,2], Paloma Ruiz-Benito[1,7]

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Keywords: Spatial autocorrelation, Spanish Forest Inventory, tree mortality and damage, forest structure, minimum SPEI, water availability index.

Summary

In the last decades, temperatures and the intensity and frequency of disturbances such as pests or fires are increasing. These increases may interact with forest structure conditions causing tree mortality and damage events in forests around the world. It is known the magnitude of tree mortality and damage rates are increasing, from local to regional scales, which may alter the dynamics and composition of species. However, our knowledge about the spatial scales of tree mortality and damage events is limited. In this study, we evaluated the spatial patterns of tree mortality and damage events in Mediterranean forests of the Iberian Peninsula. For that, we used data from the Spanish Forest Inventory for tree mortality and damage data and other databases for the climate data. The preliminary results show stronger positive spatial autocorrelation in tree damage than tree mortality. The spatial autocorrelation of tree damage was greater for gymnosperms than angiosperms at distances up to 20 km. In addition, the spatial pattern of tree mortality and damage were influenced by climatic and forest structure variables. Based on the results we suggest that climate variability and forest structure have key implications in tree mortality and damage events, and these may increase in the future due to global change. So that, the design of management tools are necessary for the mitigation of climate change in Mediterranean forests.

Catch-all no more: integrative systematic revision of the genus *Allolobophora* (Crassiclitellata, Lumbricidae) with the description of two new relict earthworm genera.

Alejandro Martínez Navarro[1], Sergio Jiménez Pinadero[1], Thibaud Decaëns[2], Marta Novo[1], Dolores Trigo[1], Daniel Fernández Marchán[1]

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Keywords: Integrative systematics, molecular phylogenetics, earthworms, taxonomy, *Allolobophora*.

Summary

The taxonomy of earthworms has been riddled by instability, scarcity of systematically useful characters, and lax diagnoses of some genera. This has led to the use of some genera, such as *Allolobophora* Eisen 1874, as taxonomic wastebaskets, blurring their evolution and biogeographical history. The implementation of molecular techniques has revolutionized the systematics of the genus; however, some of its species have not been previously included in molecular phylogenetic analyses. Thus, a set of five molecular markers were sequenced for five endemic species including several taxa of *Allolobophora* and *Aporrectodea* (another related catch-all genus). The phylogenetic relationships recovered by Bayesian inference and Maximum Likelihood analysis supported the status of two of the five studied taxa (*Allolobophora burgondiae* and *Aporrectodea icterica*) as part of *Allolobophora* sensu stricto and a putative synonymy between *Allolobophora* and *Heraclescolex*. Branch lengths and average pairwise genetic distances support the transfer of *Allolobophora satchelli* to the genus *Panoniona*, and the rise of two new monospecific, French-endemic genera to harbor *Allolobophora tiginosa* and *Allolobophora zicsii* respectively. The changes of status and the diagnosis for the two new earthworm genera are presented. These results provide more evolutionarily and biogeographically coherent earthworm groups and highlight the Maghreb and the Alpine region as potential key locations for the diversification of *Allolobophora* and several lineages of *Lumbricidae*.

Targeting the midkine/ALK axis as a therapeutic strategy in gliomas.

Elena Tovar-Ambel[1,2], Israel López-Valero[1,2], José González-Martínez[1,2], Berta Segura-Collar[5]; Adrián Sanz-Gálvez[1,2], Nélida Salvador-Tormo[1,2], Mar Lorente[1,2], Estibaliz Gabicagogeascoa[1,2], Pilar Sánchez-Gómez[4], Juan M. Sepúlveda[5], Guillermo Velasco[1,2,3].

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Keywords: ALK receptor tyrosine kinase; Midkine; SOX; autophagy; combinational therapies; glioblastoma.

Summary

Glioblastoma (GB) is the most frequent and aggressive class of malignant primary brain tumor. These tumors exhibit a high resistance to radio- / chemo-therapy and (almost in all cases) they undergo recurrence shortly after their initial surgical removal. These features could be explained, at least partially, by the presence within the tumour mass of a small subpopulation of cells termed Glioma Stem-like Cells or Glioma Initiating Cells (GICs) due to their similarity with normal stem cells and their capacity to initiate and maintain tumour growth. Therefore, GICs elimination is considered a priority to fight GBM. Our group had previously found that increased expression of the growth factor Midkine (MK) correlates with a decreased survival of GBM patients, suggesting a possible role of MK in GBM initiation and growth. Likewise, we had found that MK promotes resistance to the pro-autophagic and antitumoral action of cannabinoids via activation of the Anaplasic Lymphoma Kinase tyrosine kinase receptor (ALK). In this work we focus on investigating the role of MK/ALK axis in the GICs population.

Anurans of the High Andean ecosystems of the province of Carchi-Ecuador: a biodiverse cryptic madness.

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Keywords: Anurofauna, Andes Mountain, Cryptic species, Call, Phylogenetic.

Summary

Carchi is one of the 24 provinces of Ecuador located in the north of this country. Its location and topography mean that it is surrounded by the branches of the Andes Mountain range and spread between mountain massifs. All these characteristics have made these areas present the ecosystems that host the most endemic Anurofauna (frogs, toads) on the planet. However, much of this diversity and endemism remains unknown.

Considering these data gaps, several field trips were carried out since 2016. The samples were focused on having an integrative work, giving species a morphological, acoustic and genetic identity. A total of 26 species of anurans were registered, with recordings of 21 of them. Molecular analyses focused on determining new species and analyzing the phylogenetic relationships of the *Pristimantis myersi* species group. The *Pristimantis myersi* group, is a group of small anurans endemic to Ecuador and southern Colombia. We shown that this group presents interspecific differences in their calls with acoustic patterns that may be related to their phylogeny. We also check that this group is formed by several cryptic species, ruling out the presence of some species for this province

The use of acoustic methodologies in herpetological studies has generated discoveries of cryptic species, in species that were thought to be only one. This has long led to an underestimation of the real value of anurofauna.

Finding functional gaps: G protein-coupled signalling pathways for VIP receptors.

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Keywords: GPCRs, VIP, VPAC, ß-Arrestins, Signalling, Internalization

Summary

VIP exerts its function throughout two class B GPCRs, VPAC1 and VPAC2, which expression and cellular location have been described to be different depending on the cell activation state as well as under pathological conditions, suggesting a functional diversity between them. Despite there are several studies regarding their signalling pathways and downstream outcomes, if these differences have a role at a functional level remains unclear. Throughout Bioluminescence Resonance Energy Transfer (BRET) assays in HEK 293 cells, we described that VPAC1 interacts mainly with Gs, Gq and weakly, Gi, while VPAC2 interacts with Gs and Gq. Moreover, whereas VPAC1 builds a stable interaction with ß-Arrestins 1 and 2, VPAC2 interaction seems to be ligand-dependent. Finally, we observed an interaction pattern suggesting an early endosome internalization process with VPAC2, while results from VPAC1 assays suggest an endosome-independent pathway. These results, which address first steps of their different signalling pathways, may open a new dimension of their pharmacology and functional outcomes.

Análisis de la diversidad genética y fenotípica en la levadura *Lachancea thermotolerans.*

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Keywords: *Lachancea thermotolerans,* diversidad genética, diversidad fenotípica, acidez, fermentación vínica

Summary

La fermentación vínica es un proceso en el que intervienen multitud de especies de levaduras. Durante la fermentación se consumen los azúcares presentes en el mosto de la uva, pero, debido al cambio climático, la cantidad de azúcares aumenta mientras que los ácidos orgánicos disminuyen. Estos ácidos son fundamentales para mantener la acidez del producto. Los cambios en las condiciones pueden originar diferentes problemas, tanto microbiológicos como tecnológicos, durante el proceso de fermentación. La levadura *L. thermotolerans*, es una de las pocas levaduras empleadas en enología con capacidad de producir ácidos orgánicos durante la fermentación. La producción de ácidos láctico, unida a la menor tasa fermentativa de esta levadura, hacen de ella una alternativa para mitigar los efectos del cambio climático en el sector enológico.

En este trabajo se ha desarrollado una técnica basada en PCR multiplex para determinar la diversidad genética empleando microsatélites en 400 aislamientos de esta levadura procedentes de diferentes regiones de la Península Ibérica. Posteriormente se seleccionaron los diferentes perfiles genéticos (cepas) obtenidos además de otras cepas de colección aisladas internacionalmente. Empleando esta colección de 320 cepas se determinaron las diferencias fenotípicas presentes en la especie mediante un cribado HTS en el que se emplearon 45 condiciones de cultivo diferentes. Se ha observado que la diversidad genética de la especie es mayor a la esperada inicialmente y que además está relacionada un comportamiento fenotípico diferente, definido por grupos genéticos más que geográficos.

Este estudio se enmarca en el proyecto LowpHWine (CDTI-CIEN).

VIP promotes in vitro osteogenic differentiation of a human bone marrow mesenchymal stem cell line.

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Keywords: VIP; osteoblast, osteogenic differentiation, cytoskeletal dynamics.

Summary

Osteoblasts are bone-matrix secreting cells derived from differentiation of mesenchymal stem cells. Vasoactive intestinal peptide (VIP) has been described as a molecule involved in neuro-immuno-osteogenic interactions regulating bone homeostasis. Although VIP has been reported as an enhancer of osteoblast biology in murine models, human studies are scarce. We aimed to investigate whether VIP promotes in vitro osteogenic differentiation of hTERT-immortalized human bone marrow mesenchymal stem cells. First, we analyzed the induction of Runx2 expression, a key osteogenic factor, and selected 10−8M as optimal concentration of VIP for further experiments. We then determined alkaline phosphatase (ALP) activity, an early osteoblastic marker; bone-matrix production by Alizarin Red staining and cytoskeletal dynamics involved in osteoblast commitment. Results showed that VIP presence during osteogenic differentiation enhanced Runx2 expression and ALP activity. Moreover, increased calcium deposition and changes in focal adhesions pattern were observed. Data suggest that VIP induce pro-osteogenic effects in human bone metabolism.

Role of HER2-CB_2R heteromers in Trastuzumab resistance in HER2+ breast cancer.

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Keywords: breast cancer, heteromers, trastuzumab resistance, endocannabinoid system.

Summary

Breast cancer is the second leading cause of death among women and is a very heterogeneous disease in terms of molecular features, prognosis, and treatments. We can distinguish three main subtypes, one of them characterized by overexpression of human epidermal growth factor receptor 2 (HER2), which represents roughly 15 to 20% of all breast tumors. Trastuzumab, a recombinant humanized monoclonal anti-HER2 antibody, has significantly improved the outcome of patients. Despite its efficacy, innate and acquired resistance remains an important clinical challenge. New therapeutic approaches and diagnostic tools are therefore warranted.

It is well documented that cannabinoids, the active ingredients of the hemp plant *Cannabis sativa*, produce antitumor responses in preclinical models of cancer. Besides, previous work of our group described a new mechanism controlling the oncogenic activity of HER2: heteromerization with de cannabinoid receptor 2 (CB_2R). The expression of these heteromers correlates with poor patient prognosis and Δ^9-tetrahydrocannabinol (THC) disrupts HER2-CB_2R complexes triggering antitumor responses *in vitro* and *in vivo*.

In this work, we want to analyze the role of HER2-CB_2R heteromers in trastuzumab resistance. Our results suggest that loss of expression of heteromers after neoadyuvant treatment, could confer resistance to this treatment. Moreover, we determined that expression of CB_2 receptor or heteromer HER2-CB_2R could be used as new resistance biomarkers. Finally, we observed that combination of THC with trastuzumab antibody in a trastuzumab resistant cell line, restore its sensitivity for this treatment, suggesting cannabinoids could be use as coadyuvant to reduce resistance.

Glucagon and adrenaline: Two modulators of Nocturnin expression in goldfish?

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Keywords: Adrenaline, glucagon, goldfish, *nocturnin*, organotypic culture, paralogs.

Summary

Nocturnin (NOC) is a phosphatase involved in the transformation of NADP(H) to NAD(H), performing a key role in the metabolism regulation. However, the possible elements that modulate its expression in fish are yet unknown. Our aim was to study the possible endocrine regulation and the intracellular pathways involved in the expression of *noc* paralogs (*noc-a1*, *noc-a2* and *noc-b1*) in goldfish (*Carassius auratus*). For this purpose, we analyzed the effect of glucagon, adrenaline and different activators and inhibitors of protein kinase A (PKA) and protein kinase C (PKC) pathways on *noc* paralogs expression in hepatopancreas organotypic cultures. We found a concentration-dependent induction of *noc-a1* expression by both glucagon and adrenaline at 2 and 6-h incubation time. Forskolin, an adenylyl-cyclase activator, induced a 5-fold increase of *noc-a1* relative abundance, meanwhile a 2-fold increase was obtained with phorbol 12-myristate 13-acetate (PMA), a PKC activator. Glucagon and forskolin effects were fully blocked by a PKA inhibitor (H89), and partially by a PKC inhibitor (chelerythrine) and by a PLC inhibitor (U73122), suggesting a possible interaction between PKA and PKC pathways in the glucagon signaling on *noc-a1* expression. Regarding *noc-a2*, our results show an induction by adrenaline at 2 and 6-h incubation time but not by glucagon, forskolin or PMA. Then, *noc-b1* seems to be insensitive to both hormones, and to PKA and PKC activators. Altogether, these results suggest a possible functional specialization of *noc* paralogs in hepatopancreas and aim to investigate the role of other metabolic regulators in the control of *noc* expression in goldfish.

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Structural characterization of pulmonary surfactant at the air-liquid interface by neutron reflectometry.

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Keywords: membrane proteins, neutron reflectometry, pulmonary surfactant

Summary

Overcoming the surface tension of alveoli is crucial for sustaining breathing dynamics. This is the function of pulmonary surfactant, a lipoprotein complex that forms an interfacial film covering the alveolar air-liquid interface, minimizing the surface tension at the end of expiration, a requirement to stabilize the alveoli and prevent alveolar collapse. Pulmonary surfactant is composed by 90 % (w) lipids, mainly phospholipids, and 10 % (w) associated proteins. To ensure the proper development of breathing cycles, a rapid adsorption of surfactant into the interface is crucial. This process is facilitated by the accumulation of material in subphase reservoirs connected to an interfacial air-exposed monolayer. Surfactant hydrophobic proteins SP-B and SP-C help in the formation and dynamics of these reservoirs by mediating membrane/monolayer interactions and facilitating lipid trafficking towards and from the interface, but the molecular mechanism of surfactant hydrophobic proteins is still not fully understood. To get deeper into the interactions between these proteins and surfactant phospholipids, we have performed neutron reflectometry experiments. Films were prepared using a model lipid system mimicking pulmonary surfactant composition (DPPC/POPC/POPG 50:25:15 w:w:w) in the presence or absence of SP-B and SP-C. Reflectivity was recorded at different pressures before and after the compression-expansion cycling of the film and structural parameters obtained from the reflectivity profiles were compared. Our results indicate that SP-B would be helping to connect the interface with its associated reservoirs, as well as the different bilayers within surfactant reservoirs between them. SP-C could facilitate the exclusion of unsaturated lipids optimizing surface-active properties of surfactant at maximal compression. Additionally, the reservoir and the adsorbed material undergo structural alterations as a consequence of compression-expansion cycles.

Matching habitat choice can drive ecological divergence and adaptive evolution.

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Keywords: matching habitat choice, adaptive evolution, ecological divergence, reproductive isolation, sympatry

Summary

For a long time, natural selection has been recognized as the only mechanism able to drive adaptive evolution. The environment acts as selective agent over individuals and populations get adapted to them. However, by shifting the focus to adaptation at the individual level, additional potential mechanisms of adaptive evolution have come to light. One of them is a performance-dependent type of habitat choice, called matching habitat choice. This ecological mechanism works when individuals assess their local performance in different environments and settle where it is highest. We tested if this mechanism can cause adaptive population divergence in zebra finches (*Taeniopygia guttata*) in captivity and tree sparrows (*Passer montanus*) in the wild. To achieve this, we manipulated their local performance in two different novel habitats. We deployed birds with two sets of PIT-tags that only allowed half of each population to access (supplementary) food in one of the two areas where PIT-operated feeders were placed. Then we let the individuals assess their local performance in both areas. Finally, to evaluate their choice of settlement, we checked where each individual bred by inspecting the nest boxes in these areas. We observed that the majority of individuals bred in the area where they had access to food, generating ecological divergence. Indirectly, this biased distribution caused positive assortative mating at the population level. If PIT-tags type was heritable, this would result in a high degree of reproductive isolation between ecologically distinct populations. Thus, matching habitat choice could potentially drive adaptive evolution and sympatric speciation.

The Synaptic Organization of the Human Entorhinal Cortex: A 3D Electron Microscopy Study.

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Keywords: Human Brain, Electron Microscopy, FIB-SEM, Entorhinal Cortex, Ultrastructure

Summary

The Entorhinal Cortex (EC) is a brain region located in the middle temporal lobe. It mediates the flow of information through the hippocampal formation to the neocortex, acting as an interface between sensory areas, the hippocampus and association cortices. These circuits are essential for memory formation, consolidation, and retrieval. A network like this comprises the connection of millions of neurons through a common element: the synapse. In this work, we have used advanced Electron Microscopy (Focused Ion Bean/ Scanning Electron Microscopy – FIB/SEM) to study the synaptic organization of the human EC.

FIB/SEM analysis enabled us to 3D reconstruct 5402 synapses from the deep layers of the EC (layers V, VI) obtained from 3 human autopsies. We used a specialized imaging software (EspINA®) to determine the synaptic density, proportions of asymmetric (excitatory) and symmetric (inhibitory) synapses, spatial distribution of synapses and synaptic size.

This study represents the first detailed description of the synaptic organization of layers V and VI from the human EC. Interestingly, our results emphasize that, in this region, the synaptic organization is layer-specific. In this sense, we found differences in the size of synapses between layers: while synapses from layer V shared an almost identical size, layer VI presented smaller synapses.

The characterization of the synaptic organization is crucial to better understand how brain networks actually work. Thus, the data presented here are essential to fully comprehend one of the most important circuits that shape the human brain, both in health and disease.

POSTERS

Development of subdomains in the medial pallium of *Xenopus laevis* and *Trachemys scripta*: insights into the Anamniote-Amniote transition.

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Keywords: Hippocampus, medial cortex, pallium, evolution, amphibians, reptiles.

Summary

In all vertebrates, the most dorsal region of the telencephalon gives rise to the pallium, which in turn, is formed by at least four evolutionarily conserved histogenetic domains. Particularly in mammals, the medial pallium generates the hippocampal formation. Although this region is structurally different among amniotes, there appears to be conservation in terms of its functions, attributed to spatial memory and social behavior, as well as the specification of the histogenetic domain. Thus, the aim of the present study was to analyze this region by comparative analysis of the expression patterns of conserved markersin two vertebrate models: one anamniote, the amphibian *Xenopus laevis*; and the other amniote, the turtle *Trachemys scripta elegans*, during development and in adulthood. Our results show that, the histogenetic specification and neurogenesis of both models is comparable despite significant cytoarchitectonic differences, in particular the layered cortical arrangement present in the turtle, not found in anurans. Two subdivisions were observed in the medial pallium of these species: a Prox1+ and another Er81/Lmo4+, comparable to the dentate gyrus and the mammalian cornu ammonis region, respectively. The expression pattern of additional markers supports this subdivision, which together with its functional involvement in spatial memory tasks, provides evidence for the existence of a basic program in the specification and functionality of the medial pallium in vertebrates, or at least at the base of tetrapods. These results further suggest that the anatomical differences found in different vertebrates may be due to divergences and adaptations during evolution.

EphB3 signaling modulates bone cell lineage homeostasis.

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Keywords: Ad-MSC, Eph/Ephrin, Osteogenesis, Osteoporosis.

Summary

Bone homeostasis is a complex process in which some Eph kinase receptors and their ligands Ephrins are implicated. In the present study, we examine the role of EphB3 in the osteogenic differentiation of adipose tissue-derived mesenchymal stromal cells (MSC). Remarkably, when osteogenic differentiation was induced, MSC from EphB3-KO mice showed higher expression of pro-osteogenic transcription factors (RunX2, Osterix and Msx2) than WT MSC. Also, EphB3-deficient MSC exhibited an increased amount of alizarin red stained calcium deposits after long-term osteogenic differentiation induction. To determine the implication of EphB3 in the in vivo osteogenesis, we used two experimentally induced models of osteoporosis, ovariectomy and long-term glucocorticoid treatment. While WT mice developed the disease in both models, EphB3-deficient mice did not exhibit the typical bone loss. This was evidenced by micro-CT analysis, evaluating bone volume and trabecular thickness after the induction of the experimental osteoporosis, as well as the levels of CTX-1 and calcium in serum and urine, respectively. To further explain the absence of osteoporosis in EphB3-KO mice we evaluated their osteoblast and osteoclast populations. In EphB3-KO mice the osteoprogenitor and pre-osteoblast populations were significantly higher than in WT mice, while the osteoclast proportions were significantly lower and showed reduced differentiation capacity. We conclude that EphB3 acts as a negative regulator of the osteogenic differentiation and its lack prevents the development of experimentally induced osteoporosis.

Checklist of Vascular Plants from Rio Muni, Equatorial Guinea.

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Keywords: Tropical Africa, biodiversity, conservation, floristics.

Summary

Río Muni is the continental region of Equatorial Guinea, located in Central Tropical Africa. The map of this country is completed by two island, Annobon and Bioko. Río Muni is the last area of Equatorial Guinea pending to have a complete checklist of its plant biodiversity. We studied 11,546 specimens collected in Río Muni. Bibliographic references for all groups were also checked, most of them against the project Flora of Equatorial Guinea bibliography. In total 7,985 records from bibliographic origin. The checklist is arranged in four natural groups: *Pteridophytes*, *Magnoliids*, *Monocotyledons*, and *Eudicotyledons*.

In Río Muni we find 2,649 plant species distributed in 178 families, of which 87% belong to seed plants (*Spermatophyta*). More than half of the species are concentrated in 11 families, the most numerous being *Rubiaceae* (301 sp), which also has the genus *Psychotria*, the most numerous with 68 species, *Fabaceae* (283 sp) and *Orchidaceae* (160 sp).

Of all the taxa present in Río Muni, 70% are Not Evaluated (NE), which prevents an in-depth study of the state of conservation of the vegetation in Equatorial Guinea. The figure is much more worrying when it comes to *Pteridophyta*, since the number of NE species exceeds 98% of the total, with only 9 species Least Concern (LC) and one Vulnerable (VU).

Different management conditions affect metabolic rate in goldfish.

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Keywords: acute stress, anesthetics, goldfish, metabolic rate, temperature, respirometry system.

Summary

Ensuring the welfare of fish when kept in captivity is of vital importance. Maintenance procedures can be stressful for the animals, thus requiring improvements to ensure a good quality of life for them. The aim of this study was to determine the metabolic rate (oxygen consumption -MO_2-) by intermittent-flow respirometry as a proxy to evaluate stress responses in goldfish (*Carassius auratus*) under regular maintenance procedures. The impact of three anesthetics, at doses of deep anesthesia, was tested: MS-222 (140 mg L^{-1}), 2-phenoxyethanol (0.5 mL L^{-1}) and clove oil (0.1 mL L^{-1}). The induction time of the anesthesia and recovery phases were examined too. Acute stressors like handling and turning the lights on/off were also evaluated, altogether with the effect of a sharp increase in temperature. The best anesthetic tested was 2-phenoxyethanol since it is the one that most quickly induced deep sedation and subsequent recovery. Handling induced an increase in MO_2, with maximum values 30 min after the challenge, while recovering basal values 2 hours later. Turning lights on/off had similar results, but on a smaller scale. Increasing temperature from 21.5 to 30 °C in 90 min induced a 250% increase in MO_2. Altogether, this confirmed that standard maintenance procedures affected metabolic rate of goldfish. The search for more benign alternatives for animals is proposed, such as the gradual on/off of lights, or avoiding major environmental disturbances. This study serves to develop tools to improve the welfare of fish in captivity.

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Protective effect of a cocoa-carob blend against oxidative stress in type 2 diabetes: Molecular mechanisms and preclinical effects.

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Keywords: flavonoids, cocoa, carob, oxidative stress, type 2 diabetes.

Summary

Oxidative stress plays a fundamental role in the development of type 2 diabetes (T2D) and its complications. In this sense, dietary flavonoids are antioxidant compounds that can be a strategy in the treatment of this disease. Therefore, the objective of this work is to investigate the effect of flavonoids on T2D, in combination or not with metformin, the first-line antidiabetic in the treatment of T2D.

A cocoa-carob blend (CCB) rich in flavonoids was developed. In the *in vitro* study, the effects on the redox status of epicatechin, one of the major flavonoids in CCB, and 2,3-dihydroxybenzoic acid, a colonic metabolite derived from flavonoid intake, in H9c2 cardiomyocytes were analyzed. After 24h of incubation, reactive oxygen species (ROS) levels decreased and antioxidant defenses increased, without affecting cell viability.

In the preclinical study, an animal model of T2D was used, ZDF (Zucker diabetic fatty) rats, and the antioxidant effect of a CCB-rich diet (10%) in heart and colon homogenates was studied. The results show that ROS levels, oxidative damage (carbonyl groups) and antioxidant defenses improved in both organs.

These results show the potential protective effect of flavonoids in the oxidative stress processes of T2D. The study in humans, currently underway, will allow us to delve into the role of CAM in the treatment of T2D.

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A study of pulmonary surfactant mechanical properties using atomic force microscopy: the role of hydrophobic proteins SP-B and SP-C.

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Keywords: pulmonary surfactant, atomic force microscopy, hydrophobic proteins, membrane structure.

Summary

Pulmonary surfactant is a membranous complex that enables breathing dynamics thanks to its ability to form films coating the alveolar respiratory surface, minimizing surface tension at the air-liquid respiratory interface. This extreme reduction in surface tension is achieved thanks to a unique mixture of lipids, mainly phospholipids, and proteins. Within the lipids, the saturated phospholipid species DPPC is its major component and the only one able to pack efficiently at the interface minimizing surface tension at the end of expiration. Other lipid components are needed to modulate DPPC's properties and facilitate its transit to the interface. The hydrophobic surfactant proteins SP-B and SP-C are crucial to allow for an efficient surfactant re-adsorption and re-spreading along recurrent breathing cycles. These proteins promote membrane curvature and contacts between lipid layers, however their role at the molecular level is not completely understood.

In this study we have used atomic force microscopy to determine some quantitative parameters that provide information about the effect of the presence of pulmonary surfactant proteins SP-B and SP-C on the structural and mechanical properties of membranes mimicking pulmonary surfactant composition (DPPC/POPC/POPG, 50:25:15 w:w:w). Results show clear differences when proteins are present in the lipid system in terms of membrane thickness, lateral organization, and elasticity, which provide evidence supporting the pivotal role of both proteins in modulating the biophysical properties of surfactant along respiratory dynamics.

Gene editing of *Aspergillus niger* using CRISPR-Cas9.

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Keywords: Gene editing, CRISPR-Cas9, *Aspergillus niger*, RNP, AYG1

Summary

Conventional genetic disruption in fungi has been found to be limited in many species due to their low capacity for homologous recombination. CRISPR-Cas9 has emerged as a good alternative to solve this problem and offers some other advantages. Under the direction of a single guide RNA (sgRNA), the endonuclease Cas9 recognizes and cleaves specific and targeted DNA sequences, producing double-strand breaks (DSB) in the genome. DSB are primarily repaired by non-homologous end-joining (NHEJ), resulting in small mutations such as indels. Gene disruption using CRISPR-Cas9 is usually performed using plasmids for heterologous expression of Cas9; however, this would limit the application of mutants as biocontrol agents since they would be considered transgenic. For this reason, the aim of this work was to develop a gene editing protocol using directly an exogenous Cas9 protein with the sgRNA generated *in vitro* in the mycotoxin-producing fungi *Aspergillus niger*. Although the main objective is the disruption of genes involved in ochratoxin A (OTA) synthesis, the protocol was optimized with the pigmentation gene AYG1 to facilitate the selection of mutants.

Protoplast transformation was performed using PEG-Ca or different electroporation conditions. A single pigmentation mutant was found using electroporation transformation. The positive transformant has a 108 bp deletion in the pigmentation gene but located off-target.

These preliminary results show that gene editing of *A. niger* is possible directly transforming with the Cas9 enzyme and sgRNA. However, further research is needed to understand why off-target mutations occur and how to minimize off-target effects.

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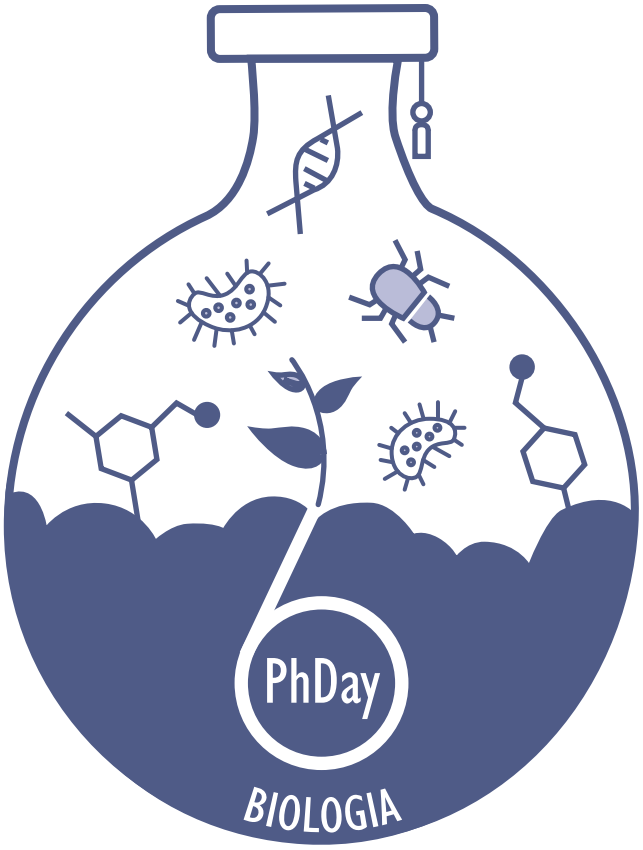

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