Oral Communications

Designing Reactivity Networks for Domino Molecular Construction

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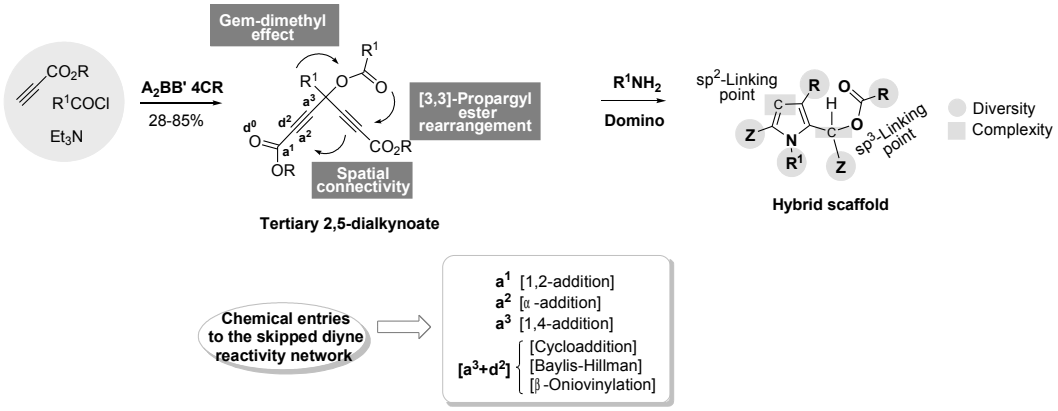
Among the most appreciated chemical complexity-generating reactions, domino processes[1] maintain a privileged status. They perform molecular complexity in a fast and efficient manner, accumulating important "green chemistry" values such as atom, time and labour economies, resource management and minimal chemical waste generation.[2] An appealing subclass of domino processes comprises the reaction of a densely and conveniently functionalized acyclic scaffold with a simple and readily accessible chemical reactant (amine, alcohol, C-nucleophiles, etc.).[3] The design and synthesis of these scaffolds constitutes a sought-after challenge in current organic synthesis and more specifically, in drug discovery research.[4] These densely functionalized structural units must be designed to accommodate three main practical requirements: a short synthesis (efficiency principle), a modular origin (diversity principle) and a defined interrelationship between functional groups (reactivity principle).

In this lecture we will describe this concept and how, conveniently designed reactivity networks, can be used as a practical chemical toolbox for the design and implementation of different domino-based complexity-generating process. As an example we will discuss the transformation of tertiary 2-5-dialkynoates into 2-chain functionalized tetrasubstituted pyrroles, which are hybrid scaffolds[5] comprising a structurally privileged pyrrole ring and a natural-occurring α-hydroxy acid motif.



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JAK-STAT-SOCS: Opportunities to develop anticancer drugs.

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Members of the Signal Transducers and Activators of Transcription (STAT) pathway, which were originally identified as key components linking cytokine signals to transcriptional events in cells, have been shown to have a critical role in cancer. Physiological activation of JAK-STAT signaling pathway is rapid and transient in nature. STAT are latent cytoplasmic proteins that form functional dimers with each other when activated by tyrosine kinases (e.g., JAK, Src, EGFR). Activated STAT proteins translocate to the nucleus to regulate expression of genes by binding to specific elements within gene promoters. Thus tyrosine phosphorylation and dimerization represent critical steps for activating STAT molecules. The JAK-STAT pathway is inactivated through both constitutive and inducible proteins. Among other negative regulatory proteins, STAT activation can result in transcriptional upregulation of Suppressor of Cytokine Signalling (SOCS) proteins that act as inhibitors of JAK. Activation of SOCS by STAT family members, therefore, results in a classic negative feedback loop to downregulate signal-transduction pathways that activate STAT molecules. Furthermore, loss of STAT function in normal cells generally has few deleterious consequences, likely reflecting the redundancies found in normal cellular signaling. Cancer cells by contrast, are often dependent on ongoing STAT-dependent gene expression for survival and proliferation. This, validate STAT as targets with the potential of displaying a very high therapeutic index. Thus, the development of inhibitors of tyrosine kinases and STAT as well as activators of natural negative regulators such as SOCS proteins might be potential pharmacological tools in cancer.

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KEYNOTE LECTURES.
INVITED SPEAKERS

Identification, characterization and validation of molecular target relevant for cancer therapeutics.

Amancio Carnero

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KEYNOTE LECTURES.
INVITED SPEAKERS

Molecular mechanisms regulating cell cycle exit: their role in cell differentiation, genomic stability and cancer

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Progression throughout the cell cycle is modulated by different E3 ubiquitin ligases that target cell cycle regulators for degradation in the proteasome. The Anaphase-Promoting Complex (APC) is an E3 ubiquitin ligase that associates with two co-activators, Cdc20 and Cdh1. APC-Cdh1 is active from anaphase until the end of G1 and promotes the degradation of cyclins A and B, Cdc20, Aurora A, Aurora B and some DNA replication regulators, such as Cdc6 and geminin. To understand the specific roles of Cdh1 and the possible overlap with Cdc20 function, we have recently generated Cdh1 conditional knock out mice and analyzed cell cycle progression in the absence of Cdh1. Cdh1-deficient mouse embryonic fibroblasts grew in culture with slower kinetics than wild-type cells. These mutant cells displayed deficient exit from mitosis and abnormal S-phase entry after stimulation with serum. The relative levels of APC-Cdh1 substrates will be presented and the consequences of deregulated APC-Cdh1 activity on DNA replication and genomic instability will be discussed.

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Characterization of cytotoxic, estrogenic and antiestrogenic properties of novel plant-derived compounds

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The classical Phytoestrogens are a large family of non-steroideal plant-derived estrogen possessing significant estrogen agonist/antagonist activity. These naturally occurring molecules includes the isoflavonoids, the lignans, the coumestanes, the stilbenes and flavonoids. The discovery of many more novel estrogen-like compounds in the plant kingdom demonstrates that the spectrum of phytoestrogens in Nature is expanding. The classical as well as the novel phytoestrogen exert pleiotropic effects on cellular signaling and show some beneficial effects on estrogen-dependent disease. Many of the biological activities, mediated via the estrogen receptor subtypes ER_α and ER_β, have been shown to be cell type/tissue specific and dose dependent. The so far known phytoestrogen may act as "natural" SERMs and may possibly be considered for the prevention of postmenopausal pathologies including osteoporosis, cardiovascular disease and cancer.

Current data demonstrate that many more novel compounds are continuously being identified in nature exhibiting estrogenic/antiestrogeninc properties, indicating that our knowledge about phytoestrogen in Nature is expanding. In this context, a screening program to characterize new phytoestrogenic compounds is being developed in our laboratory. A number natural pure compounds has been tested for their potential cytotoxicity and estrogenic/antiestrogenic activity using the NCI chemosensitivity assay and the modified MCF-7 cell proliferation assay (E-SREEN assessment system), respectively. The anticancer activity, measured as GI_{50}, against a panel of Breast cancer cell lines were in the range of 100nM-100uM. Many of the compounds induced the proliferation of the MCF-7 cell line in a dose-dependent manner with a potency and proliferative effect lower than that of 17β-estradiol (EC_{50}=10 pM and 1nM 100%, respectively) indicating a estrogenic effect in this cellular system. Three of the tested compounds showed significant antiestrogenic activity with a lower potency than that of pure antiestrogen ICI182,780, used as antiestrogenic control in the E-SCREEN bioassay

These new phytoestrogens represent new templates for future molecular modifications oriented to the synthesis and characterization of new SERM compound.

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Computational Tools in Drugs Design. From Molecular Models to the Mechanism Elucidation

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Computational chemistry techniques are routinely used in rational drug design of new and better molecules. These computational tasks are focused on structure based searching for lead compounds that complement a given binding site, suggesting how to modify known ligands to improve its affinity and/or specificity. These computational techniques are essential for data management and cost-effective development in Pharmaceutical R&D.

Theoretical molecular models led us to design specific experimental assays to gain information about the mechanism of action of drugs.

In this communication we will discuss an example which illustrates the global process of predicting the binding mode of antitumoral compounds from the molecular model previously described.

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Induction of G_2-M phase arrest and apoptosis by naturally occurring α-methylene-γ-butyrolactones in human leukemia cells

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In recent years, the use of naturally occurring agents to prevent the development or recurrence of cancers has become widely accepted as a realistic option for controlling the disease. Many natural products are known to contain an □-methylene-□-butyrolactone moiety, mainly sesquiterpene lactones (SL). These compounds have attracted attention during the last fifty years because they display a wide range of biological activities, including antitumor properties. In this study, we investigated the effect of two naturally occurring SL on viability of 5 human tumor cell lines and found that these compounds were cytotoxic against leukaemia and melanoma cells. Studies performed on HL-60 cells indicate that SL asteriscunolide A induce G2-M arrest and apoptosis through a caspase-dependent mechanism. Apoptosis was associated with (*i*) cytochrome *c* release, (*ii*) triggered in Bcl-2 and Bcl-x_L over-expressing leukemia cells, (*iii*) the activation of the mitogen-activated protein kinases (MAPKs) pathway and (*iv)* reactive oxygen species generation.

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Are Michael Acceptors undervalued anticancer drugs?

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Drug discovery continues to provide new and important leads against various diseases including cancer. Traditionally, natural products represented the most important source of inspiration for scientists in the development of new anticancer drugs. With the advent of combinatorial chemistry and molecular biology, pharmaceutical companies have synthesized several targeted libraries designed to inhibit a given class of cellular target. It was soon recognized that the quality of a library would determine its success thereby overcoming the problem of efficient hit and lead finding. In particular, libraries based on privileged structures have proved to be extremely powerful tools to aid in the discovery of selective drugs for a wide variety of cellular targets.

From the various types of chemical binding between anticancer drugs and cellular targets, covalent binding is one of the most powerful strategies in cytotoxic drug design. The traditional anticancer drugs nitrogen mustards, cisplatin and alkylsulfonate bind covalently to DNA, inducing cancer cell death. A number of natural cytotoxic compounds contain Michael acceptors (naphthoquinones) or are transformed inside the cell into Michael acceptors (mitomycin). There is a wide variety of chemical classes that bind covalently to cellular targets, including pyrrolo[1,4]benzodiazepines, nitrosoureas, quinones, etc. In this particular context, we have focused our interest in a specific group of Michael acceptors, that is, α,β-unsaturated carboxyl derivatives. Some authors claimed that Michael acceptors lack selectivity due to direct binding to various cellular nucleophiles. Despite this concern, Michael acceptors can be structurally modified, so that they can react selectively with target nucleophiles. As a consequence Michael acceptors inhibitors of cysteine protease have already progressed to clinical testing.

Within our program directed at the discovery of bioactive substances for cancer treatment, we have drawn our attention on novel Michael acceptors.

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KEYNOTE LECTURES.
INVITED SPEAKERS

The role of the tumour suppressor Hippo pathway in epithelial proliferation and axis specification

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In *Drosophila*, the body axes are specified during oogenesis through interactions between the germ line and the overlying somatic follicle cells [1-5]. A Gurken/TGF-alpha signal from the oocyte to the adjacent follicle cells assigns them a posterior identity [6, 7]. These posterior cells then signal back to the oocyte, inducing the repolarization of the microtubule cytoskeleton, the migration of the oocyte nucleus and the localization of the axis specifying mRNAs [8-10]. However, little is known about the signaling pathways within or from the follicle cells responsible for these patterning events. We show that the Salvador-Warts-Hippo (SWH) tumor suppressor pathway is required in the follicle cells in order to induce their Gurken- and Notch-dependent differentiation, and to limit their proliferation. The SWH pathway is also required in the follicle cells to induce axis specification in the oocyte, by inducing the migration of the oocyte nucleus, the reorganization of the cytoskeleton, and the localization of the mRNAs that specify the anterior-posterior and dorsal-ventral axes of the embryo. This work highlights a novel connection between cell proliferation, growth and axis specification in egg chambers.

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Genetics & Epigenetics profile of unstable endometriod endometrial cancer

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Background: tumors with microsatellite instability (MSI) have a characteristic phenotype, and patients with MSI tumors have a distinctive clinical follow-up. MSI phenotype is present in 15-20% of endometrial cancer (EC) and occurs, almost exclusively, in cancers with endometrioid histology (EEC). Aberrant Wnt signaling has been implicated in endometrial carcinogenesis. **Aim:** to get more insight about the relationship between MSI and the alteration of some Wnt signaling pathway members in EEC. **Design:** MSI (by genotyping) and promoter hypermethylation (by MSP) of SFRPs and Wif1 genes were analyzed in 197 EEC, and the results compared with the changes in others, previously studied, Wnt pathway members. **Results:** SRFP5 and WIF1 promoter hypermethylation was higher (p=0.002 and P=0.04, respectively) in tumors with MSI. Conversely, promoter hypermethylation of SFRPSs 1, 2, and 4 genes was unrelated to MSI status. **Conclusion:** our results suggest that in EEC, MSI phenotype is associated with the specific alteration in some Wnt pathway members, such as SRFP5, WIF1, and APC, but not with others, such as E-cadherin, β-catenin, and SRFPs 1, 2, and 4.

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Target genes offor microsatellite instability in endometrial cancer

Bilbao C, Ramírez R, Rodríguez G, ~~Ramírez R,~~ Falcón O, León L, Castro V, Almeida L, Díaz-Chico BN, Perucho M, Díaz-Chico JC

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Microsatellite instability (MSI) is the hallmark of cancer with DNA mismatch repair (MMR) deficiency. MSI is a widespread phenomenon throughout the genome driving to the accumulation of~~Tumors with MSI accumulate~~ hundreds of thousands of somatic insertions/deletion mutations in simple repeated sequences known as microsatellites. Among this bulk of alterations cancer arises when mutations occur within coding short repeat sequences of cancer-related genes (target genes). However because of the high background of genetic instability in these tumors it is difficult to differentiate genes that are under positive selection during tumor progression (real MSI target) from those that are not (bystander genes).Two different statistical approaches have been published to distinguish real from bystander genes based on the mutation frequency in colorectal, gastric and endometrial MSI cancers (Duval et al, 2001 and 2002; Woerner et al, 2003). These approaches were used to establish a cut-off value by means of which genes with a mutation frequency above the cut-off would be considered as real target and those below it would be bystanders. Regarding to this it has been pointed out that mutation frequency may not be reliable enough by itself and genes with infrequent mutations should not be considered as irrelevant. This is because according to the accumulative haploinsufficiency model, MSI tumors accumulate so many mutations that disruption of a cancer related pathway may be achieved in different tumors by mutations in different genes of the same pathway with a synergic effect (Koichi e tal, 2002; Perucho 2003). On this line it has been recently published a study where the authors perform the analysis of the coding mononucleotide repeat region of 25 genes involved in the major damage signaling and repair pathways (Miquel et al, 2007) in colorectal MSI cancer.

~~To get some insight about this issue I~~In the present work we analyzed 40 potential candidate genes in a series of 41 MSI endometrial tumors, and ~~expose and~~ compare ~~ntrast the~~ different approaches to identify MSI real targets. ~~among 41 potential target genes analyzed in a series of 41 MSI endometrial tumors.~~

The microsatellite length within the genes ~~microsatellite status of the potential target genes was~~ was established by PCR and genotyping (ABI Prism 3100). T11 in MRE11 (45%), G8 in BAX (34%), A11 in TAF1B (32%), A10 in AIM2 (20%) and A10 in TGFBRII (23%) were the repeated tracts ~~genes that~~ showing the highest alteration~~mutation~~ frequency.

By applying Duval's statistical approach we observed that 19 genes were real target. Considering only the genes we have studied in common (10 genes) we disagreed in three genes (BML, RAD50 and MBD4). When we used the Woerner's approach none of the 40 genes were over the cut-off line~~in our seriestwo criteria by two different research groups based on the mutation frequency~~. Twenty five of the 40 potential targets were the genes involved in damage signaling and repair pathways previously studied in colorectal cancer. We observed that 54%, 71% y 49% of the tumors presented mutations in at least one gene of the DNA damage signaling, DSB repair and MMR pathways respectively. ~~. We observed that around 49-66% of the DNA damage response, DSB and excision repair~~ Also that 37% of the MSI tumors presented mutations in at leas two genes of the DSB repair pathway. On the other hand at least half of the analyzed genes of the mentioned pathways were real targets according to the Duval approach.

The discrepancy among the results obtained by the two statistical approaches to identify real target genes may suggest that according to the happloinsufficiency model mutation frequency by itself may not be enough. On the other hand these results suggest that DNA damage and DSB repair pathways are also involved in the carcinogenesis of MSI endometrial cancer tumors. Finally the consideration of entire pathways instead of single target genes with high mutation frequency may open new possibilities for the understanding of this pathology.

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KEYNOTE LECTURES.
INVITED SPEAKERS

Determination and characteristics of MSI tumors, defective in the DNA mismatch repair system

Richard Hamelin

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Production of Natural Compounds with Antitumour Activity by Biotechnological Procedures

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Plants display an immense genotypic and phenotypic diversity and the number of higher plants is estimated to be approximately 250,000-300,000 worldwide[1-2]; with only a marginal number of them, being fully studied at the phytochemical level (8-10%). This number is even inferior when considering studies carried out at the molecular level. Higher plants are described as chemical factories able to synthesize unlimited numbers of highly complex and unusual chemical substances whose structures can be considered infinite. It is recognized that plants are sessile organisms interacting and communicating with their immediate environment *i.e.* other plants, pathogens, animals, etc. by chemical means. Accordingly, plants have acquired and evolved, through million of years of evolution, specialized biosynthetic networks also called secondary metabolic pathways, producing an extraordinary immense array of molecules. Therefore, the vast potential offered by natural resources for the discovery and development of new therapeutics of great benefit to mankind is clear and it is being exploited.

Secondary metabolites are complex low molecular weight molecules produced by diverse organisms although here mainly plant secondary metabolites are considered. Many of them are used and exploited as pharmaceuticals, flavours, fragrances, insecticides, dyes, food additives, toxins, etc. It is estimated that around 270,000 natural products are known, and each year around 4,000-5,000 new compounds are elucidated. Moreover, the importance of natural products is tremendously elevated; thus, of all drugs used in western medicine around 40-45% are natural products or compounds derived from them, and of these, 25% are obtained from plants.[3] Moreover, for anticancer compounds (60%) and antiparasitic drugs (75%), the dominant role of natural products or derivatives is even more evident.[4-5] Unmistakably, through evolution nature has been fabricating and selecting natural products which wait to be taken, assessed and exploited.

On the other hand, it is well known that plant natural products yield is frequently low and depends primarily on the physiological and developmental stage of the plant. Often the yield obtained ranges from 0.001%, to the best cases of 10-20% with the majority of pharmaceutically important secondary metabolites being obtained from wild or cultivated plants. Although some attempts have been made, their chemical synthesis in most cases has not been economically feasible, and plants remain as the major source of these molecules. In other cases, more abundant precursor molecules are obtained in large amounts from the plant, and then after simple chemical modifications through semisynthesis, the final active secondary metabolite is obtained *i.e.* the isolation of baccatin III, precursor of paclitaxel (Taxol®), which is highly abundant in the leaves of *Taxus baccata* or *T. wallichiana* is extracted and following chemical modifications, the final product is obtained in large quantities to satisfy the world demand.[6] Currently, paclitaxel is also being produced by large scale plant cell culture technology by Phyton Biotech which is one of the leading suppliers of this anticancer drug. Furthermore, the renaissance of natural products as drug candidates has been reclaimed mainly after combinatorial chemistry failed to provide the chemical entities thought to be obtained through such approach, and by emphasising their potential in drug discovery, particularly due to their extraordinary specificity and potency gained through evolutionary selection, compared to artificially designed molecules.[7]

Secondary metabolites have been produced through plant biotechnology employing different types of *in vitro* cultures, such as callus, suspended cells, organ cultures, as well as hairy roots, which has received much attention as a useful technology for the production of valuable plant bioactive metabolites reporting different degrees of success.[8-9] The current state of plant biotechnology research permits the use of a number of different approaches including high-throughput methodologies for functional analysis at the levels of transcripts, proteins, and metabolites, and methods for genome modification by both homologous and site-specific recombination. Plant biotechnology allows for the transfer of a greater variety of genetic information in a more precise and controlled manner, and these are being applied for instance to manipulate secondary metabolite biosynthetic networks, aiming at attaining larger product yields after the establishment of transgenic plants or plant cell cultures with an improved productivity of the desired compound(s).

Since Bailey[10] defined the metabolic engineering as "the improvement of cellular activities by manipulation of enzymatic, transport and regulatory functions of the cell with the use of recombinant DNA technology", this discipline has grown as a tool for manipulating biosynthetic pathways leading to secondary metabolites, and it appears feasible that a continuous production of plant natural products through metabolic engineering can be accomplished.[11-13]

As an example of the application of biotechnology for the production of bioactive compounds, the case of *Echium* research is presented. This plant genus is represented in the Canary Islands by 23 endemic species, and many of these have been considered to be a major source of polyunsaturated fatty acids (PUFAs) *i.e.* □-linolenic acid (GLA) and stearidonic acid (SDA). We have devised a new approach for the production of these compounds by means of the induction and establishment of hairy root cultures of *E. acanthocarpum* following guided infection with *Agrobacterium rhizogenes*. Our results showed for the first time that the roots are a site of synthesis of PUFAs, together with earlier reports stating that synthesis also occurs in the aerial parts[14], demonstrating the presence of the biochemical machinery, composed mainly of desaturase and elongase enzymes in this plant organ. Moreover, it was also determined that the PUFA yields were almost uniform throughout the culture period, with linoleic acid being the most abundant, followed by palmitic acid, GLA, □-linolenic acid, oleic acid and SDA. In order to improve the yield of the □-3 SDA in this culture system, the over expression of the □6-desaturase gene from *Primula vialii* which specifically converts □-linolenic acid into SDA[15] is being attempted.

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Lipid Sensing Nuclear Receptors as Integrators of Inflammation and Metabolism

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The liver X receptors (LXRα and LXRβ) are ligand-dependent transcription factors that belong to the nuclear receptor superfamily. Previous studies demonstrated that activation of LXRs can be achieved with physiological concentrations of oxidized cholesterol derivatives, and are therefore considered as endogenous cholesterol sensors. Activation of LXR promotes the expression of genes involved in cholesterol homeostasis and play a key role as endogenous inhibitors of atherosclerosis. Our previous work demonstrated that LXRs also inhibit the expression of inflammatory genes through transrepression of NF-κB signaling, suggesting that LXRs are transcription factors implicated in the resolution of inflammation.

Macrophages are multifunctional scavengers of extracellular debris, including modified LDL and apoptotic cells, and express high levels of both LXRs. Other studies have revealed that LXR is critical for cellular lipid homeostasis following ingestion of lipoprotein-derived cholesterol, but its role in apoptotic cell uptake has not been explored before. An inefficient resolution of inflammation causes the release of cellular debris and macrophage-derived cytokines that may be injurious to normal cells and tissues and leads to chronic inflammation and autoimmunity. Here we present preliminary evidence demonstrating that an intact LXR signaling is important for both apoptotic cell clearance and the maintenance of immune tolerance. Apoptotic cell engulfment or exogenous LXR

ligands activate the LXR pathway and promote transcriptional changes important for phagocytosis of apoptotic cells. LXR null macrophages exhibit a selective defect in phagocytosis of apoptotic cells and an aberrant pro-inflammatory response to them. As a consequence of these defects, mice lacking LXRs manifest a breakdown in self-tolerance and develop autoantibodies and autoimmune glomerulonephritis. These results implicate LXRs in a positive transcriptional feedback loop that couples apoptotic cell clearance with the suppression of autoimmunity.

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Proteases in Protozoans as Therapeutic Targets

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Protozoan parasites are a diverse group of unicellular organisms of the kingdom Protista. Study of parasitic proteases has received considerable attention, since their physiological role elucidation may help to develop strategies for exploiting these enzymes as novel chemotherapeutic targets (Cazzulo 2002). Identification and characterization of protease-mediated processes in parasitic protozoa are progressing at a rapid rate.

Intracellular and extracellular proteases have been reported from a number of protozoa. The malarial proteases digest proteins of the cytoplasm membrane of the red blood cells thereby affording invasion and infection by the parasite performing several functions such as mediation of the *Plasmodium* entry into host erythrocytes and formation of the parasitophorous vacuole. The aspartic proteases are transported to the food vacuole for haemoglobin hydrolysis but also are capable of exiting the parasite to act against host cytoskeletal proteins (Braun-Breton 1992). Trypanosomatid protozoa serine peptidases have been indentified and characterized and play crucial roles in host infection. This enzyme hydrolyzes components of extracellular matrix, allowing the parasites to migrate through the host tissue.

The genus *Acanthamoeba* are free-living amoeba opportunistic protozoan parasites that pervade the entire environment and can found in tap, fresh water, soil and contact lens solutions and eyewash stations. In its life cycle, Acanthamoeba has only two stages, cysts and trophozoites. Entry can occur through the eye, the nasal passages to the lower respiratory tract, or ulcerated or broken skin. Chappel 2001 showed that more than 80% of the normal human population exhibited antibodies against *Acanthamoeba*. Extracellular proteases of *Acanthamoeba* are markers for differentiating pathogenic from non-pathogenic organisms. Lorenzo-Morales et al. 2005 confirmed the importance of serine proteases in the pathogenesis of these amoebas as well as the relationship between the adaptation of *Acanthamoeba* to stress conditions and their capacity to cause disease.

We screened 32 water samples from different areas in Canary Islands. In Tenerife, *Acanthamoeba* were identified in 72,2% tap water samples, and in Gran Canaria, *Acanthamoeba* were identified in 57% samples by morphology and molecular assays. We took out extracellular proteases to characterize the pathogenic potential of the isolated strains and we can see different isolates strains exhibit different protease banding patterns.

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KEYNOTE LECTURES.
INVITED SPEAKERS

Chromatin modifying enzymes: their function and role in cancer

Tony Kouzarides

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Modifications of chromatin play an important role in the regulation of many biological processes, including transcription, DNA repair and replication. There are at least eight different classes of enzymes that modify histone and generate covalent and non-covalent changes to key residues within histones. Many lines of evidence now point to such enzymes as being implicated in a variety of cancers. Interest in the development of anti-cancer drugs that act on this class of enzyme is high, following the approval of a deacetylases inhibitor in the treatment of Cutaneous T cell Leukaemia.

Our lab is interested in characterising the function of chromatin modifying enzymes and understanding the function of different modifications in the process of transcription. We are involved in characterising the methylation of lysines and arginines, the conversion of arginines to citrulline. In addition we have a drive to identify novel modifications of chromatin. Two novel pathways will be presented that culminate a) in the phosphorylation of tyrosine within histone H3 and b) the isomerisation of prolines within histone H3. In both cases, evidence exists that the pathways are miss-regulated in cancer cells.

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Role of the plasma membrane and cell-cortex dynamics in HIV infection

Agustín Valenzuela-Fernández

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IGF-1R and vaults are new determinants of tumor radiation resistance

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IGF-1 and its receptor seem to have a central role in cancer progression. From epidemiological studies we have found a non-linear dose-response curve was observed between Total Cyclodienes Body Burden (TC; sum of aldrin, dieldrin and endrin) and IGF-I in men (p=0.024). These findings suggest that OCs could modulate the IGF-system in a way that is highly influenced by gender, age and by chemical or combination of chemicals implicated. Such circumstances may contribute to the development of a number of diseases related to IGF-I and should be taken into account in public health decisions.

In cervical cancer IGF-1R overexpression was a strong predictor of clinical outcome. High MVP (major vault protein related to chemoresistance) and IGF1-R tumour expression was strongly related to poor local and regional disease free survival (p=0,006), distant disease free survival (p=0.050) disease-free survival (p=0,006), cause specific survival (p=0.007) in patients achieving a complete response.

Response to radiotherapy is strongly influenced by hypoxia. In our studies hypoxia inhibits the NHEJ DNA repair through downregulating Ku70/80 expression combined with an increased angiogenesis and altered p53 expression. These mechanisms would be responsible for tumor progression in cervical carcinoma. Furthermore we showed for the first time that severe tumor hypoxia upregulates MVP expression but not IGF-1R in clinical cervical tumors, confirming previous preclinical studies about the role of hypoxia in favouring increased chemoresistance.

We hypothesize that an early regulating mechanism that favours homologous or NHEJ repair at first, mediated by vaults along with other factors yet to be elucidated. If vaults are overexpressed, NHEJ repair may be suppressed by several mechanisms, with resultant genomic instability. These mechanisms may be associated with the decision of damaged cells to survive and proliferate, favoring tumor progression and reducing tumor response to

oncological treatment through the development of resistant cell phenotypes. Further clinical studies will be necessary to test this hypothesis.

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KEYNOTE LECTURES.
INVITED SPEAKERS

Epidermal stem cells in tissue homeostasis and cancer

Salvador Aznar Benitah

Center for genomic regulation (CRG), Barcelona

Adult stem cells are potentially the only long-term tissue residents that can accumulate enough oncogenic mutations resulting in the development of neoplasias. Upon transformation, adult stem cells retain hallmarks of stemness such as self-renewal, high proliferation potential, and tissue-remodelling activities, among others, but loose the ability to follow the organizational cues that restrain uncontrolled growth and invasion in healthy tissues. We have previously shown that Rac1 GTPase is required to maintain epidermal stem cells quiescent and located within their niche. Upon deletion of Rac1, epidermal stem cells exit the niche through a by-functional mechanism, cell cycle entry and egression from the stem cell niche that ultimately results in loss of self-renewal of the entire epidermal unit. Mechanistically, Rac1 exerts some of its epidermal effects via PAK2-mediated phosphorylation of the transcription factor c-Myc. Here we propose a novel function of Rac1 and c-Myc in epidermal stem cells and squamous tumours. Phosphorylation of Myc, downstream of PAK2, regulates quiescence and self-renewal of skin progenitors, affects the onset of differentiation, and modulates homing to the stem cell niche. In addition, phospho-Myc changes the invading and tissue remodelling potential of epidermal progenitors, and squamous cell carcinomas in 3D and 2D assays. At the mechanistic level, phosphorylated Myc might impose a stem cell signature by regulating polycomb proteins. Thus, we propose that the Rac/PAK2/Myc axis is required to sustain epidermal stem cell homeostasis and that deregulation of this pathway might play a role in epidermal neoplasias.

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INVITED SPEAKERS

From metabolism to cáncer through choline kinase: Implications in prognosis and treatment

Juan C. Lacal, CSIC-IIB, Madrid

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