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NANOTECNOLOGIA: QUANTUM DOTS: ¿SIMPLES MARCADORES LUMINISCENTES?

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El desarrollo de las nanopartículas semiconductoras conocidas como quantum dots ha evolucionado en las dos últimas décadas del área de la ciencia de los materiales a las aplicaciones biológicas y clínicas. Los quantum dots están emergiendo como una nueva clase de marcadores luminiscentes con especial luminosidad, resistencia a la fotodestrucción y emisión multicolor. Propiedades ópticas especiales de estas nanopartículas son su emisión en forma de líneas angostas y su muy ancho espectro de excitación, que permite la observación de un elevado número de marcadores diferentes en forma simultanea. La característica única y fundamental cuando se realizan observaciones por tiempos prolongados es su alta fotoestabilidad. Esta propiedad ha permitido el rastreo de eventos biológicos a nivel celular imposibles de realizar con marcadores fluorescentes usuales. Se discutirán nuevos métodos que permiten la funcionalización, marcaje específico, y el control sobre las propiedades ópticas de estas nanopartículas, con un énfasis en las aplicaciones en biología celular y animal, así como nuevos desarrollos para estudios clínicos. Ejemplos recientes incluyen la observación de los primeros pasos en la cascada de eventos iniciada por el EGF por su interacción con la familia de receptores tirosina kinasa y la identificación de nódulos centinela en animales vivos. Las nuevas generaciones de quantum dots tienen un gran potencial para el estudio de procesos intracelulares a nivel de moléculas individuales, para las observaciones por tiempos prolongados in vivo de eventos de tráfico celular, la detección de tumores tempranos y para el desarrollo de nuevos métodos de diagnóstico. Por otra parte se discutirá un rol adicional de estas nanopartículas, la posibilidad de actuar como iniciadores de procesos biológicos, ejemplificando con estudios sobre la agregación de proteínas amiloides.

CONFERENCIA LEONARDO SATZ

LECTINAS EN INMUNIDAD INNATA: LA PRIMERA TRINCHERA (Y EL CABALLO DE TROYA)

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Las lectinas solubles y asociadas a las membranas de células fagocíticas constituyen componentes clave de las respuestas de inmunidad innata en invertebrados y vertebrados. Sus funciones biológicas incluyen no sólo el reconocimiento de patógenos y parásitos microbianos, sino también funciones efectoras, tales como aglutinación, inmobilización, y activación de las cascadas de complemento y melanización, cuyos productos son opsónicos, líticos o tóxicos. En los mamíferos, incluyendo el hombre, las lectinas no solo participan como proteínas de fase aguda en el reconocimiento de antígenos glicosilados, sino que cumplen un papel fundamental en la regulación de respuestas inmunes adaptativas. Sin embargo, estudios estructurales y funcionales de lectinas en modelos animales pertenecientes a niveles evolutivos que carecen de inmunidad adaptativa (invertebra-

dos tales como *Drosophila* sp., *C. elegans*), o poseen mecanismos inmunes adaptativos menos complejos que en los mamíferos (por ejemplo, *Danio rerio*), sugiere que los repertorios de lectinas en estos taxones también están altamente diversificados, estructural y funcionalmente. Estos incluyen no sólo representantes de las familias de lectinas y vías efectoras caracterizadas en los mamíferos, sino familias de lectinas tales como las de tipo F, descriptas recientemente en modelos animales no convencionales. Si bien la capacidad de reconocimiento inmune de las lectinas no se incrementa por mecanismos de recombinación genética como los observados en las inmunoglobulinas o los VLRs, la diversidad individual de los repertorios de lectinas basados en la expresión de múltiples miembros de cada familia (lectinas tipo C, F, ficolinas, pentraxinas, etc) y de sus respectivas isoformas (isolectinas) llevan a un sistema con alta capacidad de reconocimiento, que incluye a la mayoría de los glicanos de superficie comunes a patógenos microbianos y parásitos. Así, a través de sus funciones de reconocimiento immune que a través de mecanismos efectores llevan a la destrucción del patógeno, las lectinas contribuyen significativamente a formar una barrera defensiva temprana contra el desafío infeccioso. Sin embargo, algunos microorganismos (por ejemplo, *Perkinsus marinus*), no sólo se han adaptado para evadir o bloquear los mecanismos de defensa immune del hospedador, sino que aparentemente subvierten sus mecanismos de reconocimiento immune para facilitar su entrada en ciertos tipos celulares. (Estudios financiados a través de subsidios de National Science Foundation, Maryland Sea Grant, NOAA, and NIGMH, National Institutes of Health)

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ANGIOTENSIN AT_1 RECEPTOR SIGNALING IN PHYSIOLOGY AND DISEASE

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Activation of the AT_1 receptor (AT_1R) by angiotensin II (Ang II) initiates diverse signaling responses that mediate its physiological regulation of blood pressure and electrolyte balance, as well as its several pathogenic actions. Ang II-induced activation of $G_{q/11}$ stimulates PLCb, $InsP_3/Ca^{2+}$ signaling and activation of PKC isoforms, MAP kinases, cytoplasmic tyrosine kinases (Pyk2, Src, Tyk2, FAK), receptor tyrosine kinases (RTKs) such as the EGF-R, reactive oxygen species (ROS) and the NFkB pathway. Ang II also stimulates G protein-independent signaling pathways, including β-arrestin-mediated MAP kinases and the Jak/STAT pathway. The ability of certain GPCRs to utilize the growth-promoting actions of RTKs is exemplified by the manner in which the Ang II-activated AT_1R increases HB-EGF formation and other pathways to transactivate the EGFR and stimulate ERK phosphorylation. In addition to its regulation of cell growth, survival, and proliferation, such AT_1R activation of the EGFR is relevant to its pathogenic actions in the cardiovascular system, and in renal disease, diabetes and cancer. Recent studies have revealed reciprocal signaling from RTKs to GPCRs such as the AT_1R, in which agonist activation of either species leads to complex formation and transphosphorylation between the two types of receptors. Such interactions are dependent on co-localization of the receptors in caveolin-rich domains of the plasma membrane, which have an essential scaffolding role during agonist-induced transactivation and reciprocal signaling between the two major receptor families.

Most of the deleterious actions of AT_1R activation are caused by locally generated Ang II, and in the heart are often associated with the harmful effects of aldosterone. AT_1R-mediated overproduction of ROS causes growth-promoting, proinflammatory and profibrotic responses in vascular cells, leucocytes and monocytes. Activation of the AT_1R also accelerates the development of diabetes and contributes to tumor progression and metastasis through its growth-promoting and proangiogenic activities. The increasing recognition of Ang II-induced disorders has led to novel clinical applications of angiotensin converting enzyme inhibitors and AT_1R blockers, in addition to their established therapeutic actions in essential hypertension.

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PROLACTIN RECEPTORS: INHIBITORY SHORT FORMS IN CANCER CELLS AND ESTROGEN CONTROL OF RECEPTOR TRANSCRIPTION

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Prolactin (PRL) exerts diverse functions in target tissues through its membrane receptors, and is a potent mitogen in normal and neoplastic breast cells. PRL acts through the long form of the receptor (LF) to cause differentiation of mammary epithelium and to initiate and maintain lactation through activation of the Jak2/Stat5 pathway and subsequent transcriptional events. We have identified two alternatively spliced short forms (SF) of the human PRL receptor (hPRLR) with abbreviated cytoplasmic domains that inhibit the activation by PRL of the LF. A significant decrease in the ratio of SFs/LF is observed in the breast tumor tissue and cancer cell lines. The relatively lower expression of SFs in cancer could cause unopposed PRL-mediated LF stimulatory function, and contribute to breast tumor development/progression. Inhibition by SFs may result from hormone-independent heterodimer formation. Although SF homodimers and their heterodimers with LF bind hormone and mediate JAK2 activation, the SF heterodimer partner lacks sequences essential for activation of the STAT5 pathway and prevents the LF from mediating activation of PRL-induced genes.

The hPRLR gene has six alternative non-coding exons 1 driven by individual promoters. These include the preferentially utilized generic promoterIII/ exon1_3, (PIII/h$E1_3$). In breast cancer cells, E_2 increases PRLR h$E1_3$ transcripts directed by hPIII which lack an ERE. This promoter contains functional Sp1 and C/EBP sites that bind Sp1/Sp3 and CEBPβ, respectively. Abolition of the E_2 effect by mutation of either element indicated the cooperation of these transfactors in E_2-induced transcription of the hPRLR. E_2 activated-estrogen receptorα (ERα) through interaction with Sp1/Sp3 and C/EBPβ bound to DNA caused transcriptional activation of the promoter and of hPRLR expression in cancer cells. The ligand binding domain of ERα was essential for its physical interaction with C/EBPβ and E_2 promoted this association, and its DNA binding domain was required for transactivation of PIII. Other studies revealed tethering of C/EBPβ to Sp1 by the E_2-activated ERα, favouring interaction with its cognate element, and recruitment of coactivators to the complex, with consequent region-specific changes in histone acetylation. These hormone/receptor-induced associations and chromatin changes favored TFIIB and RNA Pol II recruitment and the activation of PIII directed hPRLR transcription. Stromal and adipose tissue, which are major sources of estrogen in post-menopausal women, could exert paracrine control of PRL and PRLR expression in adjacent mammary epithelial cells and stimulate breast tumor growth.

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SELECTIVE STEROID SIGNALING IN THE NERVOUS SYSTEM: A ROLE FOR NUCLEAR COREGULATORS

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Work over the past 10 years has revealed that the transcriptional efficiency of liganded steroid receptors is determined by nuclear coregulator proteins, comprising coactivators and corepressors. Coactivators include molecules that facilitate the access of the basal transcriptional machinery to the promoter. Among the histone

acetyltransferase (HAT) coactivators, the best characterized groups are the p160 (the Steroid Receptor Coactivators SRC-1a, SRC-1e, SRC-2 and SRC-3) and the CREB Binding Protein (CBP)/p300 families. However, only few studies have so far examined the functional relationship between steroid receptors and their coregulators in the nervous system. Nevertheless, the still fragmentary findings show that coactivators of the p160 family are critically involved in the amplification of nuclear receptor actions within the brain.

We have studied the interactions between the glucocorticoid receptor (GR) and coactivators of the p160 and CBP/p300 families in two types of glial cells: astrocytes and Schwann cells. First, we showed that the recruitment of the p160s by the GR is dependent on the promoter context. We also demonstrated by immunolocalization experiments a cell-specific intracellular distribution of the p160s, which was dependent on the duration of the hormonal induction. Overexpression and siRNA knock-down experiments allowed us to show that depending on the glial cell type, the GR differentially recruits p160 family members. Moreover, the interaction between SRC-1a and GR is unusual in glial cells: the C-terminal nuclear receptor interacting domain of SRC-1a participates in its exclusion from the GR complex in astrocytes, while in Schwann cells, SRC-1a interacts with GR via its two nuclear receptor binding domains. As a consequence of this atypical interaction between SRC-1a and GR in Schwann cells, the actions of CBP and p300 are modified. CBP is not implicated in the GR complex, and p300 unexpectedly repressed GR transactivation. Functional and pull-down assays showed that β-catenin is the coactivator replacing CBP in Schwann cells.

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HUMAN GENETIC OF PREMATURE OVARIAN FAILURE: A MENDELIAN AND CANDIDATE GENES APPROACH

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Premature ovarian failure (POF) is a disease affecting 1 to 3% of women before the age of 40 years, and 0,1 % of women aged of 30 years. Patients may present a primary or secondary amenorhea, depending on the age of occurrence. POF may result from (i) a decrease in the primordial follicle pool; (ii) an increased or accelerated follicular atresia; (iii) an alteration of the recruitment of the dominant follicle; and (iv) an interruption of the maturation of the follicle. Infertility is usually definitive and can be currently treated only by ovum donation.

Several arguments are in favor of a genetic aetiology of POF. The first one is the existence of familial cases. Even if familial cases are uncommon, they have already allowed the identification of disease loci such as the FSH-R. Other genetic alterations or mutations have been described as responsible for POF. The most frequent anomalies are chromosome X alterations, such as monosomy associated with Turner syndrome or deletions and translocations.

Unfortunately, in more 90% of POF patients, the aetiology of the disease is still unknown. For this reason, we have set up an international network gathering clinical and research teams, in order to obtain a better understanding of the genetics and physiopathology of POF. We have collected a large panel of familials and sporadic cases

Mapping approach: Genome-wide scanning in familial cases : We collected 11 families with familial cases of female infertility with POF.

With the help of the French Genome Center, the genome of the member of the families was scanned by genotyping 412 microsatellite markers at the Center National de Genotypage

Evidence for significant linkage was detected on the long arm of human chromosome 7q22-1 with a peak lod-score of 3.85. The region of interest is presently 10 Mb long and contains interesting candidate genes.

Candidate genes approach on sporadic POF: FOXL2, DMC1, MSH4, MSH5

The candidates genes involved in meiosis and follicular maturation were selected based on the fact that they are responsible of similar phenotypes in “knock out” mice.

Three hundred sporadic POF cases were sequenced for these genes, and genetic variants were found for DMC1, GDF9 and BMP15 genes. These variants where not found in control population.

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ORAL TOLERANCE ASSOCIATED WITH TOLEROGENIC ADJUVANTS TO TREAT ALLERGY AND AUTOIMMUNE DISORDERS

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The feeding of antigens leads typically to a state of unresponsiveness, known as oral tolerance. Two types of cells are playing a key role in oral tolerance, i.e. mucosal dendritic cells, which send processes probing the content of the gut lumen, and regulatory T cells, which secrete immunomodulating cytokines such as IL-10 and TGF-β. The oral intake of an antigen can induce a bystander suppression; when oral tolerance has been induced toward antigen X, the injection of antigen Y together with X in the same body site fails to induce an immune response against both antigens Y and X. Oral administration of allergens significantly improves allergic rhinitis and induces much less adverse effects than allergen injections. Increased efficacy can still be obtained by using antigen peptides instead of the whole antigen. Peptides are apparently more tolerogenic and reduce the risk of adverse effects because they cannot trigger the degranulation of mast cells.

Regarding autoimmune diseases, oral administration of autoantigens in animal models produced very promising results, but the clinical trials were disappointing. A tolerogenic adjuvant could however enhance oral tolerance. So far the main tolerogenic adjuvants are bacterial heat shock proteins (HSPs), which are molecular "chaperones". They are involved in the clearance of proteins that are improperly folded, for example under conditions of cellular stress. When bound to ATP, a hydrophobic pocket in HSPs opens and binds certain peptides. The hydrolysis of ATP into ADP encloses the peptide in the pocket, which can be reopened by ATP. In animal models, either injection or oral administration of a microbial HSP leads to the production of T cells that cross-react with self-HSPs. These T cells produce regulatory cytokines, and have disease-suppressive activity. If peptides from either allergens or autoantigens are combined to HSP, one can expect that the HSP tolerogenic effect will be extended to these antigens.

CONFERENCIA

IMMUNE TOLERANCE TO TUMOR ANTIGENS

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The spontaneous interaction between tumor cells and the immune system results in reciprocal changes leading to a less immunogenic tumor and immune cells less capable to mount an effective response against a growing malignancy[1, 2]. Although several mechanisms have been proposed to explain how tumors might influence the function of tumor-antigen specific T cells, one mechanism that has gained particular attention relates to the ability of malignant cells to induce antigen-specific T-cell tolerance[3, 4]. The immune tolerance hypothesis was first evoked following the surprising findings that most of the identified tumor antigens were not necessarily neoantigens uniquely expressed by cancer cells, but rather were lineage-specific tissue differentiation antigens also expressed in normal tissues[5, 6]. These observations raised the concern that the same mechanisms that normally prevent attack against self-antigens may also blunt the ability of the immune system to recognize and respond to antigens expressed by tumors[7]. This different view of tumor immunity profoundly changed our approach to cancer immunotherapy since the greatest obstacle to the development of successful immunotherapeutic approaches is the immune system itself, and more specifically, its complex mechanisms for tolerance induction. Thus, over the past decade, a significant amount of effort has been devoted towards answering those questions

pertaining to how tolerance towards tumor-antigens is established and maintained, and what steps can be taken to break this state of unresponsiveness in a controllable fashion.

During our presentation we will discuss therefore the experimental and clinical evidence that help identify the remarkable barrier that tolerance to tumor antigens has imposed to our efforts to effectively harness the immune system against malignancies. In particular, we will discuss the central role of bone marrow-derived antigen-presenting cells (APCs) in the induction of this state of T-cell unresponsiveness and the role of the tumor microenvironment in determining the tolerogenic properties of these APCs[8, 9]. Finally, we provide information on receptor-ligands and intracellular pathways (CD40, STAT3, *c-kit,* Histone deacetylases) that given their role in influencing the inflammatory properties of APCs are being exploited as targets to revert mechanisms of T-cell unresponsiveness in cancer.

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CONFERENCIA

THE AMPK SYSTEM - AN ENERGY SENSOR AT THE CELLULAR AND WHOLE BODY LEVELS AND A TARGET IN OBESITY AND TYPE 2 DIABETES

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The AMP-activated protein kinase (AMPK) is the downstream component of a kinase cascade that acts as a sensor of cellular energy charge. Stresses that inhibit ATP production (e.g. hypoxia, hypoglycemia, ischemia) or that accelerate ATP consumption (e.g. muscle contraction) cause an increase in ADP:ATP that is amplified by adenylate kinase into a much larger rise in the AMP:ATP ratio. Binding of AMP to the gamma subunit of AMPK triggers phosphorylation and activation of the catalytic alpha subunit by the upstream kinase LKB1. Once activated, AMPK has numerous rapid effects due to direct phosphorylation of target proteins, as well as longer-term effects on gene expression. It switches on alternate catabolic pathways that generate ATP such as the uptake and oxidation of glucose and fatty acids, while at the same time conserving ATP by switching off ATP consuming processes, including lipid, polysaccharide and protein synthesis. The ability of AMPK to inhibit cell growth and proliferation may explain why the upstream kinase LKB1 was first identified as a tumor suppressor.

As well as acting as an energy sensor at the level of the individual cell, it is now clear that AMPK also regulates energy balance at the whole body level, making it a prime target for drugs aimed at treatment of obesity, Type 2 diabetes and the metabolic syndrome. AMPK is activated by leptin and adiponectin in skeletal muscle,

stimulating glucose and fat oxidation, and hence energy expenditure. In the hypothalamus it stimulates food intake and is regulated by hormones known to affect appetite, such as leptin, ghrelin and cannabinoids. It is a target for two major classes of anti-diabetic drugs, i.e. the biguanides (e.g. metformin) and the thiazolidinediones. These drugs activate AMPK by inhibiting the respiratory chain, and the thiaziolidinediones also act indirectly by stimulating adiponectin release. Recent work from our laboratory suggests that in the carotid body and pulmonary smooth muscle the AMPK system acts as a sensor involved in the physiological responses to hypoxia that control breathing and blood flow to the lungs.

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SIGNAL TRANSDUCTION BY DIACYLGLYCEROL IN CANCER: LOOKING BEYOND PKC

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Diacylglycerol (DAG) is a key lipid second messenger generated upon activation of phospholipase C (PLC) isozymes by tyrosine kinase and G-protein-coupled receptors. Work from many laboratories, particularly comprehensive biochemical studies from Alexandra Newton's lab at UCSD, established a crucial role for DAG as a protein kinase C (PKC) activator. Our studies revealed that other intracellular receptors for DAG exist in addition to PKC isozymes that play important roles in cancer. We found that α- and β-chimaerins, "non-kinase" DAG receptors with GAP activity for the small G-protein Rac, are effectors of the epidermal growth factor receptor (EGFR) (*EMBO J.* 25: 2062-2074, 2006). Upon stimulation, EGFR activates Rac to promote a proliferative and motile response. Experiments using co-IP and Fluorescence Resonance Energy Transfer (FRET) revealed that EGF causes the translocation of chimaerins to the plasma membrane in a DAG-dependent manner, where they associate with active Rac (Rac-GTP) to promote its inactivation, as predicted from our structural studies (*Cell* 119:407-418, 2004). RNAi depletion of chimaerins led to a prolonged Rac activation in response to EGF, suggesting that they negatively modulate Rac signaling. There are several important implications for these findings. First, we can unambiguously conclude that PKCs are not the only DAG receptors and that divergence in DAG signaling via receptor activation exists. Second, we established for the first time that tyrosine kinase receptors trigger a mechanism that self-limits Rac activation via the PLC/DAG branch. Third, our recent demonstration that chimaerins are down-regulated in some types of cancers and inhibit cell proliferation and migration in response to growth factors suggests a role for chimaerins as tumor suppressors (*JBC* 280:24363-24370, 2005; *Mol. Cell. Biol.* 26:831-842, 2006). Lastly, as we have recently established using a knock-down approach in zebrafish that α-chimaerin regulates gastrulation (*PNAS* 103:5373-5378, 2006), DAG signals must play a crucial role in early development. Our challenge now is to dissect the functional relevance of these findings using knockout and knockin mouse models.