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SAIC LECTURE I

OPENING LECTURE

INSIGHTS INTO MECHANISMS OF BREAST CANCER DEVELOPMENT

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Breast carcinoma is the most frequent cancer in women with more than 1 million new cases reported in the USA each year. Although current therapeutic interventions have increased the 5-year survival rate for women with the disease, there is still an urgent need to uncover therapeutic targets, in particular for metastatic breast cancer. Our lab has taken different approaches to tackle this problem. On the one hand, we have studied breast cancer metastasis models in order to directly target disseminated disease; on the other hand, we have searched for new breast cancer driver genes. My talk will cover recent work from both areas.

Metastasis is a complex process whereby tumor cells acquire various properties allowing them to colonize and grow at distant sites. We have been studying important pathways involved in metastasis. In my presentation I will discuss targeting the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway in breast cancer models of lung and bone metastasis. Activation of STATs through JAKs is known to contribute to tumor initiation and progression at several levels and JAK inhibitors (JAKi) are currently in clinical trials for breast cancer. We showed in cancer patient biopsies that STAT proteins are active in the primary tumor and in matched metastasis, reinforcing the rational of employing JAKi to block STATs and, potentially, metastatic growth. We investigated the effect of a JAK1-JAK2i, ruxolitinib, *in vivo* by employing preclinical models of breast cancer metastasis that show activation of STAT proteins. Surprisingly, we found a significant increase in metastatic burden in mice treated with ruxolitinib, compared to vehicle treated mice, despite the fact that JAKi did block the JAK/STAT signaling pathway in the metastatic lesions. This result was unexpected. The potential clinical relevance of our finding prompted us to further investigate the biological mechanisms underlying the detrimental effect of JAKi observed in these models Reasoning that in addition to the cancer cells, other cells in the tumor environment might be affected by JAK inhibition, we designed experiments to test this hypothesis. We demonstrated that the pro-metastatic effect of JAKi is due to their immunosuppressive activity, leading to an impairment of natural killer (NK) cell-mediated anti-tumor immunity. Our study indicates that in the breast cancer models we studied, JAKi-mediated inhibition of NK cell tumor surveillance prevails over potential anti-cancer effects of blocking the JAK/STAT pathway, and that this inhibition causes enhancement of metastasis. Our findings highlight the importance of evaluating the effect of targeted therapy on the tumor environment, in addition to the cancer cells, in order to predict and potentially prevent undesired bystander effects. This work was recently published in Bottos et al 2016 Nature Comm.

In the second part of my presentation, I will discuss our work on Ret, a member of the receptor tyrosine kinase superfamily. Ret is known as the key oncoprotein in thyroid carcinomas due to gain-of-function mutations and Ret has recently been implicated in other cancer types. Indeed, Ret mutations have been reported at low frequencies in lung and breast tumors. Furthermore, we and others have reported that Ret is overexpressed in about 40% of human tumors and that this correlates with poor patient prognosis. Ret activation regulates numerous intracellular pathways related to proliferation, differentiation and inflammation, but it is not known whether abnormal Ret expression is sufficient to induce mammary carcinomas in mice. Using the doxycycline-inducible transgenic mouse model with the MMTV promoter controlling Ret expression, we show that overexpression of wild type Ret in the mammary epithelium produces hyperplasia and evokes mammary tumors displaying a morphology that recapitulates features of human ductal carcinoma in situ. Importantly, tumors rapidly regress after doxycycline withdrawal indicating that Ret is the driving oncoprotein. Using next generation sequencing we examined levels of transcripts in these tumors and have evidence that STAT signaling might contribute to Ret-driven tumorigenesis. Data on other signaling pathways that are controlled by aberrant Ret expression will be presented Ret-evoked tumors can be passaged in mice and are now being used to test novel therapeutic approaches (Gattelli et al, in preparation).

SAIC LECTURE II

THE CALYX OF HELD SYNAPSE. A MODEL FOR CHEMICAL TRANSMISSION

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Chemical synapses are the fundamental units that mediate communication between neurons in the mammalian brain. Classically, the basic concept of chemical synaptic transmission was established at the frog neuromuscular junction, and at the squid giant synapse. More recently, recordings from the calyx of Held in rodent brainstem slices have extended the classical concept to mammalian synapses providing new insights into the mechanisms underlying strength and precision of neurotransmission.

The calyx of Held synapse plays an important role in the auditory system, relaying information about sound localization via fast and precise synaptic transmission, which is achieved by its specialized structure and giant size. During development, the calyx of Held undergoes anatomical, morphological, and physiological changes necessary for performing its functions. The large dimensions of the calyx of Held nerve terminal are well suited for direct electrophysiological recording of many presynaptic events that are difficult, if not impossible to record at small conventional synapses. This unique accessibility has been used to investigate presynaptic ion channels, transmitter release, and short-term plasticity, providing invaluable information about basic presynaptic mechanisms of transmission at a central synapse.

I plan to summarize findings from our laboratory and others on the interplay between the waveform of action potentials, $Ca2^{+}$ currents and transmitter release in normal and pathological conditions.

I also plan to present our recent findings that evoked release protons are neurotransmitters and neuromodulators acting via activation of acid sensing ion channels (ASICs).

ASICs are voltage-independent, proton-gated cation-selective channels mostly permeable to Na^{+} ion. They belong to the degenerin/epithelial Na^{+} channel (DEG/ENaC) superfamily.

Protons are important signals for neuronal function. In the central nervous system (CNS), proton concentrations change locally when synaptic vesicles release their acidic contents into the synaptic cleft, and globally in ischemia, seizures, traumatic brain injury, and other neurological disorders due to lactic acid accumulation. The finding that protons gate a distinct family of ion channels, the ASIC channels, has shed new light on the mechanism of acid signaling and acidosis-associated neuronal injury.

At the calyx of Held postsynaptic neuron, ASIC channels can be activated by a drop in extracellular pH. Their activation induces transient inward currents (I_{ASIC}) in postsynaptic neurons from wild type mice. The inhibition of I_{ASIC} by the specific blocker psalmotoxin-1 (PcTx1) and the absence of these currents in *knock out* mice for ASIC-1a subunit ($ASIC1a^{-/-}$) suggest that homomeric ASIC-1a channels are mediating these currents. Furthermore, after blocking all postsynaptic glutamate receptors we detect nerve evoked ASIC1a-dependent currents strong enough to generate postsynaptic action potential suggesting an acidification of the synaptic cleft due to the co-release of glutamate and H^{+} from synaptic vesicles. A significant characteristic of these homomeric ASIC-1a channels is their permeability to Ca^{2+}. Activation of ASIC-1a by exogenous H^{+} induces an increase in intracellular Ca^{2+}. Furthermore, the activation of postsynaptic ASIC-1a channels during high frequency stimulation (HFS) of the presynaptic nerve terminal leads to a PcTx1-sensitive increase in intracellular Ca^{2+} in which is independent of glutamate receptors and is absent in neurons from $ASIC1a^{-/-}$ mice. During HFS, the lack of functional ASICs in synaptic transmission results in an enhanced short term depression of glutamatergic excitatory postsynaptic currents. These results strongly support the hypothesis of protons as neurotransmitters and demonstrate that presynaptic released protons modulate synaptic transmission by activating ASIC-1a channels at the calyx of Held synapse.

SAIC LECTURE III

CONFERENCIA PLENARIA "ALFREDO LANARI"

LO ESENCIAL NO ES INVISIBLE A LOS OJOS

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El objetivo central de mi grupo de trabajo es el estudio de la retina, tanto en condiciones fisiológicas como patológicas. Particularmente en los últimos años, nos concentramos en el estudio de distintas enfermedades visuales

prevalentes que constituyen causas de ceguera, tales como retinopatía diabética, uveítis, neuritis óptica, glaucoma e isquemia retiniana, para las cuales aún no existen terapias suficientemente efectivas. Para cumplimentar este objetivo, desarrollamos nuevos modelos experimentales o validamos modelos pre-existentes, y analizamos la viabilidad de nuevas estrategias terapéuticas. En este sentido, demostramos que el condicionamiento isquémico previene la disfunción retiniana, la pérdida de integridad de la barrera hemato-ocular y el aumento en los niveles del factor de crecimiento vascular endotelial inducidos por diabetes experimental de tipo 1 y evita la progresión de la retinopatía diabética en ratas. La uveítis es una enfermedad inflamatoria intraocular que involucra al tracto uveal (iris, cuerpo ciliar y coroides), y las estructuras oculares adyacentes (retina y vítreo). Desarrollamos un modelo experimental de panuveítis en hámster, a través de la inyección intravítrea de lipopolisacárido bacteriano (LPS) y analizamos el efecto terapéutico de la melatonina sobre las alteraciones funcionales y estructurales inducidas por uveítis experimental. En este contexto, demostramos que el tratamiento con melatonina previene las alteraciones clínicas, bioquímicas, funcionales y ultraestructurales inducidas por la inyección intravítrea de LPS. Considerando la efectividad de la melatonina en la inflamación ocular, decidimos examinar el efecto de la melatonina en otra enfermedad inflamatoria ocular que afecta primariamente al nervio óptico, la neuritis óptica. La neuritis óptica es una neuropatía aguda inflamatoria y desmielinizante del nervio óptico. Dado que no existen modelos experimentales para la forma primaria de esta enfermedad, desarrollamos un modelo a través de la microinyección de LPS directamente en el nervio óptico de ratas *Wistar*, que reproduce aspectos centrales de la neuritis óptica primaria humana y demostramos que la melatonina previene las alteraciones axogliales del nervio óptico y de la retina y evita la progresión del daño funcional inducido por neuritis óptica experimental.

El glaucoma es una disfunción ocular de alta prevalencia que se caracteriza por la pérdida progresiva de las funciones visuales, asociada a la muerte de células ganglionares retinianas (CGR) y axones del nervio óptico. En los últimos años, hemos logrado avances de consideración en cuanto a la elucidación de los mecanismos patogénicos involucrados en el desarrollo de la enfermedad y la búsqueda de nuevas estrategias terapéuticas, utilizando un modelo de glaucoma experimental desarrollado en nuestro laboratorio a través de inyecciones intracamerales de glicosaminoglicanos. En forma más reciente, analizamos un aspecto del glaucoma que ha recibido escasa atención, como es su efecto sobre el sistema visual no formador de imagen. En este contexto, demostramos que el glaucoma experimental en ratas provoca una pérdida significativa de CGR intrínsecamente fotosensibles, una disminución en el reflejo pupilar y alteraciones en el ritmo diario de actividad locomotora. En base a estos antecedentes, analizamos el ritmo diario de actividad en pacientes con glaucoma avanzado y demostramos que el glaucoma induce una disminución significativa en la calidad del sueño. En conjunto, estos resultados demuestran que el glaucoma afecta no sólo las funciones visuales formadoras de imagen, sino también las funciones visuales no formadoras de imagen, como el control de los ritmos circadianos, lo que constituye un riesgo adicional para la calidad de vida de pacientes con glaucoma. En la búsqueda de estrategias terapéuticas no invasivas, analizamos el efecto de la exposición a ambiente enriquecido sobre el daño isquémico retiniano. Para ello, luego de una isquemia retiniana deletérea, los animales fueron albergados en ambiente estándar o ambiente enriquecido por 3 semanas. La exposición a ambiente enriquecido previno significativamente el daño funcional y estructural inducido por isquemia retiniana.

Epílogo

Dice (y con razón) el saber popular que hay dos formas de "ver el vaso": medio lleno o medio vacío. A lo largo de todos estos años no hemos logrado obtener evidencias en favor de una u otra de estas opciones, pero en cambio, hemos pretendido contribuir a mejorar la visión de aquellos que padecen por no tenerla, porque después de todo, parafraseando al poeta español Ramón de Campoamor: "*Y es que en este mundo traidor, no hay verdad ni hay mentira: todo es según el color del cristal con que se mira*".

SAIC LECTURE IV

RAS-RELATED PROTEINS AS DIRECT ONCOGENIC DRIVERS

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Given the large number of mutations found in tumor genomes, it is of paramount importance to specifically pinpoint those that play driving roles during the initiation, progression, and/or posttreatment response of tumors.

Unfortunately, this task is as challenging as looking for a needle in a haystack due to the complex genetic make-up of most cancer genomes as well as the low frequency in which most mutations are found in them. Due to this, it is important to devise new genetic ways to identify genes playing proactive functions in this malignant process.

R-Ras2 (also known as TC21) is a GTPase that shows high structural similarity with the three members of the Ras subfamily. This protein attracted attention upon its discovery due to its signaling similarity with Ras proteins, high oncogenic potential in cell culture, and localization in a mutation "hotspot" in a variety of tumors. Despite this, little information exists about its physiological roles and, more importantly, about its ability to contribute to tumor development, progression, and/or maintenance in vivo. In the same context, we do not know as yet whether the mutations found in human tumors have clinical relevance or are just passenger ones. To address these issues, we have used a number of genetic (shRNA interference, ectopic expression, knockout and tamoxifen-inducible knock-in mice) and signaling techniques to address the effect of the loss-of- and gain-of-function of this GTPase in in vivo tumorigenic processes.

Using loss-of-function approaches, we have found that the elimination of endogenous R-Ras2 can abate breast cancer primary tumorigenesis and the metastatic dissemination of cancer cells. These effects are even seen when such an inactivation takes place in cancer cells bearing oncogenic mutations in classical Ras proteins. Interestingly, the contribution of R-Ras2 to this process seems to be through a PI3K-dependent route that mediates efficient protein synthesis rather than distal transcriptional events. Perhaps more importantly, we have found using a new inducible *R-Ras2* knock-in mouse strain that the somatic replacement of endogenous wild-type R-Ras2 by a mutant version found in human tumors leads to the death of the animals in a 2-3 month period. This rapid lethality derives from the development of quite aggressive T cell acute lymphoblastic leukemia that exhibit both constitutive Notch1 and PI3K signaling. We also have found the frequent development of noninvasive marginal zone splenic lymphomas, Harderian gland adenomas, skin papillomas, and several subtypes of genitourinary tumors. Collectively, these data indicate that this Ras-like protein promotes signaling events that are overlapping, but not identical, to those usually triggered by the three Ras subfamily proteins. They also demonstrate, for the first time, that genetic alterations in this gene probably act as oncogenic drivers for specific types of hematological and solid tumors. These observations suggest that the presence of such mutations in human tumors may have diagnostic, clinical, and therapeutic interest.

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SAIC LECTURE V

CONFERENCIA PLENARIA "ALBERTO TAQUINI"

LIGHTS AND SHADOWS IN CARDIAC REGENERATION

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Ischemic heart disease, the leading cause of mortality worldwide, often results in myocardial infarction, after which the surviving tissue undergoes a process termed remodelling. This process consists of myocardial hypertrophy, myocyte death, defective regeneration and progressive replacement of contractile myocytes by fibrotic tissue. The progressive loss of myocytes and its replacement by non contractile tissue leads to heart failure. Although considerable progress has been made in its pharmacological management, heart failure continues to be associated with a high mortality. Once overt heart failure develops, about 30% to 45% of patients die within 1 year, unless they receive a heart transplant. The extent of remodeling and the chances of evolving towards contractile failure are largely dependent on infarct size. Small infarcts do not lead to substantial remodelling whereas large ones do. Therefore, reducing the size of the initial infarct is of utmost therapeutic and prognostic relevance.

Currently, the most investigated strategy to meet this goal is the myocardial implantation of stem cells of diverse origin and differentiation potential including, among others, embryonic stem cells, skeletal myoblasts, bone marrow mononuclear cells, endothelial progenitor cells, mesenchymal stromal cells and, more recently, cardiac progenitor cells. Although promising achievements have been observed in small and large animal models, results from the few controlled trials in humans have been considerably poorer or even undetectable.

On account of their easy isolation and ex-vivo expansion, their amenability for genetic modification and their immunosuppressive properties which allow for allogeneic utilization, bone marrow mesenchymal stromal cells (MSCs) constitute the cell type most consistently used in pre-clinical and clinical studies of cardiac regeneration.

Although MSCs may delineate in vitro a cardiomyocyte phenotype based on morphology, automatic beating and expression of sarcomeric α-actinin, troponin I and connexin 43, the most accepted mechanism by which they afford regeneration is the paracrine angiogenic, anti-apoptotic, anti-fibrotic and pro-mitotic effect of the multiple growth factors and cytokines that they produce.

Additionally, some of these molecules may display a pro-mitotic effect on the adult cardiomyocyte, favouring not only their re-entry in the cell cycle, but also inducing advancement into mitosis.

Given that the myocardium behaves as an electromechanical syncytium, myocardial regeneration is not just a matter of repopulating the heart with cells, but also, and most importantly, to promote establishing electromechanical connections between the new cells and the remaining viable ones. Therefore, strategies aimed at encouraging the resident cardiomyocytes to exit the post-mitotic phenotype and divide into daughter cells would not only produce cardiomyocyte hyperplasia but also guarantee the physiological connection between cells that supports myocardial function.

One of such strategies would consist of genetically modifying MSCs to overexpress pro-mitotic factors that would not only increase angiogenesis but also encourage the adult cardiomyocyte to re-enter the cell cycle.

On account that in large mammals the adult cardiomyocyte can polyploidize but not advance into mitosis, an alternative approach would be removing the brake that prevents the adult cardiomyocyte to overcome de G2/M checkpoint of the cell cycle. So far, the *meis1* gene has been identified in mice as a transcription factor of inhibitor proteins, but more studies are needed both to confirm that this gene acts similarly in large mammals and to find new cell cycle-inhibiting molecules that can become targets of therapies aimed at promoting adult cardiomyocyte hyperplasia.

Despite the scientific progress achieved during the last two decades, the goal of building a new heart, or at least mending the old one, still seems remote. While new approaches have emerged, such as the use of inducible progenitor stem cells, bioresorbable scaffolds, decellularized matrices, etc., many questions remain to be answered, including how cardiomyocyte proliferation is regulated during heart development, how the adult cardiomyocytes and cardiac progenitor cells interact with the extracellular matrix and how we can attenuate the limitations of animal models of human ischemic heart disease. Only advancing our knowledge about these and other important issues will enhance the chance of partially regenerating the human heart.

SAIC LECTURE VI

CLOSING LECTURE

TARGETING SIGNALING CIRCUITRIES: NEW PRECISION CANCER TREATMENTS AND IMMUNOTHERAPIES

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The goal of our program is to exploit the emerging information on dysregulated signaling circuitries and individual genomic and molecular alterations to develop new precision therapies to prevent and treat cancer. Specifically, we have focused on the study of growth-promoting signal transduction pathways, the nature of the dysregulated signaling networks in cancer, and on the use of genomic, proteomic, and system biology approaches to study cancer initiation and progression. We have shown that human and virally-encoded G proteins and G protein coupled receptors (GPCRs) can display potent oncogenic activity. Strikingly, our recent analysis of human cancer genomes revealed an unanticipated high frequency of mutations in G proteins and GPCRs in most tumor types. Among them, mutually exclusive activating mutations in *GNAQ* or *GNA11* (encoding $G\alpha_q$ family members) occur in 5.6% of tumors, including >90% of ocular melanomas, thus providing a clear example of a human malignancy that is initiated by gain of function mutations in $G\alpha_q$ and $G\alpha_{11}$ proteins. How $G\alpha_q$ and its coupled receptors transduce mitogenic signals is still unclear, due to the complexity of signaling events perturbed upon G_q activation. Evidence will be presented that while many biological responses elicited by G_q depend on the transient activation of second messenger system, G_q utilizes a hardwired protein-protein interaction-based signaling network to achieve the sustained stimulation of proliferative pathways, thereby controlling normal and aberrant cell growth. In parallel, our team has focused on the study of oncogenic signaling circuitries driving the initiation and progression of head and neck cancer (HNSCC), a disease that results in 300,000 deaths each year worldwide, aimed at identifying novel

druggable targets for prevention and treatment. There is an urgent need to develop new precision therapies to prevent and treat HNSCC. A striking finding from the recent deep sequencing of the HNSCC genomic landscape was the multiplicity and diversity of genetic alterations in this malignancy. The emerging picture, however, is that most fall within only a few major driver biological processes, including mitogenic signaling with particular emphasis on aberrant activation of the PI3K/mTOR pathway. Remarkably, this is aligned with our early discovery that the persistent activation of the PI3K/mTOR signaling circuitry is the most frequent dysregulated signaling mechanism in HNSCC, and that PI3K/mTOR inhibition exerts potent antitumor activity in a large series of genetically-defined and chemically-induced HNSCC models, including those involving HPV-associated HNSCC. These findings provided the rationale for launching a multi-institutional Phase II clinical trial (NCT01195922), targeting mTOR in HNSCC, which was recently completed and achieved encouraging results. Emerging results from this trial and the molecular mechanisms underlying the remarkable effects of mTOR inhibitors in HNSCC and other cancer types will be discussed. A recently initiated clinical trial for HNSCC precision prevention using metformin, which decreases mTOR activity in oral premalignant lesions and their cancer initiating cells, will be presented. Recently launched efforts aimed at harnessing the full potential of immune oncology agents, including checkpoint inhibitors, to achieve durable responses (cure) in HNSCC patients will be also discussed.

SAI OPENING LECTURE

DEVELOPMENT OF AN ATTENUATED DENGUE VACCINE

PROF. JORGE KALIL

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SAI LECTURE II

REGULATORY T CELLS AND NOVEL IMMUNOMODULATORS

PROF. TIM SPARWASSER, MD.

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SAI LECTURE III

FUNCTIONAL REPROGRAMMING OF MYELOID CELLS IN CANCER: MECHANISMS AND CLINICAL SIGNIFICANCE "DR. LEONARDO SATZ CONFERENCE"

ANTONIO SICA, PHD.

Humanitas Research Hospital Milano, Italy

SAI LECTURE IV

T HELP VERSUS REGULATION BY $CD4^+$ T CELLS

STEPHEN SCHOENBERGER, PHD.

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SAI LECTURE V

TUMOR AND HOST FACTORS REGULATING ANTI-TUMOR IMMUNITY AND IMMUNOTHERAPY EFFICACY

TOM GAJEWSKI, MD, PHD.

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SAI CLOSING LECTURE

NATURAL KILLER CELLS IN HOST IMMUNITY AGAINST VIRAL INFECTION

JOSEPH SUN, PHD.

Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, USA

SAFE OPENING LECTURE

MOLECULAR AND FUNCTIONAL GENETICS OF APPETITIVE BEHAVIORS IN GENETICALLY MODIFIED MICE

MARCELO RUBINSTEIN

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A spectre is haunting the world, the spectre of obesity. This pandemia has been steadilyincreasing for the last 40 years and during the last decade its prevalence has skyrocketedparticularly in infants and adolescents, as well as in lower income families from most countries worldwide. Although pandemic obesity has been purely driven by a large increase in the consumption of ultra processed edibles of poor nutritional value, the obesogenic changes in contemporary human lifestyle do not affect all individuals at the same level revealing the importance of genetic predisposition for hyperphagia and increased adiposity. In this lecture I will present genetic and functional results that are contributing to understand how the hypothalamus orchestrates food intake and, in particular, the role of a satiety system led by a group of neurons expressing the proopiomelanocortin (*Pomc*) gene. During the past several years my group has been working in the identification of the molecular components responsible for hypothalamic *Pomc* expression. By using a large variety of transgenic and mutant mouse strains we were able to dissect the neuronal transcriptional code of Pomc, define with unanticipated resolution the cis-acting regulatory elements and the transcription factors involved in the selective expression this gene and characterize their functional importance in the regulation of food intake and body weight. I believe that these results are helping us to better understand the power and limitations of the mammalian central satiety pathways and probably will help us to improve the individual and collective strategies to reduce the overwhelming increase of this insatiable human-induced pandemia.

SAFE LECTURE II

ETHICAL ASPECTS OF HIGH COSTS OF ONCOLOGICAL DRUGS: WHEN LIFE HAS A PRICE

ERNESTO GIL DEZA

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The cost of drugs in oncology is the biggest obstacle patients face to access new treatments, and this is an ethical challenge we cannot avoid. It is so important, we use the term “financial toxicity” when talking about new drugs.

When we analyze the most common reasons given for these costs, we can see that they are unjustifiable:

a) **Inflation**: from 1970 until today, pharmaceutical products have increased their prices by a hundredfold (from an average of one hundred dollars to an average of ten thousand) in countries with stable economies (theU.S.A.), whereas other products only had a tenfold increase in that period.

b) **Breakthrough costs**: most common breakthroughs take place in universities financed by taxes and not as a consequence of money invested in pharmaceutical companies.

c) **Production costs**: most of today’s new drugs are monoclonal antibodies, discovered by César Milstein, who never patented them. Laboratories freely use the technique, but they patent their products, which results in billions of dollars of profit for their companies.

d) **Research costs:** only 15% of a company’s profits are devoted to research.

e) **Drug efficacy or security**: the cost of new drugs is similar, no matter how much safer or more efficient they are. The cost derives from novelty

f) **None of these variables (alone or combined) can explain drug prices**: this is demonstrated by the large variance in price for the same medication in different parts of the world. So much so that a medication may be up to six times cheaper in one place than in another.

g) This is even more notable when pharmaceutical companies are willing to offer **secret discounts** (allegedly, up to 90% discounts) so that the drug is used by the British NHS, or other such services in developed countries, while they refuse to do so for poorer countries, thus ensuring that the latter subsidize the former.

The only truth is that drug prices are as high as they are because someone is willing to pay those prices.

The new treatments in the US have such elevated costs that, as of 2014, there has been a move to quantify financial toxicity, since it is well known that half of all oncological patients in the US end up bankrupt due to this toxicity. Others simply abandon treatments, gain acess at a later stage, or significantly reduce the dosage.

It is necessary to shine a light on this kind of extortion, to which states, health services and patients are exposed on a daily basis. We must understand that what is happening to poor patients in rich countries is but a prelude of what will happen to the health services of poor countries if we do not act.

This idea that medication is a luxury good goes against the ethical foundations of medicine. The idea that the market will self-regulate and thus drop prices is childish, to say the least. It is the duty of society as a whole (politicians, scientific societies, doctors and patients) to preserve the rights of patients rather than the greed of pharmaceutical companies.

SAFE LECTURE III

SIGNAL TRANSDUCTION BY G PROTEINS: FROM RECEPTOR TO EFFECTOR – HISTORICAL AND STRUCTURAL VIEWS

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The Royal Society of Chemistry's web site, under Drug Development, states: *"Cell-surface receptors,such as G-protein-coupled receptors (GPCRs), are the targets of over half the drugs currently used*". Many of them "happen" to be of this kind and were developed independently of any knowledge about their mechanisms of action. But, independently of their importance, the molecular and atomic mechanism by which the GPCRs "do their thing" is fascinating and happens to involve much of my life as a scientist. This lecture is structured around the key discoveries that have led to our current understanding of how occupancy of receptors by their ligands is transduced by the G protein signal transducing machine into intracellular signals picked up by a heterogeneous mix of enzymes: 9 types of adenylyl cyclase, 4 phospholipases Cbeta, the visual phosphodiesterase, 3 guanine nucleotide exchange factors for RhoGTPases, voltage-gated Ca channels, and the GIRK K channels. The field spawned two Nobel Prices: one in Physiology (or Medicine) in 1994 to Martin Rodbell and Al Gilman for the discovery of G Proteins as Signal Transducers, and the other in Chemistry in 2012 to Robert Lefkowitz and Brian Kobilka for their work on discovery and structure elucidation of GPCRs. I participated in some of the seminal findings that led to the 1994 Nobel Price, as I was Martin Rodbell's first postdoctoral fellow and happened to pipet the ingredients he had written out that led to the discovery of the GTP-dependent activation of adenylyl cyclase, a finding that in turn led to the discovery of the GTP-binding regulatory component, separate from receptor and adenylyl cyclase, and eventually to the realization that the regulatory GTP-binding component is a GTPase (inhibited by cholera toxin) which in its GTP-bound form is active as an activator of adenylyl cyclase and loses this capacity when it hydrolyzes the resident GTP to GDP (Cassel and Selinger). The main and fundamental contribution of Gilman's laboratory to the field was to have purified the regulatory component and discovering that it was a dimer that dissociated into alpha and beta subunits upon GTP binding, which re-associated upon GTP to GDP hydrolysis. Parallel studies discovered that there were more than one GTP-binding alpha-beta protein (Sternweis and Robishaw in Dallas and Eva Neer at Harvard). At this point, 1984, we discovered that yet another G protein, this one purified by us and a substrate for the ADP-ribosylating activity of pertussis toxin (an inhibitor of inhibitory regulation of adenylyl cyclase, a form of regulation discovered by us several years earlier) was an alpha-beta/gamma heterotrimer, and that the three G proteins (Gs, Go and Gi) underwent upon GTP binding not only a conformational change but also the subunit dissociation. The three proteins shared a common pool of beta/gamma dimers. Both arms, the GTP-alpha and the beta/gamma dimer regulate effectors. It was between 1984 and 1990 that it became clear that not only adenylyl cylcase (Gs and Gi) but also the other effectors mentioned above were regulated by G protein alphas, of which there are 16, some biochemically isolated (Gq/G11 – Exton, Sternweis), the others cloned based on their structural similarity (Simon, Numa, Gilman, Kaziro, also us). Beta/gammas were found also to regulate effectors, acting on ion channels (Clapham and Neer the GIRK channels, Catterall the Ca channel, Schultz, Jakobs and Gierschik the PLC betas). An interesting curiosity in this field was that prior to the elucidation of the alpha/beta/gamma and GTPase nature of Gs and Gi in the adenylyl cyclase field, in the vision field, a light activated GTPase of subunit composition alpha/beta/gamma had been

found to be responsible for activation of the visual PDE by light-stimulated rhodopsin (Bitensky at Yale, Kuehn in Bochum, Germany), and that the parallelism, and in fact identity between the signal transduction architecture in adenylyl cyclase regulation and that of the signal transduction machinery converting light into cGMP hydrolysis had been missed by so many of us.

In my presentation I shall summarize these historical initial developments, concentrate on presenting the molecular structures of the players in adenylyl cyclase regulation, and describe in detail the importance of the Mg ion in the double GTPase and subunit dissociation-re-association cycles, at the atomic level as deduced from x-ray crystallography.

AACYTAL LECTURE I

THE CRISPR/CAS9 REVOLUTION: RAISING THE LIMITS OF FUNCTIONAL GENETICS WHILE THREATENING THE 3RS.

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Genetic engineering in live organisms is experiencing a novel revolutionary wave, the latest of the very many that have been occurring since recombinant DNA technology started almost 50 years ago. The use of molecular components of a recently discovered adaptive immune system present in many prokaryotes, known as CRISPR/Cas, is pushing forward the frontiers of transgenesis and genome editing at great strength and promise. Different to the landscape of a few years ago, it is now possible to modify the genome of a large variety of animals, plants and microorganisms, a technological breakthrough with unanticipated consequences. Animal facilities are already experiencing fast changes with more transgenic and mutant species being made, reproduced and maintained and a lot more rat and mouse models that are, once again, challenging the aims of the 3Rs, especially Reduction. During my talk I will discuss how this new CRISPR/Cas technology is changing the present and future of laboratory and farm animals.

AACYTAL LECTURE II

ANIMALES DE EXPERIMENTACIÓN: CONSIDERACIONES FRENTE A UN NUEVO PARADIGMA

CECILIA CARBONE

La ciencia de los animales de laboratorio se puede definir como una rama multidisciplinaria de la ciencia que se ocupa del estudio de todos aquellos aspectos que contribuyen al empleo humanitario de los animales en investigaciones biomédicas y a la obtención de resultados válidos, reproducibles y comparables. En ella se incluye el estudio de la biología de los animales de experimentación, su manejo y requerimientos de alojamiento como también su condición sanitaria, características genéticas y la prevención y tratamiento de enfermedades.

Los aspectos éticos y morales han sido un blanco de atención importante, sin embargo, en los últimos años se han producido cambios que conducen a reflexionar sobre las necesidades y demandas actuales para el uso y cuidado de los animales de experimentación.

A estos cambios nos enfrentamos cuando hablamos de un nuevo paradigma. Se define como paradigma a un patrón, modelo o ejemplo.

El físico, historiador y filosofo Thomas Kuhn, en su libro “La estructura de las revoluciones científicas” lo define como lo que se debe observar y escrutar, considerando a los paradigmas como realizaciones científicas universalmente reconocidas que durante cierto tiempo proporcionan modelos de problemas y soluciones a la comunidad científica.

El bienestar animal constituye uno de los conceptos que actualmente se deben considerar, irrefutablemente, a la hora de diseñar y programar investigaciones o ensayos con animales. El reconocimiento de que los animales son seres sensibles susceptibles de sentir angustia, dolor y estrés ha provocado un impacto en lo referente al uso, manejo y cuidado de los mismos. Las

variaciones que puede causar la alteración del bienestar de los modelos animales en los resultados de las investigaciones y ensayos conducen al desarrollo de estudios sobre el comportamiento, las emociones y la conciencia de dichos individuos.

A su vez, el hecho de prestar atención a la condición de bienestar de los animales de experimentación, ha hecho no solo necesario el entrenamiento y la formación del personal técnico y científico sino que también ha conducido a optimizar y, en algunos casos, cambiar las condiciones de alojamiento, alimentación y los procedimientos que se realizan.

A esto se añade el desarrollo e incremento significativo de modelos animales genéticamente modificados con los consiguientes planteos y debates éticos referidos a su creación y uso.

AACYTAL LECTURE III

RATONES CONSANGUÍNEOS: IGUALES PERO DIFERENTES. LA DIFERENCIA ESTÁ EN LOS DETALLES...

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En estos tiempos dónde la genómica y la manipulación genética del ratón y la rata de laboratorio progresan día a día, se hace imprescindible estandarizar los animales utilizados en investigación. No sólo el control y la preservación de la calidad genética deben ser prioritarios, sino también concientizar a los investigadores sobre la influencia del fondo genético en sus modelos y la existencia de diferencias genéticas marcadas entre sub-cepas. Ya a principios de los años 1970 se publicaron artículos que mostraban una gran diferencia en el fenotipo diabético de las mutaciones *diabetes* (*Lepr*^{db}) y *obese* (*Lep*^{ob}), según estuvieran en fondo C57BLKS/J o C57BL/6J. Otro caso de modificación del fenotipo según la cepa de fondo fue observado para la mutación espontánea inmunodeficiente *Prkdc*^{scid}, donde la tendencia a producir linfocitos B y T funcionales con la edad es muy variable: alta en BALB/c, baja en C3H, y muy baja en NOD. En base a estos hallazgos, empezó a prestarse más atención a la influencia que pueden tener los distintos fondos genéticos en los fenotipos, particularmente en los modelos de ratones transgénicos, KO y KI. Desde finales de la década de 1990 se han publicado muchos reportes sobre esta influencia, los cuales discutiré en mi presentación, incluyendo diferencias importantes entre sub-cepas, por ejemplo C57BL/6**N** (NIH) versus C57BL/6**J** (Jax), y la presencia de mutaciones “pasajeras” que pueden afectar el fenotipo. Finalmente, presentaré distintas formas de prevenir y solucionar esta situación.