CNIO FRONTIERS Meetings 2011

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Breast Cancer

CNIO FRONTIERS Meetings 2011

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Breast Cancer

7-9 FEBRUARY 2011

Organisers

Joaquín Arribas, Vall d'Hebron Institute of Oncology, Barcelona, Spain
José Baselga, Massachusetts General Hospital, Boston, USA
Miguel Ángel Piris, CNIO, Madrid, Spain
Lajos Pusztai, The University of Texas M. D. Anderson Cancer Center, Houston, USA
Jorge Reis-Filho, The Institute of Cancer Research, London, UK

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Spanish National Cancer Research Centre

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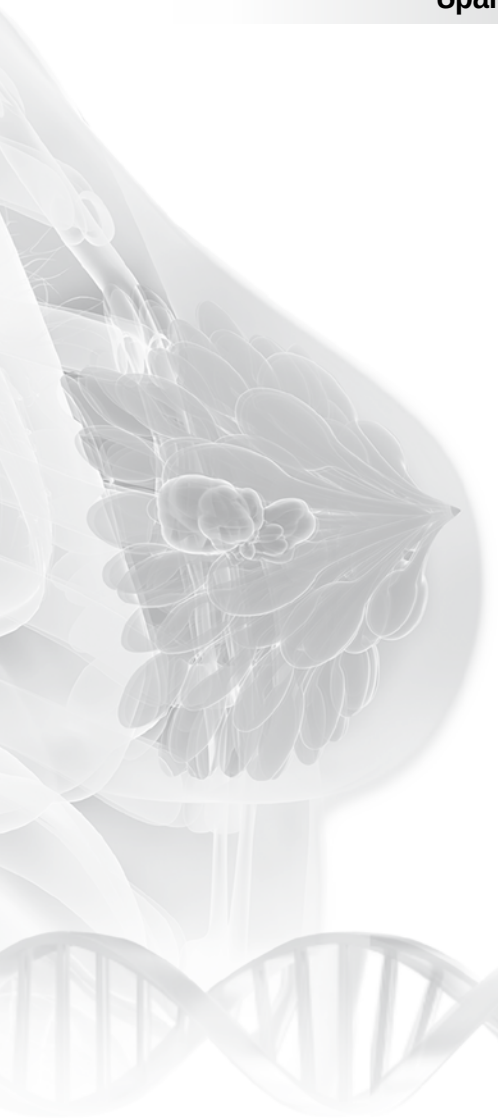

Breast Cancer

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Summary

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Detailed Programme

MONDAY, FEBRUARY 7th

9:00-9:15 **Welcome address: Miguel Ángel Piris,** CNIO, Madrid, Spain

Session I: Relevance of cancer stem cells in breast cancer biology: a new beginning or a new hype?

Chair: *Jorge Reis-Filho*

9:15-9:50 **Gabriela Dontu**, King's College London, UK
Cancer stem cells: promise and controversy

9:50-10:25 **Max Wicha**, University of Michigan, Ann Arbor, USA
Targeting self-renewal pathways in breast cancer stem cells

10:25-11:00 **Robert Clarke**, Paterson Institute for Cancer Research, Manchester, UK
Stem cells in breast cancer: treatment resistance and therapeutic targets

11:00-11:40 ***Coffee break and poster session***

11:40-12:15 **Mathew Ellis**, Washington University School of Medicine, St. Louis, USA
Cancer stem cells: a real entity or a reflection of genomic heterogeneity?

12:15-12:50 **Salvatore Pece**, European Institute of Oncology, Milan, Italy
Cancer stem cells and breast carcinogenesis: a new outlook on the molecular, biological and clinical heterogeneity of breast cancers

12:50-14:20 ***Lunch and poster session***

Session 2: Tumor stroma interactions and their prognostic and therapeutic implications

Chair: *Lajos Pusztai*

14:20-14:55 **Alex Toker**, Harvard Medical School, Boston, USA
The PI 3-K and akt signaling pathway in breast cancer progression

14:55-15:30 **Achim Rody**, Saarland University, Homburg, Germany
The distinct role of immune cells in molecular subtypes

15:30-16:20 ***Keynote lecture***
Alan Ashworth, The Institute of Cancer Research, London, UK
Synthetic lethal and high throughput genetic approaches to the development of new therapeutic approaches to cancer

16:20-16:55 **Jean Paul Thiery**, Institute of Molecular and Cell Biology, Singapore
Epithelial mesenchymal transition as a mechanism for the progression of breast carcinoma

16:55-17:15 ***Coffee break and poster session***

17:15-17:35 **Roger Gomis**, Institute for Research in Biomedicine (IRB), Barcelona, Spain
Short talk: *Unravelling breast cancer metastasis suppressors to the lung*

17:35-18:25 ***Keynote lecture***
Yosef Yarden, Weizmann Institute of Science, Rehovot, Israel
Roles for HER2 and EGFR in tumor stroma interactions and the opportunities they offer for therapeutic interventions

TUESDAY, FEBRUARY 8th

Session 3: Prognostic and predictive markers in the post-genomic era: one marker for all or different markers for each disease subset?

Chair: *Lajos Pusztai*

9:00-9:35 **Mitchell Dowsett**, The Royal Marsden Hospital, London, UK
Integration of clinical and molecular markers in ER+ disease

9:35-10:10 **Fatima Cardoso**, Champalimaud Cancer Center, Lisbon, Portugal
New prognostic markers: are they ready for clinical practice?

10:10-10:45 **Soonmyung Paik**, National Surgical Adjuvant Breast and Bowel Project Foundation, Pittsburgh, USA
Prediction of benefit from adjuvant trastuzumab – results from molecular profiling of tumor blocks from NSABP B-31

10:45-11:20 **Christos Sotiriou**, Jules Bordet Institute, Brussels, Belgium
PIK3CA mutation in estrogen receptor positive breast cancer – clinical implications

11:20-12:00 ***Group picture, coffee break and poster session***

Session 4: The next generation of drug targets and new treatment strategies to overcome drug resistance

Chair: *Mitchell Dowsett*

12:00-12:35 **Charles Swanton**, Cancer Research UK London Research Institute, UK
Prognostic impact and exploitation of chromosomal instability in breast cancer

12:35-13:10 **Lajos Pusztai**, The University of Texas MD Anderson Cancer Center, Houston, USA
Using human genomic data to define the next generation of therapeutic targets for triple negative breast cancer

13:10-13:45 **Jorge Reis-Filho**, The Breakthrough Breast Cancer Research Centre, London, UK
Identification of novel therapeutic targets through integrative genomic profiling

13:45-14:20 **Joaquín Arribas**, Vall d'Hebron Institute of Oncology, Barcelona, Spain
Hyperactivation of HER2 and anti-HER2 therapies

14:20-16:00 ***Lunch and poster session***

Short talks from selected abstracts

Chair: *Joaquín Arribas*

16:00-16:20 **Inna Kuperstein**, Curie Institute, Paris, France
Short talk: *Integrated cell cycle and DNA repair signalling network modelling for identification of key molecular regulators in basal-like breast cancer*

16:20-16:40 **Caroline Nunes-Xavier**, Centro de Investigación Príncipe Felipe, Valencia, Spain
Short talk: *Protein tyrosine phosphatase epsilon (PTPε) plays an essential role in growth and survival of human breast cancer cells*

16:40-17:00 **Ivan Plaza-Menacho**, The Breakthrough Breast Cancer Research, London, UK
Short talk: *Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance*

17:00-17:20 ***Coffee break and poster session***

17:20-17:40 **Madalena Tarsounas**, The Gray Institute for Radiation Oncology and Biology, Oxford, UK
Short talk: *BRCA2, but not BRCA1, is required for telomere integrity in mammalian cells*

Chair: *Jorge Reis-Filho*

17:40-18:30 ***Keynote lecture***
Zena Werb, University of California, San Francisco, USA
Regulation of tumor stroma during breast cancer progression

WEDNESDAY, FEBRUARY 9th

Session 5: The next generation of tissue analysis tools: promises and pitfalls of next generation sequencing

Chair: *Miguel Ángel Piris*

09:00-09:35 **Carlo Croce**, The Ohio State University, Columbus, USA
Causes and consequences of microRNA dysregulation in cancer

09:35-09:55 **Francisco Javier Gracia**, CNIO, Madrid, Spain
Short talk: *Identification of high susceptibility genes in BRCAX families by whole exon sequencing*

09:55-10:30 **Emanuel Petricoin**, George Mason University, Manassas, USA
Development and clinical implementation of functional protein pathway activation mapping of breast cancer for personalized therapy

10:30-11:10 ***Coffee break and poster session***

Session 6: Innovative clinical trials

Chair: *Christos Sotiriou*

11:10-11:45 **Fabrice Andre**, Gustave Roussy Institute, Villejuif, France
Biology driven phase II trials: which model for molecular selection?

11:45-12:20 **Miguel Ángel Quintela**, CNIO, Madrid, Spain
Trial designs aiming for biomarker definitions: no pain-no gain?

12:20-13:00 ***Keynote lecture***
José Baselga, Massachusetts General Hospital, Boston, USA
Targeting the PI3K pathway as cancer therapeutics

13:00 ***Closing remarks***
Lajos Pusztai, The University of Texas MD Anderson Cancer Center, Houston, USA
Jorge Reis-Filho, The Institute of Cancer Research, London, UK

Note: Talks: 25 minutes / Short talk: 15 minutes / Keynote lectures: 40 minutes
Discussion: 10 minutes after each talk / 5 minutes after each short talk / 10 minutes after each keynote lecture

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Speaker Abstracts

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Session 1

Relevance of cancer stem cells in breast cancer biology: a new beginning or a new hype?

Chair: Jorge Reis-Filho

Cancer stem cells: promise and controversy

Gabriela Dontu

King's College London, UK

The cancer stem cell model holds that, similar to normal tissues, tumours are hierarchically organized, containing a cancer stem cell population that gives rise to progenitor cells and differentiated cancer cells. Supporting this concept, tumours consist of a cell population that is heterogeneous phenotypically and functionally. Studies in a large variety of cancer types showed that tumourigenicty and metastasis is associated with a sub-population of cells, typically representing the minority of the tumour cell population. This concept of cancer stem cells has been subject to criticism related to the bias introduced by the experimental systems, the occurrence of clonal evolution within the cancer stem cell compartment and generation of cancer stem cell from non-cancer stem cells through genetic and epigenetic mechanisms. Solving these controversies will be critical for the development of clinical applications based on the cancer stem cell concept.

Targeting self-renewal pathways in breast cancer stem cells

Max Wicha

University of Michigan, Ann Arbor, USA

There is considerable evidence that human breast cancers are driven by a subpopulation of cells that display stem cell properties. These cancer stem cells, by virtue of their relative resistance to radiation and chemotherapy, may mediate treatment resistance and be responsible for tumor metastasis. Stem cell self-renewal is regulated by intrinsic pathways and extrinsic signals originating from the tumor microenvironment. An important component of these extrinsic pathways are cytokine loops generated by mesenchymal and immune cells. These cytokine loops involve IL-6 and IL-8. These observations suggest that a relationship between tissue inflammation and carcinogenesis involves the regulation of breast cancer stem cells by inflammatory cytokine loops. Blocking antibodies and small molecule inhibitors to these cytokine receptors are able to selectively target breast cancer stem cells. Molecules involved in both intrinsic and extrinsic cancer stem cell regulation provides novel therapeutic targets. If breast cancer stem cells indeed drive tumorigenesis and metastasis then successful targeting of these cell populations has the potential to improve the outcome for women with breast cancer.

Stem cells in breast cancer: treatment resistance and therapeutic targets

Robert Clarke

Paterson Institute for Cancer Research, Manchester, UK

There is emerging evidence that cancer stem cells (CSCs) are resistant to current therapies suggesting that CSC-specific treatments are needed. Due to their relative insensitivity to treatment, we and others have demonstrated that CSCs are enriched by radio, chemo and endocrine therapy. Increases in the proportion of CSCs after therapy is measured using cell surface markers and mammosphere colony assays of stem cell activity. DNA repair, survival and stem cell signalling pathways are strong emerging candidates for the underlying mechanisms of resistance.

However, CSCs still respond to therapy-induced changes in microenvironmental signals. One candidate pathway known to regulate normal stem cells is Notch receptor signalling. We have evidence that activated Notch plays a key role in breast tumour initiation by CSCs and that therapies targeting Notch receptor are likely to be effective in preventing treatment resistance.

Cancer stem cells: a real entity or a reflection of genomic heterogeneity?

Mathew Ellis

Washington University School of Medicine, St. Louis, USA

The cancer stem cell theory holds that tumors, similar to normal organs, have self-renewing stem cells that produce progeny that populate the bulk of the tumor and are exclusively responsible for seeding distant organs with metastatic lesions. If this were the case all cells in the tumor, as in a normal organ, would carry the same genetic complement as the stem cells, i.e. the tumor would be monoclonal. However recent studies using massive parallel sequencing to delineate complete cancer genomes challenge this theory. These technologies produce “read counts” – a frequency distribution for each mutation in the total population of DNA molecules extracted from the tumor – because the detection method starts with a single DNA molecule. In a monoclonal diploid tumor, mutant allele frequencies would be expected to follow a Mendelian distribution - 50% of the read counts will be mutant for a heterozygous mutation and 100% for a homozygous mutation. In reality, sequencing a basal-like genome revealed mutant frequencies that range from 1% (barely detectable with 30 fold coverage) to 100%, suggesting a complex mixture of dominant and subdominant clones[1]. Importantly the genomic repertoire was not static, but differed between samples taken from different sites and over time. In the case of the basal-like breast cancer genome, the brain metastasis contained 22 mutations that were significantly enriched versus the primary, in some cases very dramatically. Many of the same mutations were also enriched in a xenograft derived from the primary, suggesting that the xenograft approach is a valid model for identifying aggressively metastatic tumor subclones. If these findings are generalizable, then the stem cells may ultimately be found to represent a subdominant clone that has developed a mutational repertoire that promotes metastasis and stem cell like patterns of gene expression and function. These lethal cells may have “stemness” but they are not stem cells per se because the other cells in the tumor are not derived from them but are independently self-sustaining. Perhaps many of the cells in a primary are derived from an earlier stage in the tumors evolutionary history before the final fatal clone developed. If this is a case, accurate diagnostics may prove difficult and very deep read depths may be necessary to detect the nascent rare mutations that drive long term prognosis.

[1]Ding L, Ellis MJ, Li S, et al Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature.464(7291):999-1005. PMCID: 2872544

Cancer stem cells and breast carcinogenesis: a new outlook on the molecular, biological and clinical heterogeneity of breast cancers

Salvatore Pece[1,2,3] and Pier Paolo Di Fiore[1,2,3]

[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy; [2]Universita degli Studi di Milano, Milan, Italy; [3]Istituto Europeo di Oncologia, Milan, Italy

The emerging view of the stem cell origin of cancer holds that a handful of cells, functionally identifiable as cancer stem cells, sit at the heart of tumorigenesis representing the true responsible for the onset and development of tumors. Several lines of evidence also converge on the idea that cancer stem cells are also responsible for therapy failure and disease recurrence. A corollary of this concept, of paramount importance for its clinical implications, is that cancer stem cells also hold the key for the definitive cure of cancer. Related to breast cancer, we have recently highlighted the crucial molecular traits of normal and cancer stem cells, thus paving the way for the development of clinical strategies to improve the management of breast cancer patients. For instance, we have provided knowledge that the intrinsic content of cancer stem cells in breast tumors is proportionally higher in biologically aggressive and poor prognosis breast tumors compared to well-differentiated and good prognosis ones. Relevant to patient stratification, we found that the cancer stem cell content of tumors can be used to distinguish breast cancer patients according to their pathological, molecular and clinical features, based on the use of suitable biomarkers for the identification of cancer stem cells in routine histopathological procedures. We will discuss how these findings, once subjected to extensive clinical validation, are susceptible to refine the currently available algorithms for diagnosis, prognosis and prediction of treatment responsiveness through the introduction of novel cancer stem cell biomarkers, and therefore to guide more accurately tailored clinical choices in the clinical management of the breast cancer neoplastic disease.

NOTES

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Session 2

Tumor stroma interactions and their prognostic and therapeutic implications

Chair: Lajos Pusztai

The PI 3-K and Akt signaling pathway in breast cancer progression

Alex Toker

Harvard Medical School, Boston, USA

Akt/PKB (protein kinase B) is a proto-oncogene that plays a critical role in cell survival, proliferation and metabolism. There are three mammalian Akt isoforms: Akt1, Akt2 and Akt3. They share a high degree of amino acid similarity and are activated by similar mechanisms. The current paradigm is that all three Akt isoforms promote cancer cell survival and growth. However studies from several laboratories have recently demonstrated that Akt isoforms share non overlapping functions in breast cancer progression, whereby Akt1 inhibits breast cancer cell invasive migration. Conversely, Akt2 has been shown to promote breast cancer cell migration in vitro and in vivo. We are therefore conducting studies to explore the mechanistic basis for this selectivity. Using a combination of shRNA and phospho-proteomic approaches we have identified several novel substrates of Akt1 and Akt2 that modulate cell motility in an isoform-specific manner. shRNAs specific to Akt1 or Akt2 have been generated and delivered into human breast cancer cells. Using an antibody that recognizes phosphorylated serine/threonine residues contained within a consensus Akt motif (RXRXXS/T), we have found that palladin, a component of actin-containing microfilaments, is specifically phosphorylated by Akt1 in breast cancer cell lines. We have also found that palladin is expressed in a variety of human breast cancer cell lines, where it functions to block in vitro invasive migration. Importantly, phosphorylation of palladin is critical for its inhibition of breast cancer cell motility. More recent studies have identified additional Akt1- and Akt2-specific substrates that modulate breast cancer invasive migration. Finally, we will present analysis of phospho-proteomic screens for Akt isoforms specific substrates in cancer cells that harbor PI 3-K pathway mutations. In summary we have identified novel substrates of Akt isoforms in cancer cells and tissues and are evaluating their contribution to progression in variety of human solid tumors.

Our work is supported by NIH/NCI PHS grants, the Department of Defense Breast Cancer Research Program and the Susan G. Komen Breast Cancer Foundation

The distinct role of immune cells in molecular subtypes

Achim Rody and T. Karn

Saarland University, Homburg, Germany; Goethe University, Frankfurt, Germany

The impact of host factors such as immune cells, stromal environment and chemokines on the development and maintenance of breast cancer has frequently been hypothesized, but still remains a matter of debate.

The search for prognostic or predictive signatures using microarray analysis in bulk breast cancer specimens reveals several genes that are associated with immune cells; for example, interferon-regulated genes, B-lymphocyte marker, as well as T-lymphocyte-associated genes.

There is growing evidence that interaction of stromal and immune cells with normal or malignant epithelial cells is pivotal for the development and progression of cancer. Several reports indicate that tumor-infiltrating leucocytes may represent an essential pathophysiological factor in the development and progression of breast cancer.

A strong positive prognostic value for the T-cell surrogate marker (lymphocytespecific kinase (LCK) metagene) was observed among estrogen receptor (ER)-negative tumors and those ER-positive tumors with a HER2 overexpression. Moreover ER-negative tumors with high expression of both IgG and LCK metagenes seem to respond better to neoadjuvant chemotherapy.

Triple negative breast cancers (TNBC) are clinically heterogeneous and prognostic markers and biology-based therapies are needed to better treat this disease.

Survival of basal-like TNBC semms not to be different from non-basal like TNBC. High expression of immune cell metagenes is associated with good and high expression of inflammation and angiogenesis-related metagenes are associated with poor prognosis. A ratio of high B-cell and low IL-8 metagenes identifies 32 % of TNBC with good prognosis and is the only significant predictor in multivariate analysis including routine clincopathological variables.

Moreover, inhibition of the IL-8 pathway or other immune cell associated pathways might represent an attractive novel therapeutic target for this disease.

Keynote Lecture

Synthetic lethal and high throughput genetic approaches to the development of new therapeutic approaches to cancer

Alan Ashworth

The Institute of Cancer Research, London, UK

Many tumours harbour defects in their ability to maintain genomic integrity. This contributes to the mutational burden and likely fosters pathogenesis. We have been exploring therapeutic strategies to exploit these defects. We have used a synthetic lethal approach to target the defect in DNA repair by homologous recombination in tumours with a BRCA1 or BRCA2 mutation. This strategy using PARP inhibitors is showing considerable promise in the clinic. Here, I will describe the approach as well recent work defining determinants of sensitivity and resistance to PARP inhibitors. The application of the synthetic lethal approach to other cancer types will also be discussed. In addition, we have been using high- and medium- throughput RNAi screens to uncover new therapeutic targets for cancer as well as biomarkers of patients who might respond to specific treatments. In particular I will discuss the integration of genomic, gene expression and RNAi data to generate functional maps of cancer cell lines.

Epithelial mesenchymal transition as a mechanism for the progression of breast carcinoma

Jean Paul Thiery[1,2,3], Weng Jing Sim[1], Kelvin Chua[2], Ruby Huang[3], Yeh Shiu Chu[§], Sylvie Dufour[§], Francois Clement Bidart[§], and Jean Yves Pierga[§]

Institute of Molecular Cell Biology[1], Experimental Therapeutic Centre[2] and Cancer Science Institute[3], National University of Singapore, Republic of Singapore. Institut Curie, Paris, France[§]

Epithelial mesenchymal transition (EMT) is a major process controlling multiple events during development. EMT has been conserved throughout evolution to control morphogenetic events, such as the formation of the three primary germ layers during gastrulation. Most interestingly, signal transduction pathways have been remarkably conserved in many different species. EMT pathways are also tightly connected to determination and differentiation programs, and are reactivated in adult tissues following injury or exposure to toxic agents. EMT is likely to operate during the early stages of carcinoma invasion that lead to blood or lymph vessel intravasation. Mesenchymal-like carcinoma cells undergo a mesenchymal to epithelial transition in distant sites from the primary tumour and eventually become micrometastatic. We have characterized bone marrow micrometastases from breast cancer patients and found that the detection of micrometastatic carcinoma cells was associated with poorer distant metastasis-free survival, local relapse-free survival, and overall survival. Despite high rates of adjuvant systemic treatment and breast irradiation in this series, disseminated carcinoma cells remain a prognostic factor, in favour of the resistance to treatment of locally or distant disseminated cancer cells in bone marrow-positive patients. In addition, we detected micrometastatic carcinoma cells in patients with T1 tumours, suggesting that dissemination occurs much earlier during tumour progression than is generally accepted. Thus, bone marrow micrometastases should become a very useful prognostic indicator for relapse, and an excellent surrogate marker for patient's response to treatment. The mesenchymal-like state of carcinoma confers stemness, protection from cell death, escape from immune response and, most importantly, resistance to conventional and targeted therapies. Current strategies based on the EMT concept are aimed at designing new therapeutic approaches that interfere with the plasticity of carcinoma cells. Our laboratory has devised a high-content, high-throughput screen for EMT. Several combinations of drugs have been shown to selectively inhibit EMT. This strategy may be used to interfere with tumour progression, particularly in breast carcinomas that have acquired resistance to conventional therapies.

Short Talk

Unravelling breast cancer metastasis suppressors to the lung

Monica Morales and Roger R. Gomis

Institute for Research in Biomedicine (IRB), Barcelona, Spain

Our current understanding of the biology of breast cancer metastasis is a major barrier to identify novel therapies and improve existing therapies for the treatment and prevention of this disease. Metastasis occurs when tumor cells acquire the ability to escape their original location and invade healthy tissue and organs elsewhere in the body. This complex cellular process, virtually unique to cancer, raises fundamental questions about the role that certain genes play in determining how and why tumor cells break free and, once mobile, how they decide where to attack.

Our work focuses on breast cancer metastatic suppressor genes and their functions in the metastatic process. For this, we are using the MDA-MB-231 breast cancer cell line model and their derivatives #4175 and #1833, which have a strong metastatic capacity to lung and bone. We used these subpopulations to functionally validate a particular metastasis suppressor whose loss of expression in ER- breast cancer cells confers a selective advantage for the colonization of lung. Tumor cells under certain conditions cannot grow or survive in the absence of a supportive microenvironment. Indeed, the microenvironment may even drive tumor and metastasis development by selecting for highly invasive and resistant cancer cell phenotypes and systemically fostering the mobilization of marrow-derived progenitor cell. In particular, loss of expression of our gene of interest is selected in the primary tumor. Interestingly, how these particular gene controls the ability to subsequently colonize distant organs depends on the organ-colonizing faculties of disseminated tumor cells rather than interaction with the restrictive microenvironment of target organs. Collectively, these results show that genes selected for metastasis contribute to the different steps and represent the random accumulation of traits that provide the necessary advantage for adaptation to a different organ microenvironment and need at any time.

Keynote Lecture

Roles for HER2 and EGFR in tumor stroma interactions and the opportunities they offer for therapeutic interventions

Yosef Yarden

Weizmann Institute of Science, Rehovot, Israel

Growth factors and their transmembrane receptor tyrosine kinases regulate cellular proliferation and migration during both embryogenesis and oncogenesis. An example is provided by the ErbB/HER family of receptors, which plays essential roles in the development of neuronal and epithelial cell lineages. ErbB proteins and their EGF-like ligands play essential roles in breast cancer. One important mechanism involves autocrine loops comprising co-expression of a receptor and the respective ligands. Another mechanism entails genetic aberrations. Overexpression of the ErbB-2/HER2 protein predicts breast cancer aggressiveness, but underlying mechanisms remain incompletely understood. To study this question we employed spheroids of human mammary cells grown in extracellular matrix. HER2-overexpressing cells formed filled spheroids, which protruded invasive arms upon growth factor stimulation. Our transcriptome analyses imply a 2-hit model for invasive growth: by activating the Notch pathway, HER2-induced proliferation generates filled structures, which are morphologically and transcriptionally analogous to pre-invasive patients' lesions. In the second hit, growth factors of the EGF family escalate signaling and transcriptional responses leading to invasion. These results attribute progression of the HER2 subtype of breast cancer to growth factors acting on HER2-overexpressing premalignant lesions.

Several therapeutic strategies target ErbB signaling. The most successful strategy has been the utilization of monoclonal antibodies to ErbB-2/HER2 or to EGFR/ErbB-1. The therapeutic antibodies are thought to recruit natural killer cells to tumors, but studies in mice propose the existence of alternative mechanisms of action. For example, anti-ErbB antibodies block receptor signaling by accelerating receptor internalization. Recent studies from several laboratories propose that combinations of monoclonal antibodies directed to the same receptor can synergistically inhibit tumor growth in animals, provided that the antibodies bind with distinct epitopes. Molecular mechanisms underlying antibody synergy, as well as potential applications in cancer therapy will be discussed.

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Session 3

Prognostic and predictive markers in the post-genomic era: one marker for all or different markers for each disease subset?

Chair: Lajos Pusztai

Integration of clinical and molecular markers in ER+ disease

Mitchell Dowsett

The Royal Marsden Hospital, London, UK

Clinico-pathologic markers have been the mainstay of stratifying primary breast cancer patients for adjuvant medical therapy for the last 30-40 years. The major factors included are axillary lymph node status, tumour size and histopathological grade with some investigators including lymphovascular invasion and age. A number of prognostic algorithms have been developed to integrate these including the widely used Adjuvant! Online. It is clear, however, that particularly in ER+ disease, the disease outcome cannot be well predicted by these classical variables because of a highly heterogeneous underlying biology. In the last decade a number of successful attempts have been made to that draw together the key molecular constituents of that biology to create molecular profiles that also predict the probability of recurrence. Some of these have been sufficiently well validated for use in areas of clinical uncertainty that they have entered widespread clinical use. While each of these shows overlapping prognostic information there is little overlap with the information provided by the clinico-pathological profiles. This has allowed the integration of these 2 types of information to provide a single integrated score that performs better than either alone. The RSPC and IHC4 will be discussed as examples of this approach that use RNA and immunohistochemical molecular analyses, respectively.

New prognostic markers: are they ready for clinical practice?

Fatima Cardoso

Champalimaud Cancer Center, Lisbon, Portugal

Prediction of benefit from adjuvant trastuzumab - results from molecular profiling of tumor blocks from NSABP B-31

Soonmyung Paik

National Surgical Adjuvant Breast and Bowel Project Foundation, Pittsburgh, USA

While systemic adjuvant therapies clearly improved the clinical outcome of patients diagnosed with breast, it is also recognized that not all patients derive significant clinical benefit from them and suffer from unnecessary toxicity.

Archived formalin fixed paraffin embedded tumor blocks (FFPET) from finished clinical trials conducted by the NSABP provide the ideal clinical contexts to test predictive markers but limited by degradation or alteration of macromolecules within them. Development of a method for whole genome expression profiling of fragmented or degraded RNA extracted from FFPET allowed us to engage in discovery oriented projects using these archived materials during the past few years.

For trastuzumab, data from adjuvant trials B-31 was puzzling since even patients with HER2 negative tumors enrolled in the trial based on faulty positive HER2 testing results at local sites derived similar benefit as HER2 positive patients. Gene expression profiling revealed the following; 1) Expression level of HER2 mRNA is indeed a linear predictor of the degree of benefit from trastuzumab added to standard chemotherapy (ACT). 2) HER2 negative tumors accidentally enrolled in B-31 are truly HER2 negative with significantly lower HER2 mRNA expression. 3) There is an overlap of HER2 mRNA levels between HER2 negative and HER2 positive tumors defined by current clinical tests (IHC or FISH). 4) Significant proportion of HER2 negative patients are expected to derive some degree of benefit from trastuzumab based on HER2 mRNA threshold for benefit in B-31. Whether this low threshold for benefit from adjuvant trastuzumab is due to a lower threshold of micro-metastatic cells to trastuzumab plus chemotherapy and/or to ADCC is not clear at this point. Contrary to results from in-vitro studies and small scale clinical studies in metastatic setting, loss of PTEN or mutations in PI3kinase gene were not predictive of resistance to trastuzumab again suggesting the differences between metastatic setting versus adjuvant setting. NSABP trial B-47 will test the worth of adjuvant trastuzumab in HER2 negative patients.

Differences between metastatic and adjuvant setting underscore the difficulty of moving forward from metastatic to adjuvant setting for new targeted therapies. Post-neoadjuvant trial setting in which adjuvant trials are conducted in patients with gross residual disease after neoadjuvant therapy may provide an efficient trial platform to overcome this barrier.

PIK3CA mutation in estrogen receptor positive breast cancer – clinical implications

Christos Sotiriou

Jules Bordet Institute, Brussels, Belgium

PIK3CA mutations are reported to be present in around 25% of breast cancer, particularly the estrogen receptor-positive and HER2 over-expressing subtypes, making them one of the most common genetic aberrations in breast cancer. In experimental models, these mutations have been shown to activate AKT, induce oncogenic transformation and hence, these lesions have been hypothesized to render tumors highly sensitive to therapeutic PI3K/mTOR inhibition. In the present study, we have sought to determine whether PIK3CA mutations could be the oncogenic driver responsible for the poor prognosis of the highly proliferative ER+/HER2- breast cancer phenotype. To our surprise, we found that PIK3CA mutations were not associated with a poor clinical outcome despite their known tumorigenic effects through activation of the PI3K pathway. We observed that whilst PIK3CA mutations were associated with a distinct gene expression signature of PI3K pathway activation, the PIK3CA-GS could identify ER+/HER2- breast cancer patients, both untreated and tamoxifen-treated, with better outcomes. Moreover this ability was independent of their mutation status. We also report that PIK3CA-GS was not prognostic in ER- or in the HER2+ breast cancer subtypes suggesting that the molecular background of breast cancer is an important determinant of the functional output of a PIK3CA mt breast cancer. Finally we report for the first time that in ER+/HER2-/PIK3CA mt breast cancers, despite apparent PI3K/AKT pathway activation, downstream mTORC1 signaling was not greatly elevated at the transcriptional and biochemical levels. These patterns were observed from the gene expression data as well as corresponding protein data from multiple independent datasets of human breast cancer. This was not seen in cell lines where PIK3CA-GS correlated with high mTORC1 output. This supports the notion that pathway activation may differ in vitro compared with human PIK3CA mt breast cancer. These results could have important implications for the design of future studies involving PI3K/mTOR inhibitors and the treatment of PIK3CA-mutant breast cancers. We suggest that PIK3CA mt ER+/HER- BC may respond well to tamoxifen and probably should not be prescribed mTOR inhibitors. Hence, analysis of the corresponding gene expression profiles associated with PIK3CA mutations has been vital to understanding the phenotype produced by the aberration and has resulted in surprising findings that are contrary to that expected by previous reports from experimental models. These findings validate our approach to studying these aberrations in breast cancer and may aid drug development and clinical trial design.

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Session 4

The next generation of drug targets and new treatment strategies to overcome drug resistance

Chairs: Mitchell Dowsett / Joaquín Arribas / Jorge Reis-Filho

Prognostic impact and exploitation of chromosomal instability in breast cancer

Charles Swanton

Cancer Research UK London Research Institute, UK

Chromosomal Instability (CIN) is widely held to be associated with poor prognosis in solid tumours and to mediate phenotypic variation driving adaptation to diverse stromal pressures. However, pre-clinical and mouse cancer models have demonstrated that aneuploidy confers a negative impact upon organism biological fitness and in some situations mediates a tumour suppressor effect, analogous to bacterial population genetics where an excess of deleterious genomic events drives “mutational meltdown” and a decline in population size. We have explored the association of structural chromosomal complexity and numerical chromosomal instability with patient survival using DNA and RNA based measures of chromosome integrity and centromeric FISH analysis. We identify a complex relationship between CIN and breast cancer outcome. In light of evidence that CIN is associated with a deleterious association with cancer outcome, we have attempted to identify molecular mechanisms that trigger mitotic catastrophe in cells that have undergone aberrant mitoses initiating chromosomal instability. We previously identified CERT, a ceramide transporter, in a synthetic lethal RNA interference screen, the depletion of which enhances taxane-mediated cell death and triggers endoplasmic reticulum stress. We find that silencing CERT augments a novel mitotic catastrophe pathway that may serve to limit CIN following aberrant mitoses. We present evidence that CERT expression distinguishes poor from good risk breast cancer and is relatively over-expressed in HER2 positive disease. CIN presents a relative tumour-specific phenotype and an improved understanding of its relationship with breast cancer outcome together with novel approaches to specifically target this pattern of genome instability may provide therapeutic insights to improve the management of high-risk breast cancer.

Using human genomic data to define the next generation of therapeutic targets for triple negative breast cancer

Lajos Pusztai

The University of Texas MD Anderson Cancer Center, Houston, USA

Hight-hroughput genomic studies of human breast cancer have created large data bases that can be used to search for novel therapeutic targets for subsets of cancers. An important challenge remains to define what genomic abnormalities are “driver” events and what changes represent functionally inert “by-stander” effects. I will illustrate several approaches to identifying functionally important pathways or individual genes that may serve as drug targets in breast cancer. I will also present results from a large scale siRNA screening project that examined the functional importance of reducing the expression of 628 genes that were statistically significantly overexpressed in estrogen receptor (ER)-negative compared to ER-positive cancers but were not previously studied in the context of breast cancer. We identified a new receptor - ligand system that plays a critical role in sustaining the growth of ER-negative breast cancer.

Identification of novel therapeutic targets through integrative genomic profiling

Jorge Reis-Filho

The Breakthrough Breast Cancer Research Centre, London, UK

Breast cancer comprises a complex and heterogeneous group of diseases. Although the heterogeneity of breast cancer was known for a long time, it was only after seminal studies using high throughput transcriptomic methods that this concept was brought to the forefront of breast cancer research, and, most importantly, clinical practice. It is currently accepted that the heterogeneity of breast cancer is such that it encompasses different diseases that have distinct risk factors, clinical presentation, histopathological features, molecular characteristics, response to therapies and clinical behaviour. We have sought to identify the molecular drivers and potential therapeutic targets of breast cancers by reducing the heterogeneity of the disease a priori, by defining subgroups based on their histological and/ or immunohistochemical features. Cohorts of well characterised and relatively homogeneous tumours were microdissected and subjected to genomic and transcriptomic analysis. Potential drivers of regions recurrently amplified and overexpressed were identified in silico. These potential drivers were tested functionally using cell line models that recapitulate the phenotypic and genetic features of the primary tumours analysed, and a combination of chemical inhibitors and RNA interference. This approach has led to the identification and functional validation of i) FGFR1 as a potential therapeutic target for oestrogen receptor positive breast cancers harbouring 8p11.2-p12 amplification; ii) PPM1D as a potential therapeutic target for a subgroup of HER2-positive breast cancers harbouring 17q23.2 amplification; and iii) FGFR2 as potential therapeutic target for a subgroup of triple-negative (ER negative, progesterone receptor negative and HER2 negative) breast cancers harbouring 10q26 amplification.

Hyperactivation of HER2 and anti-HER2 therapies

Joaquín Arribas

Vall d'Hebron Institute of Oncology, Barcelona, Spain

Current classification of breast cancers depends in great part on the expression of HER2, a cell surface tyrosine kinase receptor, and ER, the nuclear receptor for estrogen. In addition to reliable biomarkers, these receptors are targets of effective and widely used anti-tumor drugs.

A subtype of HER2-positive tumors with distinct biological and clinical features expresses a series of carboxy-terminal fragments collectively known as p95HER2. One of these fragments, named 100-115 kDa p95HER2 or 611-CTF, is hyperactive because of its ability to form homodimers maintained by intermolecular disulfide bonds. Despite lacking the majority of the extracellular domain, this HER2 fragment drives breast cancer progression in vivo. The recent availability of specific anti-p95 antibodies has confirmed previous results indicating that the expression of p95HER2 is predictive of poor prognosis and correlates with resistance to the treatment with trastuzumab, a therapeutic antibody directed against the extracellular domain of HER2.

Short Talk

Integrated cell cycle and DNA repair signalling network modelling for identification of key molecular regulators in basal-like breast cancer

Inna Kuperstein[1,2], P. Vera-Licona[1,2], A. Zinovyev[1], G.C. Tucker[3], T. Dubois[2] and Emmanuel Barillot[1]

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Basal-like breast cancer (BLC) is associated with a poor prognosis and there is a lack of targeted therapy. Pathways involved in cell cycle and DNA repair are highly perturbed in BLC, thus facilitating cell survival capacity despite accumulated DNA damage. Cell cycle and DNA repair mechanisms contain a variety of signalling pathways that create molecular networks. To understand orchestration between cell cycle and DNA repair molecular mechanisms, we used a systems biology approach to represent biological processes as comprehensive models based on experimental data retrieved from literature and transcriptomic data of breast tumours. The network is created using the CellDesigner software, which is adapted to further mathematical modelling of signalling network dynamics. We have constructed an integrated cell cycle and DNA repair molecular signalling network composed of three interconnected layers. The first layer represents core cell cycle pathways and checkpoint proteins. The second layer includes DNA repair pathways. The third layer is composed of common regulators and modulator enzymes for cell cycle and DNA repair that ensure reciprocal influence between these processes. We further integrated transcriptomic data from breast tumours into the network and highlighted specific pathways modified in the disease. To verify the network, we simulated, in silico, the familial BRCA1-negative phenotype and inhibition of the base excision repair protein PARP to prove that our model recapitulates some well-described data. A comprehensive reconstruction of the cell cycle and DNA repair signalling network allows integration of multiple crosstalks between DNA repair and cell cycle. Mathematical modelling of the network will bring a better understanding of dynamic regulatory circuits. The network will be used for discovering key players in breast cancer progression to induce synthetic lethality of malignant cells.

Protein tyrosine phosphatase epsilon (PTPε) plays an essential role in growth and survival of human breast cancer cells

Caroline E. Nunes-Xavier*, Ari Elson and Rafael Pulido*

**Centro de Investigación Príncipe Felipe, Valencia, Spain and The Weizmann Institute of Science, Rehovot, Israel*

The controlled tyrosine phosphorylation in cells is coordinated by the reversing activities of tyrosine kinases (TKs) and protein tyrosine phosphatases (PTPs). Increased tyrosine phosphorylation has been correlated with human cancer, including breast cancer. In general, the increased TK activation can be antagonized by the action of the PTPs. However, in some cases the PTPs can potentiate the activation of the TKs. In this study, we report the up-regulation of PTPε in various human breast cancer cell lines in response to the phorbol ester PMA. Up-regulation of PTPε by PMA in MCF-7 cells required the prolonged activation of the ERK1/2 pathway, and correlated with the shutdown of this route. In consistence, in MDA-MB-231 cells, high endogenous expression of PTPε correlated with higher basal ERK1/2 activation. Diminishing the expression of PTPε in various human breast cancer cells abolished ERK1/2 activation by PMA, and decreased the viability and anchorage independent growth of the cells. Conversely, stable MCF-7 cell lines expressing inducible high levels of ectopic PTPε, displayed higher activation of ERK1/2, and altered cell adhesion properties and anchorage independent growth. Our results demonstrate that expression of PTPε is up-regulated in breast cancer cell lines by activation of the ERK1/2 pathway, and suggest an important role for PTPε in the control of growth and survival of human breast cancer cells.

Short Talk

Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance

Ivan Plaza-Menacho, Andrea Morandi, David Robertson, Sunil Pancholi, Suzanne Drury, Mitch Dowsett, Lesley-Ann Martin and Clare M. Isacke

Breakthrough Breast Cancer Research, London, UK

Endocrine therapy is the main therapeutic option for patients with estrogen receptor (ERα)-positive breast cancer. Resistance to this treatment is often associated with estrogen-independent activation of ERα. In this study, we show that in ERα-positive (ER+) breast cancer cells, activation of the receptor tyrosine kinase RET by its ligand GDNF results in estrogen-independent increased of ERα phosphorylation on Ser118 and Ser167, and activation of ERα transcriptional activity. Furthermore, we identify mTOR as a key component in this downstream signaling pathway as Rapamycin treatment abolished GDNF-induced ERα phosphorylation. Importantly, in tamoxifen response experiments, RET downregulation resulted in 6.2-fold increase in sensitivity of MCF7 cells to antiproliferative effects of tamoxifen, whereas GDNF stimulation had a protective effect against the drug. In tamoxifen-resistant (TAMR-1) MCF7 cells, targeting RET restored tamoxifen sensitivity. The importance of RET in tamoxifen resistance was further supported by the increase of RET protein and mRNA levels observed in long term tamoxifen and fulvestrant treated MCF7 cells and that RET overexpression in MCF7 cells resulted in a resistant phenotype. Finally, examination of two independent tissue microarrays of primary human breast cancers revealed that expression of RET protein was significantly associated with ERα-positive tumors. In addition, in primary tumors from patients who subsequently developed an invasive recurrence following adjuvant tamoxifen treatment, there was a two-fold increase in the number of RET-positive tumors. Together these findings identify RET as a potentially important therapeutic target in ERα-positive breast cancers and in particular in tamoxifen-resistant tumors.

BRCA2, but not BRCA1, is required for telomere integrity in mammalian cells

Sophie Badie[1,6], Jose M. Escandell[1,6], Peter Bouwman[2,6], Ana Rita Carlos[1], Maria Thanasoula[1], María Mercedes Gallardo[3], Anitha Suram[4], Petra Nederlof[2], Isabel Jaco[3], Javier Benítez[5], Utz Herbig[4], María A. Blasco[3], Jos Jonkers[2] and Madalena Tarsounas[1]

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BRCA2 is a key component of the homologous recombination pathway of DNA repair. Its best understood function is that of loading the RAD51 recombinase at sites of DNA double-strand breaks. Here, we demonstrate that BRCA2 associates with telomeres during S/G2 and is required for RAD51 loading onto telomeres. Conditional Brca2 deletion in mouse embryonic fibroblasts (MEFs) leads to telomere shortening and accumulation of chromosomal aberrations characteristic for telomere dysfunction. Brca2-deficient cells also accumulate fragmented telomeric signals, a hallmark of telomere fragility associated with replication defects. Both telomere attrition and fragility are recapitulated in MEFs deficient in the activities of RAD51 or RAD51C. This suggests that homologous recombination contributes to telomere length maintenance by facilitating telomere replication and implies an essential role for BRCA2 in the maintenance of telomere integrity during unchallenged cell proliferation. Indeed, BRCA2-deficient telomeres show delayed BrdU incorporation during S phase progression and high rate of damage following hydroxyurea treatment. Mouse mammary tumours lacking Brca2 accumulate telomere dysfunctioninduced foci, in contrast to Brca1 deleted tumours. Consistent with a specific requirement for BRCA2, but not BRCA1 in telomere replication, we do not detect telomere dysfunction in BRCA1-deficient MEFs. BRCA2-mutated human breast tumours have shorter telomeres in comparison to the BRCA1-mutated, suggesting that genomic instability observed in BRCA2-deficient tumours is due in part to telomere dysfunction.

Regulation of tumor stroma during breast cancer progression

Zena Werb

University of California, San Francisco, USA

Both extrinsic and intrinsic mechanisms the development of the aberrant tumor organ and regulate its progression. We have used genetic and in vivo imaging techniques to study the interaction between epithelial cancer cells and stromal cells present in the tumor microenvironment during tumor progression. The progressing tumor cells develop in an extrinsic microenvironment rich in inflammatory cells, growth factors and activated stroma that promotes neoplastic risk. All of these components communicate with each other to contribute to the aberrant tumor organ. Transcriptional regulation of epithelial differentiation, cell-cell interaction and motility is a key intrinsic event in tumor progression. We have found that GATA3, a master regulator for luminal differentiation, which is lost upon malignant conversion, allowing the malignant tumor cells to disseminate throughout the body also regulates the tumor microenvironment. Using intravital microscopy, we have observed that inflammatory myeloid cells, including M2 polarized macrophages in the microenvironment increase dramatically upon the malignant conversion of the tumor cells. The macrophages and myeloid cells are present in multiple distinct behavioral subpopulations. The inflammatory cells modulate the tumor ecology, altering vascular permeability and responses to chemotherapy. The dynamic interplay of tumor cells and host cells responding to the tumor contribute to tumor evolution and evasion of therapeutic responses. (Supported by funds from the National Cancer Institute, USA).

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Breast Cancer

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Session 5

The next generation of tissue analysis tools: promises and pitfalls of next generation sequencing

Chair: Miguel Ángel Piris

Causes and consequences of microRNA dysregulation in Cancer

Carlo Croce

The Ohio State University, Columbus, USA

Since the discovery of miR-15a and miR-16-1 deletions in CLL15, many laboratories around the world have shown miRNA dysregulation in all tumours studied, including the most common, such as lung, breast, prostate and gastrointestinal cancers. Such dysregulation, like the dysregulation of oncogenes and tumour suppressor genes, can be caused by multiple mechanisms, such as deletion, amplification, mutation, transcriptional dysregulation and epigenetic changes. As miRNAs have multiple targets, their function in tumorigenesis could be due to their regulation of a few specific targets, possibly even one, or many targets. A future challenge will be to identify all of the targets of the miRNAs involved in cancer and establish their contribution to malignant transformation. An additional challenge will be the identification of all of the miRNAs that are dysregulated by pathways that are consistently dysregulated in various types of human cancers. This point is of particular importance, as instead of focusing on specific alterations in protein-coding oncogenes or tumour suppressor genes — which may be difficult to treat — we could focus on their downstream miRNA targets. If these miRNA targets are crucial for the expression of the malignant phenotype and the cancer cells depend on their dysregulation for proliferation and survival, we can expect that the use of miRNAs or anti-miRNAs will result in tumour regression. Genomic analyses for alteration in miRNA genes or for copy number alterations in various human tumours by deep sequencing is in progress but has not been completed. These studies could provide additional information concerning the involvements of miRNAs in cancer and in many other diseases. Over the past few years, we have observed a shift from conventional chemotherapy to targeted therapies, and miRNAs and anti-miRNAs will contribute extensively to the latter.

Identification of high susceptibility genes in BRCAX families by whole exon sequencing

Francisco Javier Gracia-Aznárez[1], J.M. Rosa-Rosa[2], G. Pita[3], D. Herrero[3], O. Domínguez[4], O. Sinilnikova[5], D. Goldgar[6], P. Devilee[7] and J. Benítez[1, 3, 8]

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Non-BRCA1/2 tumors are a sub-group of familial breast tumors characterized by not presenting mutations in high susceptibility breast cancer genes, namely BRCA1 and BRCA2. In spite of that, up to 70% of familial breast tumors belong to this group, making it one of the most interesting areas of study in human genetics today. Next Generation Sequencing (NGS) represents a powerful approach that enables scientists to study complex genetic traits for which no causal genes have been identified. When set up correctly, NGS can be used for screening almost any variant type across any genomic region, regardless of the size. In the present study, four families with a minimum of 7 breast cancer cases under the age of 60 and negative for BRCA1/2 mutations were selected from an international consortium. Two affected individuals from each family and 7 selected HapMap controls were enriched for the exomic region using SureSelect Human All Exon Kit. The resulting DNA libraries were sequenced using 78 base pair paired-end technology on a Genome Analyzer II sequencer from Illumina. Sequencing data was filtered to exclude low quality reads and aligned against the whole human genome using Novoalign. Detected INDELs and SNPs were filtered against the variants detected in the 7 HapMap controls, as well as with the information in dbSNP and Ensembl databases. The remaining variants were annotated and tested for homology with other parts of the human genome before becoming final candidates. An average of 130 million reads were obtained per individual, which translated into approximately 10 gigabases of sequence read and an average deep ~125x for the whole exome. Before filtering, we obtained approximately 100000 SNPs and 30000 INDELs per sample. Serial filtering steps reduced those numbers in approximately three orders of magnitude, from which we are currently studying 6 final candidate genes. In summary, NGS has shown to be a valuable tool for the identification of candidate variants. The variety of information that can be extracted using this technique as well as its flexibility in target region selection make NGS an exceptional approach for the study of genetic disorders.

Development and clinical implementation of functional protein pathway activation mapping of breast cancer for personalized therapy

Emanuel Petricoin

George Mason University, Manassas, USA

Recently, whole genome mutational scanning analysis of a number of solid tumors has revealed that cancer is a protein pathway disease at the functional level. The description and analysis of human breast cancer has been at the forefront of the new era of molecular oncology, largely using genomic information. However, since genomic and transcript profiling likely cannot alone sufficiently predict protein pathway activation in each patient's tumor, and it is these protein signaling pathways that represent the targets for new molecular guided therapeutics, it is critical that we begin to define human cancer using the functional protein signaling activation architecture as a basis for taxonomy. These pathways contain a large and growing collection of drug targets, and govern cell function. Thus, the promise of proteomics resides in the study of molecules that are not just predictive or prognostic factors, but extend beyond correlation to causality. We have invented a new type of technology, called reverse phase protein microarrays, to generate a functional map of known cell signaling networks or pathways for an individual patient obtained directly from a biopsy specimen. This patient-specific circuit diagram provides key information that identifies critical nodes or pathways that may serve as drug targets for individualized or combinatorial therapy through the quantification of phosphorylation states of proteins. The identification of activated networks on a patient-by-patient basis can be used as both a diagnostic and a therapeutic guide to patient selection and stratification. This lecture will provide case studies for applications of this approach at the bedside in clinical trials that span a "lifetime with cancer" model: pre-malignant breast cancer applications, through neoadjuvant setting in the ISPY trial setting, to a novel clinical trial where protein pathway activation measurements are used to stratify and select metastatic breast cancer patients for treatment with all FDA approved targeted therapies.

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Session 6

Innovative clinical trials

Chair: Christos Sotiriou

Biology driven phase II trials: which model for molecular selection?

Fabrice Andre

Gustave Roussy Institute, Villejuif, France

Biology-driven trials consist in evaluating new drug in population defined by a molecular characteristic. With the identification of new therapeutic target, these trials have dramatically increased in the last years. Until recently, the model for molecular selection consisted in testing the tumor for a specific biomarker after the patient signed the informed consent for the therapeutic trial. Several priors have shown that this model is suboptimal since it is associated with a high rate of screen failure. In the present talk, we will discuss the alternative models based on current ongoing trials. Best Rx trial is a molecular screening program developed by Novartis that tests PIK3CA mutations, PTEN status and FGFR1 amplification in breast cancer. This program allows detecting genomic event in a significant number of patients, and is not linked to any phase II trial. This model is therefore associated with a lower rate of screen failure as compared to the conventional model. SAFIR01 trial is a molecular screening program based on high throughput technologies. In addition to the strengths of Best Rx program, this program presents the added value of detecting rare genomic events, and to avoid implementing new bioassay for each target.

Overall, clinical programs that aim at identifying candidate oncogenic drivers at the patient's level allow the performance of small biology driven phase II trials. These small phase II trials could speed-up development of new drugs.

Trial designs aiming for biomarker definitions: no pain-no gain?

Miguel Ángel Quintela

CNIO, Madrid, Spain

Classic paradigms of drug development do not always allow answering questions of key importance, such as definition of subpopulations of patients with higher likelihood of benefit from a therapeutic approach or anticipation to potential mechanisms of acquired resistance. Reasons accounting for this are multiple, i.e., post-hoc analysis of scarce clinical samples not necessarily harvested with the appropriate technique/processing or reliance on inadequate pre-clinical models.

However, designing solutions customized for specific drugs and objectives beyond response rate or clinical benefit is not exempt from milestones, such as cost, repeated sampling resulting in patient attrition and subsequent methodological failure, complex "bench to bedside" communication, ethical concerns, high risk of statistic "underpower" specially in the cases where high-throughput signatures are aimed for. Altogether, such factors lead often to either discovery of random associations, or lack of validation of promising biomarkers. The labor-intense activities at all the research levels to overcome the mentioned caveats, still, do not warrantee success.

We will describe/criticize our ongoing multidisciplinary project trying to address the changes at the tumor metabolism level induced by antiangiogenics; potential applications of a metabonomic signature would allow not only patient clustering but development of therapeutic strategies against acquired resistance. Activities are conducted in parallel in animal models, both representing patient heterogeneity (xenografts) and real tumor-stromal cross-talk scenarios (murine breast-cancer models), and a clinical trial, using in both platforms a pan-VEGFR/pan-PDGFR/pan-FGFR inhibitor. Cross-validation of different techniques (HRMAS-NMR and GC-MS / LC-MS) in order to ensure results validity and reproducibility is being performed. Characterization and modulation of the hits identified in the murine models will suggest therapeutic alternatives that would allow management of patients that develop resistance. The trial is designed in several steps, so that the second "round" of patients would serve as a validation set, and the third, as an "enrichment" cohort.

Keynote Lecture

Targeting the PI3K pathway as cancer therapeutics

José Baselga

Massachusetts General Hospital, Boston, USA

The phosphatidylinositol 3-kinase (PI3K) signaling pathway is integral to diverse cellular functions, including cellular proliferation, differentiation and survival. The 'phosphate and tensin homologue deleted from chromosome 10' (PTEN) tumor suppressor gene plays a critical role as a negative regulator of this pathway. An array of genetic mutations and amplifications has been described affecting key components of this pathway, with implications not only for tumorigenesis but also for resistance to some classic cytotoxics and targeted agents. Emerging pre-clinical research has significantly advanced our understanding of the PI3K pathway and its complex machinations and interactions. This knowledge has enabled the evolution of rationally designed drugs targeting elements of this pathway. PI3K inhibitors have shown to be active in preclinical models and a number of agents are now in early stages of clinical development. These agents have different degree of potency and target specificity; some agents inhibit both PI3K and mTOR whereas other are more pure PI3K inhibitors. Indirect evidence that this approach may be fruitful in breast cancer has been recently gained by a neoadjuvant study with an mTOR inhibitor in combination with anti-estrogen therapy that has demonstrated silencing of the PI3K pathway and clinical activity in patients harbouring PI3K mutations. These agents also hold promise in combination with chemotherapy and in HER2 amplified breast cancer harbouring either PI3KCA mutations of PTEN deletions. In addition to PI3K and mTOR inhibitors, targeting of other components of the pathway such as AKT is also under exploration.

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Invited Speakers' Biographies

Gabriela Dontu

King's College London
UK

Dr. Dontu's research aims to identify and characterize the normal and malignant mammary stem cells and to elucidate their role in breast carcinogenesis. Evidence accumulated in the last decade that cancer may result from transformation of stem/progenitor cells, which then drive tumorigenicity, recurrence and metastasis.

Dr. Dontu was part of the University of Michigan group who generated proof of concept for the existence of cancer stem cells in solid tumors in 2003. She established novel in vitro and in vivo experimental systems specific for the study of normal and malignant human mammary stem cells and identified markers associated with this cell population.

These experimental tools are used to investigate the mechanisms that govern stem cell fate in the human mammary gland in Dr. Dontu's group at King's College London. The ultimate goal of this research is to explore the biological and clinical implications of the cancer stem cell model, and in particular to develop new therapeutic strategies meant to eliminate cancer stem cells.

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Max Wicha

University of Michigan
Ann Arbor, USA

Max S. Wicha is a Distinguished Professor of Oncology and the Director of the University of Michigan Comprehensive Cancer Center. His laboratory was part of the team that first described stem cells in human breast cancer. Utilizing in vitro systems and mouse models, the group has elucidated pathways which regulate cell fate decisions in these cancer stem cells. This has led to the identification of potential therapeutic targets. Most recently his group has been involved in the design of clinical trials focused on targeting the breast cancer stem cell population.

Robert Clarke

Paterson Institute for Cancer Research
Manchester, UK

Dr Clarke studied the control of proliferation in the normal and neoplastic human mammary gland for his PhD at The University of Manchester. Subsequently, he undertook post-doctoral training at The Christie, Manchester. He returned to The University of Manchester as a Cancer Research UK Research Fellow in 2001, becoming a Group Leader in the Division of Cancer Studies at the Paterson Institute for Cancer Research. Currently, Dr Clarke is Senior Lecturer and Breast Cancer Campaign Research Fellow in the School of Cancer and Enabling Sciences in the Paterson Institute for Cancer Research, University of Manchester. He is also Associate Editor for the journals *Breast Cancer Research* and the American *Journal of Pathology* and serves on a number of Editorial Boards and Grants Committees.

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Mathew Ellis

Washington University School of Medicine
St. Louis, USA

Dr. Ellis specializes in breast cancer. He has research interest in insulin-like growth factor signaling, endocrine therapy, signal transduction therapy, preoperative systemic therapy, array based analysis of gene expression, and clinical trial correlative science.

He earned his M.B. from King's College, University of London, United Kingdom, in 1980; his B.Sc from St. Mary's Hospital Medical School, University of London, United Kingdom, in 1981; his M.B. and B.Chir from Queens' College & School of Clinical Medicine, University of Cambridge, United Kingdom, in 1984; his M.R.C.P. from Royal College of Physicians, London Regents Park London, United Kingdom, in 1986; his Ph.D. from Royal Postgraduate Medical School, University of London, United Kingdom, in 1991; and his F.R.C.P. from Royal College of Physicians, London, Regents Park London, UK, in 2001.

Salvatore Pece

European Institute of Oncology
Milan, Italy

Salvatore Pece is a scientist of the IFOM-IEO Campus in Milan, an internationally competitive research centre, where he carries out his research in the group of Pier Paolo Di Fiore. His work focuses on molecular pathogenesis of breast cancer, particularly on the correlation between subversion of mechanisms controlling stem cell homeostasis and breast carcinogenesis. He is also Associate Professor of General Pathology at Milan University and member of the Molecular Medicine Program recently launched at the European Institute of Oncology in Milan.

Alex Toker

Harvard Medical School
Boston, USA

Alex Toker received a BSc from King's College, University of London, in 1987 and a PhD from the National Institute for Medical Research, London, in 1991. He conducted his post-doctoral research in the laboratory of Lewis Cantley in the Department of Cell Biology, Harvard Medical School. His first faculty appointment was as Staff Scientist at the Boston Biomedical Research Institute in 1997. In 2000 he joined the faculty of Beth Israel Deaconess Medical Center and Harvard Medical School, where he is currently Professor of Pathology. His research focuses on the signal transduction pathways mediated by protein kinases and transcription factors and how they modulate cancer cell invasion and survival.

Achim Rody

Saarland University
Homburg, Germany

Achim Rody, is working as a gynecologist at the Saarland University in Homburg. Before he moved to Homburg, he spent many years as a senior consultant at the Goethe University. His scientific interest is the field of gene expression analysis and the identification of novel predictive and prognostic markers. He is also co-editor of the scientific journals "The Breast" and the "European Journal of Cancer". He has received numerous scientific awards in Germany.

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Alan Ashworth

The Institute of Cancer Research
London, UK

Professor Alan Ashworth FRS, is Professor of Molecular Biology and Director of The Breakthrough Breast Cancer Research Centre at The Institute of Cancer Research, London. The Centre contains around 120 scientists and researchers working on aspects of breast cancer ranging from basic molecular and cellular biology through to translational research and clinical trials.

Ashworth contributed to the discovery of the BRCA2 gene in 1995 and ten years later, Prof. Ashworth's team identified the synthetic lethal relationship between BRCA mutations and PARP inhibitors. The exquisite sensitivity of BRCA1 or BRCA2 mutant cells to PARP inhibitors forms the rationale behind clinical trials that are now assessing the potential of these agents. Ashworth's other research interests are wide ranging and include high-throughput genomic and functional approaches to the study of cancer.

Ashworth is an elected member of EMBO and the Academy of Medical Sciences. His contributions to mammalian genetics and identification and study of inherited breast cancer susceptibility genes saw him elected as a Fellow of the Royal Society in 2008. He has been the recipient of a number of scientific prizes and awards including in 2009, The European Society of Medical Oncology Lifetime Achievement Award and in 2010, the David T. Workman Memorial Award of the Samuel Waxman Cancer Research Foundation and also the Meyenburg Foundation's Cancer Research Award. He will take up the position of CEO of the Institute of Cancer Research in January 2011.

Jean Paul Thiery

Institute of Molecular and Cell Biology
Singapore

Jean Paul Thiery joined IMCB in 2006, from his position as Head of Translational Research at the Medical Division of the Comprehensive Cancer Center of Institut Curie, and before that, Head of the Cell Biology Department at the Institut Curie in Paris until 2003. After PhD studies at the University of Paris, his postdoctoral studies with Gerald Edelman (Nobel Laureate) at the Rockefeller University led to the discovery of N-CAM, the first intercellular adhesion molecule to be identified. His influential works include pioneering studies on cell adhesion and migration in early embryogenesis, on roles of growth factors and adhesion signalling in epithelial-mesenchymal transitions, cell spreading, discovery of widespread activating mutations in FGFR3 in bladder cancers and mechanisms of metastasis in breast cancer. Jean Paul Thiery is an EMBO and Academia Europea member.

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Yosef Yarden

Weizmann Institute of Science
Rehovot, Israel

Yosef Yarden received a Ph.D. from the Weizmann Institute of Science (1985). His postdoctoral training was undertaken at Genentech, Inc. and at the Massachusetts Institute of Technology. In 1988, he returned to the Weizmann Institute of Science and was appointed Associate Professor in 1992, and Full Professor in 1996. His administrative responsibilities at the Weizmann Institute included Vice-President for Academic Affairs and Dean of the Feinberg Graduate School. Among his honours and awards are the Michael Bruno Memorial Award (2000), the EMET Prize in Biochemistry (2007) and the 2008 Hamilton Fairly Award of the European Societies of Clinical Oncology (ESMO).

Mitchell Dowsett

The Royal Marsden Hospital
London, UK

Professor Mitch Dowsett, PhD, is Head of the Academic Department of Biochemistry at the Royal Marsden Hospital, Professor of Biochemical Endocrinology at the Institute of Cancer Research, and Professor of Translational Research in the Breakthrough Breast Cancer Centre, London.

His research focuses almost exclusively on breast cancer and predominantly on hormonal aspects of the disease and biomarkers of response. He has been closely involved with the clinical development of aromatase inhibitors and the establishment of Ki67 as an intermediate marker of treatment benefit.

He has authored over 500 published papers related to breast cancer, and was the 2007 William L McGuire Memorial Lecturer. He is a board member of the Breast International Group (BIG), and sits on the Executive/Steering Committees of several clinical trials. He is the founding chairman of the NCRI Translational Clinical Study Group and is a member of the NCRI Breast Cancer Study Group.

He was an author of both the UK and the ASCO/CAP guidelines for HER2 diagnostics. He is the international member of the ASCO/CAP Steering Committee for ER/PgR IHC guidelines.

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Fatima Cardoso

Champalimaud Cancer Center
Lisbon, Portugal

Dr Cardoso is the Head of Breast Cancer Unit of the Champalimaud Cancer Center in Lisbon, Portugal. Dr Cardoso earned her medical degree at the University of Porto in Portugal and completed fellowships in the Translational Research Unit of the Jules Bordet Institute (IJB) in Brussels, Belgium and Department of Molecular and Cellular Oncology at MD Anderson Cancer Center in Houston, Texas. She then worked for 10 years as Assistant Professor at the Medical Oncology Clinic of the IJB. She is board certified in medical oncology and internal medicine. Dr Cardoso's research interests include biology of breast cancer, prognostic and predictive markers of response to systemic therapy, and new anticancer agents. She is actively involved in a number of phase I-III breast cancer clinical trials and serves as the scientific director of the international research network TRANSBIG. Dr Cardoso is actively involved in numerous professional organizations such as ESMO, ECCO, EORTC, ASCO, and AACR where she serves on numerous committees and she is the Breast Cancer Program Coordinator of the European School of Oncology. Dr Cardoso is co-editor-in-chief of The Breast Journal, associate editor of the European Journal of Cancer, and an editorial board member of several other journals. Dr Cardoso has authored over 230 publications and has presented her work nationally and internationally.

Soonmyung Paik

National Surgical Adjuvant Breast and Bowel Project Foundation
Pittsburgh, USA

Soonmyung Paik, M.D. is a pathologist with training in molecular biology of breast cancer. He graduated from College of Medicine, Yonsei University, Seoul, Korea in 1981 and received his residency training and post-doc fellowship in USA. He has been the Director of the Division of Pathology of the NSABP since 1996. Since 2009 he has a joint appointment as the Distinguished Scientist in Medicine and the director of Samsung Cancer Research Institute, Seoul, South Korea.

He has contributed in elucidating the clinical role of HER2 in breast cancer since his post-doctoral fellowship days in 1990 when he demonstrated that patients with increased expression of HER2 protein confers poor prognosis of patients enrolled in NSABP trial B-06 (Paik et al. JCO 8:103-12). Through analyses of NSABP trials B-11 and B-12, he has demonstrated that HER2 positive tumors are more responsive to doxorubicin containing regimen and thus helped to establish ACT as a baseline regimen for NSABP trials for HER2 positive breast cancer (Paik et al, JNCI 90:1361-70 and JNCI 92:1991-8, Romond et al, NEJM 353:1673-84). Through central review of tumor blocks from the NSABP trial testing adjuvant trastuzumab, his group demonstrated the quality assurance of problems of HER2 testing in the community pathology laboratories (Paik et al, JNCI 94:852-4). This finding led to the establishment of the ASCO/CAP Guideline for HER2 Testing (Wolff et al, JCO 24:3726-34). His most recent contribution to HER2 literature was the demonstration of the potential benefit from trastuzumab even in HER2 negative patients, leading to the design of NSABP trial B-47 (Paik, Kim, and Wolmark, NEJM 358:1409-11)

Through gene expression analyses of tumor blocks procured from NSABP trials he has contributed to novel clinical trial concepts; His lab in collaboration with Genomic Health Inc has developed a multi-gene based test called OncotypeDx that is now used in the community to predict prognosis and response to chemotherapy in node negative hormone receptor positive breast cancer (Paik et al, NEJM 351:2817-26, JCO 24:3726-34). This work has led to the design of TAILORx trial (Sparano and Paik, JCO 26:721-8). Through microarray gene expression analyses of pretreatment core biopsy blocks from NSABP trial B-27, he has contributed to the concept of post-neoadjuvant trials (Rastogi et al, JCO 26:778-85).

Christos Sotiriou

Jules Bordet Institute
Brussels, Belgium

Dr. Christos Sotiriou earned a medical degree from the Université Libre de Bruxelles, Belgium in 1993. He did his internal medicine/oncology residency at the Jules Bordet Institute (Profs. J. Klastersky, M. Piccart), and he earned his specialty in internal medicine and medical oncology in July 1999 at the Université Libre de Bruxelles. From October 1999 till September 2001, he worked as basic research fellow, at the Division of Clinical Sciences, Microarray Facility, National Cancer Institute (Pr Edison Liu), National Institutes of Health, Bethesda, MD, USA. Dr. Sotiriou earned his doctor of philosophy degree (PhD) from the Université Libre de Bruxelles, Belgium in September 2004. In October 2005, he becomes associate professor at the Université Libre de Bruxelles. In March 2010 he took the direction of the breast cancer translational research laboratory J-C Heuson at the Jules Bordet Institute. Dr. Sotiriou's research is focusing on genomics and molecular biology in breast cancer. Dr. Sotiriou is a full member of ASCO, AACR, and ESMO. He is member of the TransALTTO committee, and Advisory Council Member of the Susan G. Komen for the Cures and for the National foundation for research since 2010. He received several educational and research grants. He presently has several publications in peer review journals and several book chapters.

Charles Swanton

Cancer Research UK London
Research Institute
UK

Charles completed his PhD studying the cancer cell cycle in 1998 at the Imperial Cancer Research Fund Laboratories in the laboratory of Nic Jones and completed his medical oncology and CR-UK funded post-doctoral clinician scientist training in 2008. Charles is a consultant medical oncologist in the Breast and Drug Development Units at the Royal Marsden Hospital with an interest in early phase drug development for the treatment of specific subtypes of metastatic breast cancer. Charles is a Medical Research Council Group Leader at the CR-UK London Research Institute in the Translational Cancer Therapeutics laboratory focussing on understanding mechanisms of drug resistance and genomic instability using high throughput RNA interference functional genomics approaches.

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Lajos Pusztai

The University of Texas
MD Anderson Cancer Center
Houston, USA

Dr Pusztai leads the pharmacogenomic program in the Department of Breast Medical Oncology at MD Anderson Cancer Center. He received his MD degree from the Semmelweis University of Medicine in Budapest his D.Phil. from the University of Oxford in England, he is currently Professor of Medicine. He is a practicing medical oncologist and clinical researcher who published over 150 peer-reviewed articles on the biology and treatment of breast cancer. His research focuses on the developing pharmacogenomic markers of response to therapy and identifying methods to select the optimal treatment for individual patients. His group has proposed new clinical trial designs for predictive marker evaluation, introduced new pathologic measurements of residual cancer after neoadjuvant chemotherapy, created web-based chemotherapy response prediction models based on routine clinical variables and proposed genomic markers of chemo- and endocrine-therapy sensitivity. Dr Pusztai is principal investigator of several clinical trials investigating new drugs and potential response markers. His research is supported by grants from the National Cancer Institute, the US Department of Defense, the American Society of Clinical Oncology, the Breast Cancer Research Foundation and philanthropic research grants.

Jorge Reis-Filho

The Breakthrough Breast Cancer Research Centre
London, UK

Prof Reis-Filho is the Chair of Molecular Pathology at the Institute of Cancer Research, London, UK, and the team leader of the Molecular Pathology Team at the Breakthrough Breast Cancer Research Centre. Prof Reis-Filho has published over 250 peer reviewed papers on pathology and genetics. His research interests include the development of a predictive classification system for breast cancers through a combination of traditional histopathology, high-throughput genetics, massively parallel sequencing and functional genomics, and the identification of novel therapeutic targets for breast cancers, melanomas and endometrial cancers based on the integration of high throughput genomic and transcriptomic data.

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Joaquín Arribas

Vall d'Hebron Institute of Oncology
Barcelona, Spain

Joaquín Arribas completed his graduate studies in Biochemistry at Universidad Autónoma de Madrid in 1987. At the same university he subsequently worked on the regulation of the catalytic activities of the proteasome and received a PhD in Biology in 1991. In 1192, he joined the Memorial Sloan-Kettering Cancer Center (New York, USA) as a postdoctoral fellow to work with Dr. Joan Massagué on the proteolytic processing of transmembrane growth factors. In 1997, he was recruited by Dr. Josep Baselga to join the oncology department at Hospital Vall d'Hebron (Barcelona) as a staff scientist and was promoted to lead the Oncology Research Program in 2001. Currently, Joaquín Arribas is the Director of the Basic Research at the Vall d'Hebron Institute of Oncology (VHIO).

Zena Werb

University of California
San Francisco, USA

Dr. Zena Werb received her Ph.D. in Cell Biology from Rockefeller University, New York working with Dr. Zanvil Cohn. Her postdoctoral fellowship was at Strangeways Research Laboratory in Cambridge, England. She then joined the faculty of University of California. Dr. Werb is Professor and Vice-chair of the Department of Anatomy and member of the UCSF Helen Diller Family Comprehensive Cancer Center at the University of California, San Francisco. Among many honors, Dr. Werb is a member of the Institute of Medicine and the National Academy of Sciences USA and a fellow of the American Academy of Arts and Sciences.

Carlo Croce

The Ohio State University
Columbus, USA

Dr. Croce is world-renowned for his contributions involving the genes and genetic mechanisms implicated in the pathogenesis of human cancer. During the course of his career, he discovered the juxtaposition of the human immunoglobulin genes to the MYC oncogene and the deregulation of MYC in Burkitt lymphoma, the ALL1/MLL gene involved in acute leukemias, the TCL1 gene associated with T-cell leukemias, and cloned, named and characterized many oncogenes including the BCL2 gene involved in follicular lymphoma and several tumor suppressor genes. Dr. Croce has also uncovered the early events involved in the pathogenesis of lung, nasopharyngeal, head and neck, esophageal, gastrointestinal and breast cancers. His discoveries have led to revolutionary innovations in the development of novel and successful approaches to cancer prevention, diagnosis, monitoring and treatment, based on gene-target discovery, verification and rational drug development. More recently he discovered the role of a new class of cancer genes encoding microRNAs.

A native of Milan, Italy, Dr. Croce earned his medical degree, summa cum laude, in 1969 from the School of Medicine, University of Rome. He began his career in the United States the following year as an associate scientist at the Wistar Institute of Biology and Anatomy in Philadelphia. In 1980, he was named Wistar Professor of Genetics at the University of Pennsylvania and Institute Professor and Associate Director of the Wistar Institute, titles he held until 1988. From 1988-91, he was director of the Fels Institute for Cancer Research and Molecular Biology at Temple University School of Medicine in Philadelphia. In 1991, Dr. Croce was named Director of the Kimmel Cancer Center at Jefferson Medical College at the Thomas Jefferson University, in Philadelphia. While at Jefferson, he discovered the role of microRNAs in cancer pathogenesis and progression, implicating a new class of genes in cancer causation. In 2004 he moved to The Ohio State University. Under his direction at Ohio State faculty within the Human Cancer Genetics Program conduct both clinical and basic research. Basic research projects focus on how genes are activated and inactivated, how cell-growth signals are transmitted and regulated within cells, and how cells interact with the immune system. Clinical research focuses on discovering genes linked to cancer and mutations that predispose people to cancer.

Dr. Croce, a member of the National Academy of Sciences in the US and the Accademia Nazionale delle Scienze detta deiXL in Italy, has received almost every significant award for cancer research that one can earn. He was awarded two Outstanding Investigator awards from the National Cancer Institute, the Richard and Hinda Rosenthal Foundation Award and the G.H.A. Clowes Memorial Award from the American Association for Cancer Research, the John Scott Award, the Robert J. and Claire Pasarow Foundation Cancer Award, the GM Cancer Research Foundation - Charles S. Mott Prize, the Scanno Prize for Medicine, the AACR-Pezcoller Award, the Raymond Bourgine Award and Gold Medal of Paris, President of the Republic Prize, the iwCLL Binet-Rai-Medal for Outstanding Contribution to CLL Research, the Henry M. Stratton Medal from the American Society of Hematology, the Albert Szent-Györgyi Prize for Progress in Cancer Research, the 2008 Leopold Griffuel Prize awarded by the French Association for Cancer Research, The 30th Annual Jeffrey A. Gottlieb Memorial Award and Ernst W. Bertner Memorial Award, The University of Texas M. D. Anderson Cancer Center and most recently, an Elected Membership to The American Academy of Arts and Sciences. He is principal investigator on eleven federal research grants and has more than 900 peer-reviewed, published research papers.

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Emanuel Petricoin

George Mason University
Manassas, USA

Dr. Emanuel F Petricoin has been the Co-Director of the Center for Applied Proteomics and Molecular Medicine (CAPMM) at George Mason University since 2005, where he is a University Professor. Prior to this position, he served as Co-Director of the FDA-NCI Clinical Proteomics Program from 2001-2005, and a Senior Investigator within the Center for Biologics Evaluation and Research at the US Food and Drug Administration from 1993-2005. Dr. Petricoin received his PhD in Microbiology from the University of Maryland in 1990. The focus of the CAPMM is the invention and use of proteomics technologies for signal transduction analysis, phosphoproteomics and nanoparticle-based peptidomic biomarker discovery for direct clinical applications at the bedside. He is a co-inventor on 40 filed and published patents, and has authored over 260 peer-reviewed publications and invited reviews. He has authored over 40 book chapters, is the Co-Editor-in-Chief of Human Genomics and Proteomics, is on the editorial board of Proteomics, Biomedical Microdevices, Proteomics- Clinical Applications, Proteomics- Protocols, Molecular Carcinogenesis, Journal of Personalized Medicine and is a Senior Editor for Cancer Epidemiology Biomarkers and Prevention. He was the founding Co-Editor-in-Chief of Clinical Proteomics. Dr. Petricoin is a founding member of the Human Proteomic Organization (HUPO) as well as the US HUPO, and served on the Executive Committee and Treasurer for HUPO from 2002-2004. He has received numerous awards including the NIH Director's Award, FDA Distinguished Scientist Award, American Society of Cytopathology Basic Research Award, the Roche Diagnostics/CLAS Distinguished Scientist Award and the Harvard University Leading Edge Award.

Fabrice Andre

Gustave Roussy Institute
Villejuif, France

Fabrice Andre is a MDPhD working at Institut Gustave Roussy. He is a medical oncologist involved in clinical trials, and is chairing the INSERM Unit U981, a lab dedicated to new targets and biomarkers. He is principal investigators of several phase II trials testing new targeted agents, and is leading two large multicentric trials that evaluate the medical usefulness of high throughput technologies in daily practice (REMAGUS04 and SAFIR01 trials).

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Miguel Ángel Quintela

CNIO
Madrid, Spain

Dr. Miguel Quintela-Fandino got his MD degree in Navarra on 2000. From 2000-2005 he specialized in medical oncology in the Hospital 12 de Octubre, and obtained a masters degree in statistics and his PhD at the Universidad Complutense, with a study focused on residual micrometastatic disease in breast cancer. From 2005-10, he trained as a postdoc with Dr. Tak Mak at the Ontario Cancer Institute working on invasion/metastasis mechanisms as well as on cancer metabolism, and as clinical fellowship in early drug development at the Princess Margaret Hospital. He currently leads the CNIO Breast Cancer Clinical Research Unit.

José Baselga

Massachusetts General Hospital
Boston, USA

José Baselga is the Chief of the Division of Hematology/Oncology and Associate Director of the Massachusetts General Hospital Cancer Center. Dr. Baselga received his M.D. and Ph.D. degrees from the Universidad Autonoma of Barcelona, Spain. He completed a clinical and research fellowship in Medical Oncology at Memorial Sloan-Kettering Cancer Center and subsequently stayed on as a faculty member at Memorial Sloan-Kettering. From 1996 to 2010 he was the Chairman of the Medical Oncology Service and Founding Director of the Vall d'Hebron Institute of Oncology (VHIO) in Barcelona, Spain. Dr. Baselga is currently a member of the AACR Board of Directors, the immediate past President of ESMO and a past member of the Board of Directors of ASCO and the Scientific Advisory Committee of the Ludwig Institute for Cancer Research. Dr. Baselga has received a number of awards including a Young Investigator Award (1992) and a Career Development Award from ASCO (1994), a Brystol-Myers Squibb Unrestricted Cancer Grant Award (2002-2006), and an AACR-Rosenthal Family Foundation Award (2008)

His research interests are in Clinical Breast Cancer and in Translational and Early Clinical Research. He conducted the initial clinical trials with the monoclonal antibodies cetuximab and trastuzumab and has been involved in the clinical development of several new agents including pertuzumab and PI3K inhibitors. His main focus in the laboratory and in the clinic is in the area of novel anti-HER2 agents, in the identification of mechanisms of resistance to anti-HER2 agents and therapeutic approaches to target the PI3K pathway.

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NOTES

Breast Cancer

CNIO FRONTIERS Meetings 2011

Poster Session

Myc promoter-binding protein-1 (MBP-1 transcriptionally represses ERBB2 gene, and identifies new subtypes of ERBB2 negative breast tumors

Flavia Contino[1], Claudia Mazzarella[1], Arianna Ferro[1], Mariavera Lo Presti[3], Silvia Sbacchi[1], Elena Roz[3], Carmelo Lupo[3], Giovanni Perconti[1,2], Agata Giallongo[2] and Salvatore Feo[1]

[1]University of Palermo, Internal and Specialist Medicine (DiBiMIS), Palermo, Italy; [2]CNR, Institute of Biomedicine and Molecular Immunology, Palermo, Italy; [3]La Maddalena Hospital, Anatomical Pathology Department, Palermo, Italy

MBP-1 is the product of internal translation initiation from the ENO1 gene, acts as a general transcriptional repressor and exerts an antiproliferative effect on several human cancer cells. Expression levels and localization of MBP-1, in normal breast epithelium and primary infiltrating ductal breast carcinoma (IDC), were determined using immunohistochemical analysis. MBP-1 has been found in almost all normal tissues while its expression is retained in only 35% of the matched tumours. In relation to pathohistological factors significant inverse correlations were observed between expression and nuclear localization of MBP-1 and ErbB2 and Ki-67 expression, node positivity and tumor grade. Furthermore, MBP-1 expression significantly correlates with good disease-free survival of patients with primary IDC. Transfection experiments in human breast SKBr3 cells (ErbB2+) demonstrated that MBP-1 ectopic expression results in down regulation of ErbB2 expression. Using luciferase reported assays and ChIP experiments, we demonstrated that MBP-1 directly bounds to the ErbB2 promoter and suppresses the expression of ErbB2 gene in breast cancer cells, as already reported for the MYC gene. A comparison of the gene-expression profiles between MBP-1+ and MBP-1- ErbB2-ve-IDC using on wholegenome microarrays analysis , led us to the identification of a new set of differentially expressed genes that may underlie the different clinical behaviors of these two subtypes of breast carcinoma. Together, these results support a role for MBP-1 as a tumor suppressor gene in IDCs, and MBP-1 nuclear expression could be a clinically valuable prognostic variable and a novel target gene for the therapy of breast cancer patients.

Silencing of SHP1 (SH2 domain-containing phosphatase 1) defines an important role in the inactivation of ErbB2 and ErbB3 receptors by 15 deoxi-Δ12,14-PGJ2 in breast cancer cells

Jorge Dorado[1†], Diana Aguilar-Morante[1], Miguel Pignatelli[†1], Ángel Santos[2] and Ana Pérez-Castillo[1*]

[1]Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas – Universidad Autónoma de Madrid, Spain; [2]Facultad de Medicina. Universidad Complutense de Madrid, Spain; [†]Current address: Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Previous studies have suggested that 15 deoxi-Δ12,14-PGJ2, a known ligand of PPARγ receptor, inhibits proliferation and induces differentiation and apoptosis of breast cancer cells. However, the mechanisms underlying the antineoplastic effects of 15 deoxi-Δ12,14-PGJ2 remain largely unknown. We here examined the role of SHP1 phosphatase on 15 deoxi-Δ12,14-PGJ2 effects on MCF-7 breast cancer cells. We show that 15 deoxi-Δ12,14-PGJ2 inhibits ErbB2 and ErbB3 activation through a mechanism involving ErbB dephosphorylation. 15 deoxi-Δ12,14-PGJ2 enhances the activity, but not expression, of phosphorylated SHP1 phosphatase. SHP1 interacts with ErbB2 and ErbB3 upon 15 deoxi-Δ12,14-PGJ2 treatment. Knockdown of SHP1 by small interference RNAs abrogates the 15 deoxi-Δ12,14-PGJ2-mediated ErbB2/ ErbB3 dephosphorylation. These data suggest that 15 deoxi-Δ12,14-PGJ2 disrupts ErbB2/ErbB3 signaling, at least in part, by enhancing SHP1 activity, and suggest a mechanism by which 15 deoxi-Δ12,14-PGJ2 interferes with breast cancer cell growth. Additionally, we have also shown that this compound significantly decreases mammosphere formation, pointing to a possible role also in breast cancer stem cell growth capacity. Similar results have been obtained with the newly developed TDZDs compounds, placing these drugs as possible therapeutic agents for breast cancer.

Sphere formation ability in breast tumors shows heterogeneity in histologic tumor subtypes. Novel functional model in zebrafish

Arrate Eguiara Fernández-Rivera[1], C. Quevedo[2], L. Martínez Indart[3], M. Díaz Núñez[1], K. Elorriaga[4], C. Callol[2], R. Rezola[4] and A. G. Martín[1]

[1]Fundación Inbiomed, [2]Biobide SL, [4]Sº Patología, Oncologikoa; San Sebastián, Spain; [3]Hospital de Cruces, Barakaldo, Spain

Breast cancer stem cells have been defined according to several parameters such as surface marker expression, ability to efflux dyes and growth in non-adherent conditions. Sporadic breast cancer has been classified by gene expression analysis in several molecular subtypes with distinct clinical outcomes (Basal, Her2, Luminal A and B). There is no clear evidence, however, on the cell of origin of the different subtypes.

We analyzed 180 fresh breast carcinoma fragments collected at Instituto Oncologico between 2007-2010, for which clinical-pathological data was obtained through routine diagnostic procedures. Approximately 40% of the tumors were capable of forming spheres in vitro, irrespective of the total number of cells isolated from the original tumor. The frequency of sphere forming cells was commonly between 0.1 and 1%. However we did not observe association between the ability to form spheres and clinical-pathological features of the tumor. We did not find association with the surface marker profile at the level of the previously identified breast cancer stem cell population (CD44+CD24-) or linage markers (Muc1, CD10 or ESA) either. This data suggests the presence of cancer stem cells irrespective of the tumor subtype thus pointing to an independent origin of the different subtypes. Functional analysis of cancer stem cells to date involves difficult, expensive and time consuming technology, being the gold standrad the xenograft tumor initiation assay in immunocompromised mice. We have established a simple, fast, sensitive and cost effective functional model based on xenografts in the theleost zebrafish. We have analyzed tumor mass formation and cell migration of xenografted cells and have observed that cells from continuos breast carcinoma cell lines grown in nonadherent conditions (spheres) showed significantly increased mass formation and migration to the tail of the fish compared to the unselected adherent growing counterparts.

Sox2 expression in early breast tumours and activation in breast cancer stem cells

Olatz Leis[1], Arrate Eguiara[1], Erika Lopez-Arribillaga[1], María Jesus Alberdi[2], Kepa Elorriaga[2], Ricardo Rezola[2] and Angel García Martín[1]

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The cancer stem cell model does not imply that tumours are generated from transformed tissue stem cells. The target of transformation could be a tissue stem cell, a progenitor cell, or a differentiated cell that acquires self-renewal ability. The observation that induced pluripotency reprogramming and cancer are related has lead to the speculation that cancer stem cells may arise through a reprogramming like mechanism. Expression of pluripotency genes (Oct4, Nanog and Sox2) was tested in breast tumours by immunohistochemistry and found Sox2 expressed in early stage breast tumours. No expression of Oct4 or Nanog was found. Mammosphere formation in culture was used to reveal stem cell properties, where expression of Sox2, but not Oct4 or Nanog, was induced. Over-expression of Sox2 increased mammosphere formation, effect dependent on continuous Sox2 expression; furthermore, Sox2 knock-down prevented mammosphere formation. Induction of Sox2 expression was achieved through activation of the distal enhancer of Sox2 promoter upon sphere formation, the same element that controls Sox2 transcription in pluripotent stem cells. These findings suggest that re-activation of Sox2 represents an early step in breast tumour initiation, explaining tumour heterogeneity by placing the tumour-initiating event in any cell along the axis of mammary differentiation.

Intrinsic cues and hormones control organ size in the mammary gland

Eva Diaz-Guerra, M. Angeles Lillo, Silvia Santamaria and Jose A. Garcia-Sanz

Centro de Investigaciones Biologicas, Spanish National Research Council (CIB-CSIC), Madrid Spain

Organ size control is a longstanding question in biology. In mammals, using conditional cell ablation, two mutually exclusive mechanisms involving either intrinsic or extrinsic programs have been described to control organ size. The mammary gland is an ideal model for such studies since during puberty and pregnancy undergoes large size and morphological changes. The role of stem cells in controlling mammary gland size is unclear, although mammary stem cells are able to reconstitute a functional organ upon transplantation. Here we show that mammary gland cellularity was strictly dependent of mammary stem cell number, even following a 20-fold expansion of the mammary stem cell pool at puberty and transient three-fold expansions with each pregnancy. In addition, the expansion of the mammary stem cell pool was hormone-dependent, as demonstrated by female bilateral ovariectomies during puberty, and transplants of male-derived cells into female recipients, where concomitant with a mammary stem cell expansion, the donor cells reconstituted functional mammary glands, developing alveolar structures and secreting milk after the recipient's parturition. Taken together, these data suggest that in the mammary gland there is a third organ size control mechanism, combining intrinsic cues throughout the organism's lifetime, with extrinsic hormone signals at particular developmental windows (puberty, pregnancy), where an expansion of the mammary stem cell pool occurs. This mechanism might have strong implications for the understanding of mammary tumorigenesis since expansion of the mammary stem cell pool invariably precedes the generation of breast tumors.

The role of EphB4 and ephrin-B2 in the regulation of the mammary epithelial cell fate

Philip Kaenel, Carlos Wotzkow, Rob Strange and Anne-Catherine Andres

University of Bern, Bern, Switzerland

We have previously shown that the RTK EphB4 and its ligand ephrin-B2 are differentially expressed in the mammary gland and that their deregulated expression in the mammary epithelium of transgenic mice leads to perturbations of the mammary parenchyma and vasculature. In addition, over-expression of EphB4 and expression of a truncated ephrin-B2 mutant confers a metastasizing phenotype on NeuT initiated mouse mammary tumors. We compared the expression of CK-19, Sca-1, CD24 and CD49f as markers for progenitor cells exhibiting a decreasing differentiation grade. These analyses suggest that specific progenitor cells might have been the origin of tumor formation and that this change in the tumor origin has led to the acquisition of the metastatic tumor and thus, that deregulated EphB4-ephrin- B2 signalling may interfere with the homeostasis of the stem/progenitor cell pool before tumor formation is initiated. Quantification of epithelial cell populations in the mammary glands expressing the truncated ephrin-B2 revealed a preponderance of the basal, the progenitor cell enriched and the stem cell enriched fractions. Repopulation assays indicated that transgenic epithelial cells have a higher capacity to regenerate an entire epithelial network, which could be attributed to the augmented stem cell enriched fraction. Morphological analyses revealed, however, a disturbed architecture suggesting defects during the differentiation process. In particular, the outgrowths exhibited a hampered basal/luminal compartmentalization and a preponderance of Sca-1 positive cells, supposedly the precursors of estrogenreceptor positive cells. Our results demonstrate that truncated ephrin-B2 expression leads to an accumulation of stem cells and to a shift of the differentiation pathway indicating that intact ephrin-B2 signaling is indispensable for the control of the stem cell niche and regulation of the differentiation pathway.

Role of c-Src in human MCF7 breast cancer cell tumorigenesis

Annarica Calcabrini, María Pilar Sánchez Bailón, Esther Martín Forero, José Manuel García-Martínez, María Lourdes Naranjo Valencia and Jorge Martín-Pérez

Instituto de Investigaciones Biomédicas A. Sols (CSIC/UAM), Madrid, Spain

The Src family of non-receptor tyrosine kinases is implicated in transducing intracellular signals from a large number of growth factors and cytokines. They are involved in the regulation of cell proliferation, survival, adhesion, migration, invasiveness, etc. It is therefore not surprising that their deregulations, by increasing expression or specific activity, have been associated to cancer.

We are particularly interested in deciphering the role of Src kinases in breast cancer.

Using the non-metastatic human adenocarcinoma cell line MCF7, we found that Src tyrosine kinases activity is required for proliferation. Furthermore, interfering c-Src expression by c-Src-shRNA inhibits proliferation, adhesion, spreading and migration. By conditional expression (TET-ON system) of a dominant negative form of c-Src (SrcDN = c-Src-K295M/Y527F), cells in culture also significantly reduce their rate of proliferation, adhesion, spreading and migration. When injected in nude mice conditional induction of SrcDN expression inhibits tumorigenesis and causes regression in tumors. Moreover, SrcDN also reduce tumorigenesis in enriched population of CD44+/CD24—-SrcDN, suggesting that Src functions are required for MCF7-stem cells.

Role of constitutively active JAK2 in mammary gland development and breast tumourigenesis

Maria Muñoz Caffarel[1], Rosa Zaragoza[2], Anthony R. Green[3] and Christine J. Watson[1]

[1]University of Cambridge, UK; [2]University of Valencia, Spain; [3]Cambridge Institute for Medical Research, Cambridge, UK

Signalling through the JAK/STAT pathway is required for mammary gland development and is frequently deregulated in cancer. STAT5 is essential for lobuloalveolar development during pregnancy and deletion of STAT5 results in lactation failure. In contrast, STAT3 is a critical initiator of post-lactational cell death and tissue re-modelling (involution). Paradoxically, both STAT3 and 5 have been shown to be oncogenes in the breast and other tissues such as prostate. Conditional deletion of JAK2 in the mouse results in failed lactation and protects against the onset of mammary tumourigenesis. Constitutive activation of JAK2 would be predicted to result in hyperactivation of STATs 3 and 5 with concomitant cell transformation. However, it is difficult to predict the outcome of hyperactivation of these STATs as they play opposing roles in normal mammary gland development. We have analysed the phenotype of JAK2V617F mice, which express a constitutively activated JAK2 mutant in the mammary gland. We have observed that they show increased pregnancy-induced branching, accelerated differentiation and increased cell proliferation. In addition, they show a delay in involution. All these effects seem to be mediated by STAT5 over activation. Moreover, we have shown that mammary epithelial cells transduced with retrovirus harbouring either normal JAK2 or JAK2V617F show increased proliferation, altered differentiation and resistance to cell death and to anti-growth signals. Furthermore, JAK2 V617F induces anchorage independent cell growth in the presence of a co-operating oncogene. Taken together, these results show that the constitutive activation of JAK2 in mammary epithelial cells perturbs cell signalling and affects mammary epithelial cell fate, inducing some hallmarks of cancer. Our work provides insights into signalling downstream of constitutively active JAK2/STAT5 and could be important for understanding the molecular mechanisms of breast tumourigenesis.

Role of the CB2 cannabinoid receptor in ERBB2-driven breast cancer progression

Eduardo Pérez-Gómez[1], María Muñoz Caffarel[1,4], Clara Andradas[1], Gema Moreno-Bueno[2], Juana María Flores[3], Manuel Guzmán[1] and Cristina Sánchez[1]

[1]School of Biology, Complutense University, Madrid, Spain; [2]Autonoma University, Madrid, Spain; [3]School of Veterinary, Complutense University, Madrid, Spain; [4]University of Cambridge, UK

Cannabinoid actions are mediated by engagement and activation of specific G proteinhttps coupled receptors, namely CB1 and CB2. It is well known that cannabinoids exert antitumoral effects in different models of cancer. They inhibit cancer cell proliferation, adhesion, migration and induce cell death by apoptosis. However, little is known about the role of the endocannabinoid system in tumor physio-pathology. In particular, although strong evidence point to the CB2 cannabinoid receptor as target for anti-cancer therapy, there is no information about its role in tumor generation and progression. To shed light on this issue, we have generated animals with two genetic modifications, specifically, Her2 overexpression directed to the mammary epithelium, which triggers the spontaneous generation of breast tumors, and genetic ablation of the CB2 cannabinoid receptor. These animals were analyzed in terms of tumor latency, tumor growth, number of tumors generated per animal and development of lung metastases. Our results show that the lack of CB2 receptors has a protective effect on tumor generation and progression. We are currently analyzing the molecular mechanisms involved in this tumor phenotype.

Nitrosative stress and impairment of S-nitrosothiol homeostasis modulate proliferation of breast cancer cells

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Augmented nitric oxide (NO) levels in tumors have been usually detected. Protein modifications induced by NO, such as S-nitrosylation, may constitute a significant regulating factor affecting both tumor progression and antitumoral treatment. One of the mechanisms governing protein de-nitrosylation is the system thioredoxin/thioredoxin reductase (Trx/TrxR). Manipulation or alteration of this enzymatic system may alter SNO homeostasis in tumor cells, providing new insights into the role of NO in cancer and its therapeutic significance. Materials and methods. MCF-7, MDA-MB-231 and BT-474 cells were pretreated or not with the specific TrxR inhibitor auranofin and exposed to different doses of Snitroso- L-Cysteine (CSNO). Cell proliferation was measured using the XTT assay, and phosphorylation of Akt and Erk1/2 and cyclin D1 levels were determined by westernblot using the corresponding specific antibodies.

In all the three cell lines, a high CSNO dose (500 μM) reduced cell proliferation and this effect was potentiated by pretreatment with auranofin. However, treatment with auranofin and 100 nM CSNO enhanced cell proliferation of estrogen receptor positive (ER+) MCF-7 cells, but not of MDA-MB-231 (ER-, mut p53), or BT-474 (ER+, mut p53) cells. The augmented cell growth was associated with Akt and Erk1/2 phosphorylation and higher expression of cyclin D1. Significantly, this pro-proliferative effect was abolished by the ER antagonist fulvestrant or the p53 specific inhibitor piphithrin-alpha. Besides, specific silencing of p53 or ER-alpha in MCF-7 cells abrogated the pro-proproliferative stress of TrxR inhibition.

A severe nitrosative stress promoted growth arrest in breast cancer cells, whereas a mild nitrosative stress promoted breast cancer cell growth in a ER+ and intact p53 setting. The p53 and ER status in breast cancer may dictate the tumor response to different nitrosative environments.

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Splicing analysis reveals that BRCA1 IVS18+3A>C and BRCA2 IVS22-5A>G intronic variants are deleterious

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Deleterious germ-line mutations in BRCA1 and BRCA2 confer high risk of developing breast and/or ovarian cancer. However, up to 50% of the sequence changes identified in these two genes are considered genetic variants of unknown clinical significance. This is often the case for silent, missense, in-frame indels, and intronic variants. These so-called unclassified variants represent a challenge in genetic counseling. Some of them may be true deleterious mutations associated with hereditary breast ovarian cancer syndromes. For instance, some may adversely affect the splicing process, thus generating aberrant transcripts. Several bioinformatic tools have been developed to predict the effect of sequence variants on splicing. Nevertheless, these tools are not 100% predictive and mRNA analysis is mandatory to demonstrate aberrant splicing. Here we report the identification of two intronic variants that adversely affect splicing, producing aberrant transcripts. In both cases, RNA from peripheral blood lymphocytes was isolated from index cases. After cDNA synthesis, splicing was analyzed by a combination of fragment analysis, cloning and direct sequencing. The BRCA1 variant IVS18+3A>C was identified in two high risk breast cancer families. Segregation with disease was observed in both families. In silico analysis predicted an adverse effect on splicing donor site. Our analysis demonstrates that IVS18+3A>C induces BRCA1 exon18 skipping. The BRCA2 sequence variant IVS22-5 A>G was identified in three high risk families. Segregation with disease was confirmed in one family. In silico analysis predicted the creation of a new splicing acceptor site 4bp upstream of the reference one. The study allowed us to confirm that the new acceptor site predicted by in silico analysis was indeed functional, producing messengers with the last 4bp of intron 22 incorporated into exon 23. The insertion causes a frameshift change and introduces a premature stop codon 99 bp downstream.

Taking together, the data suggest that both variants are deleterious.

A Pin1/mutant p53 axis promotes aggressiveness in breast cancer

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TP53 missense mutations dramatically influence tumor progression, however their mechanism of action is still poorly understood. Here we demonstrate the fundamental role of the prolyl isomerase Pin1 in mutant p53 oncogenic functions. Pin1 enhances tumorigenesis in a Li-Fraumeni mouse model and cooperates with mutant p53 in Ras-dependent transformation. In breast cancer cells Pin1 is required for mutant p53 metastatic properties by promoting mutant p53-dependent inhibition of p63 metastasis suppressor and by concerted induction of a pro-aggressivenes gene expression program In human breast cancer cells, Pin1 amplifies mutant p53-dependent migration and invasiveness by inhibiting the anti-metastatic factor p63 and inducing a specific transcriptional program. Accordingly, we identified a transcriptional signature associated with poor prognosis in breast cancer and, in a cohort of patients, Pin1 overexpression influenced the prognostic value of p53 mutation.

Concomitant CUL4A overexpresion and BRCA1 deficiency as indicator of trabectedin sensitivity in breast cancer cell lines

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Breast cancer is a complex and heterogeneous disease and one of the most frequent causes of cancer-related deaths in developed countries. Triple negative or basal-like breast tumours pose a significant clinical challenge mainly due to greater aggressiveness and current lack of subtype-specific therapy. Prior analysis in our laboratory of familiar and sporadic breast tumours by using array-CGH allowed the identification of an amplification at 13q34 in up to 20% of basal-like neoplasms that are closely related to BRCA1 hereditary breast tumours. Correlation of genomic amplification and expression at the mRNA and protein level together with a plausible cancer-related function allowed us to defined CUL4A ubiquitin ligase as one of the most likely putative oncogenes within the 13q34 amplicon. Among other functions CUL4A restricts the cellular repair capacity through selective degradation of damage sensors involved in the nucleotide excision repair (NER). Abnormal expression of CUL4A might have a role not only in breast tumours oncogenesis and/or progression but also implications for treatment response to DNA-damaging drugs. One of such compounds is Trabectedin (Yondelis®, ET-743) a marine-derived compound that presents antitumor activity in sarcomas and in ovarian cancer. A composite signature including low BRCA1 and either high ERCC1 and/or ERCC5 seems to identify a highly sensitive population of sarcomas with significantly improved treatment outcome. Taking this into account we seek to determine the role of CUL4A expression as a potential biomarker for Trabectedin response. We characterized the levels of CUL4A expression and of DNA-repair genes belonging to the HR (BRCA1, BRCA2 and XRCC3) and NER (ERCC1 and ERCC5) pathways in 11 breast cancer cell lines by qRT-PCR. We exposed this panel of cell lines to increasing concentrations of Trabectedin and determined IC50 values. We found that a high level of CUL4A expression might be a good indicator of Trabectedin sensitivity, particularly in combination with low BRCA1 expression. This data suggest that tumours from BRCA1 mutation carriers as well as BRCA1-like tumours (with somatic inactivation of BRCA1 function) that overexpress CUL4A might be particularly sensitive to Trabectedin treatment. Further functional analysis in BRCA1-deficcient and BRCA1-proficient cell lines with Trabectedin and other DNA-crosslinking agents will allow further evaluate the role of CUL4A amplification/overexpression in the maintenance of genome integrity and to elucidate its full potential for therapeutic intervention in breast cancer, in particular in basal-like tumours.

Effects of estrogen on the proportion of stem cells in the breast

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CIC bioGUNE, Bilbao, Spain

There is increasing evidence that breast cancers contain tumour-initiating cells with stem cell properties. Estrogen is required for the normal physiology of the mammary gland and is also implicated in tumorigenesis, but the influence of estrogen on the stem cell population is poorly understood. The regulatory gene networks controlling the function of embryonic stem cells are also found in some adult stem cell populations. We determined the expression of the embryonic stem cell genes NANOG, OCT-4 and SOX-2 in the human mammary stem cell population and investigated the role of estrogen in the regulation of self-renewal and/or differentiation. Samples of normal breast tissue, tumour samples of varying grades and normal adjacent tissue, as well as MCF-7 breast cancer cells, were used for this study. Breast cells were cultured as mammospheres in the presence of hormones, and the stem/progenitor cell populations were isolated by FACS. We found that the expression of NANOG, OCT-4 and SOX-2 was higher in the stem/progenitor cell populations than in more differentiated cells, and it was elevated in tumour cells compared to in normal cells. Estrogen treatment reduced the proportion of stem cells, inhibited mammosphere formation and down-regulated NANOG, OCT-4 and SOX-2 expression in normal cells and tumour cells. Moreover, overexpression of each stem cell gene in MCF-7 cells reduced estrogen receptor expression and increased the percentage of stem/progenitor cell populations and the capacity for invasion, properties associated with tumorigenesis and poor prognosis. Thus, our results implicate NANOG, OCT-4 and SOX-2 in the maintenance of mammary stem/ progenitor cells and associate their higher expression with a breast cancer stem cell phenotype. In addition, our work indicates that estrogen reduces the size of the human breast stem cell pool and may therefore provide an explanation for the better prognosis of ER-positive tumours.

Breast Cancer

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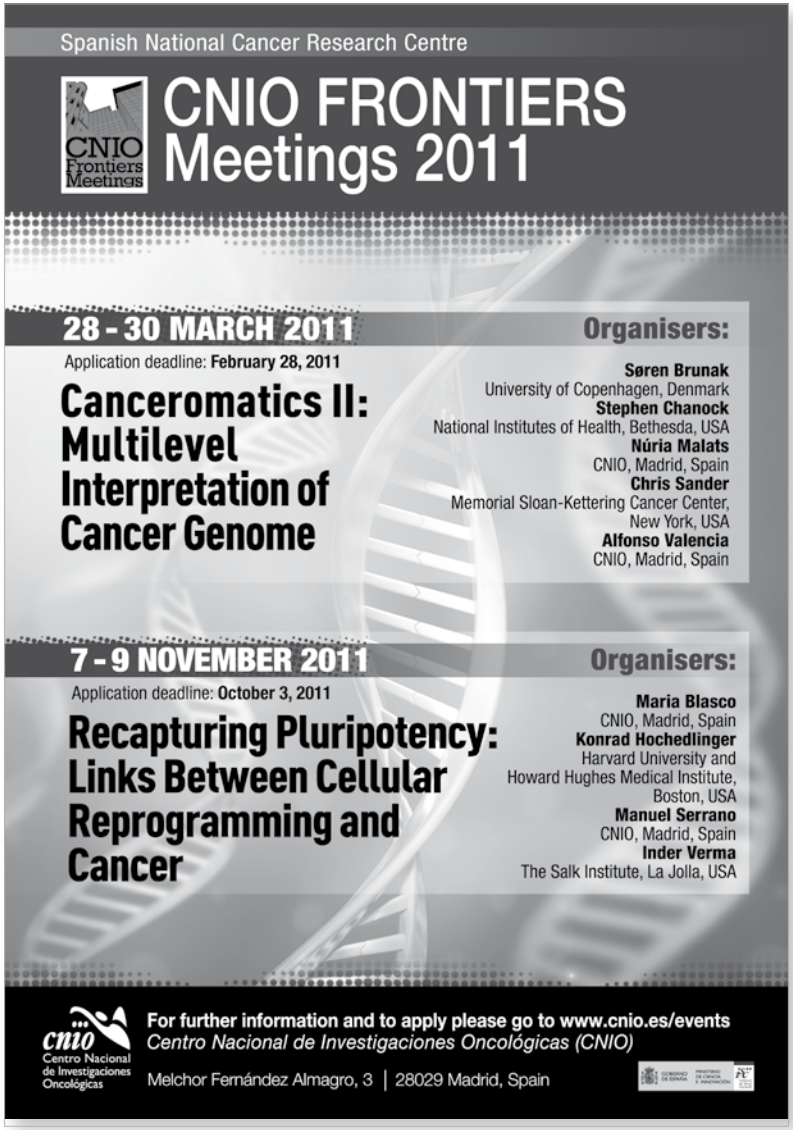

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Previous CNIO Frontiers Meetings and CNIO Cancer Conferences

2010

Cancer pharmacogenetics: Personalizing medicine
22/11/2010 - 24/11/2010
Organisers: **Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg**

Nature-CNIO Cancer Symposium on "Frontiers in Tumour Progression"
24/10/2010 - 27/10/2010
Organisers: **Douglas Hanahan, Zena Werb and Erwin Wagner**

4th ESO-CNIO Familial Cancer Conference
7/06/2010 - 8/06/2010
Organisers: **Javier Benítez, Rosalind Eeles and Hans Vasen**

Molecular cancer therapeutics
8/03/2010 - 10/03/2010
Organisers: **Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo**

2009

The energy of cancer
02/11/2009 - 04/11/2009
Organisers: **Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair**

Cancer-om-atics: Multilevelinterpretation of cancer genome data
06/07/2009 - 8/07/2009
Organisers: **Søren Brunak, Nuria Malats, Chris Sander and Alfonso Valencia**

Stem cells and cancer
23/02/2009 - 25/02/2009
Organisers: **Elaine Fuchs, María A. Blasco, Eduard Batlle and Mirna Pérez-Moreno**

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2008

Signalling upstream of mTOR
03/11/2008 - 05/11/2008
Organisers: **Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sanchez-Cespedes**

Structure and mechanisms of essential complexes for cell survival
23/06/2008 - 25/06/2008
Organisers: **Niko Grigorieff, Eva Nogales and Jose María ValpuestaDevelopment and cancer**

04/02/2008 - 06/02/2008
Organisers: **Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres**

2007

Links between cancer, replication stress and genomic integrity
05/11/2007 - 07/11/2007
Organisers: **Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig**

Myc and the transcriptional control of proliferation and oncogenesis
11/06/2007 - 13/06/2007
Organisers: **Robert N. Eisenman, Martin Eilers and Javier León**

Molecular mechanisms in lymphoid neoplasm
19/02/2007 - 21/02/2007
Organisers: **Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris**

2006

Telomeres and telomerase - CNIO / Joséf Steiner Cancer Conference
13/11/2006 - 15/11/2006
Organisers: **María A. Blasco and Jerry ShayMedicinal chemistry in oncology**

02/10/2006 - 04/10/2006
Organisers: **Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock**

Inflammation and cancer
22/05/2006 - 24/05/2006
Organisers: **Curtis Harris, Raymond DuBois, Jorge Moscat and Manuel Serrano**

PTEN and the AKT route
08/05/2006 - 10/05/2006
Organisers: **Ana Carrera, Pier Paolo Pandolfi and Peter Vogt**

2005

Cancer and aging
07/11/2005 - 09/11/2005
Organisers: **María A. Blasco, Kathy Collins, Jan Hoeijmakers and Manuel Serrano**

MAP kinases and cancer
30/05/2005 - 01/06/2005
Organisers: **Philip Cohen, Roger Davis, Worcester, Chris Marshall and Ángel Nebreda**

Animal tumour models and functional genomics
07/03/2005 - 09/03/2005
Organisers: **Allan Balmain, Mariano Barbacid, Anton Berns and Tyler Jacks**

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2004

Cadherins, catenins and cancer
29/11/2004 - 01/12/2004
Organisers: **Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy**

Structural biology of cancer targets
27/09/2004 - 29/09/2004
Organisers: **Ernest Laue, Guillermo Montoya and Alfred Wittinghofer**

2003

Apoptosis and cancer
01/12/2003 - 03/12/2003
Organisers: **Gabriel Nuñez, Marisol Soengas and Scott Lowe**

Small GTPases in human carcinogenesis
16/06/2003 - 18/06/2003
Organisers: **Juan Carlos Lacal, Channing Der and Shuh Narumiya**

Targeted search for anticancer drugs
17/03/2003 - 19/03/2003
Organisers: **Amancio Carnero and David H. Beach**

2002

Mechanisms of invasion and metastasis
18/11/2002 - 20/11/2002
Organisers: **Joan Massagué and Richard Hynes**

The cell cycle and cancer
30/09/2002 - 02/10/2002
Organisers: **Marcos Malumbres, Charles Sherr and Jiri Bartek**

Cancer epigenetics: DNA methylation and chromatin
29/05/2002 - 31/05/2002
Organisers: **Manel Esteller and Stephen B. Baylin**

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NOTES

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