Journal

June 2014

It's a great pleasure to report that the recipient of the 2014 Pezcoller Foundation-AACR International Award for Cancer Research is Elaine Fuchs , Ph.D., Rebecca C. Lancefield Professor Investigator, Howard Hughes Medical Institute Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, NY.

The Selection Committee met in Philadelphia on December 1st, 2013 and was chaired by Frank McCormick, Ph.D., FRS, Director, UCSF Helen Diller Family Comprehensive Cancer Center, CA. The other members of the Committee were: Adriana Albini, Ph.D. Responsabile Ricerca Oncologica, Polo Scientifico e Tecnologico,IRCCS MultiMedica, Milan Italy - Ruggero De Maria, M.D. Scientific Director, National Cancer Institute Regina Elena Roma, Italy - Ivan Đikić, M.D., Ph.D. Professor, Institute of Biochemistry II, Goethe-Universary Frankfurt, Germany - Raymond N. DuBois, M.D., Ph.D. Executive Director Biodesign Institute, Arizona State University - Tony Hunter, Ph.D. Director, The Salk Institute Cancer Center, La Jolla, CA - Martine F. Roussel, Ph.D. Member, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN - Josep Tabernero, M.D. Head, Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain.

Elaine Fuchs was recommended as the recipient of the Award for her many contributions to the biology and molecular mechanisms underlying development and differentiation of the epidermis and its stem cells, also having greatly advanced the understanding of epithelial biology and its cancer and provide a foundation for developing reagents for cancer diagnosis and treatment.

Dr. Fuchs systematically and brilliantly built the molecular knowledge of a tissue and elucidated how normal tissue structure goes awry in hyper proliferative disorders and cancers. The path breaking strategies that the winner has developed and employed are as impressive as discoveries she has made.

Elaine Fuchs was introduced at the 2014 Annual Meeting in San Diego C.A. where she delivered to a large audience the Pezcoller Lecture: "Stem Cells in Silence, Action, and Cancer".

Afterwards the award was solemnly given in the prestigious hall of the Buonconsiglio Castle in Trento on May 9. In this occasion Elaine Fuchs was introduced by Ruggero De Maria, member of the Selection Committee , who explained that she " has been awarded for her discoveries on skin stem cells under normal conditions and when these give rise to skin tumors. Her research excellence is well known and appreciated all over the world and is reflected by the large number of publications that have become a key reference for the international scientific community."

Two days before the ceremony in Trento Dr. Fuchs gave the "Korsmeyer Lecture" in Padova at the VIMM Venetian Institute for Molecular Medicine to honor the memory of the late Stanley Korsmeyer winner of the 2004 Pezcoller-AACR Award.

This issue of our Journal is dedicated to the 26th Pezcoller Symposium entitled "Cancers driven by Hormones" to be held in Trento from June 19 to June 21, 2014 co-chaired by David Livingston, Myles Brown, Arul Chinnaiyan, Antonella Farsetti, Massimo Loda, Roland Schuele and Enrico Mihich. The focus of the symposium will be on the hormonal influences which affect the incidence, natural history, and clinical outcomes of important and very common cancers. The molecular events that underlie these influences are increasingly apparent, and the science in this area is both fervent and incisive. Indeed, it has continued to bring about advances in clinical care that are based upon ever deeper biochemical, cell biological, physiological, and pathophysiological insights. This Symposium will explore these advances-both those arising from elegant analyses of tumor biology and those of a translational nature that have emerged from them. The five sessions are chaired by Massimo Loda, Myles Brown, Catherin Brisken, Arul Chinnaiyan, Antonella Farsetti and William Hait with the following titles :

Session I, Mechanistic Underpinnings of Tumor Cell Hormone Responsiveness

Session II, Hormonal Signaling in Breast Cancer

Session III, Translational Developments in Hormone-driven Breast Cancer

Session IV, Hormonal Signaling in Prostate Cancer

Session V, Translational Developments in Hormone-driven Prostate Cancer

The invited participants are:
Arteaga Carlos, Vanderbilt-Ingram Cancer Center - Nashville, TN
Beato Miguel, Centre de Regulacio Genomica - Barcelona-E
Bernards Rene, Nederland Cancer Center, Amsterdam, NL
Brisken Cathrin, Ecole Polytechnique Federale Lasusanne, CH
Brown Miles, Dana Farber Cancer Institute - Boston-MA
Carroll Jason, Cancer Research UK - Cambridge
Chinnaiyan Arul, Michigan Center for Translational Pathology
Clevers Hans, Utrecht Institute, Utrecht, NL
Di Fiore Pier Paolo, IFOM-IEO Campus, Milano, I
Dowsett Mitchell, Institute of Cancer Research, London, UK
Farsetti Antonella, Ist.Naz.Tumori Regina Elena, Roma, I
Freedman Matthew, Dana Farber Cancer Institute, Boston, MA
Gleave Martin, Vancouver Prostate Center, Vancouver, Canada
Hait Bill, New Brunswick, NJ
Knudsen Karen, Thomas Jefferson University, Philadelphia, PA
Livingston David, Dana Farber Cancer Institute, Boston,MA
Loda Massimo, Dana Farber Cancer Institute, Boston,MA
Mihich Enrico, Dana Farber Cancer Institute, Boston,MA
Reiter Rob, Un.of California, Los Angeles,CA
Scher Howard, Memorial Sloan-Kettering Cancer Center NY
Schuele Roland, University of Freiburg Medical center
Witte Owen, University of California, Los Angeles, CA
Yegnasubramanian Vasan, The Johns Hopkins University, Baltimore, MA
Tavazoie Sohail, The Rockfeller University, NY

The abstracts of the symposium are in the following pages.

Gios Bernardi M.D.
Editor and President Emeritus

Picture on front page: 2014 Pezcoller Foundation-AACR International Award for Cancer Research ceremony in Trento.
From the left: Walter Liber, presenter – Gios Bernardi, President Emeritus - Davide Bassi, President - Elaine Fuchs, winner - Anna Depoli, translator – Ruggero De Maria, Scientific Director Regina Elena Institute, Rome

26th Pezcoller Symposium

Cancers driven by hormones

Trento, Italy, June 19-21, 2014

ABSTRACTS OF ORAL PRESENTATIONS

The PI3K/AKT/TOR as a rational therapeutic target in breast cancer

Carlos L. Arteaga, Vanderbilt-Ingram Cancer Center, Nashville, TN

The PI3K/AKT/TOR pathway is the most frequently mutated pathway in breast cancer, with mutation and/or amplification of the genes encoding the PI3K catalytic subunits p110a (*PIK3CA*) and p110b (*PIK3CB*), the PI3K regulatory subunit p85a (*PIK3R1*), receptor tyrosine kinases (RTKs) such as HER2 (*ERBB2*) and FGFR1, the PI3K activator K-RAS, the PI3K effectors AKT1, AKT2, and PDK1, and loss of the lipid phosphatases PTEN and INPP4B. The three genes *PIK3CA*, *PIK3CB*, and *PIK3CD* encode the homologous p110a, p110b, and p110d isozymes, respectively. Expression of p110d is largely restricted to immune and hematopoietic cells, whereas p110a and p110b are ubiquitously expressed. The p110a isozyme is essential for signaling and growth of tumors driven by *PIK3CA* mutations, RTKs, and/or mutant RAS, whereas p110b lies downstream of GPCRs and has been shown to mediate tumorigenesis in PTEN-deficient cells. *PIK3CA* mutations are the most common genetic alterations of this pathway, where ≥80% occur within the helical (E542K and E545K) and kinase (H1047R) domains of p110a. Such mutations confer increased catalytic activity through different mechanisms, but both induce characteristics of cellular transformation including growth factor- and anchorage-independent growth, and resistance to anoikis.

Several drugs targeting multiple levels of the PI3K network (*i.e.*, PI3K, AKT, mTOR) have been developed (Table 1). A number of ATP-mimetics that bind competitively and reversibly to the ATP-binding pocket of p110 are in early clinical development. These include the pan-PI3K inhibitors BKM120, XL-147, PX-866, PKI-587, and GDC-0941, the p110a-specific inhibitors BYL719, GDC-0032, and INK-1117, the p110d-specific inhibitor CAL-101, and the dual PI3K/mTOR inhibitors BEZ235, BGT226, PF-4691502, GDC-0980, and XL-765. The pan-PI3K and p110a-specific inhibitors are equally potent against oncogenic p110a mutants. The rationale for the development of isozyme-specific antagonists is to allow higher doses of anti-p110a and anti-p110b drugs to be delivered without incurring side-effects caused by pan-PI3K inhibitors. Recently completed phase I trials with BKM120, BEZ235, and XL-147 showed that treatment partially inhibited PI3K as measured by levels of P-S6 and P-AKT in patients' skin or tumors, and 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) uptake measured by PET. Main toxicities were rash, hyperglycemia, diarrhea, fatigue and, mood alterations. Few clinical responses were observed in patients with and without detectable PI3K pathway mutations, although screening for genetic lesions in this pathway was not comprehensive.

Both allosteric and ATP-competitive pan-inhibitors of the three isoforms of AKT are also being developed. AZD5363, GDC-0068, GSK2141795, and GSK690693 are ATP-

competitive compounds which have shown antitumor activity in preclinical models and recently entered phase I trials. Allosteric inhibitors such as MK-2206 bind to the AKT PH domain and/or hinge region to promote an inactive conformation of the AKT protein that is unable to bind to the plasma membrane. MK-2206 inhibits AKT signaling *in vivo*, and suppresses growth of breast cancer xenografts harboring *PIK3CA* mutations or *ERBB2* amplification. Phase I data showed that treatment with MK-2206 decreases levels of P-AKT, P-PRAS40, and P-GSK3b in tumor cells, peripheral blood mononuclear cells, and hair follicles. Another approach to block this pathway has been the development of ATP-competitive inhibitors of the mTOR kinase, which block both mTORC1 and mTORC2. Several dual TORC1/2 inhibitors have been identified, including INK128 (Intellikine), CC223 (Celgene), AZD2014 (AstraZeneca), and Palomid 529 (Paloma Pharmaceuticals). Dual PI3K/mTOR inhibitors have also been developed in the hope of overcoming the loss of feedback inhibition or PI3K activation observed with rapalogs. The mTORC1 pathway is one of the prominent negative feedback regulators of the PI3K pathway; inhibition of mTORC1 can release this feedback inhibition and activate the PI3K pathway.

Drug target	Drug	Source	Mechanism	Phase of development
Pan-PI3K	BKM120	Novartis	ATP-competitive	Phase III
	GDC0941	Genentech	ATP-competitive	Phase
	XL-147	Exelixis/Sanofi	ATP-competitive	Phase I
	PX-866	Oncothyreon	ATP-competitive	
	CH5132799	Chugai Pharma	ATP-competitive	
p110a	BYL719	Novartis	ATP-competitive	Phase II
	GDC0032	Genentech	ATP-competitive	Phase II
	MLN-1117	Millennium	ATP-competitive	Phase I
p110d	CAL-101	Calistoga	ATP-competitive	Phase III
p110b	AZD6482	AstraZeneca	ATP-competitive	Discontinued
	GSK2636771	GSK	ATP-competitive	Phase I
PI3K/mTOR	BEZ235	Novartis	ATP-competitive	Phase II
	GDC0980	Genentech	ATP-competitive	Phase II
	PKI-587	Pfizer	ATP-competitive	
	PF-4691502	Pfizer	ATP-competitive	
	XL-765	Exelixis-Sanofi	ATP-competitive	Phase II
	GSK1059615	GSK	ATP-competitive	
	DS-7423	Daiichi Sankyo	ATP-competitive	Phase I
TORC1/2	MLN-128	Millennium	ATP-competitive	Phase II
	OSI-027	OSI-Astellas	ATP-competitive	Discontinued
	AZD2014	AstraZeneca	ATP-competitive	Phase I
	CC-223	Celgene	ATP-competitive	Phase II
TORC1 (rapalogs)	Everolimus	Novartis	Allosteric	FDA-approved
	Temsirolimus	Pfizer	Allosteric	FDA-approved
	Ridaforolimus	Merck	Allosteric	Phase II
AKT	MK-2206	Merck	Allosteric	Phase II
	AZD5363	AstraZeneca	ATP-competitive	Phase I
	GDC0068	Genentech	ATP-competitive	Phase II
	GSK690693	GSK	ATP-competitive	

Table 1. PI3K/AKT/TOR pathway inhibitors in clinical development

"Genome structural dynamics in hormonal gene regulation"

Miguel Beato[1], Francois LeDily[1], Guillermo P. Vicent[1], Cecilia Ballaré[1], Roni H.G. Wright[1], Gaetano Verde[1], Daniel Soronellas[1], Marc Marti-Renom[1,2], Guillaume Filion[1]
[1] Centre de Regulació Genòmica (CRG), Barcelona, Spain
[2] Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain

Breast cancer cells respond to steroid hormones by extensive changes in their gene expression program, which are the end result of hormonal activation of a complex signaling network initiated by the interaction of the hormones with intracellular receptors. The activated signaling pathways along with the hormone receptors impinge on chromatin, which is modified to enable the transcriptional response of the genome. Using as an example the response to progestins of T47D cells, we show that the organization in nucleosomes of the DNA sequences recognized by the progesterone receptor (PR) is key for the initiation of the chromatin remodeling response [1], which depends on PR associated enzymatic activities including histone modifying enzymes (kinases, acetyl transferases, deacetylases, methylases, demethylases), ATP-dependent remodelers (NURF & BAF) and PARP1 [2-5]. Moreover, the conserved division of the genome in consecutive topological association domains (TADs) contributes to coordination of the hormonal response, as the regulated genes segregate significantly into TADs that respond as a whole with either activation or repression of transcription [6]. Modeling of the 3D structure of these TADs shows that repressed TADs compact in response to hormone and the interactions among their genes decrease, while activated TADs expand and the interactions among their genes increase [6]. These unexpected findings underline the importance of the various levels of chromatin structure for gene regulation and lead to the proposal that TADs behave as "regulons" in the response of cells to external signals.

[1] Ballare C *et al* (2013) Mol Cell 49, 67-79
[2] Vicent GP *et al* (2006) Mol Cell 24, 367-81
[3] Vicent GP *et al* (2009) PLoS Genet 5, e1000567
[4] Vicent GP *et al* (2009) Genes Dev 25, 845-62
[5] Wright RHG *et al* (2012) Genes Dev 26, 1972-83
[6] Le Dily F *et al* (2014) submitted

Molecular diagnostics of breast cancer.

Rene Bernards, The Netherlands Cancer Institute, Amsterdam.

Cancer therapy is slowly but steadily changing from a morphology-centric approach to a situation in which the genome takes center stage in key treatment decisions. This is particularly true for breast cancer. One could argue that measurement of estrogen receptor was the first companion diagnostic for a targeted cancer drug. Moreover, staining for HER2 for the treatment with trastuzumab was also first used in breast cancer. Since then, a number of molecular diagnostic assays have been developed to help determine the risk of recurrence in breast cancer: including MammaPrint, OncotypeDx and Prosigna. In this presentation, I will compare the development and validation of these molecular diagnostic assays and discuss their clinical utility and validity.

Genetic and Epigenetic Determinants of Hormone Dependence

Myles Brown, M.D.
Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA 02215

Ovarian function has been recognized as critical for the growth of a large proportion of breast cancers since the late 19th century. The identification of steroid hormones, their receptors and coregulators in the 20th century facilitated a greater understanding of the pathways involved in the hormone dependent growth of breast cancer. This led to the development of specific drugs targeting both the enzymes responsible for

hormone synthesis and the steroid receptors themselves and the development of the estrogen receptor as both the first predictive biomarker for breast cancer as well as the first molecularly defined therapeutic target. Despite this progress the factors that determine whether a given breast cancer will respond to endocrine therapy and the causes of acquired endocrine resistance remain to be fully elucidated. The advent of 21st century DNA sequencing technology has revealed important new insights into the mechanisms of endocrine dependence. Several groups including our own have found that mutations in the estrogen receptor itself can explain resistance to endocrine therapy in as many as 20% of patients with advanced disease. These mutations activate the receptor in the absence of hormone and make the receptor resistant to existing antagonists. The existence of these mutations strongly supports the conclusion that in these cases the tumor initiating or "cancer stem cell" is estrogen receptor dependent. Mutations in other components of the hormone signaling machinery have not yet been validated as mechanisms of endocrine resistance. Using genome-wide approaches we have explored genetic mechanisms of resistance and potential epigenetic mechanisms involving only a change in chromatin state both in model systems and patients. Our findings strongly support the continued development of novel endocrine therapies for breast cancer.

Understanding estrogen receptor transcription in breast cancer

Jason S. Carroll
Cancer Research UK, University of Cambridge, Robinson Way, Cambridge, UK, CB2 ORE

Estrogen Receptor (ER) is the defining feature of luminal breast cancers, where is functions as a transcription factor. The traditional view of ER getting recruited to promoters of target genes is too simplistic. We have shown that ER rarely associates with promoter regions of target genes and instead associates with enhancer elements significant distances from the target genes. The genomic mapping

of ER binding events also revealed a role for the Forkhead protein FoxA1 (HNF3a) in maintaining interactions between ER and the chromatin. We have extended on these findings to explore ER and FoxA1 functional interactions in breast cancer, with a specific focus on changes in binding dynamics that occur during endocrine resistance. To address this, we have been exploiting transcription factor mapping approaches (ChIP-seq) in primary tumor material, to investigate differential ER/FoxA1 binding that occurs during tumor progression. We have functionally explored underlying variables that contribute to altered transcription factor binding and activity. In addition, we have recently established a method for rapid unbiased discovery of protein interactomes, which we have applied to discover ER and FoxA1 associated proteins. We find unexpected interactions between ER and Progesterone Receptor (PR) and show a role for PR as a functional regulator of ER activity. Our data would suggest that PR negative tumors do not necessarily reflect tumors with a non-functional ER pathway, but instead can represent tumors with copy number loss of the PR gene, which subsequently impacts ER binding and transcriptional activity. These findings flip the current paradigm around, and imply that PR regulation of ER may be more critical that the opposite. These findings help delineate the complexes that influence ER transcriptional activity and ultimately impinge on tumor progression and drug sensitivity.

"Towards Precision Medicine for Advanced Prostate Cancer"

Arul M. Chinnaiyan, M.D., Ph.D.
Director, Michigan Center for Translational Pathology
S.P. Hicks Endowed Professor of Pathology
Investigator, Howard Hughes Medical Institute
American Cancer Society Research Professor
Professor of Urology
Pezcoller Symposium

In April, 2011, the MI-ONCOSEQ clinical sequencing program was established, which prospectively enrolls patients with advanced

cancers for comprehensive mutational analysis using an integrative sequencing approach in a clinically-relevant timeframe (*Sci Transl Med*. 2011). The translation of clinical sequencing bears unique challenges including identifying patients who could benefit, developing informed consent and human subjects protections, interpreting what results should be reported and validated and how results should be reported. MI-ONCOSEQ is a unique in that it consists of three integrated projects with the following themes: Project 1) "Clinical Genomic Study" that identifies patients, consents them to the study, obtain biospecimens (tumor and germline tissues), and a multi-disciplinary Precision Medicine Tumor Board (PMTB) that deliberates on return of actionable or incidental genomic results; Project 2) "Sequencing & Analysis" processes biospecimens and perform comprehensive sequencing and analysis of tumors to identify point mutations, copy number changes, gene fusions and aberrant gene expression; Project 3) "Ethics & Psychosocial Analysis" will observe the PMTB process, examine the clinician and patient response to the informed consent process and delivery and use of genomic sequence results.

Since establishment of this protocol, over 250 adults and 40 children with cancer have been sequenced with return of results through an institutionally sanctioned "precision medicine tumor board" (PMTB). Recent discoveries stemming from MI-ONCOSEQ study include the identification of a novel recurrent NAB2-STAT6 gene fusion in solitary fibrous tumors, a rare soft tissue tumor (*Nature Genetics* 2013), rearrangements involving targetable FGFR in various cancers (*Cancer Discovery* 2013) and identification of ESR1 mutations acquired after anti-estrogen therapies (*Nature Genetics*, 2013).

Additionally, Charles Sawyers and I are leading a multi-institutional Stand Up 2 Cancer (SU2C) cohort study to sequence 500 patients with castration-resistant prostate cancer (CRPC) who will be enrolled in clinical studies testing therapies targeting androgen receptor (abiraterone and enzalutamide) and the DNA repair enzyme PARP (olaparib and ABT-888). Thus MI-ONCOSEQ will interface with and leverage ongoing and planned clinical trials to decipher the molecular mechanisms involved in cancer progression and associated resistance to therapies.

Lgr5 Stem Cells in self-renewal and cancer

Hans Clevers
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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined *Lgr5* as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of *Lgr5* in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-*LacZ* reporter strain, lineage tracing experiments were performed in adult mice. The *Lgr5*[+ve] crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar observarions were made in hair follicles and stomach epithelium.

Single sorted *Lgr5*[+ve] stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the *Lgr5*[+ve] stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the *Lgr5*[+ve] stomach stem cells.

Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the "cancer stem cell"-concept.

Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after

cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-a, Wnt3 and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Genetic removal of Paneth cells in vivo results in the concomitantloss of Lgr5 stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that Lgr5 stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

Connecting the machineries of cell fate determination and tumor suppression in mammary stem cells

Pier Paolo Di Fiore
University of Milan and IEO, Milan Italy

Numb is a cell fate determinant that by asymmetrically partitioning at mitosis controls binary cell fate decisions. In human breast cancers, there is frequent loss of Numb expression, due to its exaggerated ubiquitination and ensuing degradation. This causes alterations in two major downstream pathways. On the one hand, lack of Numb allows for unchecked signaling activity of the Notch receptor. On the other, lack of Numb causes attenuation of the p53 signaling pathway. Tumors displaying loss-of-Numb expression are addicted to this event and to its molecular consequences. Our recent results point to the mammary stem cell (MaSC) compartment as the cellular "target" of Numb misregulation in breast tumors. We have developed a technology to cultivate and purify MaSC. In normal MaSC, Numb is asymmetrically partitioned at mitosis. This in turn dictates the replicative fate, in that the Numb(+) cell remains quiescent (and retains MSC capabilities), whereas the Numb(-) cell acquires a progenitor fate and undergoes rapid symmetric divisions. The

control of Numb over MaSC fate is executed through the ability of Numb of silencing Notch signaling and maintaining high levels of p53 in the MSC. This latter event is due to Numb-mediated inhibition of the ubiquitinating function of the E3 ligase Mdm2 over p53. Lack of Numb in cancer MaSC causes a switch form the asymmetric to the symmetric mode of division, thus forcing both daughter cells to assume the same replicative fate. Our understanding of how Numb is mechanistically involved in all these aspects will be discussed.

Biomarkers of response and resistance to estrogen deprivation in primary breast cancer

Mitch Dowsett, Royal Marsden Hospital, London SW3 6JJ, UK.

Estrogen deprivation using aromatase inhibitors (AIs) are the most effective endocrine treatment for primary ER-positive breast cancer in postmenopausal women leading to an approximate 40% reduction in the risk death from the disease. Response appears to be driven primarily by reduced proliferation accompanied by rates of apoptosis that remain largely unchanged. This has underpinned the establishment of changes in the proliferation biomarker, Ki67, as an intermediate index of treatment benefit. We have recently shown (Gao et al CCR 2014) that multiple other metagenes representing proliferation (CIN70, GGI, AURKA, and Gene70) and estrogen-responsive pathways (ESR1.1, ESR1.2, and SET) were profoundly reduced by AI treatment, as were signaling pathways PTEN, CASP3, E2F3, MYC, AKT/ mTOR, and IGF-I potentially contributing to benefit from therapy. In contrast, PIK3CA, MAPK, Stroma.2-PLAU, Stroma.1, and Immune.1 expression module scores were significantly increased by treatment, suggesting that the pathways relevant to resistance are upregulated at this early time point. Markers predicting poor antiproliferative response include low ER, PDGFR/Abl, an inflammatory metagene, GDNF-RET and CDK4/Rb/E2F transcription. Further characerisation of the importance of these is required to allow their therapeutic targeting.

Estrogen/ERs, Hypoxia/HIFs and NO/eNOS signaling in hormone-driven cancers.

Antonella Farsetti, National Research Council (CNR) and Department of Experimental Oncology, Italian National Cancer Institute "Regina Elena", Rome, Italy

Our previous studies (Misiti *et al*., 2000 and Nanni *et al*. JCI 2002) assigned a crucial role to ER signaling in terms of telomerase activation in human ovary epithelial cells as well as in a traditionally androgen-dependent tumor, the Prostate cancer (PCa) and identified nuclearized endothelial NOS (eNOS) as partner of both Estrogen Receptors, ERa/ERb and Hypoxia Inducible Factors in PCa (Grasselli *et al*., 2008; Nanni *et al*., 2009; Re *et al*., 2011). Protein conformation at target promoters (e.g. hTERT) is affected substantially by formation of these complexes in response to estrogen and hypoxia stimuli that, in turn, regulate expression of genes associated with adverse prognosis in PCa. To better define the role of nuclear eNOS in the acquisition of aggressive phenotype in PCa, we performed ChIP-Sequencing on chromatin-associated eNOS from cells derived from a primary tumor associated with poor outcome and from metastatic LNCaP cells, before and after treatment with 17*b*-estradiol (E2). By this approach, we have documented the existence, on a genome-wide scale, of a considerable number of eNOS-DNA associations that define transcriptional active regions modulated by estrogen (Nanni *et al*., 2013).
 In addition, numerous potentially novel eNOS-targeted genes have been identified suggesting that eNOS participates in the regulation of large gene sets, including small non coding RNAs (miRs). In particular, we found a cluster of miRs, among which miR-34a, strongly silenced in PCa cells associated with poor outcome, suggesting a molecular link between eNOS and SIRT1, an epigenetic regulator of aging and tumorigenicity, negatively regulated by miR-34a and in turn activating eNOS. Specifically, we revealed a feedback loop involving transcriptional downregulation of pri-miR34a by the eNOS/SIRT1 complex in an estrogen-dependent fashion. As a consequence we observed induction of the miR-34a target SIRT1 that sequentially activates eNOS itself by post-translational modification. These findings revealed novel functions of eNOS and of the eNOS/SIRT1 interplay, fine-tuned by E2-activated ER signaling, favouring the concept of eNOS as critical molecular determinant in aggressive PCa.

Recent studies have placed long non coding RNAs (lncRNAs) at the leading edge of cancer research and suggested that they may serve as master drivers of carcinogenesis. However, their identity, function and deregulation in cancer are only beginning to be understood. In line with the above, we have contributed to the identification, by ChIP-Sequencing, of a consistent number of eNOS-bound complexes in the genome regions of many lncRNAs strictly associated with cancer, including PCa (Prensner JR and Chinnaiyan AM, 2011). Intriguingly, in the genomic regions overlapping with, or in the proximity of these transcripts the number of eNOS peaks significantly increases upon estrogen treatment, with a specific pattern for the primary tumor as compared to the metastatic cell line. These data are being validated by ChIP/re-ChIP and RIP assays with proteins of interest (eNOS, ERa, ERb and androgen receptor, AR) and lncRNAs emerging from the ChIP-Seq analysis. We currently aim at expanding such findings to a different microenvironment, breast cancer, by analysing, in this context, the occupancy by the eNOS complexes of genome regions of specific cancer-associated LncRNAs. Although arising in organs that are different for anatomy and physiological function, both breast and prostate cancers are typically hormone-dependent and have remarkable biological similarities. They are both driven by the sex steroid hormones, estrogen and androgen, that activate their respective nuclear receptors, ERa/ERb or AR. These nuclear receptors interact with a plethora of other transcription factors and co-factors, like eNOS, which aid ER and AR in the activation of a pro-tumorigenic gene expression program. To this end the expression of sex steroid hormone receptors (ERα, ERß or AR) and eNOS has been evaluated and compared in a large population of breast and prostate cancer cell lines, and 4 sub-groups among them have been identified on the basis of their different expression pattern. Levels of the most relevant cancer-associated lncRNAs (specifically HOTAIR, GAS5, H19, and CDKN2B AS/ANRIL and MALAT1) were quantified by *q*RT-PCR in each cell line,

before and after estrogens or hypoxia or upon interfering with the NO signaling by genetic or pharmacological approaches or upon combined treatments.

Preliminary data indicate that: *i.* lncRNAs HOTAIR and H19 basal expression in breast cancer cell lines appears highly correlated with presence of ERα, being undetectable in the ERa⁻ cells; *ii.* lncRNAs HOTAIR, MALAT1 and GAS5 have higher expression levels in metastatic prostate cancer cell lines than in primary tumors-derived PCa cells, suggesting that their induction correlates with poor patient outcome and cancer metastasis; *iii.* lncRNAs H19, HOTAIR and CDKN2B-AS/ANRIL expression significantly decreases after eNOS inhibition exclusively in ERα⁺ breast cancer cell lines, strongly suggesting that eNOS and ERa are strictly required for the transcriptional control of selected cancer-associated lncRNAs in hormone-driven cancers.

"Charting the AR cistrome in human prostate tissues"

Matthew Freedman, M.D.
Department of medicine Harvard Medical School – Medical Oncology Service, Dana-Farber Cancer Institute, Boston

The androgen receptor (AR), a nuclear transcription factor (TF), is central to prostate development, carcinoogenesis, and cancer progression. A complex interplay between AR and its co-regulators determines the genes targeted for regulation. Here we map the AR cistrome - the genome-wide set of AR binding sites - in 21 normal and tumor human prostate samples using chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) and establish that HOXB13 and FOXA1 reprogram the AR cistrome during prostate tumorigenesis. We find that the AR cistrome is dynamic during tumorigenesis. Identification of the differential AR binding sites between normal and tumor reveal key AR co-regulators as well as AR target genes that distinguish indolent from aggressive prostate cancer – findings that could only be discovered using a cohort of normal and malignant tissue. By performing ChIP-seq in primary human samples, we establish the importance of epigenetic characterization of human tissue.

Vancouver Prostate Center

Martin Gleave

Androgen receptor (AR) pathway inhibitors, including castration, abiraterone, and enzalutamide (ENZ), remain the most efficacious treatment for metastatic prostate cancer. Despite frequent responses, progression to castration resistant prostate cancer (CRPC) invariably occurs, highlighting a continued need to target alternate pathways that drive disease resistance and progression. Like many anti-cancer treatments, AR pathway inhibitors activate survival pathways that inhibit apoptosis, contribute to tumour cell plasticity and promote emergence of an acquired treatment-resistant phenotype. Adaptive survival pathways triggered by inhibition of a driver mutation, for example AR amplification in the case of CRPC, represent opportunities for conditional lethality and a high therapeutic index. Conditional lethality aims to improve therapy by combining targeted therapies under contextualized genetic and environmental conditions that specifically target tumor cells. While ongoing direct targeting of the AR will remain important, this presentation will focus on approaches to co-target the AR with cytoprotective stress response pathways that facilitate protein homeostasis and cross-talk signaling pathways that cooperatively activate the AR. Molecular chaperones are key mediators of stress responses, facilitating treatment resistance by regulating protein homeostasis (proteostasis) as well as many signaling and transcriptional survival networks. Co-targeting stress-induced chaperones regulating proteostasis may better manipulate cancer cell sensitivity to therapy; two stress-activated cytoprotective chaperones, clusterin (CLU) and Hsp27 are targets in current clinical trials of CRPC.

CLU is transcriptionally-regulated by stress-associated HSF1 and YB-1, retro-translocating from the ER to cytosol to inhibit apoptosis by suppressing protein aggregation, p53-activating stress signals, and Bax while enhancing Akt phosphorylation and trans-activation of NF-kB, YB-1, and HSF-1. Recently we defined a novel adaptor protein function for CLU, supporting tumor cell survival under stress conditions by facilitating Atg3-mediated lipidation of LC3 and autophagosome

biogenesis. In keeping with its anti-apoptotic functions, CLU confers treatment-resistance in cancer, while CLU inhibition potentiates activity of anti-cancer therapies, including ENZ, in preclinical models. The CLU inhibitor, OGX-011, is in Phase III trials of CRPC and lung cancer after a randomized phase II study in CRPC reported 7 month gain in overall survival and 50% reduced death rate when combined with docetaxel.

As a stress-activated chaperone, Hsp27 expression is induced by hormone and chemotherapy and inhibits treatment-induced apoptosis through multiple mechanisms. As a regulatory "hub" in multiple adaptive survival signaling and transcriptional pathways, Hsp27 inhibition may simultaneously suppress many pathways implicated in cancer progression and resistance to hormone- and chemo-therapies. The Hsp27 inhibitor OGX-427 has completed a randomized phase II study of in patients with mCRPC and preliminary results indicate that 82% vs 50% PSA decline and a 71% vs 48% freedom from progression at 12 weeks compared to prednisone controls. These results confirm, for the first time, single agent activity for an Hsp27 inhibitor in cancer, and phase II combination studies are ongoing in CRPC, lung, pancreas, and bladder cancer.

"Surviving Metabolic Stress: Of Mice (Squirrels) And Men"

William Hait, M.D., Ph.D. Global Head, Janssen R&D, Raritan NJ

Understanding how cancer cells survive harsh environmental conditions may be fundamental to eradicating malignancies proven to be impervious to treatment. Nutrient and growth factor deprivation, hypoxia, and low pH create metabolic demands that require cellular adaptations to sustain energy levels. Protein synthesis is one of the most notable consumers of energy. Mounting evidence implicates exquisite control of protein synthesis as a survival mechanism for both normal and malignant cells. I will discuss the role of protein synthesis in energy conservation in cancer and focus on elongation factor -2 kinase, a downstream component of the PI3 Kinase/ AKT pathway that behaves as a critical checkpoint in energy consumption.

AR-DNA repair interplay in prostate cancer: mechanisms and implications for disease progression

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Prostate cancer (PCa) remains the second most common cause of male cancer mortality in the Western world. Since the introduction of hormonal therapy sixty years ago), progress in developing new treatments has been slow despite major progress in understanding prostate carcinogenesis and disease biology. Recent development of abiraterone, MDV3100, alpharadin, cabazitaxel and Sipuleucel T has improved outcome, but metastatic prostate cancer remains a uniformly fatal disease. Moreover, while molecular subtyping has afforded therapeutic benefit and improved patient survival in other tumor types (most especially breast cancer), no such advances have been gained in the context of PCa. At present, all patients with metastatic PCa are treated identically, without any selection of appropriate therapeutic regimens based on tumor profiling.

Emerging data from our laboratory and others strongly support the concept that alterations in DNA damage repair (DDR) pathways are more common than previously thought in sporadic PCa, and that alterations in these pathways may accord new, more effective means of therapeutic intervention. *First,* alterations in genes whose functions are key for DNA repair are observed and increase in frequency as a function of disease progression. *Second,* and new findings indicate that the androgen receptor (AR) is a critical effector of DNA repair competence that alters the response to genotoxic insult in advanced PCa. This function of AR appear to be manifest through the ability

of AR to regulate expression and activity of DNAPK, an enzyme that is key for the process of repairing double-strand DNA breaks, and has a parallel role as a transcriptional modulator. *Third*, emerging data from clinical trials reveal that therapeutic agents which target the DDR pathway are contextually effective in treating advanced prostate cancer, consistent with the postulate that a significant subset of advanced tumors have altered DDR programs. Findings to be discussed strongly support a model wherein selected DDR pathways can be developed as therapeutic targets to tailor treatment for prostate cancer and improve outcome for advanced disease.

Physiology and pathology of the epigenetic drug target LSD1

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In my presentation I will summarize our current understanding of epigenetic regulation by the histone demethylase LSD1 and assess the future prospect as an epigenetic drug target. Recently it became evident that chromatin-modifying enzymes function in tight cooperation with transcription factors to regulate stemness, differentiation, metabolism, and pathological situations such as cancer. Epigenetic control mechanisms thus serve as an additional layer in the regulation of gene expression. We and others have shown that lysine-specific demethylase 1 (Lsd1; also known as Kdm1a, Aof2) selectively removes mono- and dimethyl groups from H3K4 or H3K9, thereby causing either repression or activation of gene transcription. Combining biochemical and cellular data, with the analysis of genetically modified mouse models and whole genome/bioinformatic approaches begins to uncover the molecular pathways utilized by the epigenetic enzyme LSD1 in prostate and prostate cancer. Applying this knowledge uncovered unexpected roles of LSD1 in regulating chromatin organization and androgen receptor-dependent gene expression in prostate cells, thus allowing us to develop strategies to identify chemical probes for LSD1. The use of these probes in combination with genetically modified LSD1 mouse models will further deepen our understanding of LSD1's function in physiology and pathology.

Stem Cell Targets and Pathways in Prostate Cancer Progression

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New therapeutic strategies are needed to treat prostate cancer as it progresses to the castration resistant stage following treatment with drugs to reduce androgen production or interfere with androgen receptor function. Genetic analyses of DNA alterations and RNA expression show that multiple pathways can be involved in this cancer progression. An understanding of the cellular components needed to initiate prostate cancer and sustain it during the process of metastasis may help to elucidate new pathways for therapy. We have developed techniques to transform mouse and human benign prostate epithelial basal stem cells and define them as one cell of origin which can respond to multiple stimuli to produce cancers which are capable of maturing to more differentiated cell types including luminal, squamous and neuroendocrine phenotypes. Different subtypes of cells with the properties of cancer stem cells capable of transplantation of the cancer phenotype have been defined. Current work is focusing on the activation of specific kinase driven pathways to provide new targets for therapy in metastatic prostate cancer.

Small RNAs, ApoE, and LRP's comprise a druggable anti-metastatic network in melanoma

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The impact of post-transcriptional deregulation on cancer progression is increasingly appreciated. One class of post-transcriptional regulators with robust effects on cancer progression are microRNAs. Our group has identified specific sets of microRNAs that govern metastatic progression by breast cancer and melanoma. By analyzing the expression levels of most known microRNAs across highly and poorly metastatic melanoma sub-populations we have identified miR-199a-5p, miR-199a-3p, and miR-1908 as highly over-expressed miRNAs in metastatic melanoma cells. Through loss-of-function and gain-of-function studies, we reveal these miRNAs to be metastasis promoter miRNAs in melanoma. These microRNAs convergently target the secreted protein ApoE. Molecular, genetic, and epistasis experiments reveal ApoE to be a strong suppressor of melanoma invasion, endothelial recruitment, and metastatic colonization. ApoE mediates these effects through its engagement of the LRP1 receptor on melanoma cells and LRP8 receptor on endothelial cell. We find that components of this metastasis regulatory network display significant associations with patient melanoma relapse outcomes. Moreover, therapeutic modulation of this pathway displays robust suppressive effects on melanoma progression.

The double-edged sword of androgen-induced double strand breaks in prostate cancer

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Induction of androgen receptor (AR) mediated transcriptional programs involves large scale reorganization of the nuclear genomic DNA to coordinate efficient activation of transcription at dozens to hundreds of genomic loci. Recent reports have also shown that induction of AR-mediated transcriptional programs by stimulation with androgens also involves formation of DNA damage, including DNA double strand breaks (DSB), and recruitment of DSB repair proteins. The DSB can be mediated by the class II topoisomerase TOP2B, which is recruited with the androgen receptor to regulatory sites on target genes and is required for efficient transcriptional activation of these genes. These DSB are recognized by the DNA repair machinery triggering the recruitment of repair proteins such as PARP1, ATM, and DNA-PK. We postulate that such DSBs represent a double-edged sword. On the one hand, if illegitimately repaired, such DSBs can contribute to cancer progression by promoting genetic instability and seeding formation of genomic rearrangements like the recurrent TMPRSS2-ERG fusion oncogene in prostate cancers, as we have shown previously (Haffner et al., Nat Genet, 2010). On the other hand, these androgen-induced, TOP2B-mediated DSB may also be exploitable therapeutically as an Achilles heel for prostate cancer. We propose that short pulses of androgen stimulation in the backdrop of androgen depletion therapy could selectively sensitize prostate cancer cells to DNA damaging agents such as ionizing radiation and TOP2 poisons, or to DNA repair inhibitors. Such an approach may have particular utility in the setting of high risk prostate cancer where novel therapeutic approaches are critically needed.

How progesterone promotes breast carcinogenesis

Prof. Cathrin Brisken, ISREC School of Life Sciences, Ecole Polytechnique Fédérale, Lausanne, Switzerland

Exposure to reproductive hormones affects breast cancer risk and promotes disease progression. We combine mouse genetics with tissue recombination techniques to study hormone action in vivo and provide evidence that progesterone is a major regulator of cell proliferation and stem cell activation in the adult mammary gland. Two progesterone receptor (PR) targets, Receptor activator of NfkB ligand (RANKL), and Wnt4 have distinct roles as downstream mediators of PR signaling. The relevance of the findings in the mouse model to humans could not be validated so far because of lack of a hormone responsive human model. We present a novel ex vivo model of human breast tissue microstructures and show that major signaling pathways are conserved between the two species.

PET and Optical Imaging of Prostate Cancer with Engineered Antibodies

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Prostate cancer is the most common epithelial malignancy among men in the United States and Europe. Despite major strides in translating the molecular basis of disease to improvements in treatment, the surgical and medical management of prostate cancer remains hampered by the absence of robust ways to image disease. Over the past ten years, we and others have explored the utility of engineered antibody fragments targeting major prostate cancer antigens such as Prostate Stem Cell Antigen (PSCA) and Prostate Membrane Antigen (PSMA) to image prostate cancer. Engineered antibody fragments retain the specificity of intact antibodies while providing for tunable clearance (e.g. liver vs. kidney), reduced serum half-life (enabling high contrast images at shortened intervals after tracer administration), and potentially improved tumor penetration. In this talk, we will cover our efforts to develop engineered PSCA minibodies and diabodies for both optical and PET imaging of prostate, bladder and pancreatic cancers, as well as the initial development of a PSMA targeted minibody for imaging of advanced prostate cancer. Specifically, we will discuss the use of optical PSCA probes for intra-operative visualization of prostate cancer in order to eradicate more completely tumors and reduce margin positive rates. We will also discuss preclinical and early clinical development of both PSCA and PSMA minibodies for PET imaging of high-risk and metastatic prostate cancer, which offer the potential for both more sensitive and specific staging of disease as well as potential selection tools for therapies targeting these antigens. Finally, we will discuss the potential use of molecular imaging tools as both predictive and response biomarkers to guide novel therapies.

ABSTRACTS OF POSTERS

Notch3 signaling regulates musashi-1 expression in metastatic colorectal cancer cells

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MUSASHI-1 (MSI-1) is a well-established stem cell marker in both normal and malignant colon cells and it acts by positively regulating the NOTCH pathway through inactivation of NUMB, a NOTCH signaling repressor. To date, the mechanisms of regulation of MSI-1 levels remain largely unknown. Here, we investigated the regulation of MSI-1 by NOTCH signaling in colorectal cancer cell lines and in primary cultures of colorectal cancer metastases. Stimulation by the NOTCH ligand DLL4 was associated with an increase of MSI-1 mRNA and protein levels, and this phenomenon was prevented by the addition of an antibody neutralizing NOTCH2/3 but not NOTCH1. Moreover, forced expression of activated NOTCH3 increased MSI-1 levels, whereas silencing of NOTCH3 by short hairpin RNA reduced MSI-1 levels in both colorectal cancer cells and CRC tumor xenografts. Consistent with these findings, enforced NOTCH3 expression or stimulation by DLL4 increased levels of activated NOTCH1 in colorectal cell lines. Finally, treatment of colorectal cancer cells with anti-NOTCH2/3 antibody increased NUMB protein while significantly reducing formation of tumor cell spheroids.

This novel feed-forward circuit involving DLL4, NOTCH3, MSI-1, NUMB, and NOTCH1 may be relevant for regulation of NOTCH signaling in physiologic processes as well as in tumor development. With regard to therapeutic implications, NOTCH3-specific drugs could represent a valuable strategy to limit NOTCH signaling in the context of colorectal cancers overexpressing this receptor.

Mast cells – prostate cancer stem cells crosstalk protects against neuroendocrine tumor development

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Keywords: prostate cancer, stem cells, mast cells.

Prostate cancer is the second cause of death for neoplasia in men. Its incurable variant is characterized by hormone resistance and neuroendocrine (NE) differentiation. The origin of NE tumors is still poorly understood, however studies in murine models suggest that they arise from the NE differentiation and neoplastic transformation of prostate stem cells (PSCs).

We found that infiltrating mast cells (MCs) are crucially involved in prostate cancer development. Indeed, genetic or pharmacological ablation of MCs in TRAMP

mice, a murine model closely resembling the human pathology, restrained adenocarcinoma growth while, surprisingly, increased the incidence of NE variants. As both MCs and PSCs express the cKIT receptor, we hypothesize that the competition for cKIT ligand, SCF, can maintain PSCs homeostasis, thus protecting against the genesis of NE tumors. We confirmed cKIT expression by PSCs as well as by differentiated tumor cells in our model. We found an increased SCF signaling in PSCs and luminal cells isolated from tumors of Kit[Wsh]-TRAMP mice, genetically lacking mast cells. Moreover, while TRAMP PSCs differentiated *in vitro* towards a classical luminal phenotype, Kit[Wsh]-TRAMP PSCs were more prone to differentiate toward a NE phenotype. This supports our *in vivo* evidences that proliferating cells in early neoplastic lesions of Kit[Wsh]-TRAMP mice have a prevalent NE phenotype, while in lesions from TRAMP mice, as expected, only luminal cells are proliferating. We also performed parallel experiments with PSC cell lines isolated previously (Mazzoleni et al, SCTM, 2013) from TRAMP mice with intraepithelial neoplastic lesions (called TPIN) or NE tumors (called TNE). While co-culture with MCs does not alter TPIN growth or phenotype, it seems to inhibit the differentiation of TNE cells. Thus, MCs in the prostate microenvironment probably control PSC homeostasis, preventing their differentiation towards a NE phenotype.
In this scenario, MCs seems either beneficial or detrimental depending on the type of predominant prostate tumor. We are currently evaluating the effect of Imatinib, a multitarget agent also hitting cKIT, against MCs, PSCs and differentiated tumor cells, in TRAMP mice.

Cancer stem cells from epithelial ovarian cancer patients privilege oxidative phosphorylation, and resist glucose deprivation

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Objective: Ovarian cancer is the fourth leading cause of cancer-related death in women and the leading cause of gynecologic cancer death. Moreover, it is regarded a therapy resistant tumor, because it is often associated with more aggressive recurrence of the primary tumor as a result of chemotherapy. This chemo-resistance is thought to be related to the presence of the Cancer Stem Cells (CSCs). Tumor cells are characterized by a high glycolytic metabolism even in the presence of oxygen, the so-called Warburg effect; however, it is unclear whether this condition is also shared by Cancer Stem Cells.
Material and Methods: We identified ovarian cancer stem cells (CSC), according to the co-expression of CD44 and CD117 markers, in 40 samples of ascitic effusion from ovarian cancer-bearing patients. We have analyzed their phenotipic characteristics by investigating stemness marker expression in flow-cytometry, spheroid assay, tumorigenicity *in vivo* and gene expression by RT-PCR. For the analysis of metabolic characteristics, ovarian cancer cells were FACS-sorted into CD44^{+}CD117^{+} and CD44^{+}CD117^{-} cell populations and analyzed through specific metabolic gene-cards. Results were confirmed also through Western Blot for specific metabolic enzymes and functional assays of mitochondrial activity.
Results: CD44^{+}CD117^{+} EOC cells presented high tumorigenicity and expressed stemness-associated markers and multidrug resistance pumps.
CD44^{+}CD117^{+} cell population over-expressed genes associated with glucose uptake, oxidative phosphorylation (OXPHOS), and fatty acid b-oxidation, indicating higher ability to direct pyruvate towards the Krebs cycle. Consistent with a metabolic profile dominated by OXPHOS, the CD44^{+}CD117^{+} cells showed higher mitochondrial reactive oxygen species (ROS) production and elevated membrane

potential, and underwent apoptosis upon inhibition of the mitochondrial respiratory chain. The CSC also had a high rate of pentose phosphate pathway (PPP) activity, which is not typical of cells privileging OXPHOS over glycolysis, and may rather reflect the PPP role in recharging scavenging enzymes. Furthermore, CSC resisted *in vitro* and *in vivo* glucose deprivation, while maintaining their CSC phenotype and OXPHOS profile.
Conclusion: A subpopulation of CD44^{+}CD117^{+} EOC cells fulfilling the canonical properties of CSC does not preferentially exploit a glycolytic metabolism, privileging instead the mitochondrial respiratory pathway. These observations may explain the CSC resistance to anti-angiogenic therapies, and indicate this peculiar metabolic profile as a possible target of novel treatment strategies.

Dynamic Estrogen Receptor/ chromatin interactions and transcriptomics in paired clinical specimens reveals hallmarks for breast cancer patient survival.

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Background: Around 75% of all breast cancers express Estrogen Receptor alpha (ERα) and tumor cell proliferation is though to be dependent on this hormone-dependent transcription factor. These patients receive endocrine therapy, often in the form of tamoxifen, to block ERα action and prevent metastatic outgrowth. Yet, resistance is commonly observed, and patients may still develop a relapse despite treatment. Therefore, predictive biomarkers for response to tamoxifen are urgently needed.
Our objective is to investigate prospectively whether short-term endocrine therapy may yield biomarkers for long-term benefit. Tumor samples from breast cancer patients were isolated before and after 2-6 weeks of tamoxifen treatment, and molecular changes due to treatment were assessed. Three types of data were generated for both pre-treatment (biopsy) and post-treatment (resection) material. Firstly, immunohistochemical stainings were carried out on formalin-fixed paraffin embedded tumor tissue (FFPE) sectioned material for ERα, PR, HER2 and cell proliferation marker Ki67. Secondly, ERα chromatin binding profiles were determined by chromatin immunoprecipitation, followed by massive-parallel sequencing (ChIP-seq). Lastly, microarray expression profiles were generated from FFPE tissue. These datastreams were integrated bioinformatically, and mined for potential biomarkers on treatment outcome. ERα chromatin binding patterns were highly heterogenous between patients, which was synchronized by short-term pre-operative tamoxifen treatment. This synchronization of hormonal action was reminiscent of downstream gene expression profiles, in which post-treatment gene expression data was indicative for long-term treatment benefit. These data illustrate a dynamic nature of ERα action in clinical specimens, which was directly affected by tamoxifen exposure. While pre-treatment gene expression was highly heterogenous between patients, this could be synchronized by short-term tamoxifen action. With this, our data illustrates that biomarker discovery for outcome may be better feasible after short-term exposure to the drug, instead of before.

Gene-specific methylation profiles in male breast cancer.

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Background: Male breast cancer (MBC) is rare and poorly understood. Like female

breast cancer (FBC), MBC is highly sensitive to hormonal changes, and it is recognized as being primarily a hormone dependent malignancy, specifically related to hyper-estrogenism. Indeed, epidemiological characteristics of MBC suggest that it is similar to post-menopausal estrogen/progesteron receptors (ER/PR) positive FBC.

There is growing evidence that methylation plays an important role in breast cancer (BC) development and that characterization of tumor-specific methylation profiles may allow the identification of specific biomarkers to discriminate BC subtypes. Significant differences in tumor-associated gene methylation patterns have been related to ER/PR and HER2 status.

The contribution of DNA methylation and the precise targets of epigenetic alterations in MBC have not yet well investigated.

Materials and methods: Using candidate-gene approach, we performed promoter methylation analysis of a panel of 9 BC-related genes (*hTERT, ESR1, RASSF1, AR, BCL2, MYC, WNT1, BRCA1,* and *CHEK2*), in tumors and matched normal (blood) samples from 69 MBC patients. The analysis was performed by Pyrosequencing, a highly sensitive and reproducible method, which provides absolute quantitative information on bases at each CpG site analyzed. Methylation levels were determined by calculating the average of methylation for each gene. ANOVA and Kruskal-Wallis tests were used to identify significant differences in methylation levels: 1)between normal and tumor samples; 2) among tumors stratified according to relevant clinical-pathologic features, including *BRCA1/2* mutational status.

Results: Promoter methylation of the 9 genes analyzed was found in all 69 MBC cases. A gene specific variability in methylation levels was observed, with *MYC* showing the lowest and *CHEK2* the highest methylation level. Overall, methylation levels of each gene considered were higher in tumors than in the corresponding normal samples. In particular, significant differences emerged for *hTERT* (p<0.0001), *ESR1* (p<0.0001), *RASSF1* (p<0.0001), *MYC* (p<0.0001) and *WNT1* (p=0.047). Interestingly, high-methylation levels of *RASSF1* were significantly found in HER2+ (p=0.01), Grade 3 (p=0.007) and *BRCA1/2* mutation positive (p=0.008) tumors. On the other hand, high-methylation levels of *AR* were observed in *BRCA1/2* mutation negative (p=0.008) tumors.

Conclusion: Overall, our results indicate that alterations in gene methylation profiles are common in MBC and that tumor-associated gene methylation patterns may identify specific MBC subgroups.

Gene-specific methylation profiles in hormonally treated and untreated prostate cancer cases

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Introduction: DNA promoter hypermethylation is a frequent epigenetic event in prostate cancer (PCa) and many genes have been found as aberrantly methylated in PCa. Radical prostatectomy (RP) represents an effective option for PCa treatment. In selected cases, surgery may follow neoadjuvant androgen deprivation (AD) therapy. Castration-resistant PCa remains hormonally driven despite castrate levels of circulating androgens and portends a dismal outcome. The molecular mechanisms involved in castration-resistant PCa progression are still poorly understood. The aim of this study was to characterize DNA methylation profiles of PCa in a series of surgically resected samples, in order to identify possible differences related to AD therapy.

Materials and Methods: Using the candidate-gene approach, we performed promoter methylation analysis of a panel of genes involved in hormonal (*AR, ESR1, ESR2*) and tumor progression pathways (*RASSF1, APC, CD44, CDH1, BCL2*). A series of 48 PCa cases were retrospectively collected, 25 patients had surgery alone (untreated) while 23 received AD for 3 months before surgery (treated). Clinicopathologic data, including age, histology, Gleason score, stage and margin status, were recorded. Biomolecular analysis was performed by pyrosequencing and methylation levels were assessed by calculating the average of methylation for each gene. To determine microvessel density (MVD), specimens were immunostained for CD31 and LYVE-

1. Kruskal-Wallis test was used for statistical purposes.

Results: Aberrant promoter methylation of the 8 genes analyzed was found in all the 48 PCa cases. Significant differences in methylation levels between treated and untreated tumors emerged for *CD44* (p=0.015). A significant correlation between *CDH1* methylation and positive surgical margins (p=0.03) was also noted. Regarding MVD, methylation of both *BCL2* and *CD44* was associated with LYVE-1 overexpression (p=0.05 and p=0.01, respectively), while *RASSF1* methylation was associated with CD31 overexpression (p=0.03).

Conclusion: Overall, our results showed that the methylation profiles of the genes investigated do not significantly vary in relation to hormonal therapy. Contrariwise, we observed that high methylation levels in genes involved in tumor progression were significantly correlated with tumor characteristics suggestive of a more aggressive phenotype.

The Nampt inhibitor (FK866) induces translation arrest of in solid tumors.

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Abstract: A number of cancers show increased expression of Nicotinamide phosphoribosyl transferase (Nampt), a key enzyme in Nicotinamide adenine dinucleotide (NAD) salvage pathway. Moreover cancer cells show a high rate of NAD^+ consumption compared to normal cells and Nampt is essential for the survival of tumor cells. Pharmacological inhibition of Nampt by specific agent such as FK866, reduces viability in multiple types of cancer cells, but, whether pharmacological blockade of Nampt regulates translation arrest mediated cancer cell growth remains to be determined. Here we show that inhibition of Nampt by FK866 resulted in reduced viability of cancers through reduction of cellular NAD+ and ATP levels in multiple type of solid tumors. Reduced ATP levels caused activation of 5' AMP-activated protein kinase (AMPK), inhibition of the MTOR1/4EBP1 signaling and translational arrest. Interestingly cancer

cell lines showed differential sensitivity according to the level of the FK866 induced translational arrest as measured by 4EBP1 dephosphorylation. In particular, prostate androgen independent cancer cells (PC3) showed higher sensitivity than androgen-dependent cells (LNCaP) suggesting that the presence of AR may influence cell sensitivity to FK866. These results provide a novel molecular event, in which Nampt inhibition growth inhibitory effect are strictly connected to AMPK induced translation arrest in solid tumors.

Keywords: FK866, Nampt inhibitor, Translational arrest

In vivo molecular imaging during mammary tumor progression to identify new biomarkers useful for clinical applications.

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By using two mouse models engineered to express the luciferase gene in cells undergoing proliferation (MITO-Luc reporter mice) or in cells where the estrogen receptor is activated (ERE-Luc reporter mice) we have studied the dynamics of cell proliferation and estrogen receptor activity in mouse model of sporadic and genetic carcinogenesis of the mammary gland. The results of this study demonstrated that the evolution of the majority of tumorigenic processes of breast cancer in mice share common molecular steps in their progression. *In vivo* imaging, gave us the opportunity to exactly define when this molecular switch occurs and where (i.g. if other tissues participate to the process). Since this process occurs much before the tumour appearance, we have now the unprecedented opportunity to identify the timing of the very initial molecular changes responsible for tumour onset. With the purpose to identify clinical useful biomarkers for early diagnosis, our

major aim is now to characterise the expression of a specific miRNA profile, in breast tissues and in serum, in the transformation process stages identified through in vivo imaging.

Genome-Wide Analysis of endometrial cancer Ishikawa cells: comparison to breast cancer T47D cells reveals cell-type specific mechanisms in response to progesterone and estradiol

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Progesterone (Pg) acting through the progesterone receptor (PR) is a major determinant in the development and progression of breast and endometrial pathologies. Though PR gene expression mechanisms are well described in breast cancer cells, it is still not fully understood how PR affects downstream signalling pathways and cross-talks with other intracellular signal transduction pathways in endometrial carcinoma cells. We aim to study the role of PR in cell-type specific response to Pg comparing human endometrial Ishikawa cells and the well characterized human breast cancer T47D cells. Genome-wide ChIP-seq analysis of untreated (T0) and 5 (R5), 30 (R30) and 60 (R60) min R5020 10 nM-treated Ishikawa cells revealed 256 PR binding sites (PRbs) of which the majority were located in proximal promoter regions (>=1 kb) of both treated and untreated cells. Strikingly, recent work in T47D cells (Ballaré et.al., 2013) shows substantially more PRbs than Ishikawa cells. Of the 256 sites, 181 sites were already present in untreated cells and only 55 sites only occurred in treated cells (14 in R30, 17 in R60 and 24 in both). Although none of the PRbs contained PREs, DNA motif analysis evidenced binding sites for many transcription factors (GATA, SP and ELF). Global gene expression analysis in response to 12hs R5020 or estradiol (E2) was performed with RNA-seq technology to identify 1287 genes regulated by R5020 and 506 genes by E2. Remarkably, R5020 modulates 2.25 times more genes than E2 and nearly 40% of E2-regulated genes are also responsive to R5020. Taken together, these results suggest that the genomic role of PR in regulating chromatin remodeling and gene expression takes place mostly through sites occupied by the receptor beforehand and that R5020 would have a stronger impact on gene regulation than E2 in Ishikawa cells. Tissue-specific and common patterns of genome-wide PR binding and gene regulation may determine the therapeutic effects of antiprogestins in uterine pathologies and breast cancer.
Key words: endometrium, Ishikawa, progesterone receptor, genome-wide

Combined TNFα, 17-β estradiol and Doxorubicin treatment of MCF7 cells uncovers cooperative interactions among p53, Estrogen Receptors and NFκB resulting in enhanced cell plasticity.

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Cellular responses to changes in the microenvironment often involve modulation of large transcriptional networks by sequence-specific master regulators. For breast epithelium, Estrogen Receptors (ERs), NFκB and p53 family of transcription factors exert critical functions and in part opposing functions. Alteration of various components of the associated signaling pathways and transcriptional regulatory proteins is associated and contributes to the development of cancer.

We addressed the transcriptional cooperation among those three transcription factors by expression microarrays. Human breast adenocarcinoma-derived MCF7 cells (luminal-type) were exposed to single or combinatorial treatments with the chemotherapeutic agent Doxorubicin (Doxo – able to stabilize the p53 protein), the ER ligand 17ß-estradiol (E2) and the NFκB inducer Tumor Necrosis Factor alpha (TNFα). Nearly 200 differentially expressed genes (DEGs) were identified that showed limited responsiveness to either Doxo treatment or ER ligand alone but were up-regulated in a greater than additive manner following combined treatment. Among 16 genes chosen for validation by qPCR and ChIP assays, seven (INPP5D, TLR5, KRT15, EPHA2, GDNF, NOTCH1, SOX9) were confirmed to be novel direct targets of p53 or cooperative targets of p53 and ER. 239 up-regulated and 161 repressed genes were instead synergistically regulated by the Doxo+TNFα double treatment. The addition of E2 to Doxo+TNFα resulted only in 26 selective DEGs. Transcriptome data were confirmed for 12 of 15 selected Doxo+TNFα DEGs and for seven (PLK3, LAMP3, ETV7, UNC5B, NTN1, DUSP5, SNAI1) the responsiveness was shown to depend on both p53 and NFκB. Gene ontology of Doxo+TNFα up-regulated DEGs showed enrichment for cell migration terms. Indeed, migration assays showed that the double treatment could increase the motility of MCF7 cells. Finally, a signature of 29 Doxo+TNFα highly synergistic DEGs exhibited prognostic value for luminal ER positive breast cancer patients, with adverse outcome correlating with their higher relative expression. We propose that the crosstalk between p53, ER and NFκB can lead to the activation of specific gene expression programs that may impact on cancer phenotypes and potentially modify the efficacy of cancer therapy.

In-silico identification of enhancers bound by Androgen Receptor reveals allele dependent activity

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Background: Knowledge of transcriptional regulators has increased in the last decade improving our understanding of gene expression regulation. Androgen Receptor (AR) and Estrogen Receptors (ERs) are key nuclear receptors and their role has been extensively studied also in the context of cancer development.
Aim: To identify enhancer elements responsive to AR or ER that demonstrate differential activity based on overlapping/nearby SNP.
Material and Methods: Data from ENCODE were computationally mined to identify genomic loci with the following characteristics i) chromatin signature of enhancer activity (H3K4m1, H3K4me1+H3K4me3) ii) binding by ER and AR iii) presence of a SNP. Selected loci were then validated and characterized *in vitro* by gene reporter assay with or without Dihydrotestosterone treatment in transiently transfected MCF7 and PC3 cells. Plasmids harboring the alternative alleles of the selected enhancer elements were utilized. Chromatin immunoprecipitation assays (ChIP) with AR antibody, followed by real-time PCR and sequencing analysis was performed.
Results: Forty-one loci were identified computationally and two (on 1q21.3 and 13q34) selected for *in vitro* characterization. Both regions (~1000nt in length) exhibited enhancer activity regulated by ligand-bound AR (p<0.05). The SNP variant on 1q21.3, rs2242193 (CEU Minor Allele Frequency =0.376), had an impact in the transcription regulation (p=0.028, Student's t-test) and was enriched in chromatin fragments immunoprecipitated with AR antibody. Sequencing analysis showed that AR was preferentially recruited to the A allele of the SNP rs2242193 (p-value < 0.05) confirming the results of the luciferase assay.
Conclusion: Unbiased genome-wide search proved to be an efficient methodology to discover new functional *cis*-elements. The broad coverage of ENCODE annotations allowed us for a robust investigation of the impact that SNPs have in *cis*-regulatory sequences.

Modulation of angiogenesis-related molecules by metformin on hormone-dependent breast cancer cells

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The anti-diabetic drug metformin, commonly used for treatment of type 2 diabetic patients, has gained significant attention as a potential cancer preventive agent. Here we show the effects of metformin on hormone-sensitive breast cancer cells. We analyzed expression levels of angiogenesis-associated proteins in MCF7 breast cancer cells and human umbelical vein endothelial cells (HUVEC) by Antibody array analysis. We show an opposite regulation of several mediators, including interleukin-8, angiogenin and TIMP-1 by metformin in breast cancer and endothelial cells. However, tumor cells supernatants, harvested from both breast and prostate cancer cells, upregulate endothelial cell production of the pro-angiogenic cytochrome P450 member CYP1B1 in an AMPK-dependent manner; metformin blocks this effect. Given recent findings showing conflicting results on the antiangiogenic action of metformin, we further analyzed metformin effects on endothelial cells and angiogenesis using in vitro and in vivo assays. Metformin interferes with endothelial cell cycle regulation, represses matrigel invasion *in vitro* and abrogates endothelial cells ability to organize into capillary-like networks, partially involving the energy sensor adenosine-monophosphate-activated protein kinase (AMPK). Gene expression profiling of human endothelial cells treated with metformin revealed a paradoxical short-term induction of pro-angiogenic mediators such as vascular endothelial growth factor, cyclooxygenase 2 and CXCR4 and downregulation of ADAMTS1. In vivo, metformin reduces angiogenesis in matrigel pellets and prevents the microvessel density increase observed in obese mice on a high-fat diet, downregulating the number of white adipose tissue endothelial precursor cells. Altogether, we observed a differential regulation of pro-angiogenic mediators in endothelial and breast cancer cells. Interestingly, metformin exerts an antiangiogenic activity *in vitro* and *in vivo* associated with a contradictory short-term enhancement of pro-angiogenic mediators.

Call for 2015 Pezcoller Foundation-AACR International Award for Cancer Research

The prestigious Pezcoller Foundation–AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;
- who continues to be active in cancer research and has a record of recent, note-worthy publications;
- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.

Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now have over 33,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 97 other countries around the world.

The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organiza-tions are now collaborating annually on the presentation of the Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 5-9, 2014) in San Diego, CA and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May 9, 2014). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline:
September 12, 2013
Questions about the nomination process:
Monique P. Eversley, M.S., Senior Coordinator, Scientific Achievement Awards
American Association for Cancer Research, 17th Floor, 615 Chestnut Street, Philadelphia, PA 19106-4404
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