IFOM-IEO Campus, Milan

International Workshop on

Cancer Stem Cells

10TH/12TH NOVEMBER 2005

SCIENTIFIC & ORGANIZING COMMITTEE

Bruno Amati, Luisa Lanfrancone, Saverio Minucci, Pier Giuseppe Pelicci
IFOM-IEO Campus - Milan, Italy

EVENTS COORDINATOR

Sabrina Frata
SEMM Foundation, Milan, Italy

Dear Colleague,

It is a pleasure for me to welcome you in Milan at the **International Workshop on Cancer Stem Cells**, supported and organized by the **European School of Molecular Medicine (SEMM)**.

The mission of SEMM is to foster the development of molecular medicine and associated technologies in Italy, through the promotion of world-class research, training and mobility.

Among its various activities, SEMM promotes a number of events every year on different aspects of Molecular Medicine. These include **seminars, workshops** and **international conferences**. These activities will contribute to the creation of a net in which researchers can acquire and distribute scientific culture.

This International Workshop on Cancer Stem Cells will bring together experts presenting the most innovative aspects of Stem Cell biology in tumors of major clinical relevance, including Leukemia, Breast Cancer, Lung Cancer, Melanoma and Glioblastoma.

The goal of the workshop is to offer to the scientific community a sharply focused arena of discussion and sharing of information on a rapidly evolving field in tumor biology, contributing to a better shaping of the key concepts and basic questions that remain to be addressed.

Milan has a strong tradition of being a venue for innovation on key health issues, in which this workshop finds a central place.

I wholeheartedly welcome you to Milan and wish you an enjoyable and inspiring workshop.

Umberto Veronesi
Chairman
SEMM Foundation

TABLE OF CONTENTS

SCIENTIFIC PROGRAM

Afternoon session - LEUKEMIA

h. 12.00/14.00	Registration and poster positioning
h. 14.20/15.00	**STUART ORKIN,** Boston, MA, USA *"Stem Cells and Oncogenesis"*
h. 15.00/15.15	**MYRIAM ALCALAY**, Milan, Italy *"Gene Expression Signature of Leukemic Stem Cells in Models of Acute Myeloid Leukemia"*
h. 15.15/15.30	**ZHENG XIAOMIN**, Frankfurt, Germany *"PML/RARa Blocks All-Tran Retinoic Acid Induced Differentiation in a Subset of Cells with Stem Cell Capacity"*
h. 15.30/16.00	coffee break
h. 16.00/16.40	**MALCOLM K. BRENNER**, Houston, USA *"SP Cells as Tumor Progenitors"*
h. 16.40/17.20	**CRAIG JORDAN**, Rochester, New York, USA *"Characterization of Mechanisms Controlling Survival of Human Leukemia Stem Cells"*
h. 17.20/17.35	**ANDREA VIALE**, Milan, Italy *"Role of Quiescent Stem Cells in the Maintenance of Leukaemia Cell Populations"*
h. 17.35/17.50	**WILLIAM MATSUI**, Baltimore, USA *"Regulation of Tumor Stem Cells in Multiple Myeloma by Hedgehog Pathway Signaling"*
h. 17.50/18.30	**Panel Discussion**
h. 18.30	Aperitif
	End of the session
	Night at disposal

Morning Session - BREAST CANCER

h. 08.30/09.10	**ALLAN BALMAIN**, S. Francisco, USA *"Target Cells and Target Genes in Skin Tumor Development"*
h. 09.10/09.50	**JEFFREY ROSEN**, Houston, USA *"Stem/Progenitor Cells in the Etiology and Treatment of Breast Cancer"*
h. 09.50/10.05	**GIUSEPPINA BONIZZI**, Milan, Italy *"Biological Characterization of Mouse Breast Stem Cells from Normal and Neoplastic Tissues"*
h. 10.05/10.20	**BIKUL DAS**, Toronto, Canada *"The Idea and Evidence on Tumor "Stemness Switch"*
h. 10.20/10.50	coffee break
h. 10.50/11.30	**ROBERT B. CLARKE**, Manchester, UK *"Isolation and Characterization of Human Mammary Stem Cells"*
h. 11.30/12.10	**KAY-UWE WAGNER**, Omaha, US *"The Biology of Multipotent, Parity-induced Mammary Epithelial Progenitors"*
h. 12.10/12.25	**CHIARA CAPELLI**, Milan, Italy *"Biofunctional Characterization of Human Breast Cancer-Initiating Cells Propagated in Vitro"*
h. 12.25/12.40	**SALVATORE PECE**, Milan, Italy *"Molecular Portraiting of Normal and Tumor Human Breast Stem Cells"*
h. 12.40/14.00	lunch

Afternoon Session - LUNG CANCER, MELANOMA AND GLIOBLASTOMA

h. 14.00/14.40	**MEENHAR HERLYN**, Philadelphia, USA *"Melanocyte and Melanoma Stem Cells"*
h. 14.40/15.20	**YANN BARRANDON**, Lausanne, Switzerland *"Stem Cells of Stratified Epithelia"*
h. 15.20/15.35	**ARIEL RUIZ I ALTABA**, Geneva, Switzerland *"Hedgehog-GLI Signaling in Cancer, Stem Cells and Cancer Stem Cells"*
h. 15.35/15.50	**GAETANO FINOCCHIARO**, Milan, Italy *"Neurospheres from Glioblastoma Multiforme are Enriched of Cells with Cancer Stem-Like Properties"*
h. 15.50/16.20	coffee break
h. 16.20/17.30	**Poster Session**
h. 17.30/18.10	**ANGELO VESCOVI**, Milan, Italy *"Regulation of Human Tumor Neural Stem Cells"*
h. 18.10/18.50	**CARLA F. BENDER KIM**, Cambridge, MA, USA *"Identification of Bronchio-Alveolar Stem Cells in Normal Lung and Lung Cancer"*
h. 18.50/19.05	**CORRINA KANE**, London, UK *"Side Population (SP) Cells Derived from Prostate Cell Lines"*
h. 19.05/19.20	**RICCARDO FODDE**, Rotterdam, the Netherlands *"A Role for Wnt/Beta-Catenin Signaling in Establishing Intestinal and Mammary Cancer Stem Cells"*
	End of the day
h. 20.30	**Social Dinner**

Morning Session - MOLECULAR MECHANISMS OF SELF RENEWAL

h. 08.30/09.10	**SEAN J. MORRISON**, Ann Arbor, Michigan, USA *"Stem Cell Self-Renewal and Cancer Cell Proliferation"*
h. 09.10/09.50	**STEPHEN DALTON**, Athens, Georgia, USA *"Self-Renewal of Embryonic Stem Cells"*
h. 09.50/10.05	**GABRIEL GUTIERREZ**, Boston, USA *"The Role of RB in Differentiation and the Post-Mitotic State in Bone"*
h. 10.05/10.20	**SOPHIA BRUGGEMAN**, Amsterdam, The Netherlands *"The Role of the Polycomb Group Gene Bmi1 in CNS (Stem) Cells and Cancer"*
h. 10.20/10.40	coffee break
h. 10.40/11.20	**ANDREAS TRUMPP**, Epalinges, Switzerland *EMBO Young Investigator Lecture*
h. 11.20/12.00	**SALVADOR AZNAR BENITAH**, London, UK *"Role of Rac1 in Skin Homeostasis and Cancer"*
h. 12.00/12.15	**ANTONIO COSTANZO**, Rome, Italy *"A Role for the p63-IKKalpha Pathway in the Regulation of Epidermal Stem Cells Fate"*
h. 12.15/12.30	**IGNACIO MORENO DE ALBORN**, Madrid, Spain *"c-Myc Function in Hematopoietic Stem Cells"*
h. 12.30/13.30	**Consensus/Discussion**
h. 13.30	Light Lunch
	END OF THE MEETING

NOVEMBER 10, 2005 - ABSTRACTS

ORAL PRESENTATIONS

LECTURES

Stuart Orkin
Boston, US

Michael K. Brenner
Houston, US

Craig Jordan
Rochester, US

ORAL PRESENTATIONS

Myriam Alcalay
Milan, Italy

Xiaomin Zheng
Frankfurt, Germany

Andrea Viale
Milan, Italy

William Matsui
Baltimore, US

Session 1:
Leukemia

Gene Expression Signature Of Leukemic Stem Cells In Models Of Acute Myeloid Leukemia

Gaia Scafetta*, Silvia Licciulli*, Valeria Cambiaghi, Elisa Venturini, Roberta Bergomas, Andrea Viale, Pier Giuseppe Pelicci & Myriam Alcalay.

**authors contribute equally to this work IFOM: FIRC Institute of Molecular Oncology, and IEO: European Institute of Oncology, Milan.*

Acute myeloid leukemia (AML) is characterized by the clonal expansion of myeloid precursors with increased proliferative potential and decreased capacity to differentiate into mature leukocytes. The pathogenesis of AML is linked to oncogenic fusion proteins, which are generated as a consequence of primary chromosome aberrations, and function as aberrant transcriptional regulators that interfere with myeloid differentiation, determine a stage-specific arrest of maturation and enhance survival in a cell-type specific manner. Among the most frequent chromosomal translocations in AML are the t(8;21) and the t(15;17); the translocation products encode for the AML1/ETO and PML/RAR fusion proteins, respectively.The precise nature of the hematopoietic precursor cell targeted by the leukemogenic event is not known, although several investigations indicate that transformation occurs in very early progenitors, probably in hematopoietic stem cells (HSC). There is growing interest in the role of HSC in the development of hematopoietic malignancies and in the identification of leukemic stem cells (LSC), which are believed to represent a reservoir of leukemic cells with a low proliferative rate and the ability to self-renew. To date, the isolation of LSCs is not straightforward. We have started a series of investigations aimed at characterizing the molecular signature of LSC and identifying specific markers for disease initiation and progression. Using Affymetrix technology, we have obtained gene expression profiles of selected cellular subpopulations purified from the bone marrows of normal, pre-leukemic and leukemic mice (AML1/ETO and PML/RAR transgenic). We have thus identified a set of putative stem cell regulators that are abnormally expressed in leukemia, performed functional classification, and are currently validating the expression levels of selected candidate genes that will be further investigated through functional studies.

PML/RARa Blocks All-Tran Retinoic Acid Induced Differentiation In A Subset Of Cells With Stem Cell Capacity

Xiaomin Zheng, Anita Seshire, Elena Puccetti, Hilal Gul, Tim Beissert, Dieter Hoelzer, Oliver G. Ottmann, Reinhard Henschler, Martin Ruthardt.

Department of Hematology, J.W. Goethe University, Frankfurt, Germany; Department of Transfusion Medicine and Immunhematology, J.W. Goethe University, Frankfurt, Germany

Acute promyelocytic leukemia (APL) is distinguished from other AMLs by cytogenetic, clinical, as well as biological characteristics. The hallmark of APL is the t(15;17) which leads to the expression of the PML/RAR fusion protein. PML/RAR is the central leukemia-inducing lesion in APL and is directly targeted by all trans retinoic acid (t-RA). Patients suffering from APL undergo complete hematologic but not molecular remission upon treatment with t-RA. Virtually all patients treated with t-RA-monotherapy had a rapid relapse within few months. But in the combination with an anthracycline, such as doxorubicin or idarubicin, t-RA improved the long term outcome of APL-patients dramatically. Nothing is known about why t-RA-monotherapy is unable to eradicate completely the leukemic population and how it increases the response to chemotherapy. Here we report that i) the NB4 cells engrafted in NOD/SCID mice indicating the presence of a subpopulation with stem cell capacity in NB4 cells; NB4 had a Hoechst 3342 excluding side population (SP) representing about 1% of the whole cell population; t-RA reduced but did not deplete the side population in NB4 cells; ii) the expression of PML/RAR increased CD34+/CD38- population in KG-1 cells from 75% to over 95%; t-RA reduced the CD34+/CD38- population from 75% to 3,5% in mock transfected KG-1 confirming its capacity to induce differentiation, whereas in PML/RAR-positive KG-1 cells it led only to a reduction from 98% to a 25%, which still maintain the capacity to engraft in NOD-SCID mice; also the expression of other fusion proteins, such as AML-1/ETO or PLZF/RAR, associated with t-RA-resistant AML-subtypes, increased the percentage of CD34+/CD38- KG-1 cells over 90%, which was reduced by t-RA only to 35% and 19%, respectively; iii) Atra did not reduce the replating efficiency in PML/RARa,PLZF/RARa and AML1/ETO infected Sca1+lin- cells; Atra removed differentiating and migrating colonies while it maintained small compacted ones representing stem/progenitor cells; iv) Sca1+lin-cells infected with PML/RARa cells demonstrated colonies of day-13 colony forming units-Spleen, t-RA treatment did not reduce number of day 13 CFU-S iv)4th plated Sca1+lin- cells expressing PML/RARa showed day 13 colony forming units as well and the engraftment in spleen was confirmed by RT-PCRTaken together these data suggest that a subset of early HSC expressing PML/RAR exhibit the same t-RA-resistant phenotype as HSC expressing fusion proteins associated with AML-subtypes which, in contrast to APL, do not respond to t-RA. These data may give an explanation, why APL-patients do not achieve complete molecular remission upon t-RA monotherapy and undergo early relapse.

Role of Quiescent Stem Cells In The Maintenance Of Leukaemia Cell Populations

Andrea Viale[1], Francesca De Franco[1], Annett Orleth[2], Valeria Cabiaghi[1], Simona Ronzoni[1], Saverio Minucci[1], Myram Alcalay[1,3], Pier Giuseppe Pelicci[1,3].

[1] Department of Experimental Oncology, European Institute of Oncology, Milan, - [2] University of Perugia, Policlinico Monteluce, Laboratory of Molecular Biology, Perugia, - 3 FIRC Institute of Molecular Oncology (IFOM), Milan

Stem/progenitor cells ensure for tissue and organism homeostasis and might represent a frequent target of transformation. Emerging concepts in tumor biology support the notion that tumors are hierarchically organized as abnormal tissues which originate from, and are maintained by, transformed stem cells. Stem cells are relatively quiescent, while their more differentiated progeny have dramatic proliferative ability. The quiescence of stem cells is thought to be of critical biologic importance to prevent susceptibility to myelotoxic insults and consumption of the regenerative cell pool. In the absence of the cell cycle inhibitor p21, increased cell cycle leads to hematopoietic stem cell exhaustion and, under stress conditions (serial transplantation or myelotoxic treatments) haematopoietic cell depletion. We are investigating the existence, in leukemia animal models, of quiescent leukemic stem cells (LSC) and their role in the maintenance of the leukemic clone. We found that leukemic fusion proteins (AML1/ETO, PML/RAR, PLZF/RAR) induce upregulation of p21 expression. Strikingly, the fusion protein AML1/ETO is unable to induce leukaemia in p21-/- mice and PML-RAR leukemias engineered into a p21-/- background fail to transplant into syngeneic animals. Ectopic expression of p21 into WT hematopoietic stem cells induces the expansion of the stem cell compartment, as evaluated by the long-term culture initiating-cell (LTC-IC) assay. Using a membrane stable fluorescent dye (PKH), we isolated a quiescent/slow-cycling leukemic cell population and showed that these cells have a stem cell phenotype and are able to transplant leukaemia. These data suggest that quiescient stem cells are pivotal in maintaining the leukaemia population.

Regulation Of Tumor Stem Cells In Multiple Myeloma By Hedgehog Pathway Signaling

DN Watkins, CD Peacock, Q Wang, R Jones, K McGovern*, W Matsui.

*Sidney Kimmel Comprehensive Cancer Center, John Hopkins University School of Medicine, Baltimore, MD 21231, *Infinity Pharmaceuticals, Inc., Cambridge, MA 02139*

Relapse and disease progression are inevitable for the vast majority of patients with multiple myeloma (MM) even following the induction of complete remissions. It is likely that tumor stem cells (TSC) are responsible for this phenomenon, and we recently demonstrated that clonogenic cells in MM phenotypically resemble B cells that have self-renewal potential and give rise to terminally differentiated plasma cells (PC). The hedgehog (Hh) signaling pathway has been implicated in a variety of human cancers. Moreover, its role in normal development suggests that Hh may specifically regulate the fate of TSC, but the lack of identifiable TSC in these systems has not allowed this hypothesis to be tested. We found that both MM cell lines and primary clinical samples expressed components of the Hh signaling pathway, with highest expression in MM TSC. In addition, in vitro treatment with the specific Hh antagonist, cyclopamine, resulted in PC differentiation coupled with the loss of clonogenic potential. Hh appears to regulate the self-renewal of TSC in MM, and pathway inhibition may represent a novel strategy to target MM TSC.

NOVEMBER 11, 2005 - ABSTRACTS

ORAL PRESENTATIONS

LECTURES

Allan Balmain
S. Francisco, US

Jeffrey Rosen
Houston, US

Robert B. Clarke
Manchester, UK

Kay-Uwe Wagner
Omaha, US

Meenhard Herlyn
Philadelphia, US

Yann Barrandon
Lausanne, Switzerland

Angelo Vescovi
Milan, Italy

Carla F. Bender Kim
Cambridge, MA, US

ORAL PRESENTATIONS

Giuseppina Bonizzi
Milan, Italy

Bikul Das
Toronto, Canada

Chiara Capelli
Milan, Italy

Salvatore Pece
Milan, Italy

Ariel Ruiz I Altaba
Geneva, Switzerland

Gaetano Finocchiaro
Milan, Italy

Corrina Kane
London, UK

Riccardo Fodde
Rotterdam, The Netherlands

Session 2:
Breast Cancer
Session 3:
Lung Cancer, Melanoma and Glioblastoma

Biological Characterization Of Mouse Breast Stem Cells From Normal And Neoplastic Tissues

Bonizzi G.[1], Cicalese A.[1], Giulini B.[1,2], Pesce S.[1], Gobbi A.[1,2], Pece S.[1], Di Fiore PP[1,2]. and Pelicci PG[1,2].

[1] Department of Experimental Oncology, European Institute of Oncology, Milan - [2] FIRC Institute of Molecular Oncology (IFOM), Milan

Recent findings suggest that stem/progenitor cells are frequent targets of transformation and that different tumors contain a small subset of cells endowed with the property of supporting tumor growth (cancer stem cells). We are currently investigating the biological properties of normal and transformed breast stem cells. We set-uo protocols for the in vitro propagation of breast stem cells from the mouse mammary gland (BSCs), based on their ability to survive in suspension as 'mammospheres', and to differentiate into myoepithelial and epithelial cells. Murine mammospheres do not result from passive cell aggregation and have clonal origin, as assessed by labelling cell membranes with different epifluorescent dyes. To prove the "staminality" of these cellular populations we performed in vivo reconstitution experiments, by inoculating single cell suspensions of mammospheres in the mouse cleared fat pad. Using the same experimental approach, we have generated mammospheres from adenocarcinomas of MMTV-ErbB2(cNeu) transgenic mice. The mammospheres arising from MMTV-ErbB2 tumors, as compared to matched WT samples, are bigger (1000 vs. 200-400 cells/sphere) and show a dramatically prolonged lifespan upon serial passages. To prove that cancer mammospheres are enriched in cancer BSC, we are currently performing in vivo tissue reconstitution experiments.

The Idea And Evidence On Tumor “Stemness Swith”

Bikul Das[1,3], Rika Tsuchida[3], Sylvain Baruchel[1,3], Herman Yeger[2,4]

Division of Hematology & Oncology[1], Department of Pediatric Laboratory Medicine[2], Hospital for Sick Children, Toronto, Department of Laboratory Medicine and Pathobiology[3], and the Institute of Medical Sciences [4], University of Toronto, Canada.

Recently, in a neuroblastoma model of tumor hypoxia, we have reported a hypoxia-driven internal autocrine loop between VEGF and HIF-1_. The autocrine loop was required for hypoxia-driven drug resistance and angiogenesis process [Das B et al Cancer Research, 65 (15), 2005)]. Earlier, we reported that hypoxia increases tumor side population cells in several embryonal tumors including neuroblastoma, osteosarcoma, and rhabomyosarcoma (Das B et al. abstract, 3rd annual meeting, ISSCR, San Francisco, 2005). Recent investigations suggest that tumor side-population cells may be a general source of cancer stem cell. Here, we further investigated the drug-resistance and tumorigenic activity of tumor SP and non-SP cells in a hypoxic setup. Side-Population (SP) and non-side population (non-SP) cells fraction of several embryonal tumor cell lines, including neuroblastoma, medulloblastoma, astrocytoma, teratocarcinoma, rhabdomyosarcoma, and osteosarcoma were isolated by FACS sorting. The SP cell fraction showed increased resistance to multiple drugs compared to non-SP cells in all the tumor cell line tested. However, exposure to hypoxia increased the drug resistance of non-SP cells as well. In a mouse tumor xenograft assay, the SP cells showed significantly increased tumorogenic activity as compared to non-SP cell. About 5x105 SP cells were able to form tumor xenografts, whereas non-SP fraction did not form tumors. We then investigated the tumorigenic potential of SP and non-SP cells after exposure to hypoxia in a neuroblastoma cell line, SKNBE-2. Both SP and non-SP cells obtained from SKNBE-2 were first allowed to invade in a Boyden Chamber assay against an “ injurious bone marrow stromal cell” conditioned media (human bone marrow stromal cells were exposed to 0.5mM H2O2 for 24hr. the surviving cells were washed twice and then allowed to grow in fresh, serum free media for next 3 days. The media was collected and used for the invasion assay). The invading cells were collected and allowed to grow in a serum free media containing EGF and bFGF. When cell number reached approximately 1x105, cells were exposed to 24hr hypoxia, and subsequently injected s.c. into nude/nude mouse. We found that the non-SP cells were also able to form aggressive tumor similar to SP cells. We also obtained similar result from a rhabdomyosarcoma cell line, RH4. Subsequent investigations revealed that a certain fraction of non-SP cells become SP cells after exposure to hypoxia. Most importantly, we found that hypoxia increases several “stemness” associated genes, nanog, Oct-4, CXCR4, c-kit and MELK in SP as well as non-SP populations isolated from the Boyden chamber assay. In conclusion, we found that non-SP population may become tumorigenic when exposed to hypoxia. Our results suggest that non-tumor stem cells may also be transformed into stem cell like cells depending upon the tumor microenvironment. Here we propose that similar to angiogenic switch, a “stemness switch” may make a tumor more aggressive, drug resistant and highly metastatic. Tumor hypoxia may be associated with this “stemness switch”.

Biofunctional Characterization Of Human Breast Cancer-Initiating Cells Propagated In Vitro

Dario Ponti, Chiara Capelli, Manuela Gariboldi, James F. Reid, Maria Grazia Daidone, Marco A. Pierotti

Breast cancer-initiating cells (BC-ICs) have been prospectively identified in breast carcinoma as CD44+/CD24-/low cells, which exclusively retain tumor-initiating ability and display stem cell-like properties. We recently propagated in vitro as non adherent mammospheres cells isolated from human breast cancer lesions and from the MCF-7 cell line (Ponti D et al., Cancer Res, 65:5506-11, 2005). These breast carcinoma-derived cultures encompassed undifferentiated cells capable to self-renew, to proliferate extensively as clonal non-adherent spherical clusters and to differentiate along different mammary epithelial lineages (luminal and myoepithelial), were CD44+/CD24- and Cx43-, overexpressed neoangiogenic and cytoprotective factors and the putative stem cell marker Oct-4, and were tumorigenic when injected as few as 1000 cells into SCID mice. To investigate the molecular signature of BC-ICs, we compared the gene expression profiles of BC-ICs (3 derived from breast cancers, 1 from the MCF7 cell line) with the gene expression profile of MCF7 cells, using Affymetrix technology. Unsupervised hierarchical clustering grouped BC-ICs separately from MCF7 cells. Genes up-regulated in BC-ICs involved pathways governing self-renewal and metastasis (i.e.: Wnt, Hedgehog, Sparc, MMPs), ECM components, signaling of mitogen-activated protein kinases and focal adhesion kinases. BC-ICs might represent a potentially valid experimental model for identifying diagnostic/prognostic markers and new druggable targets.

Molecular Portraiting Of Normal And Tumor Human Breast Stem Cells

[1]Pece S., [1]Pasini S., [2]Confalonieri S., [2]Vecchi M., [1]Bonizzi G., [1]Nwachukwu J., [1]Matera G., [1,2]Ronzoni S., [1,2]Pelicci P.G., and [1,2]Di Fiore P.P.

[1]Department of Experimental Oncology, European Institute of Oncology (IEO), and [2]FIRC Institute for Molecular Oncology (IFOM), Milan, Italy

Substantial evidence indicates that subversion of molecular mechanisms controlling stem cell homeostasis plays a key role in the pathogenesis of some cancers, including breast cancer. Therefore, a crucial contribution to our understanding of tumorigenesis will derive from expanding knowledge on developmental biology of stem cell systems. Over the past few years, adult breast stem cells have been the focus of intense research. However, investigations on breast stem cell biology have been hampered by the scarcity of stem cells in tissues and the lack of markers exclusively expressed on stem cells as opposed to early progenitor cells. For the human breast, isolation of stem cells using antibodies to cell surface proteins or Hoechst dye efflux have proven only partially successful to obtain sufficiently pure stem cell populations to be thoroughly genetically and functionally characterized. To circumvent these technical limitations, we devised a strategy based on the newly described technique for the prospective isolation of stem cells ex vivo, which exploits their ability to generate clonally-derived tridimensional 'mammospheres' in suspension, in combination with the use of a 'surrogate' marker, the red fluorescent cell linker PKH26, which stably incorporates a fluorescent dye into the lipid regions of the plasma cell membrane. The strength of this approach is that normal and tumor stem cells are not defined phenotypically, i..e. using cell surface markers, but functionally through their ability to form mammospheres in vitro and their intrinsic property to be slow-dividing. In fact, the expectation is that stem cells, undergoing a very limited number of divisions, should remain the most intensively fluorescent within the context of mammospheres, while their actively dividing and differentiating progeny towards the periphery of the mammospheres should loose fluorescence through dilution of the membrane-bound dye. This approach has enabled us to physically separate stem cells from their differentiating progeny of committed progenitor cells. In fact, based on the number of cells able to form mammospheres from dissociated tissues and in serial passages, we sorted and enriched the top 0.1-0.5% most highly fluorescent cells by FACS. Functional analysis of the different normal PKH26+ and PKH26- populations confirmed that only the former was endowed with the key defining features of 'stemness', such as: i) self-renewal property, assessed by the reformation of non-adherent mammospheres upon serial passages in vitro; ii) ability to differentiate into epithelial and myoepithelial histotypes under attachment conditions, as witnessed by staining with specific lineage markers; iii) formation of alveolar/ductal-like outgrowths in vitro (3D matrigel). The availability of sufficient quantities of pure fractions of stem and progenitor cells enabled us to perform global gene expression analyses. Using oligonucleotide-based arrays (Affymetrix), we obtained trascriptional profiles of two populations separated by their differential epifluorescence over a 3 log intensity scale. The comparative analysis of genes differentially regulated by a factor ≥2 uncovered many distinguishing features between the two PKH26 populations, strenghtening the notion that we indeed separated stem cells from their differentiating progeny. In fact, genes associated with an immature and quiescent state ('stemness') were associated with PKH26+ cells. In contrast, PKH26- cells overexpressed transcripts associated with proliferation, cell cycle progression and checkpoint control together with markers of myoepithelial/epithelial differentiation. Preliminary mRNA profiling results also gave indications about morphogenetic pathways that might be deregulated in breast cancer stem cells (e.g. the Notch/HES pathway) and revealed in some highly anaplastic breast cancers the expression of embryonal markers normally absent in the adult normal mammary gland. Overall, we have set up an experimental strategy suitable for molecular portraiting of normal and tumor breast stem cells, which paves the way for targeting of tumor stem cells as a therapeutic strategy in the prevention and treatment of breast cancer.

Hedgehog-GLI Signaling In Cancer, Stem Cells And Cancer Stem Cells

Ariel Ruiz i Altaba*, Virignie Clement, Pilar Sanchez, Christophe Mas and Barbara Stecca

Univ. Geneva Medical School, 8242 CMU1 rue Michel ServetGeneva, Switzerland

Signaling by Hedgehog ligands leads to the activation of GLI function, thereby regulating the GLI code. We and others* have shown that Hedgehog-GLI signaling is implicated in regulating precursor proliferation in the brain: including in the cerebellum (Dahmane and Ruiz i Altaba, 1999), tectum and cortex (Dahmane et al., 2001). Moreover, HH signaling is also required for for the behavior of brain stem cell lineages (Palma and Ruiz i Altaba, 2004; Palma et al., 2005). On the other hand, altered HH signaling can induce tumor formation (Dahmane et al., 1997) and is critical for the sustained growth of a variety of tumors (Dahmane et al., 2001; Sanchez et al., 2004; Sanchez and Ruiz i Altaba, 2005). However, to date only WNT signaling has been implicated in the control of cancer stem cells in leukemias and the nature of signaling pathways regulating cancer stem cell behavior in solid tumors remains unclear. We will present recent work on the targeting of the HH-GLI pathway for anti-cancer therapies, as well as studies deciphering its possible roles in both normal stem cells and in cancer stem cells.*Only our refs are listed due to space constraints.

Neurospheres From Glioblastoma Multiforme Are Enriched Of Cells With Cancer Stem-Like Properties

Tunici P, Suarez-Merino B, Bertolini G, Cusimano M, Valletta L, Pollo B, Caldera V, Bianchessi D, Boiardi A, Broggi G and Finocchiaro G.

Istituto Nazionale Neurologico Besta, Milano

Neurospheres from glioblastoma multiforme (GBM) can contain cells with stem like properties. We have obtained neurospheres from three adult patients with GBM and found that they can express glial and neuronal markers in vitro and generate tumors in the brain of CD1 nu/nu mice. Although these tumors were highly infiltrating, they did not completely recapitulate the features of the original GBM. Cells from two GBM were cloned and we found a positive relationship between the fraction of cloneñoriginating cells and their proliferation in vitro. Adherent cells were also grown in the absence of EGF and b-FGF and when injected into mouse brains they also caused large infiltrating gliomas, but later and less frequently than neurospheres ($p<0.02$ on Kaplan Meier analysis, log rank test). When adherent cells were shifted to EGF-bFGF medium they did not form neurospheres. Also, neurospheres obtained by growing U87 cells (a GBM established cell line) with EGF-bFGF caused tumors that were larger but phenotypically identical to those derived from U87 cells grown as a monolayer without EGF-bFGF . This observation suggests that EGF-bFGF may facilitate the growth of tumor initiating cells with cancer stem-like properties but are not directly responsible of the cancer stem cell phenotype.

Side Population (SP) Cells Derived From Prostate Cell Lines

Corrina Kane, John R W Masters and Aamir Ahmed

Prostate Cancer Research Centre, University College London, London W1W 7EJ, UK

Stem cells can be the target cells in tumorigenesis (Reya et al, Nature 414:105, 2001) but as yet no information exists regarding cancer stem cells in the prostate. We aim to use Hoechst 33342 dye exclusion (by the BCRP1 transporter) and fluorescence activated cell sorting (FACS) to isolate and characterise side population (SP) cells enriched in cancer stem cells from human prostate cancer tissue and cell lines. The first step towards achieving this aim was to identify whether the prostate cell lines PC3, DU145, LNCaP and cells isolated from cancerous human prostate tissue expressed BCRP1 at the mRNA and protein level. Preliminary results indicate that prostate cell lines (e.g. DU145) express BCRP1 (assessed by RT-PCR and immunocytochemistry). Expression of mRNA for Nanog and Oct4, two of the commonly employed stem cell markers, is also being investigated. Future studies will include (i) FACS analysis to isolate and propagate SP cells from prostate cancer cell lines and tissue (ii) characterisation of the SP cells on the basis of self-renewal capacity, pluripotency and tumorigenicity.

A Role for WNT/Beta-Catenin Signaling in Establishing Intestinal and Mammary Cancer Stem Cells

Claudia Gaspar, Patrick Franken, Ron Smits, Cor Breukel[2], Martin van Der Valk[3], and Riccardo Fodde

Department of Pathology, Josephine Nefkens Institute, Erasmus University Medical Center, Rotterdam; [1] Center for Human & Clinical Genetics, Leiden University Medical Center, Leiden; [2] Departement of Experimental Animal Pathology, The Netherland s Cancer Institute, Amsterdam.

To study how the different dosages of Wnt signaling activation may influence multiorgan tumorigenesis, we have generated several hypomorphic alleles of the Apc tumor suppressor gene by gene tergeting (Gaspar & Fodde. Int J Dev Biol 48:377, 2004). Notably, while in general Apc mutations result in intestinal cancer both in man and mouse, a novel mutation at codon 1572, Apc 1572T, resulting in a 175 kDa truncated protein lacking axin/conductin-binding motifs but still encompassing three Beta-catenin down-regulating domains, does not lead to predisposition to intestinal tumors. HOwever, the majority of Apc +/1572T females spontaneously develop multi-focal and rapidly growning mammary tumors around 8 mo. of age. Male mice also develop tumors though with decreased penetrance. Notably, Apc 1572T mammary tumors form lung metastases in considerable proportion of the animals. We used several IHC markers to identify the different cell types in the primary mammary tumors and theri lung metastases. The tumors were found to encompass myoepithelial, squamous, and luminal epithelial types, and were classified as lobular carcinomas with different degrees of metaplastic squamous differentiation. As previously observed in Apc-driven intestinal tumors, intracellular Beta-catenin accumulation, the earmark of canonical Wnt signaling activation, was found to be extremely heterogeneous within the tumor mass. IHC analysis of the lung metastases also showed a similar range of mammary epithelial types. However, when lung micro-metastases (20-100 cells in size) were analyzed by IHC and compared with their full-blown equivalent (macrometastases) and with the primary mammary lesions, differentiation was found to be significantly reduced whereas almost every cell showed cytoplasmatic and/or nuclear Beta-catenin accumulation. These results indicate that the just-right dosage of Wnt signaling activation encoded by the Apc 1572T mutation specifically affects the homeostasis of the mammary stem cell compartment and triggers tumor initiation, progression, and metastases possibly by activating cancer stem cells. These novel data will be discussed also in view of recent results from our laboratory indicating a similar role for Wnt/Beta-catenin signaling in establishing cancer stem cells in an Apc 1638N/KRASV12G mouse model for intestinal tumorigenesis and liver metastases (KP Janssen, P. Alberici, et al., submitted).

NOVEMBER 11, 2005 - ABSTRACTS

POSTERS

Session A:
Leukemia

A1	Yamazaki
A2	Smalley
A3	Porter
A4	Shing
A5	Agrelo
A6	Xiaomin Zheng
A7	Bossi
A8	So
A9	Amodio

Session B:
Breast Cancer

B1	Bonafè
B2	Peinado
B3	Pusterla
B4	Rossi
B5	Edel

Session C:
Lung Cancer, Melanoma and Glioblastoma

C1	Fuchs
C2	Tang
C3	Ortensi
C4	Clement
C5	Colin
C6	Wong
C7	Hooi
C8	Finocchiaro
C9	Muffler
C10	Stecca
C11	Magnifico
C12	Holger
C13	Piccirillo
C14	Guerra
C15	Gjerstorff
C16	Mancuso

Session D:
Molecular Mechanisms of Self Renewal

D1	d'Adda di Fagagna
D2	Bulavin
D3	Aznar Benitah
D4	Fagà
D5	Calautti
D6	Crosetto
D7	Demidov
D8	Philippe
D9	Baratta
D10	Atlasi
D11	Del Sal
D12	Cavallaro
D13	de Grey
D14	Chiaramonte

Last update October 21, 2005

Session A:

Leukemia

Identification And Characterization Of Cancer Stem Cells In Adult T-Cell Leukemia (ATL) Cell Lines

Hiroto Yamazaki and Chikao Morimoto

Division of Clinical Immunology, Advanced Clinical Research Center, Institute of Medical Science, The University of Tokyo

Recent works in stem cell biology suggest that some cancers contain stem like cells (cancer stem cells). In human cancers, they have already been identified in chronic myelogenous leukemia, acute myelogenous leukemia, breast cancer, and brain tumors. Adult T-cell leukemia/lymphoma (ATL/ATLL) is a peripheral T-cell neoplasm, and caused by an infection of human T-cell leukemia/lymphoma virus type I (HTLVñI). ATL patients have extremely poor prognosis with diverse appearance of ATL cells in peripheral blood. On the other hand, flow cytometry and Hoechst33342 dye were previously used to identify a subset of hematopoietic stem cell, termed the side population (SP), which is also thought to contain stem cells of various tissues. We examined whether ATL cell lines also contain SP cells. Among 14 ATL-derived and HTLV-I transformed T cell lines, we found that 5 cell lines contain SP cells with the range between 0.1-5.5% of total cells. Especially, ATL-2 and ATL-43Tb cell lines contain SP cells most abundantly (3-5.5%), suggesting that these cell lines are ideal for studying cancer stem cell in ATL. In the meeting, the analysis of cell surface markers, profile of gene expression, and inoculation experiments in immunodeficiency mice will be reported.

Molecular Dissection Of The Mouse Mammary Gland Identifies Stem/ Progenitor And Signal Transducing Cell Populations

Katherine Sleeman, Marjan Iravani, Howard Kendrick, *Ian Titley, Naheed Kanuga, David Robertson, Tim Dexter, Kerry Fenwick, Alan Mackay, Alan Ashworth and Matthew J. Smalley

*The Breakthrough Breast Cancer Centre and *Section of Haematological Oncology, The Institute of Cancer Research, London, UK*

Identification of breast stem cells would aid our understanding of breast biology and, potentially, breast cancer. However, such cells have not yet been definitively identified. We have used the mouse mammary gland as a model in which to molecularly dissect the relationship between putative stem cell populations and other cells in the tissue. We have separated mammary cell preparations into subpopulations based on expression of CD45, Sca-1, CD24, the 33A10 antigen and CD49f. The populations identified have been molecularly characterised and their in vitro and in vivo growth characteristics assessed. We find evidence for a luminal epithelial CD45- / Sca-1+ / CD24+ / 33A10- / cytokeratin 18+ population that does not have stem/progenitor activity but may be a steroid hormone-sensing signal transducing population. We find no stem/progenitor activity in Sca-1+ cells of the mammary gland but can detect such activity in both CD45- / Sca-1- / CD24+ / CD49fLow and CD45- / Sca-1- / CD24+ / CD49fHigh populations. Limiting dilution transplantation experiments to define this potential in more detail are in progress. This work confirms that stem/progenitor activity is a property of a specialised cell population and begins to define its identity in more detail.

Impaired DNA Replication By Chemotherapeutics Promotes Leukemogenesis

Christopher C. Porter, Ganna Bilousova, James DeGregori

Pediatrics and Biochemistry & Molecular GeneticsUniversity of Colorado Health Sciences Center

Contexts of impaired DNA replication are paradoxically associated with tumor development. Our data demonstrate that genetic and pharmacologic impairment of DNA replication promotes the development of leukemias caused by Bcr-Abl as a result of poor competition among hematopoietic stem and progenitor cells. In contrast, competent replication among hematopoietic progenitors is inherently tumor suppressive, preventing the expansion of Bcr-Abl expressing progenitors. We hypothesized that similar competitive interactions among hematopoietic progenitors are important in the development of treatment related acute myelogenous leukemia (tAML). We have found in vitro that progenitors expressing Mll-Af9, a fusion gene commonly found in tAML, have a substantial survival advantage in the presence of 6-thioguanine, a commonly used chemotherapeutic, as compared to normal cells. We have also found that treatment of mice with 6-thioguanine after bone marrow transplantation with retrovirally transduced Mll-Af9, promotes the competitive expansion of oncogene expressing progenitor cells. These findings suggest that contexts of impaired replication of hematopoietic progenitors by chemotherapeutics may promote the development of tAML by diminishing the competitive capacity of those progenitors relative to oncogene expressing progenitors. Further understanding of the role of competitive interactions among hematopoietic progenitors in leukemogenesis will help develop strategies to prevent and treat leukemias.

Role Of PRDM16 In Leukaemogenesis

Danielle C. Shing[1], Maurizio Trubia[2], Francesco Marchesi[3], Silvia Monestiroli[2], Massimo Stendardo[2] and Pier Giuseppe Pelicci[1,2]

[1] Department of Experimental Oncology, European Insitute of Oncology, Milan, Italy; [2] FIRC Institute of Molecular Oncology, Milan, Italy; [3] Dipartimento di Patologia Animale, Igiene e Sanita Pubblica Vetinaria, Universita degli Studi di Milano, Milan, Italy.

PRDM16 is a member of the SET domain family of histone lysine methyltransferases. Two protein forms can be expressed from the PRDM16 gene, differing in the presence or absence of the SET domain. We have identified three primary translocations of AML in which the PRDM16 gene is rearranged. Aberrant expression of a SET domain-minus form of the protein results from two of these translocations, t(1;3)(p36;q21) and t(1;6)(p36;q15), In the third translocation, t(1;21)(p36;q22) an AML1/PRDM16 fusion gene is formed. We have investigated the ability of PRDM16 (SET domain-plus), PRDM16 (SET domain-minus) and AML1/PRDM16 to give rise to leukaemia in mice. Overexpression of each gene was achieved in the haematopoietic stem cells of the mouse by ex vivo retroviral transduction, followed by re-inoculation into lethally irradiated, syngeneic recipient mice. PRDM16 is leukaemogenic in the presence of a second hit. A myeloid leukaemia was observed in 4/4 PRDM16 mice in the absence of p53, and in 3/4 PRDM16 mice treated with the mutagen ENU, with mean latencies of 116 and 325 days, respectively. These preliminary findings favour the hypothesis that the form of PRDM16 lacking the SET domain (PRDM16) is oncogenic, as has been suggested for other SET domain family members.

Xist Inducible Mice Model Of Non-Hodgkin Lymphoma

Ruben Agrelo and Anton Wutz

IMP Vienna

In mammals, dosage differences of X-linked genes between XX female and XY male cells are adjusted by transcriptional inactivation of one of the two female X chromosomes. Initiation of silencing is triggered by accumulation of the 17kb noncoding Xist DNA.Chromosomal silencing can be recapitulated in embryonic stem (ES) cells by expressing Xist RNA from cDNA transgenes integrated in the autosomes and X chromosome.During the process of hematopoiesis, Xist lead to gene silencing during embryonic development and in adult mice Moreover there is a exact correlation between Xist induced chromosomal silencing and ablation of the affected cell types. The most affected are the intermediate progenitors of the B- and T- cell lineage but not the stem cell as in ES ñcell development.We wanted to study the consequencesof Xist induction in the tumorigenesis process, more precisely in non -Hodgkin lymphomasTo address this issue we generated a mice model of non- Hodgkin lymphoma with an inducible Xist expression system. As a consequence we expect to gain knowledge on the behaviour of cancer cells when Xist is induced. and also to well characterize them.In conclusion the work presents a cancer mice model, Xist inducible as a powerfull tool to study the effects of Xist in the neoplasic process.

The Integrity Of The Charged Pocket In The BTB/POZ-Domain Is Essentialfor The Phenotype Induced By The Leukemia-Associated T(11;17) Fusionprotein PLZF/Rara

Anita Seshire[1], Xiaomin Zheng[1], Daria Brambilla[2], Tim Beissert[1], Martin Ruthardt[1], Clara Nervi[23] and Elena Puccetti[1]

[1] Department of Hematology, Johann Wolfgang Goethe-Universit‰t, Frankfurt, Germany; [2] Department of Histology and Medical Embryology, University iLa Sapienzai, Rome, Italy. [3] San Raffaele Biomedical Science Park of Rome, Rome, Italy.

Acute myeloid leukemia (AML) is characterized by a differentiation block and by an increased-self-renewal of hematopoietic precursors in the bone marrow. This phenotype is induced by specificAML-associated translocations such as the t(15;17) and t(11;17), which involve an identical portion ofthe retinoic acid receptora (RARa) and either the PML or PLZF genes, respectively. The resultingfusion proteins form high molecular weight complexes (HMW) and aberrantly bind the histonedeacetylase recruiting nuclear co-repressor complex (HD-NCR). The N-terminal BTB/POZ-domain isindispensable for the capacity of PLZF to form HMW. Here we studied the role of dimerization andbinding to the HD-NCR for the induction of the leukemic phenotype by PLZF/RARa and we showthat i.) the BTB/POZ-domain mediates the oligomerization of PLZF/RARa ; ii.) mutations which inhibit dimerization of PLZF do the same in PLZF/RARa ; iii) the PLZF/RARa -related block ofdifferentiation requires both dimerization and binding to the HD-NCR; iv) the mutations interfering onlywith dimerization inhibit aberrant self renewal of PLZF/RARa -positive HSC. Taken together these data provide evidence that dimerization and the formation of a functional charged pocket, essential for the binding to HD-NCR, are indispensable for the PLZF/RARa-induced leukemogenesis.

Identify The Target Cell For APL Among The Subsets Of Normal Hematopoietic Stem Cells (HSC) Committed Progenitors

Daniela Bossi and Saverio Minucci

From the European Institute of Oncology, Department of Experimental Oncology, Milan, Italy

Expression of the promyelocytic leukemia-retinoic acid receptor (PML-RAR) fusion protein - originated by the t (15; 17) chromosomal translocation – is associated with more than 95% of the cases of acute promyelocytic leukaemia (APL), one of the commonest subtypes of acute myeloid leukemia (AML). While the molecular basis of APL is relatively well understood, the cellular basis of the disease is however less clear. One of important issues that remain to be resolved is the nature of the hematopoietic cells that are the normal target of the PMLRAR fusion protein. In our group, PML-RAR Knock-In mice are available. In this mouse model, the human PML-RAR gene is under the control of the endogenous murine cathepsin G promoter. The expression of the fusion protein, during early stage of differentiation, induced a “preleukemic state” (slight alterations in myelopoiesis) that after long latency leads to development of the human-like disease. The fusion protein is the primary determinant of the leukaemia phenotype, however additional genetics events are required for the progression of the disease. During early myeloid stage of differentiation, APL may be initiated by transforming events that take place in haematopoietic stem cells (HSC) or alternatively PML-RAR may create a cellular environment favorable for the accumulation of additional genetic lesions in more committed progenitors. In order to investigate whether the disease arises among subsets of stem cells-committed progenitors, we have decided to use the hematopoietic system in a preleukemic state as a model system. Taking advantage of the normal hematopoietic lineage characterization, we have dissected a preleukemic bone marrow in different subsets. Functionally defined hematopoietic cells can be separated phenotypically through surface receptors. This set of protein markers can be tagged with monoclonal antibodies bearing a fluorescent tag. Different combinations of these antibodies can label defined hematopoietic cell subsets, isolated from bone marrow, with fluorescence-activated cell sorting (FACS). Using Lineage (Lin), Sca-1 (S) and c-Kit (K) receptor markers we have sorted out a stem-enriched hematopoietic population (Lin- K+ S+) and a stem-depleted hematopoietic populations (Lin- K+ S- and Lin- K- S-). Then we have tested the relative ability of preleukemic populations to induce APL in vivo after transplantation into lethally irradiated mice. As a control we have used the preleukemic lineage-negative population, which was demonstrated to contain the APL target cells. . All animals transplanted with control (PML-RAR expressing Lin- cells) and preleukemic HSC have been reconstituted and developed disease in 100% of the cases. Compared to Lin- PML-RAR populations, the latency time of the Lin- K+ S+ PML-RAR was shorter. Unexpectedly, all mice reconstituted with the transgenic Lin- K- S- population have been alive. It seems possible; therefore, that one effect PMLRAR expression is the induction of reconstitution ability (self-renewal) at the very restricted stage of haematopoiesis downstream of the committed compartment. In addition, 5 out of 8 mice, reinoculated with transgenic stem-depleted population, have developed an APL-like disease. Our studies establish that APL initiates through targeting of a heterogeneous population in terms of their origins (including not stem cells) and support the existence of cancer stem cells that do not overlap with HSC of the tissue of origin.

Cell And Molecular Targets Of Biphenotypic Leukemia Associated With MLL Oncoprotein

Chi Wai Eric So

Institute of Cancer Research

The unique association of MLL fusion with multi-lineage leukemia suggests the cellular transformation targets originated from primitive hematopoietic cells. Using retroviral transduction/transplantation approach, we successfully mimic the multi-lineage leukemia in a mouse model, where MLL fusion can induce acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and acute biphenotypic leukemia (ABL). To further characterize the origin of cancer stem cells, we identified early hematopoietic stem cells (HSCs) and multipotent progenitors (MPP) compartments as the cellular targets for biphenotypic leukemia associated with MLL fusion proteins. At molecular level, members of Hox family proteins that promote self-renewal are known downstream targets for MLL proteins. To investigate the functional significance of the MLL/Hox axis in MLL-mediated leukemia, we demonstrated that MLL-GAS7 oncoprotein significantly compromised its transformation ability in the absence of Hoxa9 or Hoxa7. In spite of longer disease latency, MLL transformed cells deficient in a single Hoxa gene could still induce leukemia in mice, suggesting the existence of compensatory mechanisms from other Hox members. To further characterize the molecular pathways that mediate transformation and self-renewal of MLL targeted cancer stem cells, we are currently studying the potential crosstalk and functional requirements between Hox genes and Bmi-1 in MLL leukemia.

Early Hematopoietic Zinc Finger Protein (Ehzf/Znf521): Potential Role In The Regulation Of Haematopoietic Homeostasis And Leukaemogenesis

H. M. Bond[1], M. Mesurca[1], N. Amodio[1], T. Mega1, D. Pelaggi[1], L. Bullinger[3], M. Grieco[1], M. A. S. Moore[2], S. Venuta[1], G. Morrone[1]

[1]University Magna Graecia , Catanzaro Italy - [2]Memorial Sloan-Kettering Cancer Center, New York, USA - [3]University Of Ulm, Germany

EHZF/ZNF521, a 30-zinc finger protein expressed in human CD34+ cells but not in mature leukocytes, shares high homology with the transcription co-factor OAZ, implicated in the maintenance of immaturity of progenitors of the olfactory epithelium. Like OAZ, EHZF enhances transcription of BMP-responsive genes and represses promoters responsive to EBF, a transcription factor essential for the B-cell lineage specification. EHZF is expressed in most AMLs. High expression levels strongly correlate with (del7q) and translocations involving MLL, low expression correlates with t(15;17) and FLT-3 aberrations. Enforced expression of EHZF confers proliferative advantage on human myeloid cell lines and induces progenitor expansion in primary human CD34+ cells.Co-immunoprecipitation and immunofluorescence analyses showed that EHZF: i) strongly interacts with the histone deacetilases (HDAC) 1 and 2; ii) accumulates in discrete subnuclear structures, and iii) recruits HDAC1 into such compartments. This appears of interest since aberrant recruitment of HDACs is considered a key step in the pathogenesis of several tumors, and in particular of leukaemias. These and other data suggest that EHZF may contribute to the control of the of the immature normal and neoplastic haematopoietic compartment. Ongoing studies are aimed at evaluating the expression and relevance of this factor in leukaemic stem cells.

Session B:

Breast Cancer

Malignant Progression: A Result From The Struggle For Life Of Stem Cells Exposed To Stress?

Massimiliano Bonafé, Pasquale Sansone, Gianluca Storci

Department of Experimental Pathology, University of Bologna, ItalyCRBA -Center for Applied Biomedical Research-, Bologna, Italy

The work here presented is based on the hypothesis that breast cancer stem cells are the by-product of the struggle for life of those (normal) stem cells which give raise to the mammary tissue. Normal stem cells sustain tissue structure and function while challenged by various stress (oxidation, hypoxia, inflammation, estrogens) which are known to induce apoptosis and senescence. However, these latter resemble quite inadequate stress response strategies for stem cells, since they would lead to the exhaustion of the tissue. Consequently, stem cells are forced to cope with stress while preserving their function. However, the more a stem cells acquires stress resistance, the closer it is to a malignant phenotype. To test this hypothesis, normal stem cells are isolated from mormal mammary tissue and expanded in vitro as mammosphere, These latter are cultured in presence/absence of various sources of stress (inflammatory cytokines, hypoxia mimetics, oxidants, estrogens). The cells obtained are assessed for their neoplastic potential and compared to those obtained from breast cancer tissues. So far, results indicate that chronic exposure to stress show a malignant phenotype (i.e invasiveness) and the up-regulation of molecular pathways, in which p66shc and Slug genes play a major role. The elucidation of the mechanisms involved in such a phenomenon is expected to provide information about the early steps of carcinogenesis of the mammary tissue, and thus on the preventive strategies to treat breast cancer before its expansive and invasive growth occurs.

A Molecular Role For Lysyl Oxidase-Like 2 Enzyme In Snail Regulation And Tumor Progression

Hèctor Peinado[1]#, M. Carmen Iglesias-de la Cruz[1]#, David Olmeda1, Katalin Csizsar[3], Keith S. K. Fong[3], Sonia Vega[2]&, M. Angela Nieto[2]&, Amparo Cano[1]* and Francisco Portillo[1]

**[1]Departamento de BioquÌmica. Instituto de Investigaciones BiomÈdicas ¡Alberto Solsî Consejo Superior de Investigaciones CientÍficas-Universidad AutÓnoma de Madrid. Arturo Duperier, 4, 28029 Madrid. Spain. - [2]Instituto Cajal, Avenida Doctor Arce, 37, 28002 Madrid. Spain - [3]Cardiovascular Research Center, John A. Burns School of Medicine, University of Hawaii, Honolulu, 96822 USA.*

The transcription factor Snail controls epithelial-mesenchymal transitions (EMT) by repressing E-cadherin expression and other epithelial genes. However, the mechanisms involved in regulation of Snail function are not fully understood. Here we show that lysyl-oxidase like 2 and 3 (LOXL2 and LOXL3), two members of the lysyl-oxidase gene family, interact and cooperate with Snail to downregulate E-cadherin expression. Snailís lysine residues 98 and 137 are essential for Snail stability, functional cooperation with LOXL2/3 and induction of EMT. Overexpression of LOXL2 or LOXL3 in epithelial cells induces an EMT process, supporting their implication in tumor progression. The biological importance of LOXL2 is further supported by RNA interference of LOXL2 in Snail expressing metastatic carcinoma cells which led to strong decrease of tumor growth associated to increased apoptosis and reduced expression of mesenchymal and invasive/angiogenic markers. Taken together, these results establish a direct link between LOXL2 and Snail in carcinoma progression.

Extra-Cellular HMGB1 Promotes Breast Cancer Proliferation And Invasion

Pusterla T, De Marchis F, Palumbo R, Bianchi ME

Chromatin dynamics unit, Dibit, Ospedale San Raffaele, Milan

HMGB1 is a nuclear protein that positively affects chromatin accessibility in multiple ways. All cells can release passively HMGB1 in the extracellular milieu when they die in an unprogrammed way: extracellular HMGB1 works, through its receptor RAGE, as an inflammatory cytokine and a signal of tissue damage. We have shown that HMGB1 can attract stem cells and promote their proliferation. Several cell types can secrete HMGB1 actively and without dying. Many tumors over-express HMGB1 and/or RAGE, and blockade of their interaction decreases tumor growth and invasiveness. We noticed that, in human breast cancer biopsies, HMGB1 is predominantly located in the cytoplasm of the tumoral cells only, while it is located in the nuclei of normal cells. A cytoplasmic localization is often associated to active secretion of HMGB1. In fact, we found HMGB1 present in the medium of several breast cancer cell lines. Our working hypothesis is that HMGB1 can act as an autocrine stimulus for the tumor itself. Both invasive MDA-MB-231 and non-invasive MCF7 cells proliferated when stimulated with extracellular HMGB1, but only MDA-MB-231 cells responded chemotactically to HMGB1. Responsiveness to HMGB1 might represent a threshold in the transition between invasive and non-invasive tumor phenotypes.

Stem Cell From Brest Cancer Biopsy

Dr. Perris Roberto, Dr.ssa Di Cola, Gabriella

I'm working about the possibility to extract stem cells from brest cancer biopsy.We used antibody labeled specific for cell surface protein (Miltenyi protocols) to separate stem cell from biopsy.We control the correct separation by cytofluorimeter to evaluate the specificity and the count of cells. We would to perform assay of gene expression by REAL-TIME PCR and Mycroarrays. The works have the means to estableshed the real presence of stem cells in breast cancer end the possibility to separate theme for analyses the gene expression for future research.

Control Of Mammary Cell Differentiation By P21cip1

Michael Edel and Miguel Beato

Center for Genomic Regulation (C.R.G.) BarcelonaSpain

Mammary cell differentiation is an essential event in the formation and growth of the mammary gland and during the development of carcinoma. p21CIP1 is a cell cycle kinase inhibitor that is best known for its role in inhibiting cell cycle progression by blocking cyclin E/CDK2 kinase activity. We know that p21CIP1 is regulated by steroid hormones and that it is a marker of mammary stem cell differentiaiton, but do not know the underlying mechanism.

Session C:

Lung Cancer, Melanoma and Glioblastoma

Characterization And Functional Analysis Of Cancer Stem Cells In Human Neuroblastoma

Sebastian Fuchs[1,*], Roland Meier[2,*], Elisabeth Bruder[3], Jean Marc Joseph[2], Nicole Gross[2], Lukas Sommer[1]

*[1]Institute for Cell Biology, Swiss Federal Instiute of Technology, CH-8093 Zurich, Switzerland - [2]Pediatric Oncology Resaerch Unit, Pediatric Department CHUV, CH-1011 Lausanne, Switzerland - [3]Institute of Pathology, University Medical School Basel, CH-4003 Basel, Switzerland *These authors contributed equally to this work*

Neuroblastoma is the most common solid tumor in infants. It originates from malignant sympathoadrenal cells which are derived from the neural crest, a transient population of stem cells present at early stages of development. There is increasing evidence that, at least for some cancers, the existence of malignant cancer stem cells might explain tumor appearance and growth. These cells are able to proliferate, self-renew and differentiate into other cell types within the tumor tissue. Thus, should the concept of a cancer stem cell apply to neuroblastoma, the cell at the origin of such tumors might potentially bear features of a neural crest stem cell. We have identified a population of cells within primary neuroblastoma tissue and neuroblastoma cell lines that express markers in conformity with neural crest stem cells. Moreover, differentiated cell types normally generated from neural crest cells were found in neuroblastoma tissue, including smooth muscle, neuronal and glial cells. Furthermore, using fluorescence activated cell sorting (FACS) on neuroblastoma cell lines, we were able to isolate a cell fraction expressing the neural crest marker p75, a low affinity neurotrophin receptor. To assess the tumor formation capacity of the p75+ cells we performed a xenotransplantation assay with immunodeficient mice. Strikingly, even low numbers of p75+ cells induced tumor formation upon subcutaneous injection, while equal amounts of non-sorted neuroblastoma cells were not sufficient to induce tumorigenesis. In addition, transplantation of p75- tumor cells led to drastically reduced tumor growth as compared to non-sorted tumor cells. Interestingly, preliminary data indicate that the ratio of cells expressing neural crest stem cell markers within a given tumor cell line correlates with the aggressiveness of the cell line. To further characterize the stem cell properties of this prospective cancer stem cell we are currently challenging these cells with instructive growth factors under clonal conditions and in neurosphere culture. By comparison to the already known function of specific growth factors and signaling pathways in normal neural crest stem cells, we will be able to further characterize this cancer stem cell. Ultimately the identification of molecular mechanisms underlying cancer stem cell maintenance and proliferation might open up new targets for improved cancer therapies.

Cancer Stem Cells In The Brain: Response To Temozolomide

Constance Chua[1], Sandy Lim[1,4], Norazean Zaiden[1], Wai Hoe Ng[2], Beng Ti Ang[2, 5], Siew Ju See[3], Tseng Tsai Yeo[2], Ivan Ng[2], Carol Tang[1]

1Department of Research, National Neuroscience Institute, Singapore - 2Department of Neurosurgery, National Neuroscience Institute, Singapore- 3Department of Neurology, National Neuroscience Institute, Singapore4Genome Institute of Singapore, Stem Cell & Developmental Biology5Centre for Molecular Medicine, Agency for Science, Technology and Research, Singapore

Primary malignant brain tumors have a poor prognosis and are often chemoresistant. Our group focuses on the study of chemoresistant brain tumor stem cells (BTSCs) obtained from glioblastomas. Our preliminary data using U-87MG cells (grade III astrocytoma cell line), demonstrated that a ìSide Populationî (SP) existed. Cells isolated in this SP region were capable of self-renewal in defined medium, multipotentiality, and demonstrated enhanced tumorigenic potential as defined by in vitro foci-forming assay, in comparison to cells isolated from the non-SP region. Additionally, when treated with temozolomide (TMZ) at DNA damaging concentrations, this SP percentage increased significantly over three days, despite majority cells being arrested by the drug as shown by FACS analysis. We also determined that this response to TMZ was linked to the multidrug resistance transporter, ABCG2, through interference RNA technology. We have now been able to show that such response to TMZ can be replicated in samples derived from glioblastomas. We have also identified the conditions of isolation of this chemoresistant BTSC population; and are currently engaged in defining how this SP population differs from the CD133-expressing population. We believe our study of this clinically important BTSC population will help shed light on chemoresistance in brain tumors.

Involvement Of The Neuronal Adaptor Rai (Shc-C) In Gliomagenesis

Barbara Ortensi[1], Flavia Troglio[1], Micaela Quarto[2], Maria Capra[2], Daniel Levi[3], Paolo Gaetani[3], Pier Giuseppe Pelicci[1] & Giuliana Pelicci[1].

[1]Department of Experimental Oncology, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy; [2]IFOM Institute, Via Adamello 16, 20139 Milan, Italy; [3]Department of Neurosurgery, Istituto Clinico Humanitas, Via Manzoni 56, 20089 Rozzano, Milan, Italy.

The PI3K-Akt signalling pathway is a critical determinant of proliferation and survival of mammalian cells. Constitutive activation of PI3K-Akt is frequently found in spontaneous human tumours. Rai (Shc-C), a neuronal specific member of the Shc-family of adaptor proteins, functions as an upstream activator of PI3K-Akt, stimulating neuronal cell survival upon environmental stresses (oxidative stress and hypoxia) or limited availability of GDNF, the ligand for the Ret receptor-kinase. In vivo, in adult mice, Rai expression protects against neuronal loss following ischemic brain injury. Rai is expressed in neural stem cells (NSCs), in mature neurons but not in glial cells. Preliminary results suggest that Rai expression exerts a negative effect on the growth properties of NSCs. Unexpectedly, tumors of glial origin (glioblastomas, GBMs) express high levels of Rai protein, suggesting that ectopic expression of Rai in GBM might be relevant for the transformed phenotype. Elevated Akt activity is almost invariably found in GBMs. However, the mechanisms underlying Akt activation in GBM are not completely understood. Whether ectopically expressed Rai in GBMs contributes to Akt constitutive activation remains unknown. Notably NSCs derived from GBMs express Rai proteins, which might explain the finding of Rai expression in GBMs.

Glioma Stem Cells And Glioma Growth Requires SHH-GLI Ignaling

Virginie Clement and Ariel Ruiz i Altaba

SHH-GLI pathway regulates dorsal normal brain growth and is crucial for the regulation of stem cells lineages in adult mammalian brain (Dahmane et al, 2001: Palma V et al, 2005), leading to the hypothesis that misregulation of the SHH-GLI pathway may lead to the development of brain tumors. Here, we showed that SHH-GLI is an essential pathway, required for glioma growth and glioma cancer stem cells. In addition, regarding our in vivo data, cyclopamine, the HH-GLI inhibitor, seems to be a promising therapeutic anti-cancer agent.

In Vitro Identification And Functional Characterization Of Glial Precursor Cells In Human Gliomas

C. Colin*, N. Baeza *, S. Tong*, C. Bouvier*, B. Quilichini, P. Durbecá, and D. Figarella-Branger*

**Laboratoire de Biopathologie de liAdhèsion et de la Signalisation, EA3281, IPHM, Faculté de Mèdecine Timone ; Service de Cytogènètique Oncologique, CHU Timone and Laboratoire de Neurogenese et Morphogenese dans le Développement et chez liAdulte, UMR 6156 CNRS, IBDM, Parc scientifique de Luminy, Marseilles, France.*

Human gliomas including astrocytomas and oligodendrogliomas are defined as being composed of neoplastic astrocytes and oligodendrocytes respectively. By in vitro functional assays, we show that gliomas contain a mixture of glial progenitor cells and their progeny. We have set up explant cultures from pilocytic astrocytomas, glioblastomas and oligodendrogliomas and studied antigens that characterize glial lineage, from the most precursor cells expressing the A2B5 ganglioside to the most differentiated cells. All tumoral explants contain A2B5+ cells and can generate migrating cells with distinctive functional properties according to glioma subtypes. In pilocytic astrocytomas, migrating cells are postmitotic and can differentiate in type-2 astrocytes or towards the oligodendrocyte lineage. In glioblastomas, most migrating cells are dividing, express A2B5 or GFAP and can generate oligodendrocytes and type-1 and type-2 astrocytes in appropriate medium. Oligodendroglioma explants are made by actively dividing glial precursor cells expressing A2B5 or PSA-NCAM. Only few cells can migrate and differentiation towards oligodendrocyte lineage does not occur. Isolated A2B5+ cells from both glioblastomas and oligodendrogliomas showed similar genetic alterations as the whole tumor. Therefore, pilocytic astrocytomas contain postmitotic Oligodendrocyte Precursor Cells whereas both glioblastomas and oligodendrogliomas contain neoplastic Glial Restricted Precursor Cells.

Isolation And Characterization Of Neural Crest Stem Cell-Like Cells From Human Melanoma

Christine Wong, Curdin Conrad[ß], Reinhard Dummer[ß] and Lukas Sommer

Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland, [ß]Department of Dermatology, University Hospital, Zurich, Switzerland Christine Wong, Curdin Conrad[ß], Reinhard Dummer[ß] and Lukas Sommer Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland, [ß]Department of Dermatology, University Hospital, Zurich, Switzerland

Melanoma is a very aggressive skin cancer whose incidence is increasing very rapidly. The formation of melanoma involves the transformation of melanocytes into a malignant and tumorigenic phenotype. These pigment-producing cells in the skin are derived from the neural crest, a structure formed early in development. Neural crest stem cells have the potential to generate many different cell lineages including neural and non-neural cells. Our previous results suggest, that multipotent neural crest stem cells from the melanocyte niche exist in adult skin and recent reports indicate the possibility of certain tumors arising from so called ìcancer stem cellsî, which are able to self-renew. Therefore, we will investigate whether different melanoma cell lines and primary melanoma contain cells with neural crest stem cell features. Analysis of primary melanoma tissue sections reveals a small population of cells expressing p75 and Sox10, markers found in normal neural crest stem cells. Those cells will be prospectively isolated by FACS and their capacity for self-renewal will be tested by clonal analysis. Furthermore, the responsiveness of melanoma-derived cells to instructive growth factors as compared to the behavior of normal neural crest stem cells will be assessed. We will also investigate the in vitro potential of melanoma-derived cells to differentiate into cell types derived from the neural crest. In addition we will test if neural crest stem cell-like cells isolated from melanoma cell lines have the potential to initiate tumors in mice in vivo.

Identification Of Side Population In HCT116 And Its Metastatic Derivatives

Shing Chuan Hooi*, Carol Tang^, Carol Leung*, Puei Nam Tay*

**National University of Singapore - ^National Neuroscience Institute, Singapore*

Our lab has derived metastatic cell lines from poorly metastatic colorectal HCT116 cells, by in vivo passaging in athymic nude mice (serial transplantation). Briefly, the HCT116 cells were injected intrasplenically into athymic mice, and the cells that metastasized to form tumor in the liver were harvested and expanded into derivative cell lines. Subsequent serial transplantation yielded cell lines of increasing metastatic potential, as verified by in vivo and in vitro assays. The presence of side population (SP) was studied in the parental HCT116 and 2 of its metastatic derivatives M3 and E1 using FACS analysis, based on the ability of the SP cells to efflux the Hoechst dye. The percentage of SP in the total cell population in HCT116, M3, and E1 are 0.2%, 2.56% and 3.79% respectively. Therefore, we have shown an increase in %SP in the metastatic cell lines compared to the poorly metastatic parental HCT116, suggesting that cancer stem cells may be involved in metastasis of colorectal cancer cells.

The Side Population Of Rat C6 Gbm Cells Contains Highly Tumorigenic Glioma Cells

Cusimano M, Bertolini G, Caldera V, Suarez-Merino B, Tunici P, *Delia D and Finocchiaro G.

*Istituto Neurologico C. Besta, and *Istituto Nazionale Tumori, Milano, Italy*

The side population (SP) phenotype can identify a subpopulation of stem cells that are only weakly stained by Hoechst 33342 and is conferred by the expression of the ABCG2-BCRP gene. The side population was recently identified also in tumor cells, including glioblastoma multiforme (GBM). We have sorted by flow cytometry rat C6 GBM cells. SP C6 cells were 4-5% of the whole population. After sorting SP and NON-SP C6 cells were kept in culture as neurospheres for one week during which their proliferation rate was checked by WST staining: NON-SP cells were proliferationg faster than SP cells ($p<0.03$). ABCG2 was expressed in 44%-72% of SP cells and in 0-1% of NON-SP cells, as assessed by flow-cytometry. We then injected 1x10e5 SP or NON-SP into the brain of CD1 nu/nu mice (n=12). After 15 days all mice injected with SP cells died because of a very large malignant gliomas. Gliomas in mice injected with NON-SP cells were small and not lethal until day 21, when mice were sacrificed for histology. These data suggest that C6 SP cells express critical genes for tumor initiation and may be endowed with cancer stem-like properties.

Organotypic Cultures: A Model For Human Interfollicular Epidermal Stem Cell Niches

Sonja Muffler, Hans-Jürgen Stark, Mara Amoros and Petra Boukamp

Epidermal stem cells (ESCs) are located in hair follicles and interfollicular epidermis and are surrounded by a specific microenvironment - the stem cell niche. The factors regulating this niche are not known. However, several markers are proposed to define ESCs and their location. To investigate whether organotypic cultures (OTCs) are suitable in vitro models for interfollicular ESCs, we compared the distribution of proposed ECS markers in skin and two different OTCs: OTCs with a dermal equivalent consisting of a type I collagen gel and newly developed long-term OTCs with a scaffold-stabilized dermal equivalent, both with integrated fibroblasts.
By immunofluorescence we analyzed the distribution of b1 and a6 integrins, keratin 15 and 19, p63 and melanoma chondroitin sulphate proteoglycan (MCSP). While the marker distribution in OTCs was generally comparable to skin, scaffold-OTCs were clearly superior to collagen-OTCs, particularly obvious for b1 and a6 integrins. However, both integrins, K15 and p63 were expressed by all basal cells. Only MCSP and K19 were restricted to individual cells making them best candidates for further stem cell characterization.
Collectively, our results suggest that stem cell niches re-establish in OTCs and that OTCs provide a promising model for human interfollicular ESCs under controlled in vitro conditions.

Mouse Model Of Brain Tumors: Mis-Expression Of GLI1 In Neural Progenitors

Barbara Stecca, Ariel Ruiz i Altaba

Department of Genetic Medicine and Development University of Geneva

The Sonic hedgehog-GLI (SHH-GLI) signaling pathway has been proposed to regulate numerous events during the normal development and tumorigenesis. In particular, our group has demonstrated that the growth of a variety of human tumors, including basal cell carcinoma, medulloblastoma, glioma and prostate cancer, depends on sustained SHH-GLI signal activity. In addition, we have shown that SHH-GLI pathway is critical for the control of the size of different regions of the dorsal brain and for the maintenance of stem cells and progenitor pools in neural niches. Recently, we have found that SHH-GLI also regulates clonogenic properties in a subset of tumor cells derived from gliomas, strongly suggesting the presence of SHH-GLI responsive cancer stem-like cells. In order to verify this hypothesis, we created a mouse model in which GLI1 transgene expression is spatially restricted to the CNS neural progenitors and temporally controlled by doxycycline. GLI1 mis-expression induced hyperproliferation of neural precursor cells in the brain. These hyperplastic regions are likely to be blocked in an undifferentiated state, as suggested by the expression of Nestin and the lack of neuronal or astrocytic markers. Furthermore, we observed increase in the number and in the level of BrdU incorporation in transgenic neurospheres compared to controls.

Tumor Stem Cells

Alessandra Magnifico and Sylvie Menard

Molecular Targeting Unit Department of Experimental Oncology National Cancer Institute of Milan

New anti-cancer therapies specifically targeting tumor stem cells are warranted. We grew undifferentiated spheres for 6/7 passages by culturing different carcinoma cell lines. The spheres were found to be enriched up to 30% in Side Population (SP). By comparison, the parental cell lines contained only 1-2% of SP. Strikingly, a significantly lower number of SP cells (104 cells) formed tumors in injected animals, compared to Hoechst-positive cells (106 cells). Analysis of spheres formation from 10 different tumor cell lines indicated a strong correlation between capability to form immortal spheres and p53 status.

Identification Of Metastasis-Associated Genes In Primary Tumors Of Clear Cell Renal Cell Carcinoma

Holger Sultmann[1], Bastian Gunawan[2], Florian Haller[2], L·szlÛ F¸zesi[2], Annemarie Poustka[1]

[1]German Cancer Research Center, Div. of Molecular Genome Analysis, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany; [2]Center of Pathology, Div. of. Gastroenteropathology, Georg-August-Universität Gôttingen, Robert-Koch Str. 40, 37075 Gôttingen, Germany

To explore the gene expression profile characteristic for metastasis formation in clear cell renal cell carcinoma (RCC), we selected 99 RCC samples, encompassing 68 primary tumors and 31 metastases. Gene expression profiling of the 99 RCC samples was performed with human whole genome cDNA microarrays (37,500 genes and ESTs; RZPD Unigene Set3.1). We identified genes that were differentially expressed when primary tumors and metastases were compared. In addition, a gene expression pattern in primary kidney tumors was able to distinguish tumors that gave rise to metastases from those that did not. Thus, the gene expression analysis identified new markers for the segregation of patients at high risk for metastatic disease, supporting the currently debated hypothesis that the potential to form metastases is already present in primary tumors. The gene expression profiles of primary tumors may allow for an earlier diagnosis of relapsed RCCs and suggest targets for novel therapeutic approaches in the management of metastatic renal clear cell carcinoma.

Identification Of Transformed Neural Stem Cells As Tumor-Founding Cells In The Human Glioblastoma Multiforme

Sara Piccirillo, Rossella Galli, Francesco DiMeco* and Angelo L.Vescovi

*University of Milan-Bicocca, Milan, Italy; Stem Cell Research Institute (SCRI) – DIBIT-H.S. Raffaele-Milan, Italy; *Neurological Institute C.Besta, Milan, Italy; *Johns Hopkins Medical School, Baltimore, U.S.A.*

As the most undifferentiated cell in a tissue, the stem cell (SC) bears the broadest growth and developmental potential, thus representing a prominent target for malignant cell transformation. This notion may be extended to comprise neural SCs and brain tumors. In fact, also the central nervous system (CNS) harbors reservoirs of SCs, which are multipotent self-renewing precursors and may thus constitute a sort of dormant counterpart of cancer cells. By exploiting the same methodology routinely employed to isolate and extensively expand human fetal neural stem cells (HFNSCs) in vitro, we have been able to establish ten different tumor stem cell (TSC) lines from post-surgery biopsies of glioblastoma multiforme (GBM). Notably, these cells display critical stem cell properties. In fact, steadily expanding TSC cell lines could be grown in serum-free medium containing EGF and FGF2 as mitogens, for at least 40 passages in vitro. Upon removal of growth factors, these cells gave rise to the three major CNS lineages and by clonogenic assays they displayed a wide self-renewal capacity. Karyotypic analysis revealed that all the 10 TSC lines were hyper-diploid, with polysomy of chromosome 7, 19 and 20 and the presence of 4-5 marker chromosomes. Importantly, TSCs were shown to be tumorigenic in SCID/bg mice, following subcutaneous implantation, whereas HFNSCs were not.The availability of TSC lines may represent an invaluable tool to unravel the basic mechanisms regulating cell proliferation and differentiation in normal and malignant CNS stem cells, in order to devise novel approaches to hinder their malignant growth.

Pancreatic Neoplasia Induced By An Endogenous K-Ras Oncogene Is Highly Dependent On The Developmental Timing Of Oncogenic Activation

Guerra C., Jimènez A., Lamparero M*., Gonzalez P*., Alvarez V*., Coca V*., Pèrez L*., Dubus P. and Barbacid M.

Molecular Oncology and Biotechnology Programmes, Centro Nacional de Investigaciones Oncologicas (CNIO), Madrid, Spain.*

K-ras oncogenes are activated in 90% of human pancreatic adenocarcinomas. Thus, there is significant interest in generating animal tumor models driven by endogenous K-ras oncogenes that mimic the natural history of these diseases (1-3). Endogenous K-rasG12D oncogene activation during early embryonic development (E8.5), at a time when all the pancreatic cells start to form, leads to the generation of PanINs which occasionally develop into invasive adenomas, morphologically indistinguishable from those of human origin (4). In contrast to these observations, we have reported that activation of an endogenous K-rasG12V oncogene in adult mice, a time when K-ras activation is thought to occur in human patients, does not lead to tumor formation, in spite of widespread expression of the K-ras oncogene in the pancreas (3). To determine the bases for these apparent discrepancies, we crossed our K-rasG12V mice with Elastase-tTA;tetO-PhCMV-Cre transgenics (from E.P. Sandgren) to allow expression of the K-RasG12V oncoprotein in pancreatic acinar cells in a temporally controlled manner. Untreated mice express K-rasG12V (determined by the bicistronic beta-galactosidase marker) by midgestation (E16.5). These animals develop PanIN-lesions that recapitulate the stages described for human patients, from PanIN1A to pancreatic ductal adenocarcinoma, a result highly reminiscent to that reported by Hingorani et al. However, when the endogenous K-rasG12V oncogene was activated postnatally (by exposing mice to doxycycline until P10), the number of PanIN lesions decreased considerably and basically no adenocarcinomas were observed after one year of age. These observations suggest that K-ras preferentially transforms a subset of acinar cells (stem/precursor cells?) that are significantly more abundant during embryonic development. Alternatively, K-ras transformation may require prior genetic and/or epigenetic alterations that make postnatal/adult acinar cells susceptible to K-ras transformation. 1. Johnson L, ..., Tuveson D, Jacks T. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. Nature 410:1111-6 (2001).2. Jackson EL, ..., Jacks T, Tuveson DA. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev. 15(24):3243-8 (2001).3. Guerra, C.,Ö, Barbacid, M. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. Cancer Cell 4, 111-120 (2003).4. Hingorani SR, Ö, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 4(6):437-50 (2003).

GAGE Expression In Tumorigenic Human Mesenchymal Stem Cells

Morten F. Gjerstorff[1], Jorge S. Burns[1,2], Lene E. Johansen[1], Ole Nielsen[3], Moustapha Kassem[1,2] and Henrik J. Ditzel[1,4].

[1]Medical Biotechnology Center, University of Southern Denmark, Odense, Denmark; [2]Department of Endocrinology and Metabolism, 3Department of Pathology, and [4]Department of Medicine, Odense University Hospital, Odense, Denmark.

GAGE is a member of the Cancer Testis Antigen (CTA) family. Until recently, testis germ cells was though to be the only normal cell type to express GAGE in healthy individuals, while GAGE expression is found in approximately 20% of cancers of different origin, including melanomas, lung and breast carcinomas. However, a recent study has identified GAGE in human mesenchymal stem cells (hMSC).

We have generated a panel of monoclonal antibodies to GAGE and used them to study GAGE expression, at the protein level, in normal and malignant tissues. Additionally, we examined GAGE expression in a cell line, hMSC-TERT20, derived from hMSC that had been were transduced with the telomerase hTERT gene to compensate for the lack of endogenous telomerase activity in vitro. This greatly increased their proliferative lifespan. Early cultures of hMSC-TERT20 cells (DP=148) were able to form bone when implanted in immunodeficient mice. However, late passage hMSC-TERT20 cells (DP=256) were surprisingly tumorigenic. This has provided a new model to study the stem cell hypothesis for cancer. Single cell clones derived from hMSC-TERT20 cells showed a heterogeneous tumorigenic phenotype (Burns et al., 2005). We now report that immunohistochemical analysis of these tumours demonstrated significant variation in the expression level of GAGE. Four clones (BC8, BD6, CE8, BD11) exhibited strong expression of GAGE, and the expression was confirmed in cell cultures of these clones. In contrast, the BB3 clone was only weakly positive and the DB9 negative, except for a few foci of weakly positive cells. However, we observed no correlation between GAGE expression and proliferation rate, contact inhibition, anchorage-independent growth, serum dependence, xenograft tumorigenicity, or spheroid growth.

Our results suggest that GAGE expression need not correlate with maintenance of tumorigenicity. It is hypothesised that GAGE expression may provide ontological information as whether a given cell within a tumour has a stem cell origin and/or whether the cell has maintained stemness. This hypothesis is being further investigated.

CD133 As A Cancer Stem Cell Marker In Gynecological Tumors

[1]Ferrandina G, [1]Bonanno G, [2]Pierelli L, [1]Perillo A, [1]Procoli A, [1]Mariotti A, [1]Corallo M, [1]Martinelli E, [3]Rutella S, [4]Scambia G, [1]Mancuso S.

[1] Gynaecologic Oncology Unit, Catholic University of the Sacred Heart, Rome, Italy - [2] Immunohaematology and Transfusion Service, ASL Viterbo, Viterbo, Italy - [3] Department of Haematology and Blood Transfusion, Catholic University of the Sacred Heart, Rome, Italy4 Department of Oncology, Catholic University of the Sacred Heart, Campobasso, Italy

Cancer stem cells have been identified both in haematopoietic neoplasias and solid tumors. In breast tumors the antigenic profile CD44+/CD24-/low/Lin- is associated with cancer stem cell populations, while stem cells isolated from central nervous system and prostate tumors express the CD133 surface glycoprotein, which represents a haematopoietic/endothelial stem cell marker. We evaluated the expression of the CD133-1/2 isoforms in 15 ovarian and 12 endometrial carcinomas by flow-cytometry. All CD133+/CD45- cancer cells isolated from these tumors expressed the epithelial marker cytokeratine-7 confirming the ovarian and endometrial tumor stem cells were of epithelial origin.The percentage of CD133-1+ cells ranged between 0 and 30.9% (median 0.29) and between 0.1-30.3 (median 5.5) in ovarian and endometrial cancer, respectively. The percentage of CD133-2+ cells ranged between 0.05 and 34.5% (median 0.76) and between 0.2-27.7 (median 4.7) in ovarian and endometrial cancer, respectively. There was a statistically significant direct correlation between the percentage of CD133-1+ and CD133-2+ cells in the whole series (r=+0.97, p value=0.0001). The CD133+/CD45- cell population is aneuploid (near-diploid, DNA Index=1.06-1.21; hypodiploid, DNA Index= 0.5-0.9) with a high mitotic index =5-10%). There was no difference in the ploidy as well as in the mitotic index between CD133+ versus CD133- cancer cells. RT-PCR, and immunohistochemistry confirmed the presence of CD133-1/2+ cells in the tumors. The assessment of clonogenic potential and the possible correlations between the percentage of CD133-1/2+ cells and clinico-pathological characteristics of the tumors are ongoing.

Session D:

Molecular Mechanisms of Self Renewal

Cellular Senescence And DNA Damage Response

Fabrizio d'Adda di Fagagna.

IFOM Foundation - FIRC Institute of Molecular Oncology Foundation, via Adamello 16, 20139 Milan, Italy. email: fabrizio.dadda@ifom-ieo-campus.it

Cellular senescence is a general stress-response program that restrains cellular proliferation. We recently demonstrated that telomere-initiated cellular senescence is a DNA-damage response enforced by the activation of the DNA-damage checkpoint machinery with the direct contribution of eroded telomeres. Such a response is not a transient phenomenon, but it consists of a permanent activation of the DNA damage checkpoint machinery, that, if left unperturbed, persists presumably ad infinitum. We propose that this is due to the DNA damage being irreparable in senescent cells. Its irreparability is not due to the inability of senescent cells to repair exogenous DNA damage but to the intrinsic nature of their DNA lesions.In addition to telomere shortening or uncapping, cellular senescence can be triggered by suboptimal in vitro growth conditions (culture shock) or oncogene overexpression. The molecular mechanisms that trigger cellular senescence following these events are still poorly understood. We have recently gathered new compelling evidence that strengthens the links between the establishment of senescence under these conditions and the accumulation of DNA damage. Our most novel results will be discussed.

Control Of Tumour Surveillance Mechanisms By Wip1 Phosphatase

S. Sathya, O.Demidov, O.Timofeev, E.Wong, C.Kek, DV.Bulavin

Cell Cycle Control and Tumorigenesis Group, Institute of Molecular and Cell Biology, Singapore 117609

Recent analysis of an amplified oncogene, Wip1 phosphatase, revealed its ubiquitous role in tumorigenesis. Deletion of Wip1 renders mouse embryo fibroblasts resistant to transformation and significantly delays the onset of breast cancers driven by multiple oncogenes. Tumour resistance in the absence of Wip1 is genetically linked to activation of the Cdkn2a (encodes Ink4a and Arf proteins) tumour-suppressor pathway, which in turn is dependent on p38 MAPK. Further breeding of Wip1-null mice into different tumor-prone mouse backgrounds revealed that deletion of Wip1 phosphatase almost ubiquitously delays the onset of cancer via p38 MAPK-dependent and the newly identified ATM/ATR-dependent signaling networks. Importantly, the appearance of cancer arising from a stem cell compartment such as teratomas after injecting ES cells was completely abrogated in a Wip1-null background. We propose that inhibition of Wip1 could be the basis for a therapeutic approach in a broad spectrum of human tumors.

Role of Postmitotic, Differentiated Keratinocytes in Epidermal Tumor Formation

Salvador Aznar Benitah, Masaru Honma and Fiona Watt

London Research Institute, Cancer Research UK, Keratinocyte Department.

Squamous cell carcinomas arise and progress from basal epidermal stem cells that harbour oncogenic mutations. However, deregulation of signalling pathways in suprabasal keratinocytes can influence the behaviour of basal cancer stem cells, and promote tumour onset and progression. We have previously identified that inappropriate alpha6beta4 or alpha 5 beta1 integrin expression correlates with a high risk of tumour progression in stratified squamous epithelia. Targeted expression of alpha6beta4 in the suprabasal layers of transgenic mouse epidermis dramatically increased the frequency of papillomas, carcinomas and metastases induced by chemical carcinogenesis. We have shown that suprabasal keratinocytes that express alpha6beta4 increases tumour burden by inhibiting TGFbeta-mediated growth arrest in basal cancer cells. At the molecular level we found that inhibition of TGFbeta activity in basal cells by suprabasal alpha6beta4 integrin overexpressing cells was mediated by a cytoskeletal network formed by E-cadherin, phosphoinositide 3-kinase (PI3-K) and the small GTPase Rac1. We conclude that aberrant expression of alpha6beta4 integrins in suprabasal keratinocytes can influence basal cancer stem cells in promoting tumour onset and progression.

Myc-T58 Phosphorylation By Gsk3 Is Regulated By The Apc-Axin Tumor Suppressor Complex

G. Fagá and B. Amati.

Institute of Experimental Oncology, Milan, Italy

The c-Myc transcription factor is an essential regulator of cell proliferation and apoptosis. Myc is an unstable protein and its levels are tightly regulated within the cell. Recent work in the field has started to unravel the network that controls Myc stability. Myc is degraded via the ubiquitin-proteasome pathway, in a process that is largely dependent upon the phosphorylation of Thr 58 (T58) by glycogen synthase kinase-3 (GSK-3). T58 is a mutational hotspot in Burkittís lymphomas, and Myc mutants harboring T58 alterations show prolonged half-life and increased oncogenic potential. GSK-3 also phosphorylates beta-catenin, the intracellular effector of the Wnt pathway. Phosphorylation targets beta-catenin to ubiquitination and degradation, and occurs within a multiprotein complex that comprises, among others, the tumor suppressor proteins APC and Axin. Our findings suggest that the phosphorylation of Myc by GSK-3 also relies upon the APC/AXIN complex. First, Myc and APC can be co-precipitated from transfected cells. Second, Myc T58 phosphorylation by GSK-3 is impaired in colon cancer or hepatocarcinoma cell lines that contain mutations in APC or AXIN, respectively. T58 phosphorylation is also altered when the APC protein is experimentally eliminated from a cell. Our progress in characterizing this physical and functional interaction between Myc and APC will be presented.

Phosphoinositide 3-Kinase Signaling To Akt Promotes Keratinocyte Differentiation Versus Death

Enzo Calautti[1,2], Jian Li[1], Stefania Saoncella[1,2], Janice L. Brissette[1] and Paul F. Goetinck[1]

[1]From the Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA - [2]Present address: Epithelial Stem Cell Research Center, The Veneto Eye Bank Foundation, Ospedale Civile di Venezia, Castello 6777, 30122 Venice, Italy

Epithelial carcinogenesis tightly associates with unbalances in the signaling pathways regulating cell differentiation and survival. In the epidermis, the cellular signals promoting differentiation widely overlap with those activated during apoptosis; how differentiating cells remain protected from premature death is still poorly defined. We show here that the PI3K/Akt pathway is engaged during keratinocyte differentiation both in culture, and in the intact epidermis in vivo. In keratinocytes, active Akt expression promotes growth arrest and differentiation, whereas PI3K inhibition prevents terminal differentiation and causes selective death of cells that would otherwise differentiate. Activation of the PI3K pathway in epidermal differentiation requires the activity of EGFR and Src tyrosine kinases, and the engagement of E-cadherin mediated adhesion. During this process, PI3K is recruited to cadherin-catenin protein complexes bearing phosphorylated YxxM motifs. Thus, PI3K regulates the choice between epidermal cell differentiation and death, at the cross-talk between tyrosine kinases and cadherin-associated catenins. Nevertheless, gains of PI3K/Akt activities are also implicated in epidermal carcinogenesis, process which may affect keratinocyte stem cells populations. This raises the question whether in these cells the PI3K signaling plays a distinct role, and promotes self renewal at the expenses of differentiation. Future studies will be aimed to test this hypothesys.

Modulation Of EGF Receptor Signalling By A New Family Of UBA Domain-Containing Proteins

Nicola Crosetto; Katarzyna Kowanetz; Kaisa Haglund; Mirko Schmidt; Carl Heldin; Ivan Dikic
Molecular Signaling Group - Head: Prof. Ivan Dikic

In metazoans, downregulation of stimulated Receptor Tyrosine Kinases (RTKs) ensures tight spatio-temporal control of crucial cellular functions such as growth, proliferation, migration and cell-fate determination. The E3 ubiquitin ligase Cbl monoubiquitylates activated RTKs at multiple sites, targeting them for degradation in the lysosome. We discovered a new Cbl interacting protein, Clip4/Sts2, with high sequence homology to another ubiquitous mammalian protein, named p70/Sts1. Both contain one SH3 domain and a UBiquitin Associated domain (UBA), as well as two homology regions with currently undefined funtions. Upon EGF stimulation, Clip4/p70 bound to Cbl via their SH3 domains and were recruited into a complex with activated EGF receptors. The isolated UBA domains of Clip4/p70 also bound to monoubiquitin stronger than the endocytic adaptors epsin, Eps15 and Hrs. This implicates that the UBA domains of Clip4/p70 may compete with those proteins for interacting with multiubiquitinated EGFRs, hence slowing down their degradation. Accordingly, upon transient overexpression of Clip4/p70, ligand-stimulated EGFRs were endocytosed at a slower rate and accumulated at the plasma membrane, causing prolonged signalling and increased cell proliferation.In summary, we describe a novel family of UBA-containing proteins able to modulate EGFR downregulation, whose impairment may play a role in tumorigenesis.

The Role Of Wip1 Phosphatase In WNT-Induced Tumorigenesis

Demidov ON, Timofeev O, Lee TY, Kek C, Bulavin DV

IMCB, Singapore

The WNT signaling pathway plays a critical role in self-renewal and pluripotency of stem cells and several mouse models have been established to understand the role of WNT signaling in tumorigenesis. Here we analyzed the effect of Wip1 phosphatase deletion on tumor formation in two mouse models: MMTV-WNT-driven mammary gland cancer and APC/Min-induced intestinal polyposis. We found that deletion of Wip1 from APC/Min mice leads to dramatic decrease in the number of polyps. On the other hand, Wip1-deficient mice expressing MMTV-WNT transgene developed mammary gland tumors at the same rate as wt mice expressing MMTV-WNT. We propose that effect of Wip1 deletion on WNT-driven tumor development is cell type and context-dependent.

The Intestinal Crypt Transcription Factor Sox9 Is Regulated By The Wnt Pathway And Represses Differentiation Genes

Blache P., van de Wetering M., Duluc I., Domon C., Freund J.-N., Clevers H. and Jay P.

Tcf and Sox proteins belong to the HMG-box transcription factor family. Tcfs are the transcriptional effectors of the Wnt pathway, which plays important roles in the homeostasis and cancer of the intestinal epithelium, whereas little is known about the function of Sox proteins in this organ. Here, we report that Sox9 is expressed in the intestinal epithelium, under the control of the Wnt/beta-catenin/Tcf pathway. We provide in vitro and in vivo evidence that a bipartite beta-catenin/Tcf transcription factor is required for Sox9 expression in epithelial cells. Finally, in colon-derived epithelial cells, SOX9 transcriptionally represses the CDX2 and MUC2 genes, which are expressed in the mature villus epithelial cells of the intestine.

Ras oncogene inhibition of thyroid differentiation in FRTL5 cells

M.G. Baratta [1,2], G. De Vita[1] and Roberto Di Lauro[1,3]

[1]CEINGE biotecnologie avanzate, Naples, Italy - [2]S.E.M.M. European School of Molecular Medicine, Naples site, Italy - [2,3]BioGeM, Naples, Italy 3

Activating mutation in ras genes have been associated with undifferentiated thyroid carcinomas. Accordingly in FRTL5 cells ras oncogene expression induce transformation and loss of differentiation. In order to elucidate the molecular mechanisms involved in ras oncogene-induced de-differentiation, we constructed a conditional Ras oncoprotein, fusing a tamoxifen-sensitive mutant of the estrogen receptor ligand binding domain to H-RasV12 (ER-RasV12). This inducible system allowed us to analyse the kinetic of transformation in thyroid cells.We have found that thyroid differentiation markers are downregulated by ER-RasV12 very soon after its activation. TSH receptor (TSHr), a key factor in thyroid differentiation, was rapidly decreased and its signaling pathway was inhibited. We generated stable clones expressing an ectopic TSHr. We found that even in the presence of TSHr ER-RasV12 is still able to downregulates thyroid-specific genes expression. Interestingly the inhibition of TSHr signalling still persist even restoring TSHr expression. We have found that the activity of several thyroid-specific enhancer and promoter is downregulated by ER-RasV12. We are now using these sequences to set up a fluorescence reporter system for high-throughput screening of genes invoved in ER-RasV12 mediated de-differentiation.

Oct3/4, an embryonic stem cell marker, is expressed in bladder tumors

Yaser Atlasi[1], Seyed Javad Mowla[1], Seyed Amir-Mohsen Ziaee[2], Ahmad-Reza Bahrami[3]

[1]Department of Genetics, Faculty of Basic Sciences, Tehran ,14115-175, [2]Urology and Nephrology Research Center, Labbafi-Nejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, 166666, [3] Institute of Biotechnology and Tissue Engineering, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Oct3/4 is a key regulator of pluripotency in emberyonic stem and germ cells. Recently its expression in adult human stem cells and the displastic effects of its ectopic expression in epithelial tissues has been reported. Here we have investigated the possible expression of oct3/4 in bladder cancer. Methods: We used semi-quantitative RT-PCR to examine 15 tumoral, 11 matched non tumoral and 5 healthy control tissues samples. The possible artifacts were minimized by performing the following experiments: DNAase treatment of the RNA samples, No-RT negative control, restriction digestion and sequence analysis of the PCR products.The expression of Oct3/4 at protein level was further determined by Dot blot and Western blot analysis. Results: Oct3/4 expression was detected in all examined tumors but at much lower level in some non-tumoral samples and also in few healthy controls. Dot blot and Western blot analysis further confirmed the expression of Oct3/4 in tumoral biopsies. Conclusion: regarding the anti-differentiation role of Oct3/4, the expression of the gene can be used as a potential tumor marker for diagnosis and/or prognosis of bladder tumors. Work is currently in progress in our lab to examine the correlation between the grade of malignancy and tumor recurrence with the level of oct3/4 expression.

Notch Activity Involves Phosphorylation-Dependent Interaction With Pin1

Alessandra Rustighi, Luca Tiberi, Fiamma Mantovani, Xavier Girardini, Elisa Guida, Francesca Tocco, Andrea Bisso, Anna Comel, Alice Grison and Giannino Del Sal

National Laboratory CIB, Padriciano, Trieste, Italy, and Dept. of Biochemistry, Biophysics and Chemistry of Macromolecules of the University of Triest, Triest, Italy.

The Notch signaling pathway plays a crucial role in specifying cellular fates in metazoan development and its activation has been associated with the amplification of some somatic stem cells and in cancer development.Phosphorylation of Serine or Threonine residues preceding Proline (S/T-P) is essential in diverse cellular processes and its deregulation can lead to several diseases. Pin1 is an highly conserved enzyme that isomerizes only the phosphorylated S/T-P bonds in proteins thereby inducing conformational changes required for their functions.Pin1 regulates many cellular events and is involved also in cancer and neurodegenerative disorders. Recently we have reported that Pin1 also regulates the functions of p53 and p73.To further investigate cross-talks between different signaling pathways and Pin1 we generated Pin1/p53 double knock out mice. We reasoned that the absence of p53 might unmask a role of Pin1 on other pathways. Preliminary results show that the absence of Pin1 partially rescues the p53 null phenotype providing evidence for a role of Pin1 in pathways which are involved in p53KO thymic lymphoma formation.Since Notch has been shown to control T-cell function and thymic lymphoma formation we are investigating the possibility of a role of Pin1 in regulating the Notch pathway in thymocytes. Data about the role of Pin1 in modulating some aspects of Notch pathway will be discussed.References:Atchinson et al. (2003) Development 130, 3579-3586.; Wulf et al. (2005) Nat. Cell Biol. 7, 435-441.; Zacchi, P. et al.(2002) Nature 419, 853-857.; Mantovani et al. (2004) Mol. Cell. 14, 625-636.

Novel Functions Of Ig-Cams In Tumor Progression

Chiara Francavilla, Silvia Zecchini, Luigi Maddaluno, Silvia Campanella, Sèbastien Loeffler, and Ugo Cavallaro

IFOM - FIRC Institute of Molecular Oncology, Milan, Italy

Cell adhesion molecules of the immunoglobulin superfamily (Ig-CAMs) are frequently dysregulated concomitant to the malignant conversion of various tumor types. However, whether this aberrant expression and/or function of Ig-CAMs during cancer progression is an epiphenomenon of tumor development itself or it rather contributes causally to the disease remains elusive.We have addressed this issue by focusing on two IgCAMs, the neural cell adhesion molecule (NCAM) and L1, which exhibit altered expression in different solid tumors. The functional role of these Ig-CAMs in tumor progression has been studied in animal models of multistage tumorigenesis as well as in appropriate cellular systems and in patients' samples.Our results point to novel and crucial functions of NCAM and L1 in various steps of cancer development, ranging from tumor cell adhesion and migration to metastatic dissemination. Moreover, we have unraveled novel signaling properties of these two Ig-CAMs, in particular due to their ability to establish functional interactions with receptor tyrosine kinases. Finally, we have obtained preliminary evidence that the expression of L1 is regulated by nuclear beta-catenin. Besides its role in tumorigenesis, nuclear beta-catenin has been implicated in stem cell formation, thus raising the interesting hypothesis that L1 may be involved in cancer cell stemness.Taken together, our data implicate NCAM and L1 as novel players in cancer progression, and offer additional targets to be validated in pre-clinical tumor models.

WILT: An Ambitious, But Possibly Uniquely Powerful, Potential Cancer Prevention Therapy

Aubrey D.N.J. de Grey

Department of Genetics, University of Cambridge

The intrinsic genetic instability of cancer cells makes age-related cancers more difficult to postpone or treat than any other age-related diseases. Cancer cells that retain (or acquire, by mutation) the characteristics of stem cells are especially formidable, given their potential for self-renewal. Any treatment that a cancer can resist by activating or inactivating specific genes is unlikely to succeed over the long term, because pre-existing cancer cells with the necessary gene expression pattern will withstand the therapy and proliferate. Whole-body Interdiction of Lengthening of Telomeresî (WILT) is a proposal to pre-empt this problem by deleting from as many of our cells as possible the genes needed for telomere elongation. Cancers lacking these genes can never reach a life-threatening stage by altering gene expression, only by acquiring new genes, which is far more unlikely. Continuously-renewing tissues can be maintained by periodic reseeding with telomere elongation-incompetent stem cells that have had their telomeres lengthened in vitro with exogenous telomerase; evidence suggests that this need be done no more than once per decade for any tissue. I will describe why WILT might prove to be an exceptionally powerful anti-cancer modality.

The Notch Pathway In Tumor Cell Migration

Raffaella Chiaramonte, Leonardo Mirandola, Andrea Basile, Massimo Locati*, Paola Comi

*Dept. Biomedical Science and Tecnology, Universitá degli Studi di Milano, via Fratelli Cervi 93, 20090, Segrate, Italy.*Istituto di Patologia Generale, Universitá degli Studi di Milano, via Mangiagalli 31, 20132, Milano, Italy.*

This work aims to elucidate the relationship between two pathways which play a role both in stem cells and when deregulated in cancer: the pathway of Notch1 (Annu.Rev.Immunol. 23: 945) and the CC Chemokine Receptor 9 (CCR9) signaling. CCR9 is expressed in thymic multipotent precursors (TMP) which have the potentiality to give rise to T, B and dendritic cells (J.Exp.Med. 202: 21). The CCR9 agonist CCL25 is expressed by thymic stromal cells and mediates TMP localization in the thymic sub-capsular zone. CCL25 expression in the gut is also responsible for mature CCR9+ lymphocytes localization at this site and explains relapse location of CCR9+ leukemic cells in the gut (Blood 103: 2806); furthermore CCR9 affects prostate cancer cell migration and invasion (Clinical Cancer Res. 10: 8743). Through the siRNA technique and the use of DAPT, as inhibitor of Notch1 activation, we demonstrated that CCR9 expression in T-ALL cell lines is sustained by Notch1 signaling, and that Notch1 plays a role in CCR9-mediated biological effects such as CCL25-induced chemotaxis and anti-apoptotic effect. This evidence suggests that the integrated activities of these pathways during oncogenesis may allow leukemic stem cells to escape apoptosis and migration at specific sites.

NOVEMBER 12, 2005 - ABSTRACTS

ORAL PRESENTATIONS

LECTURES

Sean J. Morrison
Ann Arbor, US

Stephen Dalton
Athens, Georgia, US

Andreas Trumpp
Epalinges, Switzerland

Salvador Aznar Benitah
London, UK

ORAL PRESENTATIONS

Gabriel Gutierrez
Boston, US

Sophia Bruggeman
Amsterdam, The Netherlands

Antonio Costanzo
Rome, Italy

Ignacio Moreno de Alborn
Madrid, Spain

Session 4:

Molecular Mechanisms of Self Renewal

Prb Loss Alters Progenitor Potential

Gabriel M Gutierrez, David M. Thomas, Hai-SuYang, Elizabeth Kong, Pedro Santiago, Amit Desphande, Volkan Gunduz, and Philip W Hinds Tufts

New England Medical Center, Molecular Oncology Research Institute

Our lab has shown that pRb acts as a transcriptional co-activator for the bone master regulator, Runx2, necessary for bone terminal differentiation. Further, upon inactivation of pRb both the tumor suppressor and differentiation pathways are compromised. Individuals inheriting mutant RB1 will likely develop retinoblastoma and then osteosarcoma later in life. Interestingly, many such childhood cancers have a dedifferentiated status. Further, loss-of-heterozygosity at the RB locus correlates with dedifferentiated liposarcomas. Taken together, these data implicate pRb in masterminding a switch from a proliferating to differentiating cell, disruption of which leads to greater proliferative potential and stem cell like characteristics.We show that osteoblasts lacking pRb are highly proliferative and express high levels of early bone markers. However, these cells fail to properly differentiate and grow to very high density. These traits are consistent with accumulation of osteoprogenitors. These cells in turn may serve as precursors to an osteosarcoma cancer stem cell. Similarly, we have examined the hair cells of pRb null mice and shown that these cells continue to proliferate despite expression of differentiation markers. Further, we have analyzed an intestinal defect in pRb null mice during development. We see increased proliferation and abnormal expression of differentiation markers analogous to bone.

The Role Of The Polycomb Group Gene Bmi1 In CNS (Stem) Cells And Cancer

Sophia Bruggeman[1], Merel Valk-Lingbeek[1], Petra van der Stoop[1], Carly Leung[2], Danielle Hulsman[1], Ellen Tanger[1], Tessa Buckle[3], Olaf van Tellingen[3], Yvan Arsenijevic[4], Silvia Marino[2] and Maarten van Lohuizen[1].

[1]Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands - [2]Department of Pathology, Institute of Clinical Pathology, University of Zürich, Zürich, Switzerland - [3]Department of Clinical Chemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands - [4]Laboratory of Oculogenetics, Opthalmic Hospital Jules Gonin, Lausanne University, Lausanne, Switzerland

The Polycomb group (PcG) genes, constituting a family of chromatin modifiers, were originally identified as repressors of the Homeobox genes determining body plan and segmentation. They play important roles during normal development, but can contribute to cancer when improperly expressed. Recently, we and others have shown that the PcG protein Bmi1 is required for Sonic hedgehog-induced proliferation of cerebellar granule neuron progenitors and for self-renewal of subventricular zone-derived adult neural stem cells (Nature 425:6961; Nature 428:6980; J. Neurosci. 25:24). Previously, our group identified the Ink4a/Arf tumor suppressor locus as a target of Bmi1 repression in the protection of cells from premature senescence. Here we addressed to what extent this locus is involved in restricting neural stem cell self-renewal and proliferation of cerebellar progenitors downstream of Bmi1, and more specifically whether Ink4a or Arf is the critical target. We find that Arf de-repression is largely responsible for Bmi1 null phenotypes, however, Ink4a upregulation significantly contributes in some but not all cell systems indicating cell-type specific requirements for Ink4a and Arf repression (Genes Dev. 19:12). Interestingly, the Ink4a/Arf locus together with EGF signaling, the latter being essential for neural stem cell maintenance, are frequently found mutated in gliomas. We are currently investigating if Bmi1 plays a role in the formation of these tumors independently from the Ink4a/Arf locus.

A Role For The P63-Ikkalpha Pathway In The Regulation Of Epidermal Stem Cells Fate

Barbara Marinari[1], Maranke Koster[2], Maria Laura Giustizieri1, Costanza Ballar[3], Stefano Alemá[3], Michael Karin[4], Dennis R Roop[2] and Antonio Costanzo[1].

[1]Department of Dermatology University of Rome Tor Vergata - [2]Department of Molecular and Cellular Biology, Baylor College, Houston TX - [3]Istituto di Biologia Cellulare, CNR, Italy - [4]Department of Pharmacology, UCSD, La Jolla, CA.

The P63 gene belongs to the p53 gene family of tumor suppressor genes and encodes for sequence specific transcription factors. The DNp63 protein, lacking the canonical transactivation domain, has been proposed to identify the epidermal stem cells and to regulate their survival and self renewal. In an attempt to identify new potential p63 targets, we have found that the epidermal morphogenesis modulator IKKalpha is tightly regulated at the transcriptional level by different isoforms of p63. We have observed that IKKa mRNA expression increases at the onset of the stratification program. The selective downregulation of DNp63 by siRNA in primary keratinocytes abolishes IKKa activation and the onset of terminal differentiation and increases keratinocyte proliferation rate. This is consistent with transcriptional assay data performed with an IKKa-promoter reporter gene showing a strong activation of IKKa transcription by DNp63 isoforms and a repressory activity of TAp63 isoforms. On the other hand, the downregulation of IKKa in primary keratinocytes hampers the ability of differentiating cells to exit the cell cycle inducing the formation of a K1 expressing proliferative layer that is reminiscent of the embryonic intermediate layer and of squamous cell carcinoma. Our results indicate IKKa as an important p63 target potentially involved in the control of epidermal stem cells proliferation in skin development and in cell transformation.

C-Myc Function In Hemotopoietic Stem Cells

Esther Baena and Ignacio Moreno de Alborn

Dept. Immunology & Oncology National Center for Biotechnology. CNB-CSICC/ Darwin 3 Madrid 28049, SPAIN

The basic helix-loop-helix transcription factor c-myc has been implicated in the regulation of cellular proliferation, cell growth, cell differentiation, and apoptosis. Deregulated c-myc expression are common to a wide of murine and human malignancies. Germline inactivation of c-myc gene in mice results in embrionic lethality. To elucidate c-Myc function in Hematopoietic Stem Cells (HSC) we inactivated c-Myc in the Bone Marrow using a condicional approach (c-mycfl/fl;mx-cre+ mice). We have seen that perinatal inactivation of c-Myc in bone marrow causes severe impairment of hematopoiesis in c-mycfl/fl;mx-cre+ mice. We observed loss of HSC (lin-Sca-1+c-Kit+) and accumulation of lin-Sca-1+c-Kit- cells overtime. C-Myc deficient lin-Sca-1+c-Kit- cells show normal apoptosis, increased proliferation and overexpress the cell cycle inhibitor P21. In vitro assays revealed the inability of c-Myc deficient Bone Marrow to generate colonies of different lineages. In vivo the mice showed severe anemia and cytopenia and die after two months. These results show a new role of c-Myc in the maintenance/generation of HSC.

FACULTY

Salvador Aznar Benitah
Keratinocyte Laboratory,
London Research Institute, Cancer Research UK,
London, UK
salvador.aznar-benitah@cancer.org.uk

Allan Balmain
Barbara Bass Bakar Professor of Cancer Genetics,
Cancer Research Institute,
San Francisco, US
abalmain@cc.ucsf.edu

Yann Barrandon
Lab. of Stem Cell Dynamics, School of Life Sciences,
Swiss Federal Institute of Technology Lausanne and Dept. of Experimental Surgery,
Lausanne University Hospital,
Lausanne, Switzerland
yann.barrandon@epfl.ch

Malcolm K. Brenner
Center for Cell & Gene Therapy,
Houston, US

Robert B. Clarke
Breast Biology Group, Division of Cancer Studies,
University of Manchester, Christie Hospital (NHS) Trust,
Manchester, UK
robert.clarke@manchester.ac.uk

Stephen Dalton
Dept. of Biochemistry and Molecular Biology,
University of Georgia,
Athens, Georgia, US
sdalton@uga.edu

Meenhard Herlyn
The Wistar Institute,
Molecular and Cellular Oncogenesis Program,
Philadelphia, US
herlynm@wistar.org

Craig Jordan
James P. Wilmot Cancer Center,
University of Rochester School of Medicine,
Rochester, NY, US
craig_jordan@urmc.rochester.edu

Carla F. Bender Kim
Jacks Laboratory, MIT Center for Cancer Research,
Cambridge, MA, US
cfkim@mit.edu

Sean J. Morrison
Howard Hughes Medical Institute/Dept. of Internal Medicine,
University of Michigan,
Ann Arbor, US
seanjm@med.umich.edu

Stuart H. Orkin
Harvard Medical School,
Dept. of Pediatric Oncology, Dana Farber Cancer Institute,
Boston, US
stuart_orkin@dfci.harvard.edu

Jeffrey Rosen
Molecular and Cellular Biology and Medicine,
Baylor College of Medicine,
Houston, US
jrosen@bcm.edu - www.bcm.edu/rosenlab/

Andreas Trumpp
Genetics and Stem Cell Laboratory,
ISREC Swiss Institute for Experimental Cancer Research,
Epalinges, Switzerland
andreas.trumpp@isrec.ch

Angelo Vescovi
Istituto di Ricerca per le Cellule Staminali (SCRI),
DIBIT, H. S. Raffaele
Milan, Italy
vescovi.angelo@hsr.it

Kay-Uwe Wagner
Eppley Institute for Research in Cancer and Allied Diseases,
University of Nebraska Medical Center,
Omaha, US
kuwagner@unmc.edu

AUTHORS' INDEX

ORAL PRESENTATIONS & POSTERS

AUDITORS

IMPORTANT ADDRESSES

WORKSHOP VENUE

IFOM-IEO CAMPUS
Via Adamello, 16
20139 Milan, Italy
Phone +39.02.574303200
Fax +39.02.574303231
www.ifom-ieo-campus.it

FOR GENERAL INFORMATION

Sabrina Frata
Events Coordinator for SEMM Foundation
IFOM-IEO Campus
Via Adamello, 16
20139 Milan, Italy
Phone +39.02.574303296
Fax +39.02.574303231
events@semm.it

ORGANIZING SECRETARIAT

CQ Travel Srl
Via Pagliano, 3
20149 Milan, Italy
Phone +39.02.4804951
Fax +39.02.43911650
congress@cq-travel.com

TOURISTIC INFORMATION ABOUT MILAN

HOTELS OF THE WORKSHOP

GRAND VISCONTI PALACE (4 star sup.)
Viale Isonzo, 14 - Milan, Italy
Phone +39.02.540341 Fax +39.02.54069523
www.grandviscontipalace.com

BEST WESTERN HOTEL MAJOR (4 star)
Viale Isonzo, 2 - Milan, Italy
Phone +39.02.55188335 Fax +39.02.55183140
www.bwhotelmajor-mi.it

TAXI IN MILAN

Phone 02/8585 - 02/4040 - 02/6767

ATM

In Milan all the public transportation is managed by **ATM.**
In the Milan area an integrated system enables users to travel with a single ticket or pass on all urban and suburban routes. ATM provides different kinds of tickets for all needs: tourists, occasional users, workers, etc. (*http://www.atm-mi.it*)
ATM Information Office
For information on scheduling, routes, tickets and fares you can apply also to the information/pass office at the Duomo metropolitan railway station (red and yellow lines)
ATMPOINT
Galleria del Sagrato, mezzanino stazione M1/M3 Duomo
from 7.45am to 8.15pm - Mondays to Saturdays (Sundays and public holidays excluded)
Toll-free number 800-80.81.81
from 7.30am to 7.30pm - Mondays to Sundays

UTILITIES

Touristic Information

A.P.T., Azienda di Promozione Turistica del Milanese
Via Marconi, 1 Milan – Ph. 02.72524301/2/3
www.milanoinfotourist.com

T.C.I., Touring Club Italiano
C.so Italia, 10 Milan – Ph. 02.85261
www.touringclub.it

BANKS

Banks in Milan are open Mondays-Fridays from 8.30 am to 1.30 pm and one hour in the afternoon between 3.00/4.00 pm. Many banks offer the service where you can automatically change your money through your credit cards.

HEALTH

Free first aid services are available to all EU citizens who have a special form (E111) issued by the health authority of their own home country. For no EU citizens we suggest to open a special insurance for the duration of the trip in order to avoid the cost of possible medical treatments.
Medicines can only be bought at the pharmacy (*"Farmacia"*). They are identified by a red or green cross.
They are opened from 8.00 am to 12.30 pm and from 3.00 pm to 7.00 pm.

EMERGENCY NUMBERS

Police - 113
Firemen - 115
Ambulance - 118
Traffic police - 02/77271
Malpensa airport - +39.02.74852200
Linate airport - +39.02.74852200
Lost luggage *(Malpensa)* - +39.02.58580069/70
Lost luggage *(Linate)* - +39.02.70124451