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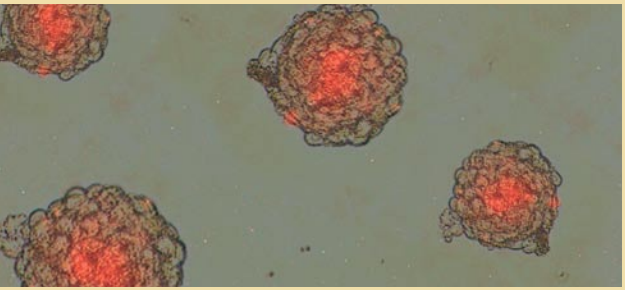
Università degli Studi di Milano

International Workshop on

Cancer Stem Cells

2nd edition

1st – 3rd December 2007
IFOM-IEO Campus
Milan, Italy



Abstract Book

International Workshop on

Cancer Stem Cells

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Organization

SCIENTIFIC & ORGANIZING COMMITTEE

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Robert B. Clarke, Manchester, UK

h. 9.45/10.00 am
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h. 10.30/10.45 am
HERCEPTIN TARGETS TUMOR INITIATING CELLS OF HER2 POSITIVE TUMORS
Alessandra Magnifico, Milan, Italy

h. 10.45/11.15 am - Coffee break

h. 11.15/11.45 am
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h. 11.45/12.00 am
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END OF THE WORKSHOP

December 1, 2007

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Jeffrey Rosen
Houston, USA

Meenhard Herlyn
Philadelphia, USA

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Palo Alto, USA

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Oral Presentations

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Singapore, Singapore

Salvatore Pece
Milan, Italy

Christopher Heeschen
Münich, Germany

Claudia Gaspar
Rotterdam, The Netherlands

Marina Pajic
Amsterdam, The Netherlands

Session I and Session II

Cancer stem cells in solid tumors - Model systems

REGULATION OF HUMAN BREAST CANCER STEM-LIKE CELLS

ROB CLARKE

Breast Biology Group, University of Manchester

We have been investigating human breast cancers for the presence of a stem-like cell population. Using both adherent and non-adherent culture methods known to enrich for normal tissue stem cells, we have demonstrated that breast cancer cell lines and primary tumours contain a self-renewing, colony-forming population that can be enriched for by cell surface markers such as CD44, alone or in combination with other markers. In order to discover novel stem cell markers, we have been investigating novel antibodies raised against neural stem cells and have discovered that several of these enrich for breast cancer stem-like cells. We have demonstrated the cancer-initiating stem-like cell population to be highly regulated by the oestrogen, epidermal growth factor (EGF) and Notch receptor signaling pathways. Recently, we have applied retroviral short hairpin (sh) RNA libraries to discover novel pathways involved in the control of breast cancer stem-like cells. This has revealed the importance of several pathways not previously known to regulate stem cell survival and self-renewal. Inhibitors of signalling pathways that regulate cancer stem-like cells could represent a new therapeutic modality in breast cancer, perhaps through combination with current treatments.

THERAPEUTIC RESISTANCE OF BREAST CANCER TUMOR-INITIATING CELLS

M Zhang, X. Li, M T Lewis, J C Chang and J M ROSEN

Departments of Molecular & Cellular Biology and Medicine, Baylor Breast Center, Baylor College of Medicine, Houston, TX 77030

We have identified a tumor initiating subpopulation within a unique p53 null mouse mammary tumor model by limiting dilution transplantation experiments in syngeneic mice. The functional activity of the tumor initiating subpopulation was correlated with its ability to generate secondary mammospheres. We compared gene expression profiles in the tumorigenic subpopulation with the comparable stem/progenitor cell population from the normal mammary gland of Balb/c mice. These analyses have suggested that the p53 tumors may have arisen from a bipotential progenitor. Furthermore, gene expression signatures identified for polycomb gene family members and their gene targets, suggest that epigenetic modifications may play an important role in regulating the cell fate of these tumor-initiating cells. Finally, increased expression of genes involved in DNA damage response pathways may help explain the hypothesized resistance of CSCs to chemo- and radiation therapy. To provide support for this hypothesis, we have analyzed by flow cytometry paired breast cancer core biopsies before and after neoadjuvant chemotherapy or lapatinib. The tumorigenic breast cancer cells were intrinsically chemoresistant □ chemotherapy led to an increase in the relative proportion of CD44+/CD24-/low cells, and increased self-renewal capacity in mammosphere assays. Conversely, in patients with HER2 overexpressing tumors, lapatinib (EGFR/HER2 tyrosine kinase inhibitor) decreased the relative proportion of tumorigenic cells and mammosphere formation. These studies provide the first clinical evidence for a subpopulation of chemotherapy-resistant breast cancer-initiating cells, and suggest that specific pathway inhibitors in combination with conventional chemotherapy may provide a therapeutic strategy(Supported by CA16303 and U01-CA84243 from the National Institutes of Health (JMR), and grant PDF0504283 from the Komen foundation (MZ).

MELANOCYTE AND MELANOMA STEM CELLS

MEENHARD HERLYN, Ling Li, Susan Zabierowski, Nga Nguyen, Jennifer Marmion, Kathrin Sproesser, Trish Brafford, Ben Himes, Mizuho Fukunaga-Kalabis

The Wistar Institute, Philadelphia, PA 19096

Using culture conditions for human embryonic stem cells, we have isolated cells with stem cell-like characteristics from both human hair follicles and the dermis. The cultured cells grow as spheres and single cell-derived clones can be differentiated to melanocytes, neuronal cells, smooth muscle cells, adipocytes and chondrocytes when appropriate culture conditions are being used. We are testing the hypothesis that melanocyte stem cells are the primary target for transformation to melanoma. Using a complex model of skin equivalents mimicking the human skin microenvironment the laboratory is reconstructing each step in the melanoma progression cascade. Genes associated with melanoma are overexpressed or expression is silenced with shRNAi constructs in lentiviral vectors. Our recent experiments suggest that as few as two genetic 'hits' can induce malignant transformation of melanocytes if the microenvironmental conditions are supporting cells to survive the initial crisis. We are then characterizing melanoma stem cells (tumor-initiating cells) from melanoma spheres that represent a subpopulation in malignant lesions and that have characteristics of stem cells. We are distinguishing four populations of melanoma cells with stem cell-like characteristics: a. CD20+ cells, b. side population cells with increased drug resistance, c. label-retaining cells that turn over very slowly, and d. CD133 positive cells. Each of the tumor stem cells (tumor-initiating cells) show properties of self-renewal, differentiation (towards melanocytic cells, adiopocytes, chrondrocytes, and osteoblasts) and at least 10-fold higher tumorigenicity than control melanoma cells from adherent cultures. Investigations of cancer stem cells will help us to understand tumor dormancy, recurrence, metastasis, and therapy resistance.

"CANCER STEM CELLS": OPERATIONAL DEFINITION AND CLINICAL IMPLICATIONS

PIERO DALERBA & Michael F. Clarke

Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Palo Alto, CA 94304

A growing body of evidence is increasingly lending support to the concept that cancer can be modeled and interpreted as a stem-cell disease. This hypothesis is supported by a set of three experimental observations: 1) only a minority subset of cancer cells within each tumor is capable of initiating tumor growth when transplanted into immunodeficient mice; 2) cancer cells endowed with tumorigenic properties are characterized by a distinctive profile of surface markers and can be differentially and reproducibly isolated from non-tumorigenic ones; 3) tumors originated from purified tumorigenic cancer cells contain mixed populations of tumorigenic and non-tumorigenic ones, thus recreating the full diversity of the parent tumor's phenotypic repertoire. Currently, the phenotypic subpopulation of cancer cells that is selectively endowed with tumorigenic capacity is operationally defined as the "cancer stem cell" (CSC) subpopulation. Initially developed in leukemias, the CSC working model has been subsequently extended to solid tumors, including breast, brain, head & neck and pancreatic cancer. Recently, we have developed a very robust and highly reproducible CSC model for the study of human colorectal carcinoma, based on a novel set of three independent surface markers (EpCAM/CD44/CD166), thus confirming the validity of this experimental approach in another major form of human epithelial cancer. The CSC hypothesis has several implications for both the basic modeling of tumor biology and the design of anti-tumor treatments, as it suggests that the CSC subset, although relatively small in number, might be preferentially endowed with the capacity to self-renew and therefore be primarily responsible for the long-term maintenance of tumor growth of both primary and metastatic tumor lesions. In accordance with this prediction, analysis of the transcriptional profile of human "breast cancer stem cells" (Br-CSC) by gene-expression arrays has recently led us to the identification of a 186-gene Br-CSC gene-expression signature, which could be used to stratify breast cancer patients in different prognostic categories. Patients whose tumors' transcriptional profile had the highest similarity to the Br-CSC signature displayed substantially reduced 10-year overall and metastasis-free survival. The prognostic role of the Br-CSC signature proved to be independent of several known clinical and pathological prognostic criteria. Interestingly, the Br-CSC signature could be used to predict reduced survival and/or increased risk of relapse across different types of human tumors, including lung cancer, prostate cancer and medulloblastoma. Taken together, these observations suggest that analysis of the CSC subset could provide important insights in the processes of tumor relapse and metastasis and that these processes could be associated to a core set of CSC functional properties shared across different tumor types.

THE BIOLOGY OF THERAPEUTIC RESPONSE IN MOUSE MODELS OF BRAIN TUMORS

ERIC C. HOLLAND

Genetic models of tumors can provide experimentally manipulatable tumors arising in the host with similar tumor host interactions found in human tumors. We have used models of gliomas and medulloblastomas to understand the biology of response to standard therapy. We find that the cells that survive radiation and repopulate the tumor have stem cell properties and depend on the activation of specific signaling pathways. Blockade of these pathways cooperates with radiation to reduce the survival of these tumor-repopulating cells.

PROLIFERATION AND SELF-RENEWAL IN DROSOPHILA ADULT INTESTINAL STEM CELLS

ALLISON BARDIN, Carolina Perdigoto and Francois Schweisguth

Ecole Normale Superieure, CNRS UMR8542 - Paris, France

A major challenge in stem cell research is to understand how different strategies are utilized in different biological contexts to allow both self-renewal and proper differentiation of stem cell progeny. Drosophila melanogaster provides several models of stem cell systems and has extensively developed genetic tools with which to address mechanisms of self-renewal and differentiation. The adult Drosophila melanogaster instestine harbors stem cells (ISCs) that provide newly differentiated cells throughout the adult lifetime, likely replacing those which turnover (Micchelli and Perrimon, Nature, 2006; Ohlstein and Spradling, Nature, 2006). The ISCs have been shown to employ the Notch signaling pathway to control the differentiation of their progeny. The Notch pathway is known to play important roles in many stem and progenitor cell systems (reviewed in Wilson and Radkte, Febs Lett., 2006). We are using the ISC to address how the Notch pathway is regulated during homeostasis in the adult intestine. In particular we are trying to understand how asymmetric fate of the daughter cells is controlled and what regulators of the Notch pathway contribute to this. We will present our ongoing work to understand the maintenance of stem cell identity, self-renewal and proliferation in the adult Drosophila intestine.

LONG-TERM CULTURE AND PROLIFERATION OF CD44+/CD24_/LOW CELLS DERIVED FROM HUMAN BREAST CARCINOSARCOMA

KYUNG-MIN LEE[1*], Jong Bin Kim[1*], Eunyoung Ko[2], Incheol Shin[3], Jeong Eon Lee[2], Wonshik Han[2], Jihyoung Cho[2], Dong-Young Noh[1,2]

1 Cancer Research Institute and 2 Department of Surgery, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea and 3 Department of Life Science, College of Natural Sciences, Hanyang University, 17 Haengdang-dong, Sungdong-gu, Seoul 133-791.

The CD44+/CD24-/low cells have been recently identified as breast cancer-initiating cells. They retain tumorigenicity in vivo and display stem cell-like properties. We have obtained CD44+/CD24-/low (> 90%) cells from carcinosarcoma (metaplastic carcinoma) using mammospheres culture method which was previously used to isolate breast stem/progenitor cells. While most of the primary cells were adherent and terminally differentiated within a few passages in vitro, the mammosphere-forming cells could be maintained as floating spheres for more than 50 passages in vitro. The mammospheres were stained positive for fibronectin while negative for epithelial markers CK14 and CK18. Interestingly, nestin and tuj-1 were also expressed in these mammospheres suggesting that they may posess multipotency to differentiate into other cell types. In differentiating medium containing FBS, floating cells became adherent and their CD44 expression levels were significantly decreased. This might imply that CD44 may be responsible for maintaining self-renewal of the mammospheres. We have also found that both mammospheres and derivative adherent cells could efficiently form tumors in NOD/SCID mouse. Taken together, our results suggest that our mammospheres could be a suitable in vitro model to study breast cancer-initiating cells.

HERCEPTIN TARGETS TUMOR INITIATING CELLS OF HER2 POSITIVE TUMORS

ALESSANDRA MAGNIFICO[1], Luisa Albano[1], Sylvie Ménard[1] and Elda Tagliabue[1]

1 Molecular Targeting Unit, Department of Experimental Oncology, National Cancer Institute, Foundation IRCCS, Milan; 1 Supported by Associazione Italiana per la Ricerca sul Cancro.

HER2-positive tumors represent a particularly aggressive set of breast carcinomas with high proliferation index and metastatic potential. Evidence for the existence of tumor stem cells (TSC) in solid tumors including breast cancer has profound implications for cancer therapy because it is likely that eradication of TSC is the critical determinant in achieving cure. We set out to isolate and characterize TSC derived from HER2 positive tumors with the aim to probe TSC response to conventional therapies (doxorubicin and herceptin) used for adjuvant treatment of HER2 positive tumors. To analyze the cellular nature of doxorubicin and herceptin targets we treated xenotrasplants of MDA-MB361, a breast carcinoma cell line presenting HER2 overexpression. Tumors cells were subsequently explanted and analyzed for TSC presence using mammospheres formation efficiency (MFE) assay. We found that Herceptin-treated tumors had a statistically significantly lower MFE than doxorubicin-treated and untreated tumors. Biochemical analysis confirmed the presence of TSC markers as Oct4, Bmi1 and deltaNp63 only in mammospheres of untreated or doxorubicin-treated tumor cells, but not in Herceptin-treated tumors. Serially transplants showed that control and doxorubicin-treated tumor cells could be transplanted multiple times, while herceptin treated tumor cells could not be transplanted at any cellular doses. We found that the mammospheres express higher levels of HER2 mRNA compared to the differentiated parental cell line. HER2 expression control seems to be controlled by the Notch pathway, since selective inhibition of Notch signaling blocked the self renewal/proliferation and HER2 expression of TSC of such carcinomas. We propose that herceptin treatment might be particularly efficient in targeting cancer stem cells responsible for aggressiveness of HER2 positive breast, while doxorubicin could be used as an efficient de-bulking agent. Therefore, our data rationalize the use of combined chemotherapy in the treatment of HER2 positive tumors.

WIP1 DELETION PREVENTS THE CONVERSION OF INTESTINAL STEM CELLS INTO CANCER STEM CELLS

OLEG N.DEMIDOV, Oleg Timofeev,Hnin NY Lwin, Calvina Kek, Ettore Appella [2] and Dmitry V. Bulavin.

1 Institute of Molecular and Cell Biology, Singapore. - 2 Center for Cancer Research,NCI,NIH, Bethesda, MD, USA.

The intestinal epithelium renewal is controlled by intestinal stem cells (ISC) that give rise to rapidly proliferating progenitors that differentiate and migrate to be shed after a total lifetime of 5-7 days. Only ISC escape this flow. The long life and rapid rate of cell division make ISC primary candidates for the accumulation of genetic defects associated with cancer induction. We found that the Wip1 phosphatase was highly expressed in ISC and could serve as potential ISC marker. We investigated the role of Wip1 using a tumor mouse model of APC(Min)-driven polyposis. Wip1 removal increased the life-span of APC(Min) mice through a drastic suppression of polyp formation. This protection was dependent on the p53 tumor suppressor, which plays a putative role in the regulation of apoptosis of intestinal stem cells. Activation of apoptosis in stem cells of Wip1 deficient, but not wild-type, APC(Min) mice increased when the Wnt pathway was activated. We propose that Wip1 modulates APC(Min)-driven polyposis by setting a threshold for p53-dependent apoptosis of stem cells, thus preventing their conversion into cancer stem cells.

MOLECULAR PROFILING OF NORMAL HUMAN BREAST STEM CELLS: INSIGHTS INTO MAMMARY CARCINOGENESIS

[1]PECE S., [2]Confalonieri S., [1]Tosoni D., [1]Matera G., [2]Vecchi M., [2]Garrè M., [1]Ronzoni S., and [2]Di Fiore P.P.

1 European Institute of Oncology and 2 FIRC Institute for Molecular Oncology, Milan, Italy

Mounting evidence suggests that hijacking of signalling pathways governing normal mammary stem cell homeostasis are often subverted in human cancers. Therefore, thorough understanding of the molecular identity and biology of normal mammary stem cells is expected to profoundly impact on our current knowledge of human breast carcinogenesis as well. In this study, we described the comprehensive molecular profile of human mammary stem cells isolated from human normal breast epithelium taking advantage of their ability to survive and generate spheroid structures, termed as 'mammospheres' in appropriate culture conditions. We used the lipophilic fluorescent dye PKH26 to functionally label stem cells within mammospheres, based on the prediction that, likewise other adult regenerative cells, normal mammary stem cells are slow dividing or quiescent, while retaining the property to originate actively dividing and differentiating precursors. Therefore, we used FACS analysis to sort the most highly fluorescent cells, putatively comprising the strict-sense stem cells, from the progeny of committed progenitors. Functional analysis of the different cell fractions confirmed that only the PKH26+ cells exhibited the key defining features of 'stemness', such as: i) self-renewal property (re-formation of mammospheres upon serial passages in vitro); ii) ability to generate both epithelial and myoepithelial histotypes, as determined with specific lineage markers; iii) in vitro formation of organotypic outgrowths. Using oligonucleotide-based arrays (Affymetrix), we obtained trascriptional profiles of the two populations separated by their differential epifluorescence. Our transcriptomic screen identified genes seemingly differentially regulated when the immature stem cells enter the morphogenetic program to originate differentiating progenitors. In view of the emerging concept of the stem cell origin of cancers, we investigated the clinical and prognostic significance of this "stemness" signature. Supporting the intimate connection between molecular mechanisms involved in normal mammary gland morphogenesis and breast carcinogenesis, gene clustering of different collections of human breast tumors based on this newly identified normal 'stemness signature' defined clinically relevant associations with the degree of differentiation and prognosis in respective patient subgroups.

DISTINCT POPULATIONS OF CANCER STEM CELLS DETERMINE TUMOR GROWTH AND METASTATIC ACTIVITY IN HUMAN PANCREATIC CANCER

Patrick C. Hermann, Stephan L. Huber, Tanja Herrler, Alexandra Aicher, Joachim W. Ellwart, Markus Guba, Christiane J. Bruns, CHRISTOPHER HEESCHEN

Department of Surgery, Ludwig-Maximilians-University, Munich, Germany (P.C.H., S.L.H., T.H., M.G., C.J.B., C.H.), the Institute of Molecular Immunology, Helmholtz Center for Environment and Health, Munich, Germany (J.W.E.), and the Department of Internal Medicine III, J.W. Goethe University, Frankfurt, Germany (A.A.)

Pancreatic adenocarcinoma is currently the fourth leading cause for cancer-related mortality. Stem cells have been implicated in pancreatic tumor growth, but the specific role of these cancer stem cells in tumor biology including metastasis is still uncertain. We found that human pancreatic cancer tissue contains cancer stem cells defined by CD133 expression which are exclusively tumorigenic and highly resistant to standard chemotherapy. In the invasive front of pancreatic tumors, a distinct subpopulation of CD133+ CXCR4+ cancer stem cells was identified which determines the metastatic phenotype of the individual tumor. Depletion of the cancer stem cell pool for these migrating cancer stem cells virtually abrogated the metastatic phenotype of pancreatic tumors without affecting their tumorigenic potential. In conclusion, we demonstrate that a subpopulation of migrating CD133+ CXCR4+ cancer stem cells is essential for tumor metastasis. Strategies aimed at modulating the SDF-1?CXCR4 axis may have important clinical applications to inhibit metastasis of cancer stem cells.

APC1572T: A MOUSE MODEL ENCODING FOR WNT/B-CATENIN SIGNALING DOSAGES AFFECTING MAMMARY BUT NOT INTESTINAL STEM CELL HOMEOSTASIS AND MALIGNANCY

CLAUDIA GASPAR, Patrick Franken*, Joana Monteiro*, Lia Molenaar, Cor Breukel[1], Martin van der Valk[2], Ron Smits, Riccardo Fodde

Dept. of Pathology, Josephine Nefkens Institute, ErasmusMC, Rotterdam; 1 Human and Clinical Genetics Center, LUMC, Leiden; 2 Dept. of Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

**these authors equally contributed to this work*

We report the generation and molecular characterization of a novel mouse model, Apc1572T, carrying a truncating mutation that differs by only 66 residues from Apc1638T, a previously reported targeted allele that does not result in tumor predisposition in hetero- and homozygous animals. The hypomorphic Apc1572T allele results in an intermediate level of Wnt/b-catenin signalling activation and in multifocal mammary adenocarcinomas and pulmonary metastases in both female and male Apc+/1572T mice. Apc1572T/1572T animals die in utero. Notably, these animals do not develop intestinal tumours, thus indicating the tissue-specificity of the Wnt signalling defect encoded by the Apc1572T allele for the mammary gland. The histology of the Apc1572T lung metastases recapitulates that of the primary tumours and is highly heterogeneous with different mammary and skin epithelial lineages indicative of the Wnt-driven activation of a pluripotent stem or early progenitor cell in the mammary niche. Sorting of Apc1572T tumor cells for the Lin-/CD24+/CD29hi combination of surface antigens, previously shown to enrich for normal stem cells in the mouse mammary gland, results in the isolation of a relatively small subpopulation of cancer cells earmarked by intracellular b-catenin accumulation. Expression profiling of the different cellular populations from both tumor and normal tissues reveals a close similarity between tumor and normal stem cell compartments characterized by differential regulation of genes involved in Wnt, TGF-b, Notch and Hedgehog pathways.

A GENETICALLY ENGINEERED MOUSE MODEL FOR BREAST CANCER TO TEST THE CANCER STEM CELL HYPOTHESIS AS AN EXPLANATION FOR LACK OF TUMOR ERADICATION BY CHEMOTHERAPY

MARINA PAJIC, Sven Rottenberg, Ariena Kersbergen, Eline van der Burg, Jos Jonkers, Piet Borst.

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Although many cancers respond to anti-cancer drugs, lack of tumor eradication is a central clinical problem preceding the development of drug resistant tumors. In order to characterize therapy-surviving remnant cells in a "humanized" mouse model, we studied the response of mammary tumors generated in the K14cre; Brca1flox/flox; p53flox/flox mouse model for hereditary breast cancer to clinically relevant anti-cancer drugs. Treatment of these mice with the maximum tolerable dose yields resistant tumor "remnants". After repeated treatment with doxorubicin and docetaxel, tumors eventually become fully resistant to treatment. Tumors growing from the cisplatin "remnants", however, remain sensitive to the drug. Our findings indicate that these remnants are not resistant for reasons such as inaccessibility to drug, but consist of cells that differ from the bulk of the tumor cells. These remnants may therefore be enriched in tumor-initiating cells that can have certain properties in common with normal mammary stem cells. Our work focuses on testing the cancer stem cell hypothesis and determining factors causing the drug-resistant phenotype in these remnants.

Poster session I

A1	Maddalena Adorno
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Last update: November 13, 2007

A1

INTEGRATION OF TGF-BETA AND RAS/MAPK SIGNALING THROUGH P53 PHOSPHORYLATION

Cordenonsi M, Montagner M, ADORNO M, Zacchigna L, Martello G, Mamidi A, Soligo S, Dupont S, Piccolo S.

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During development and tissue homeostasis, cells must integrate different signals. We investigated how cell behavior is controlled by the combined activity of TGF-beta and RTK signaling, whose integration mechanism is unknown. We find that RTK/Ras/MAPK activity induces p53 N-terminal phosphorylation, enabling the interaction of p53 with the TGF-beta activated Smads. This mechanism confines mesoderm specification in Xenopus embryos and promotes TGF-beta cytostasis in human cells. These data indicate a mechanism to allow extracellular cues to specify the TGF-beta gene expression program. In line with these results, we are investigating also the role of loss of p53 and of the presence of other p53 family members in events of cancer malignancy induced by TGFbeta. Indeed, the integration of Ras and TGFbeta signals mediated by p53 suggests a new way for explaning many events occuring in cancer.

Cordenonsi M, Montagner M, Adorno M, Zacchigna L, Martello G, Mamidi A, Soligo S, Dupont S, Piccolo S. Integration of TGF-beta and Ras/MAPK signaling through p53 phosphorylation.Science. 2007 Feb 9;315(5813):840-3. Epub 2007 Jan 18.

Dupont S, Zacchigna L, Adorno M, Soligo S, Volpin D, Piccolo S, Cordenonsi M. Convergence of p53 and TGF-beta signaling networks. Cancer Lett. 2004 Sep 30;213(2):129-38.

A2

PROGNOSTIC CENTROSOME ABERRATIONS IN STEM CELLS AND THEIR DIFFERENTIATED PROGENIES CONNECT CHRONIC ESOPHAGITIS AND ADENOCARCINOMA

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Mechanisms leading from chronic inflammation to cancer are largely unknown. Barrett's Esophagus (BE) is a genetically unstable pre-cancerous metaplasia induced by chronic esophagitis. In patients with BE-derived adenocarcinoma (EA), we found prominent centrosome aberrations (CeAbs) with early prognostic value in normal and metaplasic tissues and, importantly, pluripotent esophageal stem cells. CeAb-dependent chromosomal instability play a crucial role in tumor formation and aneuplody of BE cells is a key prognostic marker. Therefore a new mechanism connecting chronic inflammation and cancer might be mediated by CeAbs of stem cells. We present a model where CeAbs favour both the formation of cancer stem cells and tumor development. We also discuss how inflammation-induced CeAbs contribute to the balance of clonal diversity and clonal expansion characterizing the genetic evolution of adenocarcinomas. Therefore, the understanding of the origin, composition, interactions and functional consequences of esophageal CeAbs could provide new insights into the mechanisms of tumor initiation and progression. We propose a new pathological condition, called inflammatory centrosomeopathy, characterized by the presence of CeAbs in stem, progenitor and differentiated cells of any tissue before the possible formation of (pre)neoplastic cells. Detection of centrosomeophaties may be crucial for the development of new protocols of cancer prevention.

A3

ISOLATION OF A NOVEL HUMAN ADULT PERIPHERAL BLOOD CELL POPULATION WITH IN VITRO HEMATOPOIETIC AND ENDOTHELIAL POTENTIAL

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Accumulating studies support the idea that a common progenitor, termed the hemangioblast, generates both hematopoieitc and endothelial cell lineages. Here, we identify a novel subset among mononuclear peripheral blood (PB) cells depleted of lineage commitment markers (Lin-) that are devoid of CD45 expression. Functional examination of Lin-CD45- cells also lacking cell surface CD34 revealed they were capable to differentiate into either hematopoietic and endothelial cells. The cells stained and sorted to yield Lin-CD45-CD34-cells represented approximately 0.02% of total cells analyzed. PCR analysis of the cell population revealed that do not express CD45 and CD34, but express Wnt, Notch1, EphB4. Directly after sorting, the cells not containd clonogenic cells that gave rise to hematopietic colonies in progenitor cell assays. The CD45-cells failed to adhere into plastic colture and to proliferate under mesenchymal stromal cells condition. To test whether this population also contains individual precursors with both capacities, the defining characteristics of the adult hemangioblast, we developed a culture system that allows single-cell analyses of differentiation. The Lin-CD45-CD34- cells were cultured in the presence of Stem Cell Factor (SCF), FLT-3 ligand, trombopoietin (TPO), interleukin-(IL)16, vascular endothelial factor (VEGF), SDF-1, and co-culturated with murine stromal cell line MS-5 for 6 weeks. These culture conditions resulted in the formation of a myeloid (CD33+CD45+ cells) and lymphoid cells (CD56+ cells) (hematopoietic cells lineages) or endothelial cells (CD105+ KDR+CD45-cells), analyzed by FACS. These data demonstrate that CD45-Lin-CD34- may be precursors of both hematopietic and endothelial cells lineage

A4

IDENTIFICATION OF HUMAN LUNG TUMOR INITIATING CELLS

BERTOLINI G., Pratesi G., Roz L., Livio A., Tortoreto M., Fabbri A., Fontanella E., Andriani F., Delia D., Pastorino U., Sozzi G.

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There is increasing evidence that tumors are organized as a hierarchy originating from rare stem-like cells responsible for tumor maintenance. Brain, prostate and colon cancer initiating cells can be identified through the expression of CD133 marker which is expressed by normal primitive cells of hematopoietic, neural, endothelial and epithelial lineages. To investigate the existence of a population of tumor initiating cells in non- small cell lung cancer (NSCLC), we analyzed the expression of CD133 antigen in A549 cell line, primary tumors and tumor xenografts established in immunocompromised mice. Phenotypic cancer cell subsets were purified by FACS : double positive cells CD133+/ EpCAM+ (epithelial cell adhesion molecule) were detected in tumors (mean = 4.9%) and CD133 expression was confirmed by immunohistochemistry on paraffin sections of tumor tissues. A decrease in CD133 expression was observed in short term adhesion cell cultures during differentiation. The ability to engraft in vivo in SCID mice appeared increased in the CD133+ cell fractions from A549, primary tumors and tumor xenografts. These initial observations indicate the presence in lung tumors of CD133+ cells which may be endowed with increased tumorigenic potential and might represent a new target for treatment of this lethal malignancy.

A5

PRELIMINARY STUDY FOR THE IDENTIFICATION AND CHARACTERIZATION OF RENAL CANCER CELLS WITH STEM/PROGENITOR PROPERTIES

BOMBELLI SILVIA*, Invernizzi Lara*, Angeloni Valentina*, Torsello Barbara*, Brambilla Paola*, Strada Guido^, Bovo Giorgio^, Cattoretti Giorgio°^, Bianchi Cristina*, Perego Roberto A*

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Renal cell carcinomas (RCCs), 3% of all human malignancies, are therapy resistant, the diagnosis is difficult and the tumor growth might be sustained by "cancer stem cells" that, however, have not yet been described. We tried to identify and characterize these cancer stem cells. Single cells obtained after collagenase digestion of RCC tissue were cultured, in specific DMEM-F12 serum-free medium supplemented with EGF, bFGF2 and B27, at low density on nonadherent plates for 12 days to form spheres. The RCC CAKI-1 cell line was also cultured in the same conditions. Clonal origin of the spheres was evaluated by staining the cells with PKH dye before plating and by limiting dilution assay. The dissociated single cells of primary spheres formed filial secondary spheres. Immunofluorescence on the secondary spheres against RCC markers (cytokeratin, vimentin and E-cadherin) and stem cells markers (CD133, CD24 and CD44) was performed to characterize the spheres' cellular composition. The isolation and characterization of renal cancer stem cells will provide a powerful tool to investigate the tumorigenic process, to identify molecular neoplastic markers and to develop therapies targeted to cancer stem cells.

A6

IN VIVO/IN VITRO AGED FIBROBLASTS TRIGGER MALIGNANT FEATURES IN NORMAL AND CANCER STEM/PROGENITOR CELLS OF THE MAMMARY GLAND

Gianluca Storci[1,2], Pasquale Sansone[1,3], Raffaella di Micco[4], Marzia Fumagalli[4], Simona Tavolari[5], Tiziana Guarnieri[5], Mario Taffurelli[6], Claudio Ceccarelli[7], Donatella Santini[7], Pasquale Chieco[1], Fabrizio d'Adda di Fagagna[4], and MASSIMILIANO BONAFÈ[1, 2].

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The age-related increase in cancer incidence has been taken as a proof of the mutation accumulation theory of cancer in humans. Recent data suggest that long-living stem cells are the most likely target for oncogenic mutations. Nevertheless, a plenty of data point out for a major role of stromal cell in cancer growth. These data suggest that the study of the interaction between aged stromal cells and cancer initiating cells (i.e. cancer stem cells) may be of primary importance to better understand cancer biology.In this investigation we employed human mammospheres (MS) from normal and tumor tissues as a model for normal and cancer stem/progenitor cells of the mammary gland. We here report that, compared to fibroblasts from young individuals, fibroblasts from aged people and nonagenarians show an enhanced capacity to induce invasive behaviour of normal and tumor MS. Similarly, we show that in vitro senescent fibroblasts induce invasiveness of MS at a higher extent than non senescent cells. We also provide details about the molecular pathway involved in such a phenomenon, by showing that the capacity of aged/senescent fibroblasts to enhance the invasive behaviour of stem/progenitor cells is pivoted by p66Shc gene. These data support the notion that in vivo/in vitro aged stromal cells create a pro-tumorigenic environment for stem/progenitor cells of the mammary gland.

A7

IDENTIFICATION OF A TUMOR-INITIATING SUBPOPULATION IN ESCC CELL LINES

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Drawing from the discovery of cancer stem cells in a number of solid tumor types, we hypothesized that Esophageal Squamous Cell Carcinoma (ESCC) contain a sub-population of self-renewing, highly tumorigenic cells that drive tumor progression. Because there is no marker for the ESCC tumor-initiating cell population, we exploited the slow-cycling nature of stem cells to isolate and study the potential tumor-initiating cell. ESCC cell lines were labeled with fluorescent membrane dye PKH67 and cultured until only a small population retained the dye, due to its dilution during subdivisions. Asymmetric cell division and self renewal was demonstrated by serially passaging flow cytometry sorted label-retaining (LR) cells and re-seeding at clonal cell densities; Resultant clones contained one labeled cell. A preliminary tumorigenicity study in NOD/SCID mice indicated LR cells generated faster-growing and less-differentiated tumors. To further characterize LR cells, microarray analysis was performed and OCT4 and CD133 were identified as potential markers. OCT4 knockdown caused an increase in percentage of cells in S-phase and elimination of the CD133+ population. Tumors generated from OCT4 knockdown cells were primarily composed of keratinized cells suggesting that OCT4 maintains the stem cell population within ESCC cell lines. This slow cycling subpopulation is characterized by, asymmetric self-renewal, increased tumorigenicity, and expression of stem cell markers CD133 and OCT4.

A8

THE ROLE OF OPA1 IN DIFFERENTIATION OF EMBRYONIC STEM CELL TO NEURONS AND CARDIOMYOCYTES

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OPA1 is a dynamin-related protein located in the inner mitochondrial membrane and mutated in dominant optic atrophy. OPA1 plays two genetically distinct roles in promoting mitochondrial fusion by cooperating with MFN1, a large GTPase of the outer mitochondrial membrane; and in regulating apoptosis, controlled by the mitochondrial rhomboid protease PARL. PARL operates upstream of OPA1, by participating in the production of a soluble form of OPA1 required to keep in check the pathway of cristae remodeling and cytochrome c redistribution. Thus, OPA1 likely affects complex functions, as substantiated in overexpression studies that show a role for this protein in lymphocyte movement and dendritic spine formation. We therefore reasoned that levels of OPA1 are likely to affect development and function of multiple organs, by regulating mitochondrial fusion or apoptosis. To dissect the roles of OPA1 we decided to generate an OPA1 conditional knock-out mouse model, required to study the function of OPA1 in specific tissues and at different development stages. At the same time, we decided to study whether ablation of OPA1 influenced differentiation of embryonic stem (ES) cells in vitro using a hanging-drop differentiation system. To this end, we analyzed an ES cell line where Opa1 had been gene trapped (Opa1gt), resulting in an Opa1+/- genotype. We compared the differentiation potential into cardiomyocytes and neurons of this Opa1gt ES cell line to its relative wt ES cell line. Opa1gt ES cells display a decreased capacity to differentiate into beating cardiomyocytes, while they retained an almost normal neuronal differentiation potential. We aim at understanding the molecular mechanism by which levels of Opa1 influence differentiation into cardiomyocytes.

A9

ONCOGENE-INDUCED SENESCENCE IS A DNA DAMAGE CHECKPOINT RESPONSE TRIGGERED BY DNA HYPER-REPLICATION

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Early tumorigenesis can be associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation have been shown to be restrained by cellular senescence. Whether DDR activation and oncogene-induced senescence (OIS) are causally linked is unclear. We will provide evidence that senescence, triggered by the expression of an activated oncogene (H-RasV12) in normal human cells, is the consequence of the activation of a robust DDR. Experimental DDR-inactivation abrogates OIS and promotes cell transformation. DDR and OIS establish after a hyper-replicative phase occurring immediately after oncogene expression. Senescent cells arrest with partially replicated DNA and with DNA replication origins having fired multiple times. In vivo DNA labelling and molecular DNA combing reveal that oncogene activation leads to augmented numbers of active replicons and to fork progression alterations. We also demonstrate that oncogene expression does not trigger a DDR in the absence of DNA replication. Finally, we will demonstrate that oncogene activation is associated with DDR activation in an in vivo mouse model. We therefore propose that OIS results from the enforcement of a DDR triggered by oncogene-induced DNA hyper-replication.

A10

ACUTE MYELOID LEUKEMIA IS PROPAGATED BY A LEUKEMIC STEM CELL WITH LYMPHOID. CHARACTERISTICS IN A MOUSE MODEL OF CALM/AF10 POSITIVE LEUKEMIA

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We recently demonstrated that leukemic stem cells with lymphoid characteristics can propagate myeloid leukemia in a mouse model of the t(10;11) translocation. In a murine bone marrow transplantation model of the C/A fusion gene, we observed that mice injected with C/A transduced bone marrow (BM) cells succumbed to AML showing IGH DJ rearrangements. In the BM of leukemic mice, the majority of cells expressed myeloid markers. Interestingly, a minor fraction of cells lacked myeloid markers and displayed the B220 lymphoid antigen. Intriguingly, limiting dilution transplantation assays of leukemic BM into secondary recipients showed that the B220+/Mac1? cells had the highest frequency of leukemia propagation, > 548 fold higher compared to the Mac1+/ B220? bulk population. Single IGH DJ rearranged B220+/Mac1? cells gave rise to the Mac1+/ B220? populations with clonal DJ rearrangements, and induced identical leukemias in transplanted mice. Furthermore, depletion of the B220+ cells effectively prevented development of leukemia in secondary recipients. This murine model closely recapitulated C/A+ human AML; BM cells of the majority of patients tested displayed C/A+ cells with clonal IGH DJ rearrangements and B220 expression. These results demonstrate that AML can be propagated by LSCs with lymphoid characteristics which could potentially be targeted using specific antibodies.

A11

CHARACTERIZATION OF HUMAN GLIOBLASTOMA-DERIVED CANCER STEM CELLS

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Glioblastomas are the most common and aggressive adult brain tumours. There is overwhelming evidence that the tumour is maintained by a small subpopulation of brain tumour stem cells (BTSCs) with marked capacity for self-renewal, multipotency and tumorigenic potential. The neural stem cell marker CD133 has been used for the enrichment of BTSCs from human glioblastoma, but CD133 negative cells from primary human glioblastoma with stem cell properties have been also described. Thus, the actual identity of BTSCs and the role of CD133 in human glioblastoma remains to be established. We have obtained 6 cell lines from primary human glioblastomas and addressed the question whether proliferation, differentiation and tumorigenic capacities correlate with CD133 expression. All tumour-derived cells, cultured as neurospheres, showed extensive proliferative ability. FACS analysis of the expression of cell surface antigens, including CD133, CD44, CD24, CD29 and CXCR4 was performed. Expression of stemness markers such as SOX2, OCT4, Nanog, and nestin was also evaluated by immunofluorescence. Under appropriate conditions all cells exhibited different capacities to differentiate in neural cell lineages. To evaluate the malignant behaviour of the cells, tumorigenic assays are been performed. The correlation between the phenotype of the cells and their neoplastic properties will be discussed.

A12

MOLECULAR CYTOGENETIC CHARACTERIZATION OF TUMOR INIZIATING CELLS

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There is direct indication of a tumor-initiating or progenitor cell origin for at least some solid tumors like glioblastoma, breast cancer and colon carcinoma. However, little is known on the genomic characterization of tumor initiating cells and on their genetic stability. We isolated cells cultures growing as “spheres” in serum-free medium from 6 established epithelial cell lines (A549, MCF7, 734B, MDAMB 361, BT474, JR8) and three primary glioblastoma surgical specimens. Spheres and serum-cultured adherent cells were analyzed for stem-like properties including sphere-forming efficiency, expression of phenotypic markers and tumorigenesis in vivo. A detailed molecular-cytogenetic characterization by Spectral Karyotyping Imaging (SKY) and fluorescent in-situ hybridization (FISH) was performed. Overall, SKY and FISH analyses showed a more rearranged karyotype in spheres compared to adherent cells with additional, complex chromosomal rearrangements with marks of karyotypic evolution. Moreover, a certain degree of genetic instability was observed in these cells during consecutive rounds of ?in vitro? differentiation-sphere formation. Overall, these results suggest the existence of heterogeneous and genetically unstable subpopulations of cells within cell-lines and tumors. These populations, due to their genetic instability, are endowed of an intrinsic ability to generate highly tumorigenic cells with stem-like features.

A13

CML STEM CELLS SURVIVE HIGH DASATINIB CONCENTRATIONS IN THE ABSENCE OF GROWTH FACTORS FOR 12 DAYS IN VITRO

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We have previously shown that cancer stem cells exist in all patients with newly diagnosed chronic myeloid leukaemia (CML). These cells are primitive, quiescent, transplantable in NOD/SCID mice and resistant to the tyrosine kinase inhibitor imatinib. This resistance may in part be explained by high expression of Bcr-Abl in the most primitive cells. To overcome this high level Bcr-Abl expression we cultured CML CD34+ cells in vitro in a high concentration of the most potent Bcr-Abl inhibitor available - dasatinib at 150nM - in serum free medium in the absence of growth factors. The drug was replenished every 3 days and cells remaining at 12 days were analysed. 10% of the starting CD34+ cells were recovered. These cells were exclusively Ph+/Bcr-Abl+ and included colony forming cells, long-term culture initiating cells and cells capable of proliferation in growth factors after drug wash-out. The resistant population showed high levels of phosphorylation of Crkl suggesting failure to fully inhibit Bcr-Abl using dasatinib rather than Bcr-Abl independent resistance. Our next approach will be to combine Bcr-Abl knock-down using siRNA with dasatinib to fully inhibit Bcr-Abl in the stem cell population in an effort to determine whether these cells are dependent on Bcr-Abl for survival and proliferation.

A14

SKP2 ELIMINATION PREVENTS P21WAF1/CIP1 STABILIZATION IN VIVO

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Many tumours being refractory to genotoxic stimuli are characterized by high levels of the cell cycle inhibitor p21WAF1/Cip1. To understand apoptotic resistance and p21WAF1/Cip1 regulation in vivo after ionizing radiation, the developing cerebellum of the mouse was used. While after gamma-irradiation p21WAF1/Cip1 mRNA can be detected throughout the cell cycle, p21WAF1/Cip1 protein can only be detected in G1 and G2 cells being refractory to radiation treatment. However, during S- and M-phase p21WAF1/Cip1 is degraded by the ubiquitin/proteasome system. Using Skp2 knockout mice we tested the role of this ubiquitin ligase subunit for p21WAF1/Cip1 and p27Kip1 degradation in vivo. During normal development and also after radiation p27Kip1 can be detected when cells leave the cell cycle at G0. In Skp2-/- mice, however, p27Kip1 is maintained in mitotic granule cells and, in addition, replaces p21WAF1/Cip1 in G1. When analysed for apoptosis after radiation, cell death was initially absent in Skp2-/- mice, even in S and M phase cells. However, apoptosis as detected by DNA fragmentation occurred at later timepoints, strikingly in cells that usually are apoptotic resistant. Further questions will address whether Skp2 elimination can be exploited to target apoptotic resistant cancer stem cells into moribund cells.

A15

ANCHORAGE-INDEPENDENT SPHERE FORMATION WITH STEM-LIKE PROPERTIES AND SILENCED INK4A-ARF LOCUS GENES IN RAT SARCOMA CELL LINES

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The presence of cancer stem, in both solid and hematopoietic malignancies, cells has been recently linked to their pathogenesis. Sarcomas are rare, and diversely characterized by degrees of mesenchymal differentiation. The current study demonstrated that the rat osteosarcoma and malignant fibrous histiocytoma cells, both of which were induced by 4-hydroxy (amino) quinoline 1-oxide, possessed an ability to form spherical, clonal expanding colonies in anchorage-independent, serum-starved conditions at the frequency of 3-4.5 x 10-2. The spheres from both sarcomas showed the stem-like properties with the ability of self-renewing, and expressed the stem cell related STAT3 and Bmi1 genes. More interestingly, spheres from both sarcomas remarkably decreased the expression of INK4a-ARF locus genes, p16INK4a and p19ARF, which are related to cell senescence and apoptosis. Spheres showed strong tumorigenicity in vivo via the inoculation into syngeneic rats. These results suggest that these sarcoma cells possess the ability to form tumorigenic spheres with stem-like properties lacking INK4a-ARF locus gene expression in anchorage- independent condition, and this might contribute to the tumor development such as metastasis via the resistance to apoptotic stimuli.

A16

DEFINING THE MALE GERMINAL STEM CELL NICHE BY EXPRESSION PROFILING IN RATS

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Stem cells have the capacity to self-renew or differentiate into multiples lineages. These stem cell fates are controlled by extracellular signals from the niche and by intrinsic genetic programs within the stem cell. In the germ line, the notion of stem cell niche was intensely studied in the past in C. elegans and Drosophilia and led to identification of essential genes. However, little is known of mammalian germ stem cells. Therefore, our genomic approach of analyzing the expression profile of spermatogonia stem cells and somatic support Sertoli cells in rats provides a valuable tool for the characterization of the niche. In prepuberal rat testis (9 dpp) both types of cells proliferate, following a mitotic program initiated during embryogenesis. Af puberty, spermatogonia and Sertoli cells follow different cell fates. Somatic cells enter into quiescence and form a tight hematotesticular barrier. While germ cells divide to maintain the stem cell pool and differentiate to produce spermatocytes, which proceed through meiosis. We compared the transcript profiles of purified spermatogonia from pre-puberty and post-puberty animals, the former representing pure spermatogonia type A and the latter spermatogonia type I and B, and preleptotene spermatocytes. A similar comparison was performed for the Sertoli cells from both stages. Interestingly, the largest group of differentially expressed genes at the two stages, in spermatogonia and Sertolis cells, related to the extracellular environment, including cell adhesion, secreted proteins, extracellular matrix genes, receptors, and channel and transporter proteins. Furthermore niche to stem cell signaling pathways can be proposed, based on the expression of specific factors in the niche and the respective receptor in the stem cells. Thus the identification of new markers in spermatogonia and Sertoli cells will help to define the molecular basis of the germ line stem cell niche in mammals.

A17

CANCER STEM CELLS IN OVARIAN TUMORS

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The existence of cancer stem cells was discovered in several solid tumors, including breast, brain, prostate, liver, lung, and colon tumors. So far, the presence of cancer stem cells in ovarian cancer tumor has not been demonstrated. The aim of the present study was to indentify cancer stem cells within primary ovarian cancer cell culture. The specimen was derived from an ovarian tumor and cultured in vitro. Although a large number of human ovarian carcinoma cell lines have been established in vitro, most of them are serous cystadenocarcinomas or poorly differentiated adenocarcinomas. We cultured the primary cell line (YMOV5) derived from an ovarian mucinous cystadenocarcinoma tissue which apparently had developed through in vivo clonal selection. Our results suggested that YMOV5 cell line might be useful for studying ovarian cancer stem cell. At time of writing, YMOV5 cells have been passed 6 times in vivo, respectively,fully maintanining their tumourigenicity. We plan to compare ABCG2+ and ABCG- cells purified from YMOV5 by flow cytometry, confirm the expression of ABCG2, Notch-1, b-catenin, SMO, Oct-4, CD133 and Sca-1 by real time RT-PCR and immunostaining in SP cells, and compare protomics of ovarian cancer stem cells.

A18

ACQUISITION OF ANOIKIS RESISTANCE IN HUMAN BREAST CANCER CELLS ALTERS INVASIVENESS AND SENSITIVITY TO DOXORUBICIN

EUNYOUNG KO[1], Kyung-Min Lee[2], Jong Bin Kim[2], Wonshik Han[1], Incheol Shin[4], Sangmin Kim[3], Jong Won Lee[1], Jihyoung Cho[1], So-Youn Jung[1], Eunkyu Kim[1], Dong-Young Noh[1,2]

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Some cancer cells can survive without affinity to ECM and migrate to other tissues, which are named anoikis-resistance. Acquisition of anoikis resistance is important in metastasis of cancer, and some authors suggested that anoikis resistance may be characteristic of cancer stem cell. We study to prove relationship between invasiveness and doxorubicin-response in breast cancer cell lines cultured on polyHEMA-coated dish, which is used to induce anoikis in in vitro culture system. Breast cancer cell lines (MCF-7 and MDA-MB-231) were cultured on dishes coated with or without polyHEMA for a long time. We observed that suspened cells decreased proliferation about 50% compared to adherent cells. In contrast to proliferation, suspended cells increased invasiveness about 50% and resistance to doxorubicin. Cell cycle, apoptosis, and expression of FAK-p are not different. However suspended cells showed decreased adhesion ability and expression of integrin beta 1, but highly increased expression of MMP. We show that suspended anoikis resistant cell using PolyHEMA are more invasive and resistant to doxorubicin. Suspension culture system using PolyHEMA may be a useful method in studying anoikis resistance and characteristics of cancer stem cells.

A19

HPV16 TRANSFORMED CELLS PROGRESSIVELY LOSE THEIR ABILITY TO UNDERGO APOPTOSIS AND DIE BY NECROSIS

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1 Unit of Cellular Signaling, Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel.

To follow changes during HPV16-induced transformation, we used a model system consisting of primary keratinocytes (K) and papilloma-transformed keratinocytes (HF1 cells) from early (E) and late (L) passages, and from benzo[a]pyrene treated L (BP). L and BP cells display many of the properties of transformed cells, with gene expression patterns approaching those of cervical cancer. The number of cellular pathways active under basal growth conditions contracted strikingly during the evolution from K to BP cells, as shown by microarray analysis. Pro- and anti-apoptotic pathways figure prominently among the contracted pathways. Upon CDDP-induced DNA damage, L and BP cells do not activate the caspase cascade. The contraction in apoptotic pathways enables the cell to maintain its transformed phenotype, but renders it vulnerable to stress. While K and E cells respond to CDDP by apoptosis, L and BP cells possess enhanced sensitivity and undergo necrosis, which becomes the default response of the cells to genotoxic stress. The shrinkage of the apoptotic network is part of an overall contraction in pathways not directly involved in proliferation.

A20

VARIATION OF HOECHST 33342 STAINING PARAMETERS TO ENABLE AND OPTIMIZE SP DETECTION

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Side population (SP) detection has been proven a useful tool to narrow down cancer stem cells (CSCs) utilizing their enhanced expression of efflux plasma membrane transporters that render them less fluorescent for the soluble, cell-permeable dye Hoechst 33342. By varying staining parameters in various cancer cell lines we found out that standard conditions are not always usable to detect an SP. Following the Hoechst staining process in "real-time" by flow cytometry the incubation time turned out to have a great influence on the detectability of the SP in some cell lines. In addition, the Hoechst concentration could affect the distinctiveness of the SP and even the incubation buffer had a strong effect on the SP size and also on the viability of cells. Furthermore we tested the influence of three serum-free media and FCS culture on the SP of a neuroblastoma cell line. Preliminary data showed that the resulting Hoechst staining profiles were significantly distinct. In conclusion, varying the parameters investigated can help to optimally detect and display Hoechst excluding cells. On the other hand it emerges that due to the relative sensibility of the technique, for comparison studies tightly controlled conditions are indispensable.

December 2, 2007

Lectures

Lukas Sommer
Zürich, Switzerland

Allan Balmain
San Francisco, USA

James Sherley
Watertown, USA

Jürgen Knoblich
Wien, Austria

Andreas Trumpp
Epalinges, Switzerland

Max S. Wicha
Ann Arbor, USA

Oral Presentations

David Virshup
Singapore, Singapore

Patricia Molina-Ortiz
Madrid, Spain

Luca Tiberi
Trieste, Italy

Günther Schütz
Heidelberg, Germany

Elisabetta Marangoni
Paris, France

Giuseppina Bonizzi
Milan, Italy

Session III and Session IV

Basic mechanisms

NEURAL CREST-DERIVED STEM CELLS IN THE SKIN AND THEIR POTENTIAL ASSOCIATION WITH MELANOMA STEM CELLS

LUKAS SOMMER

Stem cells play a critical role in normal tissue maintenance, and mutations in these stem cells may give rise to cancer. According to the cancer stem cell hypothesis, only a subpopulation of cells within a cancer has the capacity to sustain tumor growth. This subpopulation of cells is made up of cancer stem cells, which are defined as the population of cells within a tumor that can self-renew, differentiate, and generate a phenocopy of the cancer when injected in vivo. Cancer stem cells have been prospectively isolated from human cancers of the blood, breast, and brain. Several evidences strongly suggest that melanocytic neoplasias are derived from immature melanocytic cells, which are neural crest derived, and we thus hypothesize that melanoma develops from mutated neural crest stem cells. Accordingly, at least a fraction of melanoma cells should display features of neural crest stem cells. Here, we present evidence that metastatic human melanomas, as well as established human melanoma cell lines, contain a cell subpopulation resembling neural crest stem cells. These cells are potent in tumor formation when a limited number of cells are injected into an orthotopic *in vivo* microenvironment. Very importantly, this cell population is able to self-renew and can generate melanoma after serial transplantation when re-isolated from secondary recipients. Moreover, these tumors contain the cell types observed in the original cancer. These results all together strongly support that melanomas are generated by cancer stem cells with characteristics similar to neural crest stem cells. The identification of cancer stem cells in melanoma has fundamental implications for the development of new therapeutic agents. Eradication of cancer may require the targeting and elimination of cancer stem cells.

MECHANISMS OF GENETIC FIDELITY IN ADULT STEM CELLS

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The cellular and molecular properties of cancer stem cells (CSCs) are of great interest for cancer therapeutics. CSCs are tumor cells responsible for the propagation capacity of tumors. Characteristic features of cancer stem cells are likely to reflect, in part, properties of their normal cell of origin. Among the tissue cell types proposed as frequent targets for carcinogenesis are adult stem cells (ASCs). ASCs are postulated to be rare, long-lived, tissue cells that undergo asymmetric self-renewal divisions to replenish mature differentiated tissue cells. Among their unique features is non-random mitotic chromosome segregation, a mechanism proposed for ASC genetic fidelity. Several recent in vivo and in vitro studies demonstrate the ability of rare cells in anatomical stem cell compartments or cell culture, respectively, to co-segregate chromosomes that contain "immortal DNA strands". Immortal DNA strands are a genomic set of conservatively inherited template DNA strands. The unique inheritance is accomplished by non-random chromosome segregation after semi-conservative DNA replication. Theoretically, this mechanism could reduce the rate of ASC mutation by 1000-fold. Loss of immortal DNA strand co-segregation is, therefore, predicted to be a carcinogenic catastrophe. Our research with cultured cell models and ex vivo expand human adult stem cells implicates the TP53 gene as an essential maintenance factor for immortal DNA strand co-segregation in ASCs. Thus, tumors that arise with homogeneous p53 mutations may derive from ASCs with greatly compromised genetic fidelity. This hypothesis leads to specific predictions for the CSC profile of such tumors.

DROSOPHILA AS A MODEL FOR STEM CELL DERIVED TUMOR FORMATION

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Stem cells can generate self-renewing and differentiating daughter cells at the same time. We are using Drosophila as a model system to understand, how they control the balance between these two fundamentally different types of progeny. Using a proteomics approach for proteins that segregate into one of the two daughter cells in neuroblasts (stem cell like precursors of the central nervous system) we have found the growth regulator brat (brain tumor). During mitosis, Brat segregates into one of the two daughter cells, where it downregulates protein synthesis, stops proliferation and prevents cell growth. In brat mutant animals, all daughter cells of the stem cell undergo self renewal and continue to proliferate. This leads to dramatic overproliferation and the formation of a stem cell derived tumor which grows indefinitely and kills the animal. Tumors will continue to proliferate indefinitely, even when transplanted into other flies, thus indicating that cells become immortalized. Very similar phenotypes are observed in flies mutant for Lethal (2) giant larvae (Lgl), where Brat is present but does not segregate asymmetrically. Thus, the asymmetric segregation of Brat into one of the two daughter cells regulates proliferation in Drosophila neural stem cells. Brat is a member of a conserved protein family charaterized by a similar domain composition. We have analyzed the function of other family members and find that they regulate self renewal in other types of stem cells. Like in brat mutants, stem cells overproliferate and form of tissue specific tumors. Our results indicate that this role might also be conserved in vertebrates suggesting that brat-like proteins have a conserved role in regulating stem cell self renewal.

DORMANT AND ACTIVATED STEM CELLS DURING HOMEOSTASIS INJURY AND CANCER

Marieke Essers[1], Anne Wilson[2], Gabriela Oser[1] and ANDREAS TRUMPP[1]

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Somatic stem are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Many of the mature cell types in these tissues have short half-lives and must therefore be continuously produced by the activity of long-lived stem cells. Stem cells are maintained during the life of the organism through self-renewal, while simultaneously generating all the mature cell types of a particular tissue through differentiation. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cell to date, and serve as a model for understanding other adult stem cells present in the mammalian body. Using two types of label-retaining assays we have identified a long-term quiescent/dormant population within the most primitive HSCs. These dormant HSCs appear to have higher repopulation activity compared to actively self-renewing stem cells. They are not involved in the maintenance of homeostasis, but are activated and driven into a self-renewing program in response to injury signals. We hypothesize that these dormant HSCs serve as a stem cell reservoir, and are specifically activated and recruited to sites of injury. Immunohistochemical studies show that dormant HSCs are distributed in the bone marrow as individual cells indicating that dormant HSC niches might be very small, comprising only one stem cell. One of the reasons cancer stem cells are thought to escape anti-proliferative chemotherapy is their relative quiescence. I will provide evidence that treatment of mice with a member of the Interferon (IFN) family leads to the activation and proliferation of dormant HSCs in vivo. This novel role of IFN can be used to eliminate the entire stem cell pool by short-term priming of dormant HSCs with IFN followed by treatment with chemotherapy drugs. The implications of these results for the design of strategies to target dormant cancer stem cells will be discussed.

BREAST CANCER STEM CELLS: IMPLICATIONS FOR PREVENTION AND THERAPY

MAX S. WICHA, M.D., Christophe Ginestier, Ph.D., Suling Liu, Ph.D., Hasan Korkaya, Ph.D., Emmanuelle Charaffe-Jauffret, Ph.D., and Gabriela Dontu, M.D., Ph.D.

Research in a variety of tumor systems have given support to the cancer stem cell hypothesis which holds that tumors originate in tissue stem or progenitor cells, as a result of disregulation of the normally tightly regulated process of self-renewal and as a result, tumors contain and are driven by a cellular subcomponent that retains key stem cell properties. Our laboratory has developed both in vitro and mouse models to isolate and characterize stem cell populations from the normal mammary glands as well as from mammary carcinomas. We have utilized a series of markers including aldhyde dehydrogenase, CD44/CD24 and Bmi1to characterize these cell populations. Utilizing in vitro mammosphere-based culture systems, we demonstrate a role for both Notch and Hedgehog signally as well as the polycomb gene Bmi1 in regulating stem cell self-renewal. Furthermore, we provide evidence that both the hereditary breast cancer gene BRCA1 as well as genes involved in sporadic breast cancer including HER2 and PTEN also regulate stem cell self-renewal. These studies provide a conceptual link between hereditary and sporadic breast cancers by suggesting that both may be initiated by disregulation of stem cell self-renewal resulting in accumulation of abnormal stem/progenitor cells which provide targets for further transforming events. Tumor heterogeneity, in turn, is driven by a small component of cells retaining stem cell properties. These cancer stem cells are relatively resistant to chemotherapy and may contribute to therapeutic resistance and tumor relapse. Strategies aimed at targeting stem cell self-renewal pathways represents a rational approach to eliminate this key cell population which may improve clinical outcomes.

SMALL MOLECULE INHIBITORS OF THE WNT SIGNALING PATHWAY

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Wnt signaling is required for stem cell maintenance and dysregulation occurs in cancer. In breast cancer and some leukemias, cancer stem cell maintenance has been shown to depend on Wnt signaling. Both beta-catenin dependent and independent signaling pathways are implicated in stem cell maintenance. It is unclear in most cases exactly how Wnt signaling is upregulated in cancer stem cells. However, in the more overtly Wnt dependent cancers, it is clear that intervention at multiple points in the signaling pathway(s) is required for effective treatment of a broad range of cancers. We have developed and validated a cell based screen for inhibitors of Wnt signaling and characterized a series of secondary assays which can place the function of each inhibitor within one of six defined regions or compartments of the signaling pathway. Through high throughput screening, we have identified small molecule inhibitors in each compartment and characterized them in a broad range of cancer cell based assays. Ongoing studies are examining the role of these compounds in cancer cell apoptosis, growth inhibition, and stem cell self-renewal and/or differentiation.

FUNCTIONAL CHARACTERIZATION OF SNAIL2 MEDIATED E-CADHERIN REPRESSION

PATRICIA MOLINA-ORTIZ, Matthew McPherson, Amparo Cano and Francisco Portillo.

Snail2, also called Slug, is a member of the Snail-family of zinc-finger transcription factors that plays a significant role both during development and carcinogenesis, by controlling epithelial-mesenchymal transition (EMT) processes. Snail2 has been also described as a direct transcriptional repression of E-cadherin during EMT being implicated as a prosurvival factor during tumorogenesis. Snail1 and Snail2 are highly homologous factors, containing a common N-terminal transrepressor domain (SNAG), a C-terminus DNA binding domain of four (Snail1) or five (Snail2) zinc fingers, and a divergent central region, which in Snail2 is formed by a unique domain called `Slug domain´ whose function remains to be elucidated. Snail1 repressor activity has been shown to be dependent on SNAG-mediated interaction with a repression complex formed by the corepressor mSin3a and histone deacetylases 1/2 (HDAC1/2). Importantly Snail1 transcription factor is further regulated through phosphorylation by various kinases. However, at date little is known about the control of Snail2 repressor activity. Here, we present interesting data shedding light into the regulation and function of Snail2 as a E-cadherin repressor. For this purpose we have performed ectopic expression of several Snail2 deletion mutants and examined the contribution of the specific domains to protein stability, localization and E-cadherin repressor activity. These data reveal a key role for the `Slug domain´ to repress E-cadherin expression. Furthermore, in vivo phosphorylation analysis revealed that specific phosphorylation on Snail2 protein is implicated in Snail2 function as a transcriptional repressor whose functional significance is currently being investigated.

NOTCH1 AND PIN1 ESTABLISH A POSITIVE FEED BACK LOOP TO ENHANCE TUMOR GROWTH

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Phosphorylation-directed prolyl-isomerization has recently emerged as a potent post-translational signaling mechanism. Triggering these reactions is an enzyme called Pin1, that transduces specific phosphorylation events into conformational changes of important cellular proteins (1) and among them p53 and p73 (2-4). Pin1 regulates many cellular events and is involved also in cancer and neurodegenerative disorders. Recently we have turned our interest on the role of Pin1 in the oncogenic functions of the Notch pathway in mammary tumor. Notch signaling pathway plays a crucial role in specifying cellular fates and its activation has been associated with the amplification of some somatic stem cells and in cancer development (5). We have identified human Notch1 as an interactor of Pin1, and showed that Pin1 affects Notch1 signaling. Remarkably Pin1 potentiates Notch1 cleavage by gamma secretase, leading to an increase of the active intracellular domain, ultimately enhancing Notch1 tumorigenic activity. Moreover we found Pin1 as a novel Notch1 target gene and postulated a feed forward loop between these two factors. Consistently, in a breast cancer tissue microarray we find a significative correlation between Notch1 nuclear staining and overexpression of Pin1 suggesting relevance to human carcinogenesis.

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THE ROLE OF THE NUCLEAR RECEPTOR TAILLESS IN NEUROGENESIS AND MAINTENANCE OF BRAIN TUMOR FORMATION

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The tailless (Tlx) gene encodes an orphan nuclear receptor which is expressed in the developing eye and brain and in adult neural stem cells. We show that in the adult brain it is exclusively expressed in astrocyte-like B cells of the subventricular zone whereas C and A cells do not express Tlx. These cells have the ability for self-renewal and are multipotent. Use of a genetic approach allowed the establishment of a cell lineage relationship of neural stem cells from neural epithelial to radial glial cells and finally to adult neural stem cells. Tlx expressing cells express CD133, a marker of brain tumor stem cells, and loss of Tlx leads to upregulation of PTEN in the subventricular zone compatible with its function in control of neural stem cell proliferation. Since Tlx has been found to be overexpressed in brain tumors we have generated mice by BAC transgenesis with two additional copies. These mice develop brain neoplasia. To define the function of Tlx in the adult brain we have generated an inducible Tlx mutation with the Cre/loxP system. This mutation leads to loss of neurogenesis, even though astrocyte-like B cells still exist in the subventricular zone. These findings establish Tlx as an indicator for and regulator of adult neural stem cells.

CD44-TARGETING INHIBITS TUMOR GROWTH AND PREVENTS POST-CHEMOTHERAPY RELAPSE IN HUMAN BREAST CANCER XENOGRAFTS

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Breast cancer is the most common cancer of women. Despite recent progresses in therapy, mortality remains high due to local recurrences and development of distant metastases. It is therefore essential to develop new targeted therapies to reduce recurrences and consequently breast cancer mortality. “Tumor initiating cells” have been recently discovered in breast cancers and characterized as CD44+/CD24- . These cells are thought to be resistant to conventional treatments like chemotherapy or radiotherapy and therefore responsible of post-treatment recurrences. To test whether CD44-positive cells are involved in tumor growth and tumor recurrence after chemotherapy, we evaluated the antitumor activity of the CD44-targeting antibody (H90) in three different human breast cancer xenografts. We found that the anti-CD44 treatment strongly decreases tumor growth in both estrogen-receptor (ER) negative and positive xenografts. Importantly, the H90 antibody strongly decreases tumor recurrence after chemotherapy-induced remission. Immunohistological analysis of xenografts showed heterogeneous CD44 staining, with isolated positive cells or small positive islets found in the vicinity of blood vessels or in necrosis areas. Moreover, tumor relapse is associated with strong CD44 enrichment as showed by immunohistology. Mechanism of action could relate to anti-proliferative cytokines that have been found to be more expressed in treated tumors and to a decreased proliferation of target cells.

INCREASED SELF RENEWAL PROPERTIES OF BREAST CANCER STEM CELLS

BONIZZI G.[1], Cicalese A.[1], Pasi C, Giulini B. [1,2], Scanarini M., Pece S., Di Fiore PP and Pelicci P.G.[1,2].

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Recent findings support the concept that cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of human cancer, and that eradication of cancer stem cells (CSC) may be essential to achieve cancer cure. However, direct proof of these concepts is still lacking, mainly due to poor knowledge of the biological differences between normal and cancer stem cells and the relevant underlying molecular pathways. To investigate the growth properties of normal and transformed breast stem cells (BSC), we have set-up robust protocols for the in vitro cultivation of mouse BSCs, based on their property to survive in suspension and generate floating spheroids (mammospheres). Using, as model system, transgenic mice where mutated ErbB2 is expressed in the breast tissue by the estrogen-inducible MMTV promoter. Mammospheres can be propagated serially (to study self renewal) and differentiated when cultured on collagen. Using serial spheroid cultures, we adapted the classic 3T3 protocol for quantization of SCs vs. progenitors growth (validated by transplantation experiments into syngenic mice). To isolate homogenous populations of normal and cancer BSCs, we have set-up a strategy based on the biological property of SCs to remain quiescent or undergo few cells divisions. Cell suspensions from normal or transformed mouse breast tissues are stained with the red fluorescent dye PKH26, which stably incorporates into membranes, and grown as spheroids. Quiescent/rarely dividing SCs remain intensely fluorescent, while actively proliferating progenitors loose fluorescence through dilution of the membrane-bound dye. After culture, we FACS-sort PKH+ and PKH- cells. Using these assays, we showed that: *i)* the number of SCs increases progressively during serial passages of ErbB2 mammospheres, while it decreases in wt mammospheres; *ii)* ErbB2 mammospheres contain more SCs then normal mammospheres (1:100 vs 1:300, determined by limiting dilution transplantation assays); *iii)* the ErbB2 breast cancer tissue contain more SCs than the normal breast (1:4.000 vs 1:30.000). These differences are due to increased self renewal of cancer BSCs, as shown by the finding that only (slowly dividing) PKH+ cells from wt mammospheres form breast tissue upon transplantation, while both PKH+ and (the rapidly dividing) PKH- cells from ErbB2 mammospheres initiate tumors *in vivo*.

Poster session II

B1	Carla Micucci
B2	Ola Myklebost
B3	Sarit Mizrachy-Schwartz
B4	Giovanni Morrone
B5	Barbara Ortensi
B6	Francesca Orzan
B7	Laura Pedranzini
B8	Jay Philippe
B9	Valeria Poli
B10	Ugo Rovigatti
B11	Cristina Santoriello
B12	Lucia Santoro
B13	Sveva Sanzone
B14	Danielle Shing
B15	Verónica Romina Sobrado
B16	Margherita Turco
B17	Andrea Viale
B18	Maria Vias
B19	Federica Zunino
B20	Elena Longobardi

Last update: November 13, 2007

B1

IDENTIFICATION AND ISOLATION OF NORMAL AND TUMOR LUNG STEM CELLS

CARLA MICUCCI[1,2], Elena Belloni[1,2], Giulia Veronesi[3], Giada Matera[1,2], Soheil Javan[1,2], Lorenzo Spaggiari[3], Mariano Barbacid[4], Giuseppina Bonizzi[1,2], Salvatore Pece[1,2], Pier Paolo Di Fiore[1,2], and Pier Giuseppe Pelicci[1,2].

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The definition of cancer as a stem cell disease, is based on the isolation of those cells, which presumably are cancer initiating cells (cancer stem cells). We applied this hypothesis to the study of lung cancer. We tested the hypothesis that normal and tumor lung stem cells do exist, developing an in vitro model system to isolate lung normal and cancer stem cells, in human and mouse. Similarly to what has been evidenced for breast and neural stem cells, after dissociation of the lung tissue, we isolated an undifferentiated multipotent population of cells that can be grown in suspension, forming floating spherical structures, which we call pneumospheres. We could demonstrate that normal pneumospheres: i) are clonogenic; ii) grow serially; and iii) contain bi-lineage cells. Using PKH26 staining, we could further enrich for cells with stem properties, among those constituting the pneumospheres. We also obtained human lung tumor pneumospheres, and we are studying their features, comparing them with their normal equivalent. Then, we intend to test the existence and study the properties of mouse lung tumor pneumospheres, derived from a K-ras inducible tumor model. Our final goal will be the identification of a normal and tumor lung stem cell-specific signature.

B2

NORWEGIAN CANCER STEM CELL INNOVATION CENTRE

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With the partners University of Oslo, Ullevål University Hospital, Oslo, Alpharma AS, Oslo, InVitrogen Dynal AS, Oslo, Affitech AS, Oslo, PCI Biotech/Photocure, Oslo. The Oslo Cancer Stem Cell Innovation Centre is an academic-industrial consortium for research-based innovation comprised of eleven academic groups and four industrial partners, with top expertise in cancer research, genomics, bioinformatics, cellular and molecular biology, proteomics, stem cell, animal and patient studies, molecular imaging, immunotherapy, and cellular pharmacology and pathway targeting, as well as biotechnological and pharmaceutical research, development and production. The central goal of the CSCIC is to find methods for selected tumour types to (i) identify and characterize cell populations (?side-populations?) with cancer stem cell potential, and to subsequently (ii) develop innovative approaches to develop new small drugs, cancer vaccines and antibodies that destroy specifically the cancer stem cells and to (iii) visualize at high resolution cancer stem cells in the body and track therapeutic success by advanced imaging. The work is divided into 5 work packages: 1) Tumour sub-populations with stem cell characteristics and activity of stem cell pathways in tumours, 2) From cancer stem cells to therapeutic antibodies, 3) Searching for pathway-specific drugs that selectively affect cancer stem cells, 4) Training the immune system to eradicate cancer stem cells: experimental immunotherapy, and 5) Targeting cancer stem cells by photochemical internalization.

B3

OPTIMIZATION OF ENERGY-CONSUMING PATHWAYS TOWARDS RAPID GROWTH IN HPV-TRANSFORMED CELLS

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Cancer is a complex, multi-step process characterized by misregulated signal transduction, and altered metabolism. Cancer cells divide faster than normal cells and their growth rates correlate with increased metabolic flux during cell transformation. Here we report on progressive changes in essential elements of the biochemical network, in an in vitro model of transformation, consisting of primary human keratinocytes, human keratinocytes immortalized by HPV16 and passaged repeatedly in vitro, and the extensively-passaged cells subsequently treated with the carcinogen benzo[a]pyrene. The more transformed cells were smaller and divided faster, but the cellular energy flux was unchanged. During cell transformation the protein synthesis network contracted, as shown by the reduction in key cap-dependent translation factors. Moreover, there was a progressive shift towards IRES-dependent translation. The switch from cap to IRES-dependent translation correlated with progressive activation of AMPK, which controls energy-consuming processes, including protein translation. As cellular protein synthesis is a major energy-consuming process, we propose that the reduction in cell size and protein amount provide energy required for cell survival and proliferation. The cap to IRES-dependent switch seems to be part of a gradual optimization of energy-consuming mechanisms that redirects cellular processes to enhance cell growth, in the course of transformation.

B4

EARLY HAEMATOPOIETIC ZINC FINGER PROTEIN ? ZINC FINGER PROTEIN 521: A CANDIDATE REGULATOR OF NORMAL AND LEUKAEMIC STEM CELLS

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EHZF/ZNF521 is a 30-zinc finger transcription co-factor whose expression in the human haematopoietic system is restricted to CD34+ cells and particularly abundant in CD133+ and SCID-repopulating cells. EHZF mRNA levels rapidly decrease in response to conditions that induce differentiation and become undetectable in precursor cells and mature leukocytes. EHZF shares high amino acid identity with OAZ/ZNF423, another 30-ZF protein implicated in the maintenance of immaturity in olfactory epithelium progenitors. Like OAZ, EHZF enhances the transcriptional activity of BMP-responsive elements and represses promoters whose transcription is activated by EBF, a master factor in the specification of the B-cell lineage and in neuronal differentiation. Enforced expression of EHZF inhibits the differentiation of myeloid cell lines and of primary haematopoietic progenitors in response to chemicals and haemopoietins, and results in progenitor accumulation in human stromal co-cultures. Transfection or transduction of EHZF in B-cells inhibits the expression of EBF target genes and enhances apoptosis.The EHZF mRNA is detectable in most AMLs and T-ALLs but not in B-ALLs. In AMLs, discrete levels of EHZF expression correlate with specific genetic aberrations. High expression levels are associated to the (t9;11)(p22;q23) translocation that generates an MLL-AF9 fusion protein. RNAi-mediated silencing of EHZF in THP1 cells, that express MLL-AF9, resulted in impaired growth and clonogenicity. High expression of EHZF is found in all the leukeamic stem cells analysed compared to the stem cells-depleted population, strongly suggesting that EHZF may be implicated in the homeostasis of the immature compartment in AMLs as well as in normal haematopoiesis. EHZF interacts with histone deacetilases 1 and 2 and other components of the NuRD complex through a 12-AA N-terminal repressor motif shared with other transcriptional co-repressors including friend of GATA (FOG)-1 and 2. This domain appears to be required for some of the biological effects of EHZF, in particular the inhibition of myeloid differentiation. Taken together, our data suggest that EHZF/ZNF521 contributes to the homeostasis of the immature compartment of the human haematopoietic system, and may be a relevant factor for the development and/or maintenance of leukaemic clones. The identification of molecular interactors and downstream targets of EHZF/ZNF521 in normal and leukaemic stem cells will shed important insight into its mechanism of action and help define its role in the control of normal and malignant haematopoiesis.

Work supported by funds from AIRC and MIUR (Cluster C-04, Interlink).
TM is the recipient of an AIRC fellowship, and DP of a fellowship from Regione Calabria

B5

RAI (SHC C) IS EXPRESSED IN CANCER NEURAL STEM CELLS AND IS REQUIRED FOR BRAIN TUMOR DEVELOPMENT

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Rai (Shc C) belongs to the family of Shc adaptor proteins and is expressed in brain, neural stem cells , neurons but not in glial cells. In our previous works we showed that Rai has a pivotal role in neuroprotection after ischemic damage by activating the PI3K/Akt pathway, thus leading to an increase in cell survival. In a tissue-microarray screening of human tumors arising from a wide range of organs we found that Rai is expressed in glioblastoma multiforme (GBM). In cancer stem cells, isolated from human GBM specimens, Rai is always expressed regardless of its level in the total tumor. Therefore we analyzed the behavior of Rai stably interfered cancer stem cells in vitro and in vivo, by intrathecal injection in nude mice. Reduced gliomagenesis after Rai silencing suggests that the presence of RAI in cancer stem cells confers them a selective advantage. The obtained results provide important insights into the molecular mechanisms of tumor development, identifying Rai as a new potential diagnostic/prognostic marker.

B6

OVER-EXPRESSION OF THE POLYCOMB GENE EZH2 IN PRIMARY GLIOBLASTOMAS AND CORRESPONDING CANCER STEM-LIKE CELLS

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Polycomb group (PcG) proteins are epigenetic gene silencers implicated in the maintenance of embryonic and adult stem cells and in cancer development. EZH2 is a member of the PcG repressive complex 2 and it is upregulated in prostate and breast cancer. We have investigated EZH2 expression in a panel of 48 gliomas of different grade using real time PCR. In 12 grade II gliomas EZH2 expression was found 4.8± 3.2 fold upregulated than in normal brain. In glioblastoma multiforme (GBM; grade IV glioma) EZH2 expression was 22.9±17.2 fold higher than in normal brain ($p<0.0008$). Recent data show that culture conditions can select in vitro two GBM subpopulations: neurospheres (NS), expressing stem cell markers, and adherent cells (AC). We have found that NS from 8 GBM express 8.2±10.2 fold (fold change 1.3 to 30.9) EZH2 than corresponding AC. These results show for the first time the over-expression of EZH2 in malignant gliomas and that, coherently with its role in stem cell maintenance, it is particularly abundant in NS enriched with cancer stem-like cells. Based on these findings EZH2 appears as an interesting marker of glioma progression and an appealing target for treatment.

B7

IMMUNOPHENOTYPIC AND CYTOGENOMIC CHARACTERIZATION OF THE KASUMI-1 CELL LINE: A MODEL SYSTEM FOR THE STUDY OF AML STEM CELLS

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The Kasumi-1 cell line was established from the peripheral blood of a 7-year-old boy with acute myeloid leukaemia (AML), subtype M2, in relapse after bone marrow transplantation. It presents the t(8;21)(q22;q22) translocation, a cytogenetic hallmark of M2 AML, originating the AML1-ETO fusion protein which expression has been shown to exert a dominant-negative activity on myeloid differentiation. Moreover Kasumi-1 cell line is characterized by the amplification of a mutated allele (N822K) of the c-kit gene encoding for a constitutively activated form of the stem cell factor receptor. The cooperation of a loss of function and an activating tyrosine kinase receptor mutation render the cell line a paradigm of the ?two-hits? model for AML pathogenesis. Kasumi-1 cells were previously shown to express myeloid and macrophage markers and the CD34 stem cells marker, and were therefore proposed to originate from an early myeloid stem cell. On the basis of the above observations we reasoned that early passage Kasumi-1 cell line might contain a subset of primitive multipotent/progenitors cells and could be therefore a good system for the identification and study of AML stem cells. To investigate this hypothesis early passage Kasumi-1 cell line was first subjected to a refined immunophenotypic characterization: surface markers analysis showed that cells were positive for CD34 (93.3%), CD38 (40.8%), CD33 (31.9%), CD117 (c-kit) (89.2%), CD4 (32.7%), CD8 (3%), and CD13 (75.4%) and negative for CD3, CD16, CD19 and CD20. Double staining analyses revealed that CD34+CD38- cells, the only capable of transplanting leukaemia in NOD/SCID mice for most AML subtypes, are 52%, confirming the presence in the Kasumi-cell line of a consistent population of cells with a stem cell-like immunophenotype. In order to better characterize the Kasumi-1 cell line at the cytogenomic level we used SKY and Array-CGH (Agilent 244K oligo arrays), two complementary high-resolution Fluorescence In Situ Hybridization techniques. The presence in all cells of both the t(8;21) and a variable number (1 to 3) of chromosome 4-derived markers was confirmed by SKY, while a-CGH precisely indicated a 4q11-specific gain, consistent with our previous FISH findings that the minute markers represent 4cen-q11 isochromosomes duplicating the 4q11 region, where the c-kit gene is located. In addiction a number of other chromosomal abnormalities were detected including t(2;8), t(9;15), t(13;16) and a trisomy of the chromosome 10. To assess whether the CD34+CD38-LIN- subpopulation is indeed enriched of

cells with stem cell properties it will be subjected to the classical assays designed for the detection and characterization of haematopoietic stem cells, namely long-term culture initiating cells assay, colony forming cells assay and transplantation in NOD/SCID mice. Cytogenomic characterization will be then performed on the isolated CD34+CD38-LIN- cells and compared to that obtained on the bulk Kasumi-1 cell population.

Supported by CARIPLO Nobel Project.

B8

SOX9 REGULATES CELL PROLIFERATION AND IS REQUIRED FOR PANETH CELL DIFFERENTIATION IN THE INTESTINAL EPITHELIUM

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The HMG-box transcription factor Sox9 is expressed in the intestinal epithelium, specifically in stem/progenitor cells and in Paneth cells. Sox9 expression requires an active beta-catenin/Tcf complex, the transcriptional effector of the Wnt pathway. This pathway is critical for numerous aspects of the intestinal epithelium physiopathology but processes that specify the cell response to such multipotential signals still remain to be identified. We now inactivated the Sox9 gene in the intestinal epithelium to analyse its physiological function. Sox9 inactivation affected differentiation throughout the intestinal epithelium, with a disappearance of Paneth cells and a decrease of the goblet cell lineage. Additionally, the morphology of the colon epithelium was severely altered. We detected general hyperplasia and local crypt dysplasia in the intestine, and Wnt pathway target genes were upregulated in such lesion. This correlated with downregulation of Wnt pathway inhibitors such as ICAT and Grg/TLE mRNAs. The number of putative stem cells (positive for Musashi-1) was increased in Sox9-deficient crypts. These results highlight the central position of Sox9 as both a transcriptional target and a regulator of the Wnt pathway in the regulation of intestinal epithelium homeostasis.

B9

IN VIVO ONCOGENIC PROPERTIES OF CONSTITUTIVELY ACTIVE STAT3

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STAT3 is constitutively active in many primary tumors and is considered an oncogene, triggering cell proliferation, survival, angiogenesis, tissue invasion and immune evasion. In addition, STAT3 is both essential and sufficient to maintain indifferentiated mouse embryonic ES cells, and is a potential player in promoting renewal of cancer stem cells as well. However, direct oncogenic activities in vivo have not been proven and the mechanisms involved are not fully understood. We have generated mice expressing only the constitutively active form STAT3C. These display slightly enhanced basal STAT3 activity that is prolonged upon cytokine stimulation, similar to the continuous but low activity observed in tumours. STAT3C mice develop myeloid and plasma cell hyperplasia, and STAT3C/C MEFs display increased proliferative potential. STAT3C can cooperate with ErBb2-mediated mammary tumorigenesis, since MMTV-Her2Neu (NeuT) transgenic mice carrying the STAT3C allele develop faster growing, more invasive tumours. STAT3C-NeuT mammary tumor cells display increased migratory and invasive capacity. These data demonstrate for the first time in vivo oncogenic properties of STAT3, which promotes cell survival as well as motility and invasivity, suggesting enhanced metastatic potential. This idea is also supported by the observation that STAT3 knock-down in NeuT stabilized cell lines completely abolished anchorage-independent growth.

B10

MICRO-FOCI INDUCING VIRUS (MFV) TARGETS AND TRANSFORMS STEM CELLS

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Micro-Foci inducing Virus (MFV), a dsRNA virus, was initially isolated from children with neuroblastoma diagnosed in a "cancer-cluster" situation and in both sporadic neuroblastomas and paediatric lymphomas (Burkitts type, [1]). Infection/transformation of neuroblasts and/or mesenchimal cells suggests that a Stem Cell (SC) is the target for MFV:

I. MFV-infected cells display 10-20 fold higher telomerase levels than mock-infected controls and transformed cells have activities 100-150 fold higher than controls.
II. Numbers of transformed colonies are not proportional to virus titers, but to the number of plated cells, indicating a probable SC target.

Terry Hamblin recently suggested that an infectious agent may trigger CLL onset, where SC targeting is also hypothesized [2]. Preliminary data on CLL will be presented for the following reasons:

1. microRNAs are deregulated by MFV infections (high dsRNA levels) and also in CLL [3].
2. CLL stereotyped Ig gene rearrangements suggest presence of a "common" infection [2].
3. Antigen selection and auto-antigen triggering are also consistent with an agent like MFV [2].

[1] U. Rovigatti, Conference Childhood Leukaemia. Section P1-18 pp I-IV, September 2004.

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B11

GENE-TRAP SCREEN TO DEVELOP ZEBRAFISH CANCER MODELS

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Zebrafish has already proven to be a model organism suitable to study cancer related processes and in recent years it has become clear that multiple research platforms are required to elucidate the nature, mechanisms and biological interactions that occur during cancer progression. In order to develop one of these platforms we decided to create a panel of transgenic lines that develop cancer in different organs/ tissues using the tol2 transposon gene trap system [(1)]. In order to generate tumors in these lines we used an activated form of Ras (H-RasV12) fused to green fluorescent protein (EGFP) as reporter, because the Ras gene family is frequently (30%) mutated in human cancer. The construct used to generate these lines was able to induce different kind of neoplasia (in internal organs and generated f-nevi in the skin) when expressed in somatic cells. After screening 146 fish for germ line integration we observed that 22% of the injected fish carried integrations of the EGFP-RasV12, but that only 1% displayed EGFP-RasV12 expression. This probably suggests that the EGFP-Rasv12 expression may be toxic for the germ line. We then decided to switch to an inducible transgenic approach using an Hsp70 promoter driving EGFP-RasV12 expression. We will present the phenotypic and genetic characterization of the transgenic lines generated so far, which will eventually be used for genetic and pharmacological studies on cancer development and therapy.

1 (Kawakami et al., Dev Cell. 2004 Jul: 133-44)

B12

CD133 CELL POPULATION FROM HUMAN ADENOCARCINOMA CELL LINES AND FROM ADENOCARCINOMA PATIENTS

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We developed a procedure to enrich for prostatic cancer stem cells through spheres formation and to analyze prostatospheres for cancer stem cells properties. Starting from PC3 and 22RV1 cell lines we report that both cell lines can produce prostatospheres. Non-adherent spheres grown from PC3 cells demonstrate their self renewal ability by the formation of secondary spheres from dissociated primary sphere cells and forming tumours in NOD-SCID mice. 22RV1 cell line was able to form secondary spheres from primary sphere cells but showed lower tumorigenic potential. This different behaviour correlated with a different CD133 expression in two cell lines. On this background, we isolated and characterized the CD133 cell population from human primary prostatic adenocarcinomas. We report a topographic CD133 gradient within the prostatic tumour. In the six patients analysed so far, the tissue district with highest CD133+ cells percentage (CD133 niche) was either the tumoural or the peritumoural (identified by bioptic analysis) area. The co-culture of spheres and of the adherent counterpart from parental primary culture derived from the same donor reproduces in vitro the adhesion dependence and the differentiating capacity of cancer stem like cells and this interaction between tumour and microenvironment can become now target of therapeutic intervention.

B13

A RAT MAMMARY GLAND CANCER CELL WITH STEM CELL PROPERTIES OF SELF-RENEWAL, MULTI-LINEAGE DIFFERENTIATION AND TUMOR SUSTAINABILITY

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The cancer stem cell hypothesis posits that tumors are derived from a single cancer-initiating cell with stem cell properties. The task of identifying and characterizing a single cancer initiating cell with stem cell properties has proven technically difficult because of the scarcity of the cancer stem cells in the tissue of origin and the lack of specific markers for cancer stem cells. Here we show that a single LA7 cell, derived from rat mammary adenocarcinoma has: the differentiation potential to generate all the cell lineages of the mammary gland; the ability to generate branched duct-like structures that recapitulate morphologically and functionally the ductal-alveolar-like architecture of the mammary tree; the capacity to initiate heterogeneous tumors in NOD-SCID mice. In addition, we show that cultured cells derived from the tumors generated by a single LA7 cell-injection, have properties similar to LA7 cells, can generate all the cell lineages of the mammary gland and recapitulate the ductal-alveolar-like architecture of the mammary tree. The property of self-renewal, extensive capacity for proliferation, multi-lineage differentiation potential and single-cell tumor-initiation potential suggest that LA7 cells are cancer stem cells and that LA7 cells can be used as a model system to study the dynamics of tumor formation at the single cell level.

B14

SPRDM16 AND P53-LOSS COOPERATE IN EXPANDING HEMATOPOIETIC STEM AND PROGENITOR CELLS AND INDUCING MYELOID LEUKEMIAS

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Transgenic expression of the abnormal products of acute myeloid leukemia (AML)-associated primary translocations in hematopoietic stem/progenitor cells initiates leukemogenesis in mice, yet additional mutations are needed for leukemia development. We found aberrant expression of PRDM16 in AMLs with translocations of 1p36 or with normal karyotype, carrying, respectively, a relatively high prevalence of mutations in the TP53 tumor suppressor gene or in nucleophosmin (NPM), a gene that regulates p53. Two isoforms are expressed from the PRDM16 gene, differing in the presence or absence of the PR domain. Overexpression of the short isoform, sPRDM16, in murine bone marrow induces AML with full penetrance, but only in the absence of p53. The murine leukemias are characterised by multilineage dysplasia and dysmegakaryocytopoiesis, a common feature of human AMLs with 1p36 translocations or NPM mutations. sPRDM16 overexpression increases the pool of hematopoietic stem cells in vivo and, in vitro, blocks myeloid differentiation and prolongs progenitor life span. Loss of p53 augments the effects of sPRDM16 on stem cell number and induces immortalization of progenitors. Thus, overexpression of sPRDM16 induces abnormal growth of stem cells and progenitors and cooperates with disruption of the p53 pathway in the induction of myeloid leukemia.

B15

NEW INSIGHTS IN THE REGULATION OF E-CADHERIN AND EMT: THE ROLE OF BHLH FACTORS E2-2A AND E2-2B

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Downregulation of E-cadherin is a key event for epithelial-mesenchymal transition (EMT), allowing cell migration in the developing embryo and tumor invasion. Several E-cadherin transcriptional repressors have been characterized, including Snail, Slug, E47 and Twist. All are capable of inducing EMT, and are expressed during embryonic development in regions undergoing this transition. We have identified the class I bHLH transcriptional regulator E2-2 (ITF2/TCF4), in a yeast one-hybrid screen designed to identify repressors interacting with the murine E-cadherin promoter. The E2-2 gene encodes two isoforms, E2-2A and E2-2B (differing in their N-termini), whose specific functions remain unknown. Here we show that both E2-2A/B repress E-cadherin at the level of transcription, and drive a complete EMT when stably expressed in epithelial MDCK cells. Promoter activity assays indicate a direct action of both isoforms on the E-cadherin promoter, dependent on the integrity of the E-pal element and HDAC activity. RT-PCR analysis reveals increased levels of E2-2 transcripts in highly invasive cell lines, and confirms their upregulation in MDCK cells overexpressing Snail, Slug or E47. These results support a new role for E2-2A/B on E-cadherin regulation and EMT, and suggest a functional relationship with other E-cadherin repressors, which is currently under investigation.

B16

RALP, A NEW MEMBER OF THE SHC FAMILY, IS EXPRESSED EARLY DURING DEVELOPMENT AND IN ADULT NEURAL STEM CELLS IN THE MOUSE

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RaLP is a newly identified member of the Shc family of adaptor proteins that encodes a cytosolic protein highly expressed in aggressive and metastatic melanomas. When ectopically expressed in non-metastatic melanoma cells, it functions as a substrate of activated IGF-1 and EGF receptors, increasing Ras-MAPK signaling and cell migration; while its silencing in RaLP-positive melanoma cells abrogates migration in vitro, without affecting MAPK signaling, suggesting that RaLP activates both Ras-dependent and-independent migratory pathways in melanomas. Silencing of RaLP expression in melanoma cell lines by RNA interference inhibits tumorigenesis in vivo and migration in vitro, thus suggesting that RaLP could be a critical determinant in the acquisition of the migratory phenotype in melanoma cells. Analysis of RaLP expression in the mouse by in situ hybridization has evidenced its presence in the sites of neurogenesis during embryonic development and continues to be expressed in the olfactory lobe, cerebral hemisphere and cerebellum in the adult. To investigate the role of RaLP in neural migration and differentiation, neural stem cells were derived from adult mouse brains of RaLP wild type and KO animals. RaLP is expressed in neural stem cells, as well as in neural progenitors and migrating melanoblasts, while its expression decreases in the differentiated normal melanocytes and during the in vitro differentiation of neural stem cells, suggesting that RaLP may be necessary for the differentiation process. Preliminary experiments in RaLP KO-derived neural stem cells show a defective pattern of differentiation in these cells. To dissect the role of RaLP in self renewal, migration and differentiation, we have recently derived embryonic stem (ES) cells from RaLP wild type and KO animals and induced to differentiate in vitro in several lineages. Results will be discussed.

B17

ONCOGENIC FUNCTION OF THE CELL CYCLE INHIBITOR P21 IN THE REGULATION OF SELF-RENEWAL OF LEUKEMIC STEM CELLS

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Rare cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of cancer. A defining characteristic of SCs is their capacity to self-renew, which is markedly increased in cancer SCs. The underlying molecular mechanisms, however, are largely unknown. We demonstrate that two fusion proteins of myeloid leukemias upregulate expression of the cell cycle inhibitor p21 and that p21 is indispensable for their ability to extend self-renewal of SCs. In the absence of p21, fusion proteins fail to initiate leukemogenesis or to support self-renewal of leukemic SCs. Cell cycle analysis of SC-enriched populations showed that p21 expression is necessary to maintain the pool of quiescent leukemic SCs, thus preventing premature SC depletion during leukemia outgrowth. These data unravel the oncogenic potential of the cell cycle inhibitor p21 and provide the first molecular target for anti-leukemic strategies aimed at eradicating quiescent cancer SCs.

B18

NEUROENDOCRINE DIFFERENTIATION IN ANTI-ANDROGEN RESISTANT PROSTATE CANCER CELLS

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Anti-androgens are administered as a principal treatment for prostate cancer. Aggressive hormone refractory disease is characterised in some cases by the development of a neuroendocrine phenotype. We show here using a cell-line resistant to non-steroidal anti-androgens, LNCaP-Bic, that the upregulation of the neuropeptide Neurotensin/Neuromedin N (NTS) confers increased invasiveness and proliferation on the line. Neurotensin stimulation of the parental LNCaP cell-line results in the activation of Rac1 and JNK. Rac1 activation has previously been implicated in increased cell migration and signalling pathway emanating from the neurotensin receptor may prove to be treatment targets in recurrent neuroendocrine prostate cancer. We also demonstrate that a number of transcription factors including HES6 (hairy and enhancer of split 6 (Drosophila)) and Achaete Scute Complex Homolog (Ascl1) are overexpressed in the anti-androgen resistant cell-line compared to the parental line. These pro-neural factors have been implicated in the generation of neural progenitors during tissue differentiation. Through an analysis of published datasets in a number of other tumours at different organ sites we propose that pro-neural factors drive the development of aggressive, potentially drug resistant tumours elsewhere. We suggest that identifying the target genes for pro-neural factors will in future enable the development of new biomarkers and therapeutics to target this niche.

B19

NEW INSIGHTS INTO THE DIFFERENTIAL ROLE OF CD133 ISOFORMS IN BRAIN TUMOR STEM CELLS

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Many words have been written about CD133 and its role as a stem cell marker in physiological and pathological conditions, none yet being definitive. CD133 was originally identified as a novel cell surface marker expressed on a subpopulation of the CD34+ hematopoietic stem and progenitor cells. CD133 belongs to a family of pentaspan membrane glycoproteins. Almost nothing is known about its function, except that it is associated with microvilli and binds cholesterol, wherefrom the speculation of a role in cell-cell adhesion. Its role as a cancer cell stem marker in brain tumors has been widely investigated. A recent paper appeared in Cancer Research 2007 seemed to contradict the whole of existing literature, by showing that the CD133 negative fraction is capable of forming tumors in nude mice [Beier D. et al. 2007]. We do not think their work to be conclusive on the subject, since the authors did not analyse the possibly different role played by the two isoforms, CD133-1 and CD133-2. In our laboratory we aim at unraveling the functions of the two isoforms and their causal relation to glioblastoma formation, seeking for a final tenet for CD133 role within the cancer stem cell theory in the human brain.

B20

THE HOMEODOMAIN PROTEIN PREP1: A NOVEL ONCOSUPPRESSOR IN HUMAN AND MICE

ELENA LONGOBARDI, Giorgio Iotti, Elisa Lenti and Francesco Blasi.

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Prep1 is an homeodomain protein that is essential in embryonic development at the gastrulation stage. An hypomorphic mutation (*Prep1*$^{i/i}$) has an leaky embryonic phenotype, with 80% of the homozygous embryos dying before birth. These embryos have a pleiotropic phenotype, including hypoplasy of essentially all organs and more specific defects in angiogenesis, oculogenesis and hematopoiesis. The hematopoietic phenotype affects all lineages, and its Long Term Repopulating Hematopoietic Stem Cells have an extremely low competitive repopulation capacity (0.5%). Twenty % of the embryos escape death *in utero* and live a normal-length life. However they display a variety of pathological phenotypes. In particular, more than 40% of the homozygous and a few heterozygous mice develop various types of tumors after one year of age: lymphomas (mainly), adenocarcinomas. The tumor phenotype can be observed in normal mice transpolanted with fetal liver cells of the homoxygous *Prep1*$^{i/i}$ or heterozygous *Prep1*$^{i/+}$ genotype. The tumors have both genotypic and phenotypic properties of the transplanted cells. In addition, they tend to appear somewhat earlier than the spontaneous ones. Preliminary data show that the transplanted fetal liver cells have deficient proliferation, DNA damage response and chromatin organization. The phenotype of the *Prep1*$^{i/i}$ mice suggests that *Prep1* may be a novel tumor suppressor gene. This is supported by tumor micro-array analysis of a total of 416 tumors in which *Prep1* expression has been analyzed (with over 75% concordance) by both immunohistochemistry and *in situ* hybridization. In agreement with the tumor suppressor hypothesis, over 70% of the tumors show no or very low Prep1 expression.

This work has been made possible thanks to the collaboration of P.P. Di Fiore (IFOM), A. Doglioni, F. Sanvito and M. Ponzoni (Pathology, S. Raffaele Scientific Institute), P. Nuciforo and Marco Bianchi (IFOM, Pathology Service), M. Caniatti (Pathology, Univ. of Milano, School of Veterinary Science). This research was supported by AIRC (Ass. Ital. Ricerche sul Cancro).

December 3, 2007

Lectures

Emmanuelle Passegué
San Francisco, USA

Stuart H. Orkin
Boston, USA

Tessa L. Holyoake
Glasgow, UK

Michael Cleary
Stanford, USA

Chia-Lin Wei
Singapore, Singapore

Oral Presentations

Stefano Campaner
Milan, Italy

Oksana Bereshchenko
Monterotondo, Italy

Session V
Leukemia stem cells

DEREGULATION OF STEM CELL FUNCTIONS IN LEUKEMIA STEM CELLS

EMMANUELLE PASSEGUÉ

Understanding the fundamental mechanisms regulating stem cell functions has clear implications for tissue replacement and cancer prevention. Many human cancers have now been shown to arise from specific subsets of cancer cells with stem cell-like properties called cancer-initiating stem cells (CSCs), which escape normal regulation and drive the formation and growth of the tumors. Recent evidence indicates that CSCs might not be efficiently killed by current cancer treatments, and suggests that their persistence could be responsible for disease maintenance and cancer recurrence. Developing therapeutic interventions that will specifically target these disease-causing cells is an appealing approach to improve cancer treatment; yet much remains to be learned about how CSCs escape normal stem cell regulatory mechanisms and become malignant. The blood – or hematopoietic – system provides a unique tractable experimental model to investigate the functional regulations of specific cell populations. In the laboratory, we are using a mouse model of human leukemia - blood cancer - that we have developed and in which we have identified the leukemia-initiating stem cell (LSC) population as arising from the hematopoietic stem cells (HSC) compartment. Our current experiments investigate the contribution of deregulated HSC proliferation and interactions with the bone marrow microenvironment – or niche – in LSC generation.

INDUCTION OF APOPTOSIS IN CANCER STEM CELLS IN CHRONIC MYELOID LEUKAEMIA

TESSA L. HOLYOAKE

Section of Experimental Haematology, University of Glasgow UK

CML is a haemopoietic stem cell disorder characterised by the Philadelphia chromosome and Bcr-Abl oncogene. The recently introduced tyrosine kinase inhibitors (TKIs) imatinib, dasatinib and nilotinib target Bcr-Abl and induce apoptosis. However in the clinic two problems are encountered – development of Bcr-Abl mutations than impair drug binding and stem cell persistence. We have developed methods to purify CML stem cells and shown that these cells are primitive, transplantable in immunosuppressed mice, quiescent and relatively resistant to growth factor stimulation. Of importance CML stem cells express high levels of Bcr-Abl which may contribute to their inherent resistance to TKIs. Based on Bcr-Abl signalling we have investigated a range of novel agents for induction of apoptosis within the stem cell population. Of these rationale drug combinations dasatinib, a second generation multi-targeted TKI, with BMS-214662, a cytotoxic farnesyltransferase inhibitor, are synergistic, leading to eradication of long-term culture initiating cells (a stringent assay for primary human haemopoietic stem cells) in vitro and of Philadelphia positive xenografts in vivo. This drug combination is now being pursued in clinical trial through CR-UK New Agents Committee with the Phase I approved Sept 2007.

LEUKEMIA STEM CELLS: LESSONS FROM ANIMAL MODELS

Tim C. P. Somervaille[1], Christina J. Matheny[1], John L. Rinn[1], Daniela M. Witten[1], Howard Y. Chang[1], Robert J. Tibshirani[1], Sheila A. Shurtleff[2], James R. Downing[2] and MICHAEL L. CLEARY[1].

1 Stanford University School of Medicine, Stanford, CA, USA; 2 St. Jude Children's Research Hospital, Memphis, TN, USA.

We have identified and characterized leukemia stem cells (LSCs) in a mouse model of AML initiated by MLL oncogenes, which are frequently associated with the FAB-M4 or M5 subtypes of human AML. In this model, LSCs are remarkably frequent, accounting for up to one-quarter of malignant myeloid cells at late-stage disease. They are organized in a phenotypic and functional hierarchy, and express myeloid lineage-specific antigens, placing them downstream of the known hematopoietic progenitor compartments. Thus, the LSCs are not synonymous with normal upstream progenitors that are targeted for initiation, but rather constitute myeloid lineage cells that have acquired an aberrant self-renewal program as well as other biologic features of hematopoietic stem cells including substantially altered micro-environmental interactions. Global gene expression profiling confirms the downstream myeloid character of LSCs in this model, and further demonstrates the aberrant expression of a stem cell associated Hox/Meis transcriptional subprogram. Loss of LSC self-renewal, however, occurs despite persistent expression of the latter. We have derived a transcriptional signature for LSC maintenance based on the observation that LSC frequencies vary in AMLs initiated by different MLL oncogenes. The biological and clinical significance of the LSC maintenance signature will be discussed.

TOWARD THE UNDERSTANDING OF TRANSCRIPTIONAL NETWORKS AND CHROMATIN STRUCTURES IN EMBRYONIC STEM CELLS

CHIA-LIN WEI, Genome Institute of Singapore

Understanding the genetic and epigenetic regulation of "stemness" property of ES cells and their differentiation is fundamental for the comprehension of the proper lineage specification and appreciation of their therapeutic potential. To explore the chromatin modification landscapes in human ES cells, we profiled the two key histone modifications H3K4me3 and H3K27me3 by ChIP-PET analysis. H3K4me3 was found to be a prevalent mark. Among the H3K27me3 loci identified, 56% are associated with promoters and the vast majority of them are co-modified by H3K4me3. By deep transcript digital counting, we found that active transcription only initiates at K4me3 promoters and the K27me3 co-modification provides a "repressed but transcriptional ready" state. Remarkably, the target genes associated with different categories have distinct biological functions. Building from the global histone methylation maps, we have constructed the whole genome target genes and global regulatory networks of over 15 key transcription factors and chromatin modifiers by ChIP-seq analysis. Furthermore, we have currently investigated the global long range chromatin interactions mediated by key TF binding, namely insulator CTCF in ES cells. I will elaborate on using the enabling next generation sequencing capability for chromatin interaction analysis in ES cells. Our plan is to comprehensive decipher the global long range interactions, determine their associations with gene expression and understand the effects of chromatin organization in the pluripotent cell genomes.

FUNCTION OF CYCLIN E1 AND CDK2 IN HEMATOPOIETIC STEM CELLS

STEFANO CAMPANER, Andrea Viale, Serena De Fazio, Francesca De Franco, Mirko Doni, Domenico Sardella, *Mariano Barbacid, Pier Giuseppe Pelicci and Bruno Amati

The Cyclin E/CDK2 kinase complex is activated at the G1/S transition. Mammals have two genes encoding related cyclin E species (cyclin E1 and E2). Analysis of knockout mice has demonstrated that these genes are largely redundant, while deletion of both genes led to embryonic lethality due to placental defects. Of note, quiescent embryonic fibroblasts derived from cyclin E1/E2 double-knockout (dKO) mice failed to re-enter the cell cycle when stimulated with growth factors, hinting to a specialized function of cyclin E when quiescent cells are challenged to proliferate. Hematopoietic stem cells (HSC) represent a relatively quiescent population of cells supporting blood cells production, whose regulated cell cycle entry is fundamental for maintaining a tight balance between differentiation and self-renewal. Given the role of cyclin E in regulating cell cycle entry, we decided to explore its function in HSCs. Bone marrow mononucleated cells (BMMNCs) were derived from mice lacking cyclin E1, cyclin E2 or CDK2, and compared with control cells from wild-type (WT) littermates. None of the KO genotypes affected colony formation in short-term cultures (CFC-assay), indicating proliferation of committed progenitors was unaffected. Instead, in long-term culture (LTC-IC) assays, cyclin E1 and CDK2 KO cells formed less colonies, indicating a defect in HSC self-renewal. This was particularly pronounced in the cyclin E1 KO BMMNCs, where colonies were not only much fewer in numbers, but also reduced in size. To further assess HSC repopulation efficiency in Cyclin E1 KO mice, we subjected a cohort of mice to weekly anti-metabolite (5-fluorouracil) treatments and followed survival over time. Cyclin E1 KO mice showed a significantly reduced overall survival compared to WT littermates. Altogether, these data suggest that Cyclin E1 regulates self-renewal in a cell-autonomous and non-redundant manner. Our progress in analyzing this function of Cyclin E1 and the underlying mechanisms will be discussed.

C/EBPA LEUKEMOGENIC MUTATIONS COOPERATE IN HEMATOPOIETIC STEM CELLS EXPANSION AND TUMORIGENESIS

OKSANA BERESHCHENKO, Elke Kurz, Elena Mancini & Claus Nerlov
EMBL Mouse Biology Unit, Monterotondo, ITALY

C/EBPa is a tumor suppressor gene found mutated in up to 9% of human AML cases. It is expressed in two translational isoforms: p42 full-length and Lp30 shorter form unable to induce growth arrest. Two types of C/EBPa biallelic mutations are frequently found in the same AML patient: first allowing expression of only Lp30 form and a second targeting C/EBPa?s C-terminal part. We generated a C/EBPa-K313KK knock-in mutation (KK) that corresponds to AML-associated C/EBPa mutation resulting in inframe insertion of an extra lysine residue DNA binding. Homozygous C/EBPa-K313KK/K313KK mice die perinatally, therefore fetal liver cells carrying different C/EBPa mutant alleles were transplanted into irradiated recipients to address the role of individual mutations and their combinations in leukaemogenesis. First, KK/KK and KK/Lp30 cells reveal a block in myeloid lineage differentiation at the step of GMP formation. Moreover, the KK/Lp30 mutant combination results in a strong increase in frequency and number of hematopoietic stem cells. Later on, all KK/Lp30 cells-transplanted animals develop myeloid leukemia, producing a more aggressive phenotype than that of Lp30-Lp30, as judged by the time of the disease development, penetrance and tumor cells dissemination. This model provides the tool to identify leukemia-initiating cells and dissect the molecular mechanism providing the K/L cells with advantage in tumorigenesis.

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Last update: November 13, 2007

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