

SPEAKERS

CAROLINE M. ALEXANDER, PH.D.

Associate Professor, Department of Oncology, University of Wisconsin-Madison

WADE BUSHMAN, M.D., PH.D.

Robert F. and Dolores Schnoes Professor of Urology and Vice Chair for Research, Department of Surgery, University of Wisconsin School of Medicine and Public Health (Moderator)

GEORGE Q. DALEY, M.D., PH.D.

Associate Professor of Pediatrics, Biological Chemistry and Molecular Pharmacology, and Medicine, Children's Hospital Boston, Harvard Medical School; Investigator, Howard Hughes Medical Institute

PETER DIRKS, M.D., PH.D.

Staff Neurosurgeon and Principal Investigator, The Hospital for Sick Children; Scientist, Research Institute; Associate Professor of Neurosurgery, University of Toronto

WILLIAM F. DOVE, PH.D.

George Streisinger Professor of Experimental Biology and Professor of Oncology and Medical Genetics, University of Wisconsin-Madison (Moderator)

CARL E. GULBRANDSEN, PH.D., J.D.

Managing Director, Wisconsin Alumni Research Foundation

CARLA BENDER KIM, PH.D.

Assistant Professor, Children's Hospital Stem Cell Program; Department of Genetics, Harvard Medical School; Harvard Stem Cell Institute

JOHN S. KUO, M.D., PH.D.

Assistant Professor, Neurological Surgery and Human Oncology and Director, Comprehensive Brain Tumor Program, University of Wisconsin School of Medicine and Public Health

MICHAEL T. LEWIS, PH.D.

Assistant Professor, Baylor College of Medicine

LUIS F. PARADA, PH.D.

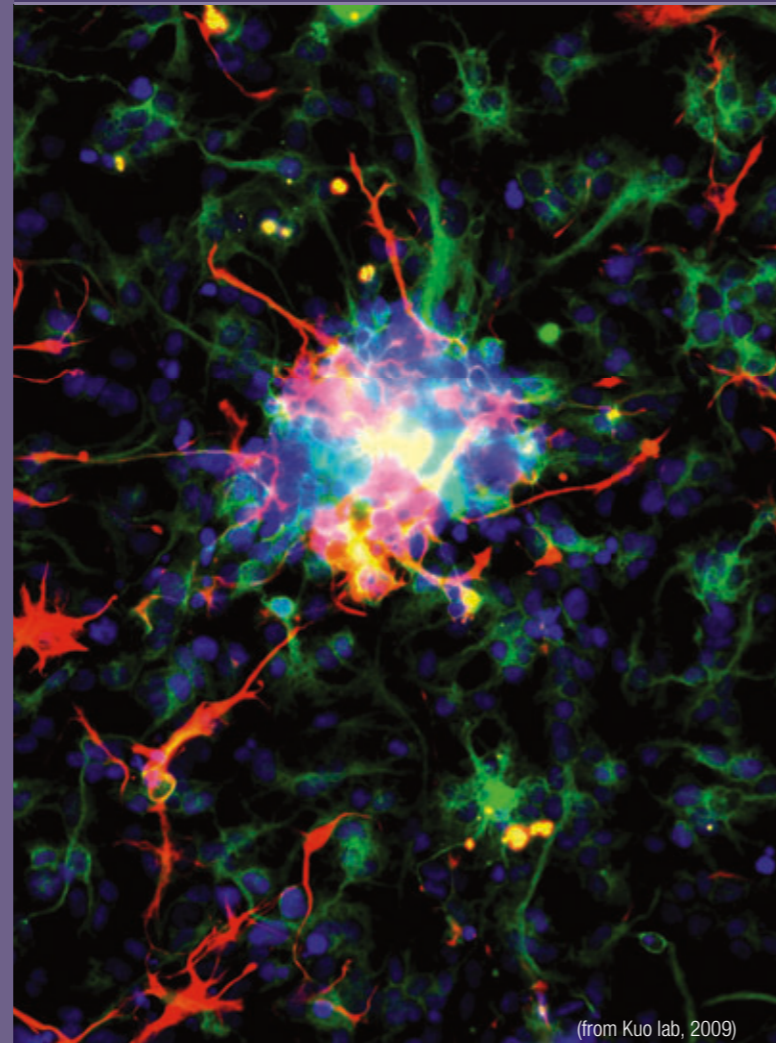
University of Texas Southwestern at Dallas: Chairman, Department of Developmental Biology; Diana and Richard C. Strauss Distinguished Chair in Developmental Biology; Director Southwestern Ball Distinguished Chair in Basic Neuroscience Research; American Cancer Society Research Professor

MAX S. WICHA, M.D.

University of Michigan: Professor, Department of Internal Medicine; Director, Comprehensive Cancer Center, Distinguished Professor of Oncology; Cancer Center Member

4th Annual Wisconsin Stem Cell Symposium:

Cancer, Stem Cells & Cancer Stem Cells



April 15, 2009
BioPharmaceutical Technology Center
Madison, Wisconsin

OVERVIEW

Coordinated by the University of Wisconsin Stem Cell & Regenerative Medicine Center and the BioPharmaceutical Technology Center Institute (BTCI), this symposium brings together world leaders in the area of cancer stem cells, and recruitment of tumor precursor cells. The focus is on basic cellular and molecular mechanisms that govern the cell growth potential of tumors, and whether there is a relationship between the long-lived/immortal cells of tumors and the long-lived/immortal cells of somatic tissues.

HIGHLIGHTED ISSUES ARE:

- Breast cancer - is it a stem cell based disease?
- How do somatic stem cells relate to lung tumor development?
- How do these systems compare to the rapidly regenerating lineages such as the hematopoietic lineage?
- What do we need to know about stem cells to understand brain tumors?
- Does this information need to be incorporated into therapeutic strategies?

PLATINUM SPONSOR

Promega Corporation

GOLD SPONSORS

The Automation Partnership
BioSpherix, Ltd.
Invitrogen Corporation
Millipore Corporation
Quarles & Brady, LLP
WiCell Research Institute, Inc.
WTN Media LLC

SILVER SPONSORS

Applied Biosystems
BioForward
CEDARLANE
Cellular Dynamics International, Inc.
Clontech Laboratories, Inc.
Master of Science in Biotechnology Program,
UW-Madison
R&D Systems, Inc.
STEMCELL Technologies
Stemina Biomarker Discovery, Inc.
Takara Bio B USA
Thermo Fisher Scientific Inc.
Wisconsin Technology Council

SCHEDULE OF EVENTS

8:00am	Registration and Continental Breakfast
8:45am	Welcome <i>Carl E. Gulbrandson</i> <i>William F. Dove (moderator)</i>
9:10am	<i>Luis F. Parada:</i> Glioma Mouse Models: Tumor Cell of Origin and Stem Cell Nexus
9:50am	<i>Peter Dirks:</i> Characterizing Cancer Stem Cells in Human and Mouse Brain Tumors
10:20–10:40am	BREAK
10:50am	<i>John S. Kuo:</i> Glioblastoma Cancer Stem Cell Biology
11:20am	<i>Carla Bender Kim:</i> Examination of Stem Cells in Normal Lung & Lung Cancer
Noon–1:00pm	LUNCH
1:00pm	<i>Caroline M. Alexander:</i> Wnt Signaling and Mammary Growth Regulation
1:30pm	<i>Michael T. Lewis:</i> Hedgehog Signaling, Mammary Stem Cells and Breast Cancer
2:20–2:40pm	BREAK
2:40pm	Max S. Wicha Self-renewal Pathways in Normal and Malignant Breast Stem Cells
3:20pm	<i>George Q. Daley:</i> Patient-Specific Pluripotent Stem Cells
4:00–5:00pm	RECEPTION

ABSTRACTS (IN ORDER OF PRESENTATION)

Glioma Mouse Models: Tumor Cell of Origin and Stem Cell Nexus

Luis F. Parada, Department of Developmental Biology & Kent Waldrep Foundation Center for Basic Neuroscience Research on Nerve Growth and Regeneration, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9133

It is only in the last decade that the existence of self-renewing cells in the brain has become fully appreciated. As a consequence classic models of gliomagenesis entertained dedifferentiation as a requisite for tumor initiation. New concepts have arisen with the continuing study of “adult” neural stem cells in vivo and in vitro. Preparation of neurosphere cultures from primary glioma tissue from human tumors and from mouse genetic models permits detailed analysis and the hope for a full molecular understanding of these cells and how they compare to normal stem cells. A central question remains the identification of the cell of tumor origin. Our own work using tumor suppressor models of gliomagenesis lead us to propose that the cell of origin in gliomas is an early progenitor cell or possibly the primary stem cell. I will discuss the nature of these mouse models, recent advances, how we hope to resolve our current working hypothesis, and implications for sporadic human glioma. If indeed stem or progenitor cells give rise to glioma, it becomes important to fully understand their properties and physiological roles.

Characterizing Cancer Stem Cells in Human and Mouse Brain Tumors

Peter B. Dirks, Staff Neurosurgeon and Principal Investigator, The Hospital for Sick Children; Scientist, Research Institute; Associate Professor of Neurosurgery, University of Toronto

Human brain tumors appear to be organized as a hierarchy. We have recently derived adherent cell lines with high efficiency from malignant glioma that display stem cell properties and initiate high grade gliomas following orthotopic transplantation. Significantly, these cell lines from different tumors exhibit divergent gene expression signatures and differentiation behaviour that correlate with specific neural progenitor subtypes. The diversity of gliomas may therefore reflect distinct cancer stem cell phenotypes. Adherent brain tumor stem cell lines offer significant experimental advantages compared to ‘sphere’ based cultures, and provide a simplified model enabling refined studies of cancer stem cell behaviour. In addition, it remains unclear if genetically engineered mouse models of cancer recapitulate the functional heterogeneity observed in their human counterparts. We demonstrate medulloblastomas arising from Patched1 (Ptc1 +/-) deficient mice contain a subpopulation of cells that demonstrate a neural precursor phenotype, show clonogenic and multilineage differentiation capacity, wild-type Patched-1 expression, and the ability to initiate tumors following allogeneic orthotopic transplantation. The normal neural stem cell surface antigen CD15 enriches for the in vitro clonogenic and in vivo tumorigenic potential from uncultured medulloblastomas supporting the existence of a cancer stem cell hierarchy in this clinically relevant mouse model of cancer. Interestingly, the cell that drives the growth of these tumors has a stem cell phenotype, lending further support to a potential cell of origin of these tumors from normal cerebellar neural stem cells, or acquisition of a stem cell phenotype as part of the neoplastic transformation process from lineage restricted progenitors.

Glioblastoma Cancer Stem Cell Biology

John S. Kuo, Assistant Professor, Neurological Surgery and Human Oncology and Director, Comprehensive Brain Tumor Program, University of Wisconsin School of Medicine and Public Health

Glioblastoma multiforme (GBM) is the most common, primary malignant brain tumor in adults. Patients succumb to rapid GBM re-growth despite the standard regimen of maximal surgery, followed by radiation and chemotherapy. With a median survival of 15 months, GBM patients and their families suffer from an incurable disease with devastating neurological consequences. Recently, a small subset of GBM cells has been identified that demonstrates the cancer stem-like properties of self-renewal, multipotent differentiation and high efficiency tumor initiation. We utilized a method of isolating and cultivating normal neural stem cells to isolate and propagate multiple independent lines of GBM cancer stem cells (CSC) from original tumor specimens. Their CSC properties were verified by a) continued growth in non-adherent sphere culture, b) demonstrated ability for multipotent differentiation into multiple neural-derived lineages, and c) efficient initiation of tumor xenografts after injection into immunocompromised mouse brains. Comparative analyses of GBM

CSC with normal neural stem cells and the patient-specific GBM lines are in progress. In vitro characterization of GBM CSC revealed relative growth independence from exogenous growth factors, and has yielded several epidermal growth factor-independent CSC lines for further study as a potential model of therapeutic resistance to EGF-targeted therapies. The in vivo tumor xenograft model derived from CSC injections is being used to test novel therapeutic agents. Initial work also shows that GBM CSC may be involved in mediating tumor-related disruption of the blood-brain barrier. This data suggest multiple ways that GBM CSCs are involved in cancer recurrence and resistance to current therapies. Elucidating the molecular biology of GBM CSC in comparison with normal neural stem cells will likely yield new therapeutic strategies for this deadly cancer.

Examination of Stem Cells in Normal Lung & Lung Cancer

Carla Bender Kim, Assistant Professor, Children’s Hospital Stem Cell Program; Department of Genetics, Harvard Medical School; Harvard Stem Cell Institute

Whereas recent evidence supports the cancer stem cell (CSC) hypothesis in a variety of cancers, a CSC population in lung cancer has yet to be isolated. Our previous identification and characterization of Bronchioalveolar Stem Cells (BASCs), putative resident stem cells of the distal lung, has suggested that these cells are important in normal lung homeostasis, lung tumor initiation and cancer progression. We hypothesize that BASC-like cells within lung tumors play a critical role in tumor maintenance and may function as cancer stem cells. To address this question, we developed an assay to test for lung CSCs. Orthotopic transplantations of primary murine lung adenocarcinoma cells via intratracheal injections into nude mice yield secondary tumors that recapitulate the features of the primary lung tumors. This lung CSC assay provides an important tool for future studies to compare tumor cell populations from murine and human lung tumors in a physiologically relevant setting. Using this assay, the cell surface markers used to isolate normal BASCs have differing success in enriching for CSCs from tumors initiated by activating oncogenic K-ras compared to those with additional loss of p53. These results suggest a previously unexplored idea that the phenotype of a CSC population depends on the genetics of the primary lesion, a concept that could be important for the effective design of therapeutics to target CSCs in patients.

Wnt Signaling and Mammary Growth Regulation

Caroline M. Alexander, McArdle Lab for Cancer Research, University of Wisconsin

Twenty years ago dysregulated Wnt signaling was shown to be highly oncogenic for mouse mammary epithelia. Ever since then, this pathway has been of interest as a potential etiology for human breast cancer. We have previously shown that ectopic Wnt signaling is associated with increased ductal mammary stem cell activity (measured in vivo) early in the tumorigenic process, and that loss of one of two LRP receptors (Lrp5) confers resistance to Wnt1-induced tumor development. Here, we show that the absence of Lrp5 results in the specific loss of mammary regenerative capacity. Thus, though mammary stem cell activity is reduced by 100x in the absence of Lrp5, primary mammary development is sustained, and canonical Wnt signaling readouts are normal (maintained by Lrp6). In stem cell-deficient glands, markers associated with cellular senescence increase, suggesting that stem cell activity is an important determinant of average cellular function. Furthermore, high expression of cell surface Lrp5 protein is a biomarker of mammary stem cells, and can be used to isolate cell fractions that are 500x enriched in stem cell activity.

Hedgehog Signaling, Mammary Stem Cells and Breast Cancer

Michael T. Lewis, Assistant Professor, Baylor College of Medicine

The hedgehog signaling pathway is essential for embryonic development where it controls stem/progenitor cell maintenance and self-renewal, differentiation, and patterning in many organs. It also functions in post-embryonic tissue homeostasis wound healing. Deregulated hedgehog signaling causes developmental defects, and is implicated in approximately 25% of all cancers, including breast cancer. We and others have demonstrated a key role for hedgehog network genes (Patched-1 (Ptch1), Smoothened (Smo), and Gli2) in regulation of mammary epithelial stem/progenitor cell behavior, and in ductal elongation and morphogenesis. However, the mechanism(s) by which these genes function in the gland is not known. The best understood mechanism for SMO-mediated hedgehog signaling involves activation of the Gli family of transcription factors to induce hedgehog target genes. Recent controversial reports suggest that a non-canonical signaling route exists, in which Smo couples to G i family members of the heterotrimeric G-protein superfam-

ily. Consistent with these data, our results suggest Smo couples to G i proteins in the mammary gland of MMTV-SmoM2 mutant mice. In breast cancers, Ptch1 expression is lost or reduced frequently, while Smo is ectopically expressed in about 70% of ductal carcinoma in situ and 30% of invasive breast cancers. Both expression patterns are consistent with activated hedgehog signaling. Other data suggest a direct role in regulating tumor-initiating “cancer stem cells”. These tumor-initiating cells display intrinsic resistance to chemotherapeutic agents and other current systemic therapies. If, in fact, hedgehog signaling regulates tumor-initiating cells in some breast cancers, hedgehog signaling inhibitors may prove to be useful clinically to target tumor-initiating cells – either alone, or in combination with other systemic therapies.

Self-renewal Pathways in Normal and Malignant Breast Stem Cells

Max S. Wicha, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI

There is increasing evidence that many cancers, including breast cancer, may be driven by a cellular subcomponent that displays stem cell properties. These properties include self-renewal which drives tumorigenesis as well as differentiation that generates cellular heterogeneity. An understanding of self-renewal is thus important for understanding carcinogenesis, as well as developing strategies to target the cancer stem cell population. A number of developmental pathways, including Notch, Hedgehog and Wnt play important roles in the regulation of mammary stem cell self-renewal. Furthermore, HER2 overexpression in breast carcinomas may drive tumorigenesis and metastasis through its regulation of the cancer stem cell components. This occurs via the P13 kinase Akt pathway and is primarily mediated via GSK3 phosphorylation and Wnt activation. In addition to intrinsic signals, normal and malignant breast stem cells are regulated by elements in the microenvironment. One important cellular element involves mesenchymal stem cells recruited from the bone marrow. These cells modulate cancer stem cells via cytokine network loops. In order to target this interaction, we have developed strategies to interfere with cytokine signaling and demonstrated that these are able to selectively target breast cancer stem cells in mouse xenograft models. These studies demonstrate the feasibility of targeting self-renewal pathways in breast cancer stem cells. Since these stem cells drive tumor growth and metastasis as well as contributing to treatment resistance and relapse, these strategies may ultimately lead to more effective treatments for women with breast cancer.

Patient-Specific Pluripotent Stem Cells

George Q. Daley, Children’s Hospital Boston, Department of Biological Chemistry, Harvard Medical School, Harvard Stem Cell Institute, Howard Hughes Medical Institute

Pluripotent stem cells isolated from embryos (ES cells) or generated by direct reprogramming of somatic cells (induced Pluripotent Stem or iPS cells) represent an inexhaustible source of precursor cells that can be differentiated into specific cell lineages. As with conventional organ transplants, cell-based therapies will face immunologic barriers. Genetically matched pluripotent stem cells generated via nuclear transfer (ntES cells), parthenogenesis (pES cells), or direct reprogramming (iPS cells) are a possible source of histocompatible cells and tissues. In a proof of principle experiment, we have shown that customized ntES cells can be used to repair a genetic immunodeficiency disorder in mice (Rideout et al., Cell 2002). However, generation of ES cells by nuclear transfer remains inefficient, and to date has not been achieved with human cells. ES cells with defined histocompatibility loci can be generated at much higher efficiency by direct parthenogenetic activation of the unfertilized oocyte (Kim et al., Science 2007). Subsequently, cell lines can be genotyped and selected for MHC identity to the oocyte donor. Cell lines with homozygous MHC haplotypes can also be identified, and tissues from such cells engraft in MHC heterozygous recipients. Compared to ES cell lines from fertilized embryos, pES cells display comparable in vitro hematopoietic activity, and blood derivatives can repopulate hematopoiesis in irradiated adult mouse recipients. These experiments establish murine models for generating histocompatible ES cell-derived tissue products, and suggest the theoretical feasibility of ES cell banking to enable off-the-shelf cell therapies. We have generated human iPS cells by direct reprogramming of human somatic cells with retroviruses carrying OCT4, SOX2, MYC, and KLF4 (Park et al., Nature 2008). We have used this platform to generate disease-specific iPS cells for research (Park et al., Cell 2008), but future use of patient-specific cells for therapeutic ends must await methods to produce viral-free cells. Applications of disease-specific cells for investigating the mechanisms of reprogramming and for probing aspects of human bone marrow disorders will be discussed.

4th Annual Wisconsin Stem Cell Symposium:

Cancer, Stem Cells & Cancer Stem Cells

REGISTRATION FORM

On-line registration option also available: www.btcj.org

Name: _____

Address: _____

City: _____ State: _____

Zip Code: _____ Country: _____

Phone: _____ Fax: _____

E-mail: _____

Profession: _____

REGISTRATION FEE:

\$90 – faculty, academic staff, and all non-academic attendees

\$45 – students and post-doctoral candidates

Please make check out to: WSCS/BTCI
or pay by credit card on-line at: www.btcj.org

Send completed form to:
BTCI
5445 East Cheryl Parkway
Madison, WI 53711

For more information please visit: www.btcj.org
or call: 608-273-9737.

BTCI is pleased to announce the
**9th Annual International Bioethics Forum:
Sustainability**

April 23–24, 2009

Please visit www.btcj.org for more information.