

Day 3: Thursday, February 12th
Tissue-based Applications

9:00 – 12:00 Morning Symposium: Grace Auditorium

Keynote presentation:
**Spatial Analysis of Hematopoietic Stem
and Progenitor Cells in Bone Marrow**

Presenter: Leslie Silberstein, M.D., Harvard Medical School, Boston, MA



Dr. Silberstein is currently Professor of Pathology at Harvard Medical School and Director of the Joint Program in Transfusion Medicine at Children's Hospital, Boston, Brigham and Women's Hospital, and the Dana-Farber Cancer Institute, integrating research, clinical practice and education with a focus on development of cell-based therapies. Additionally, he is Director of the Center for Human Cell Therapy (CHCT) at the Immune Disease Institute, whose mission is to rapidly translate novel cell therapy protocols from the laboratory to the clinic, providing cell therapy resources for the Harvard community and its affiliated hospitals. Dr. Silberstein is also a Senior Investigator at the Immune Disease Institute and Head of the Harvard Stem Cell Institute's Translational Research Program.

Abstract

Sustained production of all mature blood cell types relies on the continuous proliferation and differentiation of a rare population of self-renewing, multipotent hematopoietic stem cells (HSCs). HSC maintenance and lineage differentiation are strictly regulated by distinct microenvironments, termed niches, defined by cellular components, soluble regulators, and by the extracellular matrix.

Upon entering the differentiation pathway, HSCs progress from primitive, multi-lineage potential progenitors through more restricted progenitors, finally giving rise to fully functional mature blood cells. Therefore the BM constitutes an extremely complex and diverse environment harboring a vast array of hematopoietic cells at all stages of differentiation, distinct populations of stromal cells of mesenchymal origin as well as cells involved in bone metabolism and an intricate vascular network. A detailed understanding of the spatial organization of BM tissue underlying the sophisticated regulation of hematopoiesis, has not been achieved to date due to limitations of conventional imaging techniques. Among the best characterized multi-step developmental pathways occurring in BM cavities is the one leading to B cell production. Thus, as an initial validation for the use of Laser Scanning Cytometry (LSC) to study BM tissue sections, we have analyzed the anatomical localization of progenitor B cells at distinct stages of development. Analysis of whole femoral longitudinal sections showed that B lymphopoiesis in BM is not strictly spatially compartmentalized, however, early B cell progenitors reside preferentially in niches/microenvironments in the periphery of the BM cavity near bone surfaces. These studies lead the way to the current focus of our work which is to investigate hematopoietic stem and progenitor (HSPC) localization in BM cavities. Preliminary data generated using LSC suggestive of a non-random preferential localization of HSPCs to metaphyses of long bones, will be presented. Our future effort will be directed to identify HSPC niche cellular constituents. A comprehensive description of niche-derived signals regulating unique properties of HSPCs will certainly prove relevant in human HSPC transplantation and cellular therapies.

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Laser Scanning Cytometry in Autism Research

Presenter: Janine M. LaSalle, Ph.D., UC Davis School of Medicine, Davis, CA



Dr. LaSalle is a Professor of Microbiology and Immunology with a cross appointment in the Rowe Program in Human Genetics. She serves on the editorial board of the journal Human Molecular Genetics, is a member of the NIH study section on Developmental Brain Disorders, and reviews for a number of national and international funding agencies and journals. The research focus in Dr. LaSalle's laboratory is on epigenetics of neurodevelopmental disorders, including autism, Rett syndrome, Angelman syndrome, and 15q proximal duplication syndrome. Dr. LaSalle's laboratory has developed multiple innovative approaches for epigenetic investigations, including the use of T cell cloning for separating X-inactivation and heterogeneous methylation patterns, the use of laser scanning cytometry for quantitating immunofluorescence on brain tissue and tissue microarrays, and the use of fluorescence in situ hybridization to investigate neuronal nuclear organization in human brain. Dr. LaSalle's laboratory has been successful in the use of genomic and epigenomic technologies to investigate the role of MeCP2 in the pathogenesis of Rett syndrome and autism spectrum disorders.

Abstract

Autism is a complex genetic disorder likely caused by interactions between multiple genes and environmental influences. Epigenetics is the study of heritable and reversible modifications to nucleotides or chromosomes that do not alter the genetic sequence but can modify gene expression and phenotype. Epigenetic marks are therefore at the interface of genetics and environment and are found to be of increasing importance in pre- and postnatal neurodevelopment in mammals. The importance of epigenetic mechanisms in regulating human brain development has been recently revealed by the discovery of the genetic bases of several human neurodevelopmental disorders, including Rett syndrome (caused by mutation in the X-linked gene *MECP2* encoding a methyl CpG binding protein) and Angelman and Prader-Willi syndromes (caused by deficiencies in imprinted genes within 15q11-13).

Current molecular methods are often inadequate for investigating epigenetic changes of individual cells in complex tissue such as brain. Over the past decade, we have quantified immunofluorescence on tissue microarrays using laser scanning cytometry in order to detect epigenetic changes in human and mouse brain samples relevant to autism and autism spectrum disorders. Applications have included investigating mosaic X chromosome inactivation patterns in females with Rett syndrome, showing reduced MeCP2 levels in the majority of idiopathic autism brain samples, and comparing wild-type and *Mecp2*-mutant mouse brain samples for histone acetylation and MeCP2-target gene changes.

Using the iCys[®] Imaging Cytometer (CompuCyt Corporation, Westwood, MA), we are currently testing the hypothesis that perinatal exposure to persistent organic polybrominated diphenyl ethers (PBDE) may result in epigenetic changes in developing neurons that impact the development of social and cognitive behavior. Preliminary investigations of epigenetic changes using immunofluorescence and laser scanning cytometry have revealed several alterations to global DNA methylation, MeCP2, and histone acetylation levels and localization patterns in cerebral cortical neuronal nuclei from BDE-47 perinatally exposed pups.

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**Application of Laser Scanning Cytometry in
Biomarker Drug Development: Challenges and Opportunities**

Presenter: Gloria Juan, Ph.D., Clinical Immunology, Amgen, Thousand Oaks, CA

As a graduate student at the University of Valencia, Spain, Dr. Juan's research focused on the development of flow cytometric methods to assess metabolic toxicity in whole cells. As a Postdoctoral Fellow in Dr. Darzynkiewicz's laboratory at New York Medical College, and later as an Assistant Professor there, she was involved in research projects primarily focused on the analysis of cell cycle regulation and apoptosis, work that continued at Memorial Sloan Kettering Cancer Center, centering on translational cancer studies. Since 2005, Dr. Juan has led the imaging cytometry group at Amgen Inc., where in close collaboration with a cross-functional team of scientists, she is involved in developing and validating pharmacodynamic biomarkers using state-of-the-art microscopy, with the purpose of enabling clinical decisions.

Abstract

Many transformed cells are hypersensitive to Trail-induced apoptosis. Anti-Trail Receptor immunoglobulin therapies, such as AMG655, currently in clinical development, are designed to exploit this characteristic by crosslinking Trail Receptor2 and initiating the cascade of cysteinyl aspartate proteases (caspases) within the tumor that ultimately lead to DNA fragmentation and cell death. Laser Scanning Cytometric quantitation of activation of caspases 3 and 8 *in situ* allowed us to explore, at the preclinical level, mechanism-specific biological activity of AMG655. Our quantification by LSC correlated with independent pathologist-scoring and confirmed that caspase activation is an early event that precedes tumor regression.

We further developed this platform to explore the usefulness of monitoring caspase activation as a predictive biomarker to quantitatively assess the intensity of caspase-related staining in tumor fine needle aspirate (FNA) biopsies. To evaluate assay feasibility and determine whether this can become a robust predictive factor and mechanistic endpoint for the clinical testing of AMG 655, human mock FNAs were generated from freshly resected colorectal tumors and analyzed by LSC for caspase activation. In addition, murine mock FNAs were collected and compared to their corresponding xenograft tumor sections to examine reproducibility of AMG 655-induced caspase 3 and 8 activation by LSC. The assay has been implemented in a Phase 1 dose expansion clinical trial.

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**Mapping of Tissue Architecture for Cells with
Shorter Telomere Lengths**

Presenter: Alexei Protopopov, Ph.D., Dana-Farber Cancer Institute, Boston, MA

Alexei Protopopov is Senior Scientist at Dana-Farber Cancer Institute, where he is Director of the Molecular Cytogenetics Laboratory, Associate Director of Belfer Cancer Genomics Facility at the Center for Applied Cancer Sciences, Senior Scientist Dana-Farber Cancer Institute, Boston, MA. Dr. Protopopov graduated from Novosibirsk State University, Russia in 1983 and received extensive training in the State Optical Institute, St. Peterburg and Institute of Cytology and Genetics. He obtained his Ph.D. in Biophysics and Molecular Biology in 1990. In 1995, he was offered a position in Prof. George Klein's Microbiology and Tumor Biology Center at the Karolinska Institute, Stockholm, Sweden where his work was dedicated to tumor biology. In 2003, he joined Department of Medical Oncology, Dana-Farber Cancer Institute and established the Molecular Cytogenetics Laboratory as part of the Multiple Myeloma Program and CACS. Dr. Protopopov is an Associate in Medicine at Harvard Medical School and a member of multiple associations of researchers. He is the author of more than 60 scientific publications.

Abstract

Continued age-dependent epithelial renewal has been linked to telomere erosion. Telomere erosion, coupled with loss of DNA damage checkpoint function, results in genomic instability that promotes the development of cancer. The critical role of telomere dynamics in cancer has motivated the development of technologies designed to monitor telomere reserves in humans and model organisms in a highly quantitative and high-throughput manner. To this end, we have adapted and modified two established technologies: telomere-FISH and laser scanning cytometry. Specifically, we have produced a number of enhancements to the iCys[®] Imaging Cytometer (CompuCyte) package including software updates, use of 60X dry objectives, and increased spatial resolution by 0.2 μ m size of stage steps. In addition, the 633 nm HeNe laser was replaced with a 532 nm green diode laser to better match the viewing options. Utilizing telomere-deficient mouse cells with short dysfunctional telomeres and matched telomerase reconstituted cultures as a model system, we demonstrated significantly higher median/mean integral specific fluorescence values for mTR transfectants relative to empty vector controls. The validation of LSC approach derives from a strong correlation between iCys LSC values and Southern blotting. In the context of organismal aging, we are investigating the role of telomere dysfunction and DNA damage checkpoints in degenerative disease and organ homeostasis. Our model combines telomerase-deficient mice with those lacking some other target genes. These mice exhibit many of the hallmarks of accelerated cellular and organismal aging, particularly affecting tissue stem cell populations. Further, we are embarking on an ambitious project to test the ability of telomerase re-expression to stem the aging process in these mice. In this setting, we require the ability to effectively map complex tissue architecture for cells with different telomere lengths in intact organs, such as liver, intestine, brain, and others – and correlate data with histological and physiological assessments of organ and cellular dysfunction that we have already noted in these mice. Laser Scanning Cytometry greatly increases our experimental interpretations, throughput and objectivity.

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**Laser Scanning Cytometry Analysis of Rare Circulating Tumor Cells
in Pre-Clinical Models of Cancer**

Presenter: Alison L. Allan, Ph.D., University of Western Ontario, London, ON, Canada



Dr. Alison Allan is an Oncology Scientist in the London Regional Cancer Program at the London Health Sciences Centre; a Scientist in the Lawson Health Research Institute; and an Assistant Professor of Oncology and Anatomy & Cell Biology in the Schulich School of Medicine and Dentistry at the University of Western Ontario. She holds a B.Sc. in Molecular Biology and Genetics and a Ph.D. in Biomedical Sciences, both from the University of Guelph. Her translational research program is focused on cellular and molecular mechanisms of breast cancer metastasis, in particular the study of circulating tumor cells and cancer stem cells in patients and animal models. Dr. Allan has been the recipient of numerous awards and honors and has authored and co-authored several journal articles, book chapters, abstracts, and posters on topics relating to oncology and cancer stem cells. She is a member of the American Association for Cancer Research, the Metastasis Research Society, and the Clinical Cytometry Society. She has also served as a reviewer for the U.S. Army Department of Defense Breast Cancer Research Program and the Canadian Breast Cancer Foundation grant programs, and is an Editorial Board member for the journals Biochemistry and Cell Biology and Stem Cell Reviews.

Abstract

Over the last several decades, tremendous advances have been made in breast cancer detection and management of primary tumors. Despite this, breast cancer remains a leading cause of morbidity and mortality in women, mainly due to the propensity of primary breast tumors to metastasize to regional and distant organ sites and the subsequent failure of most therapies in the metastatic setting. Given the multi-step nature of the metastatic cascade, there should be several opportunities for early therapeutic targeting of metastatic cells before they become a clinical problem for the patient. However, imperfect techniques to detect, quantify, and analyze small numbers of cells in the peripheral blood or distant organs of breast cancer patients have been a significant obstacle to accurate prognosis, proper adjuvant treatment decisions, assessment of treatment efficacy, and long-term monitoring of disease recurrence. Similar challenges in detecting and quantifying rare metastatic cells in pre-clinical mouse models of breast cancer have hindered our ability to properly study metastasis in experimental systems and to test new therapeutics in the metastatic setting.

Our current research focuses on development of less artificial, more sensitive, and more clinically relevant techniques to address this problem. We have developed several cytometry-based methods for detecting and characterizing circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) in the blood, lymph node, and bone marrow of mouse models of breast cancer. These include the use of flow cytometry, laser scanning cytometry, and the CellSearch system. We have successfully used these methods to quantify early steps in metastasis and determine the timing and location of metastatic spread of cells in our pre-clinical mouse models of breast cancer metastasis. Ongoing studies are aimed at implementing these methods for determining tumor cell response to therapy and the development/screening of new anti-metastatic agents.

Cytometry-based methods provide the opportunity to elucidate the mechanistic details of early steps in metastasis and determine how these steps relate to the development, monitoring, and treatment of metastatic disease. The detection of CTCs and DTCs in breast cancer has been recognized to have prognostic value, but many methodological issues remain to be resolved and are a major limitation to widespread application in the clinic. The assessment of different techniques in the context of pre-clinical mouse models is therefore a significant step towards validation of this process in the clinical setting.