Liposarcoma Genome Project

July 2015

Submitted by:

John Mullen, MD
Edwin Choy, MD, PhD
Gregory Cote, MD, PhD
G. Petur Nielsen, MD
Brad Bernstein, MD, PhD
**Liposarcoma Background**

Liposarcoma is the most common soft tissue sarcoma in adults, accounting for 20% of all sarcomas (1). They usually arise in deep soft tissues, most commonly the thigh or retroperitoneum (2). According to the World Health Organization, liposarcomas are subclassified as well-differentiated, dedifferentiated, myxoid, and pleomorphic (3). De-differentiated liposarcoma is a high grade sarcoma that arises from well-differentiated liposarcoma via an unknown process. Although genetic studies have provided some insight in the mechanisms of liposarcoma development, the detailed mechanisms and drivers of de-differentiation remain largely unknown.

Oncogenesis is typically a multistep process involving the activation of oncogenes and inactivation of tumor suppressor genes. Prior studies have identified MDM2 amplification and overexpression as a frequent abnormality in dedifferentiated liposarcoma (4). In a zebrafish model, constitutive activation of AKT2 in mesenchymal progenitors leads to the development of well-differentiated liposarcomas (5). Recent studies have also identified PIK3CA mutations in 14% of liposarcomas (6). In a dedifferentiated liposarcoma xenograft mouse model, PTEN down-regulation has been shown as a malignant signature and response to PI3K pathway inhibition. In the team’s research laboratory, we have found that targeting PI3K synergizes with chemotherapy in liposarcoma cell lines. We also identified CDK11 as an important gene in liposarcoma proliferation. A separate group has shown that the JUN oncogene is amplified and overexpressed in de-differentiated liposarcoma and that it serves to block adipocytic differentiation (7). Despite these recent advances, a comprehensive approach to understand and tie together all of the genetic and epigenetic factors involved in the de-differentiation of a well-differentiated liposarcoma has not yet been undertaken.

**Project Goals**

The team proposes to comprehensively identify the genetic and epigenetic factors that distinguish dedifferentiated liposarcoma from their well-differentiated precursor lesions. First, the team will catalogue genetic mutations in DNA sequence for both well-differentiated and de-differentiated liposarcoma. Next, they will use newly collected fresh tumor samples to interrogate the transcriptome and epigenetic changes to genome organization and chromosome structure in the respective tumor types. The team will also investigate inter- and intra-tumoral transcriptional heterogeneity in de-differentiated liposarcomas by sequencing DNA and RNA from different sites within each tumor (DNA) or from multiple individual cancer cells from the same tumor (RNA). To achieve these goals, Drs. Mullen, Choy, Cote, Nielsen and Bernstein will combine their unique clinical access to liposarcoma tumor samples, including extensive banked samples and freshly resected tumors, with cutting-edge genomic technologies spearheaded by members of the research team.
Our group hypothesizes that a discrete set of genetic changes is driving de-differentiation of well-differentiated liposarcoma and that this process involves new regulatory programs due to transcriptional and epigenetic changes. We believe that the complete constellation of genetic, transcriptional and epigenetic changes involved in de-differentiation will allow better understanding of liposarcoma de-differentiation and generate a putative list of therapeutic targets. Here, we describe an experimental approach that seeks to fully characterize these changes and create models for testing potential targets.

A critical feature of this approach is the team’s unique ability to collect fresh tumor samples representing well-differentiated and de-differentiated tumor from the same patient in order to perform the following genomic analyses: (1) whole genome and whole-exome DNA sequencing, (2) genome-wide chromatin profiling and (3) single-cell RNA-sequencing to investigate intra-tumoral heterogeneity. Dr. Mullen and Dr. Nielsen will provide fresh, histologically characterized tumor samples suitable for rapid cell dissociation and preparation for RNA-sequencing and chromatin analysis. Dr. Bernstein will provide the laboratory and scientific expertise to implement our analysis. This project will provide critical information regarding the genetic mutations, epigenetic drivers and inter- and intra-tumoral heterogeneity in liposarcoma.

**Aim 1: Genomic sequencing of paired well- and de-differentiated liposarcoma**

**Overview:** To date, liposarcoma genomic sequencing efforts have been limited by small sample size and lack of careful distinction between well-differentiated and de-differentiated tumor. Given the significantly increased morbidity and mortality of de-differentiated liposarcoma, an optimal approach involves comparative sequencing of paired samples from patients with both well-differentiated and de-differentiated tumor. This will enable identification of changes that drive de-differentiation and provide a list of putative therapeutic targets. Given the tendency of these tumors to de-differentiate over a time, we hypothesize that the tumors acquire a limited number of genetic lesions that drive de-differentiation and that these can be identified by comparative sequencing of both components. The team will also obtain DNA sequencing data for multiple foci within each of the fresh tumors to evaluate intra-patient genomic heterogeneity and RNA sequencing data on individual tumor cells to characterize transcriptional heterogeneity.

**Aim 1.1: Collect formalin-fixed, paraffin-embedded (FFPE) and fresh liposarcoma samples.**

The team will curate tumor samples for all liposarcoma patients seen at MGH over the prior 15 years. This will involve careful histological examination of the tumor resections in order to identify candidate FFPE blocks that can be used for sequencing. Additionally, the team will collect 10 samples of paired well-
differentiated and de-differentiated tumors. This process requires careful collaboration between the surgeon, pathologist and researcher in order to obtain high-quality, pure, fresh samples intra-operatively.

**Aim 1.2: Perform genomic sequencing on FFPE and fresh samples.**

To elucidate genetic differences between well-differentiated tumors and de-differentiated tumors, the team will perform whole-exome sequencing of the entire set of FFPE samples. By performing this analysis on a large set of tumor samples from both paired well-differentiated and de-differentiated tumors, both more and less common genetic events that are involved in de-differentiation can be identified. In order to also query intra-genic events that are associated with de-differentiation, whole genome sequencing will be performed on the set of fresh tumors that are obtained.

**Aim 1.3: Catalogue tumor heterogeneity through genome sequencing.**

Tumor heterogeneity can play a significant role in driving disease progression and therapy resistance. The team will investigate inter-patient and intra-patient heterogeneity in these tumors by obtaining (a) genomic sequencing data for multiple sites within each tumor and (b) single cell RNA sequencing data for each fresh tumor sample. This analysis will elucidate the extent of both genomic and transcriptional heterogeneity within liposarcoma.

**Aim 2: Catalogue transcriptional and epigenetic changes to genome organization and chromosome structure in tumor samples.**

**Overview:** While Aim 1 seeks to understand the genetic events that are involved in de-differentiation, it is also important to characterize the transcriptional and epigenetic changes that are taking place during de-differentiation. These data will provide insight into the biological transitions taking place during de-differentiation and provide additional therapeutic targets. Emerging tools to modify both the transcriptional environment and the epigenetic landscape may allow researchers to both differentiate de-differentiated liposarcomas and prevent de-differentiation in patients with only well-differentiated tumor, which is so frequently a lethal step in the disease progression.

**Aim 2.1: Perform RNA sequencing on fresh tumor samples.**

RNA sequencing will be obtained for each of the ten fresh samples, representing both the well-differentiated and de-differentiated components. This will allow the transcriptional network distinguishing the two tumor types for each patient to be compared, with control for inter-patient variability and evaluation of intra-tumoral heterogeneity.

**Aim 2.2: Catalog epigenetic landscape of de-differentiated liposarcoma tumor samples.**

The chromatin environment is critical for regulation of transcription and provides a potentially reversible therapeutic target. Presently the epigenetic changes that
occur in liposarcoma are uncharacterized. In order to interrogate the epigenetic landscape, we plan to perform genome-wide ChIP-seq of histone modifications for both components of the liposarcoma.

**Aim 3: Generate liposarcoma cell lines and validate potential therapeutic targets.**
*Overview:* Aims 1 and 2 will generate lists of factors associated with dedifferentiation. In order to test these putative therapeutic targets, we will explore their functional role in liposarcoma cell lines. We will also make use of xenograft models to explore tumor growth and the role of these factors.

**Aim 3.1: Obtain liposarcoma cell lines.**
We will collect a number of pre-existing liposarcoma cell lines, as well as generate new cell lines from the set of patients for which fresh tissue is obtained.

**Aim 3.2: Test effect of perturbation of targets in cell culture.**
A wide range of changes will be identified through this study. These will include oncogene expression, loss of tumor suppressor expression, aberrant signal pathway activation and alterations to the genome through transcription factor modulation and chromatin-based changes. We will select a subset of these to explore how the alterations acquired in de-differentiated tumors may drive oncogenesis. Doing so will involve genetic alterations of cell culture lines as well as use of known drugs that can modulate a given target. This will allow us to identify a subset of these changes that play critical biological roles in de-differentiation. This set will be an important group of putative therapeutic targets and merit additional efforts to both understand their biology and explore through small molecule libraries.

**Aim 3.3: Explore role of target modulation in xenograft tumor models.**
After using an initial screen in cell lines, a subset of targets will be evaluated in xenograft mouse models using tumor cell lines established from our characterized, fresh tumor samples.

**Summary and Conclusion**

By comprehensively profiling the mutations and epigenetic alterations in DNA, RNA, and chromatin in both well-differentiated and dedifferentiated liposarcoma samples, both across tumor samples and in single-cells within a tumor sample, the team will gain unprecedented systematic insight into the genetics and epigenetics of de-differentiated liposarcoma. Therapeutic targets identified in this comprehensive analysis will be readily tested in liposarcoma cell lines and xenograft models. This proposal will be a first of its kind in terms of its exhaustive characterization of liposarcoma genomics. A critical and unique aspect to our study design is to have well-characterized well-differentiated and
de-differentiated tumor. This approach will allow us to more confidently gain insight into the clinically-critical process of de-differentiation. Our study design makes use of inter-disciplinary expertise at our institution and has the capacity to profoundly impact our understanding of liposarcoma and ideally treatment.
Reference:

3. Fletcher et al., WHO Classification of Tumours of Soft Tissue and Bone. IARC 2013.
4. Ware et al., MDM2 Copy Numbers in Well-differentiated and De-differentiated Liposarcoma. AJCP 2014; 141: 334-341.
**Physician Biographies**

**John T. Mullen, M.D., FACS**

Dr. John Mullen is a Visiting Surgeon in the Department of Surgery at Massachusetts General Hospital and the Director of the General Surgery Residency Program. He received his MD from the University of California, Davis Medical School and completed his residency and fellowship at Massachusetts General Hospital. Dr. Mullen has developed a national reputation for surgical excellence in the management of complex soft tissue and retroperitoneal sarcomas and upper gastrointestinal cancers. He is an active participant in prospective clinical trials for sarcoma, and has been an author of several printed and online review articles and book chapters defining the standard of care for the surgical treatment of sarcoma and gastric cancer. He has developed prospective clinical databases of sarcoma and gastric cancer patients to answer important clinical questions, as well as to audit outcomes and the quality of cancer surgical care delivered at MGH.

Dr. Mullen has been an invited speaker and discussant at major national and international surgical meetings to present his research, and serves as a member of the program committees of the surgical societies that plan these meetings. Dr. Mullen serves on the editorial board of two major peer review journals in the field of cancer surgery, the *Annals of Surgical Oncology* and the *Journal of Gastrointestinal Surgery*, and conducts ad hoc reviews for a number of other prestigious journals.

In addition, Dr. Mullen has made substantial contributions as a teacher and mentor to surgical oncology fellows, general surgery residents, and Harvard medical students as the Program Director of the General Surgery Residency at MGH. He is an active member of national committees in developing curricula, assessment tools, and innovative training paradigms for general surgery trainees.

**Edwin Choy, MD, PhD**

Dr. Edwin Choy was raised in Los Angeles, CA and graduated from Yale College in 1993 with a B.S. in Molecular Biophysics and Biochemistry. He received his MD and PhD in 2000 from New York University School of Medicine and then completed residency training at Massachusetts General Hospital. This was followed by a fellowship in medical oncology at the Dana Farber Cancer Institute and Massachusetts General Hospital. He then completed a postdoctoral research fellowship at the Broad Institute.
Institute of MIT and Harvard. Upon completion of his fellowship, Dr. Choy joined the faculty in the Division of Hematology Oncology at Massachusetts General Hospital.

Dr. Choy has spent more than 20 years studying the genetics and biology of cancer, and he is currently the Director of Sarcoma Research at the MGH Cancer Center. His clinical practice and research focuses exclusively in the medical management of patients with sarcomas, gastrointestinal stromal tumors, chordomas, giant cell tumors, and desmoid tumors. He works closely with a team of world class surgical, orthopedic, and radiation oncologists as well as connective tissue pathologists and radiologists to provide optimal care for his patients. He directs a clinical trials program at the MGH Center for Sarcoma and Connective Tissue Oncology that includes over a dozen active phase I-III clinical trials. He also maintains an active scientific research program that investigates diverse areas of sarcoma therapy, ranging from the use of nanotechnology and other translational research tools aimed at developing new targeted therapies for treating cancers to preclinical studies using cell lines and animal models to better understand the molecular basis of sarcoma biology. Dr. Choy is currently Assistant Professor at Harvard Medical School.

**Gregory T. Cote, MD, PhD**

Dr. Greg Cote is a physician in Sarcoma and Connective Tissue Oncology at Massachusetts General Hospital and an Instructor at Harvard Medical School.

Dr. Cote specializes in sarcoma and connective tissue oncology at the Mass General Cancer Center, including osteosarcoma and chordoma. His research and clinical focus is in the management and treatment of sarcomas, gastrointestinal stromal tumors (GIST), chordomas and desmoid tumors. Dr. Cote received his M.D. and Ph.D. in the field of Biochemistry from the Boston University School of Medicine. He completed his residency at Massachusetts General Hospital and then completed a fellowship in Hematology and Oncology at the Dana-Farber Cancer Institute/ Partners Cancer Care Fellowship Program.

**Gunnlaugur Petur Nielsen**

Dr. Gunnlaugur Petur Nielsen is an Associate Pathologist and the Director of Bone and Soft Tissue Pathology at Massachusetts General Hospital. He has served as a pathologist at MGH for the past 20 years, with an interest in bone and soft tissue pathology. Dr. Nielsen has written extensively on neoplastic and non-neoplastic diseases and of bone and soft tissue. He is the co-author of several book
chapters, has participated in writing a number of sections in the current World Health Organization (WHO) Classification of Tumours of Soft Tissue and Bone and recently finished a book on bone tumors. He is also contributing to several sections of the upcoming WHO Classification of Tumours of Soft Tissue and Bone to be released in 2013. Dr. Nielsen is a regular speaker at national and international pathology and orthopedic courses. He is currently an Associate Professor of Pathology at Harvard Medical School.

Bradley E. Bernstein, MD, PhD

Dr. Bradley E. Bernstein is a professor in pathology at Massachusetts General Hospital and Harvard Medical School and an Early Career Scientist of the Howard Hughes Medical Institute. Bernstein co-directs the Broad Institute’s Epigenomics Program and oversees data production centers for the ENCODE project and the NIH Common Fund for Epigenomics. His research focuses on epigenetics – changes in gene activity governed by influences outside the genes themselves – and specifically how the organization of genomic DNA into chromatin influences development and disease. His work is notable for the discovery of epigenetic mechanisms in pluripotent stem cells and for the systematic annotation of enhancer-like elements in the human genome that coincide with DNA sequence variants associated with human diseases. Recent studies have characterized epigenetic mechanisms underlying cellular transformation and therapeutic resistance in cancer.

Bernstein received his B.S. in Physics from Yale University and his M.D. and Ph.D. from the University of Washington School of Medicine. He completed a residency in clinical pathology at Brigham and Women’s Hospital and carried out postdoctoral research at Harvard University. Bernstein’s honors and awards include a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund, a junior faculty award from the Culpeper Foundation, an Early Achievement Award from the University of Washington Alumni Association, the Howard Goodman Award, and the Martin Prize for Basic Science from the Massachusetts General Hospital.