Sixth International Chordoma Research Workshop

CHORDOMA FOUNDATION

Speaker abstracts

TABLE OF CONTENTS

Epigenetic control of brachyury and metabolic stress response: novel therapeutic targets for chordoma

Dr. Lucia Cottone
University College London

Despite recent advances, novel targeted therapies are still required to improve survival of patients with chordoma. The transcription factor brachyury (T), the diagnostic hallmark of chordoma strongly implicated its pathogenesis, is regulated during embryonic development at the epigenetic level suggesting that epigenetic inhibitors may be novel therapeutic tools able to block T expression. To test this hypothesis, we have undertaken a focused compound screen using validated small molecule inhibitors (n=91) of enzymes involved in chromatin biology and metabolic pathways. Screening revealed activity in a number of compounds targeting the jumonji domain-containing lysine demethylases, including GSK-J4 and KDOBA67, two structurally closely related KDM6 inhibitors. These compounds reduced cell proliferation in all five chordoma cell lines tested and efficiently reduced T at the transcriptional and protein level. ChIP-seq experiments showed an increase in H3K27me3 and decrease in H3K4me3 histone modifications around T transcription start site, consistent with reduced transcription. Transcriptomic analysis also revealed a strong activation of the ATF4 pathway, a well-known mediator of an integrated metabolic stress response. Notably, we found that Halofuginone, a highly specific inhibitor of the enzyme glutamyl-prolyl tRNA synthetase already tested in phase I autoimmunity clinical trials, promoted a metabolic stress response similar to KDM6 inhibitors in all chordoma cell lines and partially controlled T expression. Halofuginone treatment of a chordoma PDX model demonstrated 44% tumour growth inhibition (p=0.0052). In conclusion, we have demonstrated the efficient control of T at the epigenetic level by jumonji domain-containing lysine demethylases, and we have identified epigenetic and metabolic pathways that represent potential novel targets for the treatment of chordoma.

Systematic discovery of novel vulnerabilities in chordoma

Dr. Tanaz Sharifnia
Broad Institute

This presentation will describe our efforts to systematically identify chordoma dependencies through the application of genetic, epigenetic, and small-molecule sensitivity profiling approaches.

Rationale for the advancement of PTEN/AKT pathway inhibitors and combinations for personalized chordoma therapy

Brenner J.C., Michmerhuizen N.M., Owen J.H., Mann J.E., Leonard E, Prince M.E.P.
University of Michigan Medical School

Chordomas are rare and serious tumors with few effective treatments outside of regimens that include surgery and radiation. Personalized targeted therapies may present a more effective option for a subset of patients with lesions possessing certain genetic biomarkers. Here, we performed targeted exome sequencing of two recently developed cell lines derived from chordoma patients treated at the University of Michigan, UM-Chor1 and UM-Chor2. Copy number alterations and/or mutations in *PTEN*, *CDKN2A*, and *EGFR* were among the most common alterations in these models. Hypothesizing that these changes might be essential drivers of cell growth and survival, we treated the chordoma cell lines with targeted inhibitors of the phosphotidylinositol 3-kinase (PI3K), cyclin dependent kinase (CDK) and epidermal growth factor receptor (EGFR) pathways and examined their responses in proliferation, apoptosis, and cell signaling. Pan-PI3K inhibitor BKM120 was the most effective targeted treatments generating a differential response in cell proliferation and apoptosis assays. Xenograft assays demonstrated a strong effect of PI3K inhibition on in vivo tumor growth. Further, using our UM-Chor1 cell line, we evaluated the effectiveness of several thousand di-therapy combinations with PI3K inhibitors in a high throughput small molecule screen. This was used to prioritize potential combination therapies that will be evaluated in additional assays in future work. Overall the effects of the PI3K, CDK, or EGFR targeted therapies suggest a baseline utility as monotherapies for Chordoma therapy that may have durable effects in matched personalized medicine settings. Future work will determine if personalized combinations can further improve the efficacy of these agents in vitro.

The role of philanthropy in transforming cancer research

Michele Cleary
The Mark Foundation for Cancer Research

Although cancer research receives billions of dollars of funding from government, non-profit and biopharma sources worldwide, critical gaps remain in delivering optimal options for all cancer patients. Along with the need for new treatments and combinations regimens with higher efficacy and lower toxicity, improved outcomes for patients can be gained through the discovery and development of optimal biomarkers for early detection, predicting response to therapeutics, forecasting resistance to treatment, and diagnosing recurrence. Unfortunately, high risk and limited prospects for financial return often diminish investment in rigorous efforts to develop platforms that can be transformative, especially when such approaches are not needed to move a blockbuster therapeutic forward commercially. In general, support for many innovative ideas or concepts falls “between the cracks” of the typical funding mechanisms for basic research and industrial investment. Philanthropic organizations play an important role in accelerating advances that can change the outcome of a cancer diagnosis. Most life science philanthropists are inspired by the opportunity to advance scientific knowledge and fill research funding deficits. They also aspire to integrate technology innovation with basic science. Moreover, they seek better prospects for patients as their primary return on investment. These goals are aligned with the mission and vision of The Mark Foundation for Cancer Research. As we build our organization, we are establishing a portfolio of funded projects that will benefit patients in the nearest term and that are centered around novel technology-driven approaches for gaining insights that have been difficult to achieve with conventional experimental strategies. To be successful, we will identify critical unmet needs in cancer research, fund highly innovative proposals for solutions to these challenges, create a robust and dynamic network of collaborators who share our passion to deliver breakthroughs to patients, and continually invest in the next generation of revolutionary science.

Brachyury in chordoma and carcinomas: biology and potential targeting approaches

Claudia Palena, PhD
Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH

Expression of the embryonic transcription factor brachyury is consistently found at high levels in the nucleus of chordoma cells in almost all chordoma tumors. The ability of brachyury to drive the proliferation of chordoma cells in preclinical models, and the identification of brachyury gene copy amplification in association with hereditary and a fraction of sporadic chordomas support a role for this protein in the pathogenesis of this disease. Brachyury is also aberrantly expressed in certain tumors of epithelial origin, including in lung, triple negative breast, colon and prostate carcinomas where variable levels of the protein can be found either in the nucleus and/or the cytosol of the carcinoma cells, at the primary or metastatic tumor site. In contrast to chordoma, brachyury has been associated with poor prognosis or tumor progression in carcinomas, with levels of brachyury in the primary tumor being predictive of poor clinical outcome. In line with these observations, brachyury expression has been shown to promote the acquisition of mesenchymal features by epithelial cancer cells (i.e., epithelial-mesenchymal switch) in preclinical models, a phenomenon that results in enhanced tumor cell motility, invasiveness and resistance to multiple anti-cancer therapeutics. Elucidation of the genetic or epigenetic mechanisms involved in the control of brachyury expression, both in chordoma and carcinomas, has been a major focus of recent investigations, as blockade of such mechanisms could afford reduction of brachyury and subsequent tumor control. Several studies have now implicated IL-8, TGB-β, and EGFR-mediated signaling, among others, as brachyury regulators, and efforts involving the use of specific inhibitors of these pathways both in preclinical and clinical studies are ongoing. Moreover, brachyury has proven to be a highly immunogenic target, and several immunotherapeutic approaches directed against this protein are currently being evaluated in the clinic in the form of cancer vaccines. Future combinatorial approaches based on the use of immunotherapy and other conventional or small molecule targeted therapies are also being investigated, which could potentially improve the clinical outcome of patients with chordoma or carcinomas that express brachyury. The various aspects of the biology of brachyury and its potential targeting will be discussed in this presentation.

Charting Brachyury-mediated developmental pathways during early mouse embryogenesis

Dr. Zhe Liu
Howard Hughes Medical Institute's Janelia Research Campus

To gain insights into coordinated lineage-specification and morphogenetic processes during early embryogenesis, here we report a systematic identification of transcriptional programs mediated by a key developmental regulator—Brachyury. High-resolution chromosomal localization mapping of Brachyury by ChIP sequencing and ChIP-exonuclease revealed distinct sequence signatures enriched in Brachyury-bound enhancers. A combination of genome-wide in vitro and in vivo perturbation analysis and cross-species evolutionary comparison unveiled a detailed Brachyury-dependent gene-regulatory network that directly links the function of Brachyury to diverse developmental pathways and cellular housekeeping programs. We also show that Brachyury functions primarily as a transcriptional activator genome-wide and that an unexpected gene-regulatory feedback loop consisting of *Brachyury*, *Foxa2*, and*Sox17* directs proper stem-cell lineage commitment during streak formation. Target gene and mRNA-sequencing correlation analysis of the *Tc* mouse model supports a crucial role of Brachyury in up-regulating multiple key hematopoietic and muscle-fate regulators. Our results thus chart a comprehensive map of the Brachyury-mediated gene-regulatory network and how it influences in vivo developmental homeostasis and coordination.

Crystal structures of Brachyury: a prelude to drug discovery

Dr. Opher Gileadi
University of Oxford

We have recently solved crystal structures of the DNA-binding domain of human Brachyury protein in complex with a cognate DNA fragment. We have investigated in parallel the structure of the G177D variant, which is strongly associated with chordoma. The mutated amino acid is located far from the DNA binding surface of the protein and has no measurable effect on DNA binding affinity. However, the variant affects the overall shape of the full-length protein, and may affect the binding of Brachyury to partner proteins. Most recently, we have obtained crystals of Brachyury protein alone, which allow to interrogate the DNA-binding surface. I will discuss the prospects of using high-throughput crystallographic methods to identify binding sites for small molecules and initial chemical hits.

A brachyury transcriptional reporter to guide drug discovery

Dr. Slim Sassi
Massachusetts General Hospital

We constructed a sensitive brachyury transcriptional reporter expressed stably in several chordoma and non-chordoma cell lines. With the aim of identifying effective chordoma drugs, we set up a screening platform based on these reporter lines to identify inhibitors of the brachyury pathway. Screening of an FDA approved panel and novel libraries constructed in Boston University (BU) identified several hit compounds that have been validated in secondary screens. Functional investigation of these compounds to better understand their mechanism of action is continuing. We have also taken a selection of these molecules to test in chordoma PDX murine models where a statin is showing promise. The BU library screens have identified two broad categories of promising compounds Rocaglates and Secalonic acids.

Investigating brachyury gene regulation to identify therapeutic targets in chordoma

Hadley Sheppard
Baylor College of Medicine

Chordoma is a rare primary tumor that develops in the skull base and spine. Genetic and functional data have implicated the developmental transcription factor brachyury as a central dependency of chordoma tumors and motivate efforts to directly drug its activity. Here we present the first strategies to model direct pharmacological brachyury inhibition using a degradation epitope (dTag) that can be triggered by cell permeable small molecules. We have generated dTag brachyury transgenes and have achieved the first direct endogenous tagging of brachyury in chordoma cell lines using CRISPR/Cas9. With these systems, we show rapid degradation of brachyury within hours that is reversible and phenocopies the growth arrest observed in brachyury knockout cells. These data establish a novel system to model direct brachyury inhibition, which we will use to better understand brachyury's function in chordoma and to guide efforts for brachyury drug discovery.

Delineating and targeting the brachyury-YAP regulatory axis in cancer

Sagar R. Shah, Justin M. David, Nathaniel D. Tippens, Ahmed Mohyeldin, Juan C. Martinez-Gutierrez, Sara Ganaha, Paula Schiapparelli, Duane H. Hamilton, Claudia Palena, Andre Levchenko, Alfredo Quiñones-Hinojosa

Department of Neurologic Surgery, Mayo Clinic and Department of Biomedical Engineering, Johns Hopkins University School of Medicine

Malignant neoplasms exhibit uninhibited and dysregulated growth coupled with acquisition of stem-like properties that are integral to the development and progression of disease. Molecular factors that define stem cell identity have recently emerged as oncogenic drivers. For instance, brachyury, a key developmental transcriptional factor, is also implicated in carcinogenesis, most notably of chordoma, through mechanisms that remain elusive. Hence, it is imperative to identify the transcriptional drivers of cancer stemness. Here, we demonstrate a critical role of Brachyury in regulating stemness in central nervous system (CNS)-derived cancers by activating YAP, a key regulator of tissue growth and homeostasis. We found that brachyury-based regulation of YAP can occur through direct transcriptional or post-transcriptional mechanisms leading to enhanced YAP-dependent oncogenic activity in various cancers where it correlates with tumor aggressiveness. By binding to the proximal region of the YAP promoter, brachyury was found to transactivate YAP-dependent signaling in chordoma. Furthermore, we find that brachyury expression is elevated in glioblastomas (GBM), the most common and aggressive type of primary brain cancer, and in a majority of brain metastases derived from various carcinomas where it transactivates YAP signaling. Surprisingly, we also observed that brachyury enhanced YAP signaling in lung cancer cells by increasing protein stability instead of transcriptional activation, suggesting a dual role of brachyury in modulation of YAP expression. These results elucidate a mechanism of controlling both tumor stemness and aggressiveness through regulatory coupling of two developmental factors. Moreover, this regulatory mechanism can shed further light on phenotypic plasticity in various cancers and inform new directions for treatment of chordoma and more common aggressive cancers. To that end, our novel proprietary nanotechnology-based brachyury-YAP targeting system offers hope for the management and treatment of a wide range of malignant neoplasms.

Open access T1-weighted dynamic contrast-enhanced MR perfusion imaging characterizes tumor response to radiation therapy in chordoma

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Perelman School of Medicine at the University of Pennsylvania | Memorial Sloan Kettering Cancer Center

Background: Chordomas notoriously demonstrate a paucity of changes following radiation therapy on conventional MR imaging. We hypothesized that dynamic contrast-enhanced MR perfusion imaging parameters of chordomas would change significantly following radiation therapy.

Methods: Eleven patients with pathology-proved chordoma who completed dynamic contrast-enhanced MR perfusion imaging pre- and postradiation therapy were enrolled. Quantitative tumor measurements were obtained by 2 attending neuroradiologists. ROIs were used to calculate vascular permeability and plasma volume and generate dynamic contrast-enhancement curves. Quantitative analysis was performed to determine mean and maximum plasma volume and vascular permeability values, while semiquantitative analysis on averaged concentration curves was used to determine the area under the curve. A Mann-Whitney U test at a significance level of $P < .05$ was used to assess differences of the above parameters between pre- and postradiation therapy.

Results: Plasma volume mean (pretreatment mean = 0.82; posttreatment mean = 0.42), plasma volume maximum (pretreatment mean = 3.56; posttreatment mean = 2.27), and vascular permeability mean (pretreatment mean = 0.046; posttreatment mean = 0.028) in the ROIs significantly decreased after radiation therapy ($P <.05$); this change thereby demonstrated the potential for assessing tumor response. Area under the curve values also demonstrated significant differences ($P <.05$).

Conclusions: Plasma volume and vascular permeability decreased after radiation therapy, suggesting that these dynamic contrast-enhanced MR perfusion parameters may be useful for monitoring chordoma growth and response to radiation therapy. Additionally, the characteristic dynamic MR signal intensity–time curve of chordoma may provide a radiographic means of distinguishing chordoma from other spinal lesions.

Developing endpoints and outcome measures for NF1: Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) working group

Dr. Brigitte Widemann
National Cancer Institute

REiNS is an international working group of clinicians, researchers, advocates, and patient representatives covering a wide range of interests and expertise initiated by Dr. Scott Plotkin (Massachusetts General Hospital, Boston MA). The overarching goal of REiNS is to develop meaningful endpoints and outcome measures for clinical trials targeting neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. REiNS has 7 working groups: Patient reported outcomes, tumor imaging, whole body MRI, visual outcomes, neurocognitive outcomes, biomarkers, and cutaneous neurofibromas. Proposed outcome measures and endpoints are presented to the larger research community, published and incorporated in new clinical trials to allow for a meaningful comparison of trial results.

Patient outcomes and clinical endpoints: Quality of life insights from AO Spine Knowledge Forum Tumor (AOKFT)

Dr. Charles Fisher
University of British Columbia

AOKFT is a group of dedicated spine oncology surgeons, radiation and medical oncologists whose principle mission is to advance patient care through multi-centre research and high impact peer review publications.

AOKFT has developed a reliable and validated Spine Oncology Disease Specific HRQOL Outcome Instrument for use in Primary and Metastatic Spine Tumors.

In 2017 AOKFT received a 2 year grant to study patient expectations and perceptions regarding treatment decisions and received treatment for primary and metastatic tumours.
AOKFT has studied the long term HRQOL in patients who undergo these complex long surgeries with high adverse event profile and often neurological sacrifice and determined Oncologic resection is associated with HRQOL that more closely approximates normative values with increasing disease free survival. If disease recurrence occurs, HRQOL will deteriorate, making HRQOL a potential clinical endpoint.

PRO and clinical endpoints for skull base QoL

Dr. Erin McKean
University of Michigan

Patient reported QoL, neurocognitive outcomes and endocrinologic outcomes are important endpoints for skull base chordoma. The anterior skull base questionnaire (ASBQ) is a tool to consider for assessing QoL and performance in patients with skull base chordomas, and the EORTC-HN 35 could be another option. Each has limitations. Neurocognitive testing is not routinely performed, and there are no standards for endocrine screening for patients who have been treated for skull base chordomas. Patient reported QoL/performance, as well as objective neurocognitive and endocrine outcomes, are important endpoints for clinical trials.

Methodological considerations for patient reported outcomes in chordoma clinical trials

Dr. Antonia Bennett
University of North Carolina

This talk will provide an introduction to the major components of developing and validating patient-reported outcome (PRO) endpoints for clinical trials. The particular challenges of conducting PRO endpoint development work in rare diseases will be discussed. Key references and guidance documents will be highlighted, and a recommended timeline for PRO endpoint development work to support phase II / phase III trials will be presented.

Regulatory Perspective on Efficacy Endpoints in Studies of Rare Tumors

Ashley Ward, MD
Food and Drug Administration

Although the demonstration of an improvement in overall survival remains the gold standard for drug approval, this endpoint may not be practical in cancers such as chordoma that are rare, or that have long natural histories, due to the time required and large patient numbers needed to power a trial to detect a survival improvement. Fortunately, innovation in cancer research has led to the use of other endpoints in regulatory decision-making. These endpoints include substantially delaying tumor progression or extending progression-free survival, substantially reducing tumor size for a prolonged time, improving objective response rate and duration of response, and, potentially, improving cancer-related symptoms and patient function. Dr. Ward will provide an overview of the FDA's perspective on clinical trial endpoints currently accepted for oncology drugs, and give context for regulatory consideration of alternative (e.g., patient-reported or functional outcomes) endpoints.

Investigation of in vivo synergistic effect of checkpoint blockade and radiation therapy against chordomas in a humanized mouse model

Wataru Ishida, M.D., & Hui Wang, M.D., PhD., Kyle L. McCormick, B.A., Eric M. Feldstein, B.S., Aayushi Mahajan, M.S., Jeffrey Bruce, M.D., Peter Canoll, M.D., PhD., Yong-Guang Yang, M.D., PhD., Sheng-fu L. Lo, M.D.

Johns Hopkins University | Columbia University Medical Center

Introduction: With the advent of immunotherapy (IT) against various cancers and its clinical success, its applications to other cancers have been extensively investigated. However, it has been a challenge for us to apply IT to chordomas, despite evidence supporting its efficacy in in vitro studies, partly due to lack of clinically-translatable in vivo models. Here we aimed to develop a humanized mouse model to study interaction between human immune system and human cancer cells. We also sought to utilize it to study synergistic effect between checkpoint blockade and radiation therapy (RT) against chordoma.

Materials and methods: Fifteen 10-12-week-old NSG mice were sublethally irradiated and then implanted with fetal thymic tissue and CD34+ stem cells that had been harvested from a fetus, whose HLA-type is partially-matched with that of the U-CH1 chordoma cell line. Reconstitution of immune cells in NSG mice was confirmed 8 weeks post transplantation and then each animal was injected with U-CH1 cell suspension bilaterally and subcutaneously. Next, they were treated for 4 weeks as follows: A) control (n=3), B) anti-PD1 antibodies (n=4), (C) RT only (n=3, unilaterally to the left tumor, 8Gy x 4), and (D) anti-PD1 and RT (n=5). During and after the treatment, anti-tumor activities were monitored via tumor size, flow cytometry, qRT-PCR, and immunohistochemistry. $P<0.05$ was defined as statistically significant.

Results: On average, human PBMCs of 43.8% among all PBMCs, human T cells of 23.4% among human PBMCs, human CD8+ T cells of 24.3% among human T cells, and other lymphocytes such as B cells, macrophages, and NK cells were observed in blood via flow cytometry. One week after the treatment, on the irradiated side, (D) demonstrated lowest tumor volume, highest number of human PBMCs, highest % of CD8+ human T cells, highest % of CD45RO+CD4+ human T cells, and lowest % of PD-1+CD8+ human T cells in tumor via flow cytometry, and highest IFN-gamma both in blood and tumor via qRT-PCR, compared to the other three groups with statistical significance. On the non-irradiated side, similarly D) harbored the smallest tumor compared to the others, although the difference between B) and D) was not statistically significant ($P=0.09$)

Conclusions: We demonstrated that this humanized mouse model can be a platform to investigate IT against chordoma. Direct synergism between IT and RT against chordoma as well as potential abscopal effect were observed.

Clinicopathologic characteristics of poorly differentiated chordoma

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Massachusetts General Hospital

Chordoma is a rare malignant tumor of bone with high morbidity and mortality. Recently, aggressive pediatric poorly differentiated chordoma with SMARCB1 loss has been described. This study summarizes the clinicopathologic features of poorly differentiated chordoma with SMARCB1 loss in the largest series to date. A search of records between 1990-2017 at MGH identified 19 patients with poorly differentiated chordoma. Immunohistochemical stains were evaluated. Kaplan-Meier survival statistics and log-rank (Mantel Cox) tests compared survival with other subtypes. The patients (n=19) were diagnosed at a median age of 11 years. Tumors arose in the skull base and clivus (n=10/19; 53%); cervical spine (n=6/19; 32%); and sacrum or coccyx (n=3/19; 16%). The clinical stage of these patients (AJCC 7e) was Stage 2A (n=7/16; 44%); Stage 2B (n=6/16; 38%); Stage 4A (n=1/16; 6%); and Stage 4B (n=2/16; 13%). The tumors were composed of sheets of epithelioid cells with nuclear pleomorphism, abundant eosinophilic cytoplasm, and increased mitoses. Tumors were positive for cytokeratin (n=18/18; 100%) and brachyury (n =18/18; 100%). Patients were treated with a combination of excision, radiation therapy, and chemotherapy. Clinical follow-up reveals that six patients had local recurrence at a median of 11 (range: 3-26) months from initial diagnosis. The mean overall survival (OS) is 53 months (SD=9 months); mean progression free survival (PFS) is 28 months (SD=8 months); mean local control (LC) time is 39 months (SD=9 months); and mean metastasis free survival (MFS) is 37 months (SD=8 months). Compared to other subtypes, poorly differentiated chordoma has a statistically significant decreased mean overall survival ($p=0.012$), mean progression-free survival ($p<0.0005$), mean local control time ($p=0.002$), and mean metastasis free survival ($p<0.0005$). Pediatric poorly differentiated chordoma has a distinct clinical and immunohistochemical profile, with characteristic SMARCB1 loss and decreased survival compared to conventional/chondroid chordoma. Recognition of this subtype is important because these malignancies should be treated aggressively with multimodality therapy.

A series of 62 Chordomas in children and adolescents patients: clinical characters, surgery treatments and outcomes

Dr. Jiwei Bai
Beijing Tiantan Hospital, Capital Medical University

Introduction: Clivial chordomas are rare tumors in the children and adolescents population and are challenging to manage due to their difficult accessibility, proximity to important anatomy and extension into adjacent structures. In order to understand of the natural history, treatment options and outcomes of chordomas in the younger population we retrospectively analyzed the surgery treatments and outcomes of children and adolescents patients with clival chordomas (CAPCC) in our hospital.

Methods: The CAPCC who underwent surgical treatment between July 2007 and March 2016 were included. Medical records and imaging were retrospectively reviewed for the following information: patient age, sex, past medical history, signs and symptoms, chordoma location, imaging characteristics, management, and outcome.

Results: A total of 62 pediatric patients with skull base or craniovertebral junction chordomas underwent 79 resections, including 56 open approaches and 23 endoscopic endonasal or transoral approaches. There was a female predominance, with a male-to-female ratio of 1:1.38. The median age was 14.5 years (range, 2-20 years). The average duration of clinical symptoms before diagnosis was 6 months (range, 0.5 months to 96 months). Headache and diplopia are common. The tumors located mainly in midline area of skull base. The number of total resection, near-total resection and partial resection were 13(16.5%), 31(39.2%) and 3(3.8%), and resection extent of the other 2 cases were unavailable. The surgical complication was found in 38 patients (48.1%). Eighteen（29%）patients underwent radiotherapy as adjuvant therapy postoperatively. During follow-up (median 45 months), tumors recurred in 30 patients (48.4%). Five-year PFS were 36.8%.

Conclusions: Our data support the safety and efficacy of endoscopic endonasal approaches (EEA) in children and adolescents patients. Despite advancing treatment options, the prognosis continues to be bad in children and adolescents patients with skull base chordoma.

Optimizing precision medicine therapies for pediatric brain tumors

Dr. Carl Koschmann
University of Michigan

The number of targeted therapies utilized in precision medicine is rapidly increasing. Neuro-oncology offers a unique challenge due to the varying blood brain barrier (BBB) penetration of each agent. Neuro-oncologists face a difficult task weighing the growing number of potential targeted therapies and their likelihood of BBB penetration. Along with collaborators at multiple institutions around the country, we developed the UM Brain Tumor Precision Medicine Conference in which we discuss pediatric brain tumors cases with sequencing results. As well, we have developed new algorithms for the use of molecularly targeted therapy for children and young adults with brain tumors, based on selection of drugs with the highest likelihood of crossing the blood brain barrier. In particular, our group created the CNS-TAP (Targeted Agent Prediction) tool, which is an algorithm for predicting the CNS activity of targeted therapies in pediatric neuro-oncology by evaluating the likelihood of blood brain barrier (BBB) penetration of targeted agents with patient-specific tumor sequencing data. As well, I am exploring new ways to monitor precision medicine therapies. My lab is using spinal fluid and plasma to help inform diagnostics and therapeutic monitoring of precision medicine in pediatric brain tumors patients.

UPMC Pediatric chordoma experience

Dr. Paul Gardner
University of Pittsburgh Medical Center

Introduction: Chordoma of the skull base in the pediatric population is rare. These tumors tend to be extremely aggressive and prone to recurrence. There is an absence in the literature regarding large treatment series in the pediatric population.

Methods: We retrospectively reviewed all pediatric patients with skull base chordomas at our institution from 2004 to 2015 and found 10 patients who underwent surgical treatment.

Results: Our series included 8 male and 2 female patients with a mean age of 12 years (range 4-18). The most common complaint was neck pain (n=4) followed by swallowing difficulty (n=3), double vision (n=2) and nasal congestion (n=2). A total of 12 surgeries were performed on these patients and included predominantly an endoscopic endonasal approach (EEA) which was combined with a transcervical approach in two cases. Two cases were part of a two-stage operation. Two patients had prior surgery and radiation treatment before our intervention. Six patients underwent postoperative proton beam therapy. Two patients underwent treatment with chemotherapy. Four patients developed tumor progression. Two of these underwent further resection, one is scheduled for further treatment and one patient's family elected to stop treatment. Six patients did not require further surgical treatment upon follow up. The average follow up was 55.5 months (range 1.5-106 months). Complications included one carotid artery injury resulting in hemiparesis that improved on follow up. Four patients had post-operative cerebrospinal fluid (CSF) leaks that required further operations for treatment. One patient developed seeding of the inferior turbinate which was resected and and one patient presented with intradural cervical spine metastasis and developed another. One patient developed a post-operative hematoma requiring an evacuation.

Conclusions: Pediatric skull base chordoma is a rare entity that presents multiple challenges to treatment. A multispecialty team is required for proper treatment, but endoscopic endonasal approaches provide a good option for resection; long term tumor control is possible with combined therapy. Pediatric patients are at increased risk for postoperative CSF leak.

Base editing: Chemistry on a target nucleotide in the genome of living cells

Dr. David Liu
Broad Institute

Point mutations represent the majority of known human genetic variants associated with disease but are difficult to correct cleanly and efficiently using standard genome editing methods. In this lecture I will describe the development, application, and evolution of base editing, a novel approach to genome editing that directly converts a target base pair to another base pair in living cells without requiring DNA backbone cleavage or donor DNA templates. Through a combination of protein engineering and protein evolution, we recently developed two classes of base editors (BE4 and ABE) that together enable all four types of transition mutations (C to T, T to C, A to G, and G to A) to be efficiently and cleanly installed at target positions in genomic DNA. The four transition mutations collectively account for most known human pathogenic point mutations. Base editing has been successfully performed in a wide range of organisms including bacteria, fungi, plants, fish, frogs, mammals, and even human embryos. We have characterized and substantially improved key molecular features of both classes of base editors, including their efficiency, product purity, targeting scope, and DNA specificity. We also show that base editing can function in vivo in post-mitotic cells that do not support efficient homology-directed repair. Base editing can be used to correct pathogenic point mutations, introduce disease-suppressing mutations, and create cell and animal models of human disease. These studies suggest the potential of base editing to serve as therapeutics for many diseases with a genetic component.

NCI Rare Tumors Research: Applying lessons learned to chordoma

Dr. Brigitte Widemann
National Cancer Institute

The NCI Center for Cancer Research (CCR) intramural research program is uniquely positioned to contribute to the understanding of rare tumors and the development of effective therapies. Examples for advances in rare tumor research at the NCI include the development of a clinical trials program for neurofibromatosis type 1 related tumors and the establishment of a wild-type gastrointestinal stromal tumor (GIST) clinic. Two NCI initiatives will further advance rare tumors research: The NCI CCR Rare Tumors Initiative (RTI) fosters focused collaborations between basic and clinical researchers at NCI, as well as extramural investigators. The Rare Tumor Patient Engagement Network (RTPEN), supported by the Cancer Moonshot, aims to connect patients and investigators through shared infrastructure and networks, accelerate the understanding of rare tumors and develop clinical trials for rare tumors through these national and international collaborations of patients, advocates, clinicians, clinical and basic researchers, and other stakeholders. The long-term goal of the RTPEN is to effectively study the biology and clinical course of rare tumors, translate these findings to improve care and treatment and to ensure that all patients have access to clinical trials which may benefit them.

Opportunities to accelerate research from AO Spine Knowledge Forum Tumor

Dr. Charles Fisher
University of British Columbia

AOKFT is a group of dedicated spine oncology surgeons, radiation and medical oncologists whose principle mission is to advance patient care through multi-centre research and high impact peer review publications. AOKFT is funded by AO Spine, part of AO International; providing a project manager, and data capture and analysis through AOCID, an in house CRO. AOKFT's initial large-scale project was a multicenter Spine Primary Tumor Study evaluating histology specific prognostic factors associated with local control and survival in patients diagnosed with primary spinal column tumors. 1500 cases were collected and 14 manuscripts, including 3 on Chordoma, were published.

AOKFT has now established a Primary Tumor Research and Outcomes Network (PTRON). The network has an established electronic data collection system, with all centers sharing the same CRF's and CRO. Data fields can be added and then removed once a prospective study is completed. The network also does bio banking. The 2 key objectives of PTRON are to do multicenter prospective cohort studies and maintain an ongoing registry of primary spine tumor patients to assess PRO's, morbidity, local control, and survival. The highest priority objective is clinical outcome and genomic correlational studies.

AOKFT feels there is tremendous potential for collaborative research opportunities with the Chordoma Foundation. By operating jointly, rather than in parallel, we should be able to accelerate Chordoma research and cure by expanding the network and utilizing adaptive study design around medical, surgical and radiation interventions.

The Center for Data Driven Discovery in Biomedicine (D^3b)

Dr. Adam Resnick
Children's Hospital of Philadelphia

The Center for Data Driven Discovery in Biomedicine (D^3b) is a transformative healthcare discovery ecosystem at Children's Hospital of Philadelphia (CHOP) Research Institute, one of the largest pediatric research institutes in the U.S. D^3b's multi-disciplinary team brings together experts in basic science, precision medicine, bioinformatics and genomic research to provide innovative and personalized care for children through collaborative, data-driven science.

Under its "open-science" model, the center drives the secure generation and integration of complex genomic and clinical patient data, and develops the open-access platforms that support collaborative discovery. Researchers worldwide are able to access this information and work together to fully empower and share novel approaches to biological targets for precise, less toxic clinical treatments on behalf of children.

The Chordoma Foundation has partnered with D^3b, transferring all specimens from the Chordoma Foundation Biobank as well as genomic data generated on chordoma cell lines and animal models. Through this partnership we will use the D^3b established infrastructure to advance chordoma research.

Clonal chromosomal changes associated with patient outcome

Dr. Paul Gardner
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Background: There are currently no reliable means to predict the wide variability in behavior of clival chordoma so as to guide clinical decision-making and patient education. Furthermore, there is no method of predicting a tumor's response to radiation.

Methods: A molecular prognostication panel, consisting of Fluorescent In Situ Hybridization (FISH) of the chromosomal loci 1p36 and 9p21, as well as immunohistochemistry for Ki-67, was prospectively evaluated on 105 clival chordoma samples from November 2007 to April 2016. The results were correlated with the overall progression free survival after surgery (PFSS), as well as the progression free survival after radiation (PFSR).

Results: Although Ki-67, and the percentages of tumor cells with 1q25 hyperploidy, 1p36 deletions, and homozygous 9p21 deletions were all found to be predictive of PFSS and PFSR in univariate analyses, only 1p36 deletions and homozygous 9p21 deletions were shown to be independently predictive in a multivariate analysis.

Using a prognostication calculator formulated by a separate multivariate Cox model, two 1p36 deletion strata (0-15% and >15% deleted tumor cells) and three 9p21 homozygous deletion strata (0-3%, 4-24%, and 25+% deleted tumor cells) accounted for a range of cumulative hazard ratios of 1 to 56.1 for PFSS, and 1 to 75.6 for PFSR.

Conclusion: Homozygous 9p21 deletions and 1p36 deletions are independent prognostic factors in clival chordoma, and can account for a wide spectrum of overall progression free survival after surgery, and progression free survival after radiation. This panel can be used to guide management after surgical resection of clival chordomas.

Single cell RNAseq to investigate chordoma cell heterogeneity

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We applied three single cell RNAseq technologies to investigate cellular heterogeneity of chordoma cell lines and primary tumors. Both examined cell lines (U-CH1 and U-CH2), and tumor cells showed a mixture of transcriptionally distinct cell populations. The functional relevance of these groups is not clear at this point. We examined differences under the light of proliferation state, EMT status, and common chordoma markers. One small but notable cell population lacks detectable T transcripts. Given the limitations of single cell sequencing this does not necessarily mean T is completely absent from this small group but it does indicate significantly lower levels of the mRNA compared to the bulk of cells. Targeting a tumor could mean targeting all cells, targeting a specific proliferative cell population, or in rare occasions influencing the cellular program to favor a terminal differentiated or senescent state. Understanding single cell transcriptional heterogeneity is an invaluable tool towards this goal.

Cell culture models of tumor diversity in chordoma

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Chordoma is a malignancy with a very high degree of diversity both concerning the location of the tumors and the intratumoral heterogeneity. Although occurring all along the spine, the primary tumors are predominantly located in the clival and sacral regions. Clival and sacral chordomas show differences in size and tendencies to recur and to metastasize.

We established and characterized the novel cell line U-CH14 to increase the number of clival chordoma cell lines available. Thus, we were able to compare three clival chordoma cell lines with nine cell lines originating from sacral tumors on the level of gene expression patterns. In cluster analyses, the individual cell lines formed two separated groups indicating distinct differences in the gene expression patterns of clival and sacral chordoma cell lines. Interestingly, several developmental genes of the HOX gene family were found to be downregulated in the clival lines.

To gain an insight into tumor evolution of chordoma, we established three cell lines from the same chordoma patient. U-CH17P was derived from the primary sacral chordoma, the other cell lines, U-CH17M and U-CH17S, originated from a soft tissue and a skin metastasis, respectively. Using gene expression profiling, array comparative genomic hybridization, and proliferation assays, the cell lines were characterized in depth. We identified several genes associated with the occurrence of metastases, proliferation and migration like MAGEC2 and SEMA6A to be differentially expressed in the metastases cell lines. Furthermore, compared to the cell lines originating from primary tumor, the cell lines derived from metastases clearly show differences in growing behavior in soft agar and in spheroid growing assays indicating that the clonigenicity, tumorigenicity and capability of anchorage-independent proliferation vary between the primary tumor and metastases derived cell lines. Therefore, the U-CH17 cell culture model can be used to identify treatment options for metastasizing chordoma and to elucidate the underlying mechanisms of chordoma oncobiology.

Molecular characterization of available preclinical models

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In the past, lack of access to preclinical models including cell lines and xenografts posed a major barrier to advancing chordoma research. To overcome this barrier, the Chordoma Foundation invests in developing new models and operates repositories to make them easily accessible to the research community. The major goal is to develop a diverse collection of models that represent the many clinical and molecular variations of the disease. Cell lines derived from chordoma tumors that have undergone more than 50 population doublings were acquired from collaborating investigators and validated using criteria established by the Chordoma Foundation's Scientific Advisory Board. Additionally, the Foundation has partnered with South Texas Accelerated Research Therapeutics (START) to establish, expand and maintain patient derived xenograft (PDX) models from tumor tissue of patients undergoing surgical resection and consented through the Foundation's IRB approved biobanking protocol. Whole genome sequencing (WGS) and RNASeq were performed on DNA and RNA isolated from both cell lines and PDX models to determine whether these models harbor molecular alterations similar to those identified in primary chordoma tumors. A total of 14 chordoma cell lines have been validated and are available to the research community. Eleven of the fourteen cell lines are of sacral origin and represent primary, recurrent and metastatic disease states, whereas the remaining 3 are of skull-base origin. In contrast to the cell lines, 7 of the 9 validated PDX models are of skull-base origin. Three of the PDX models were developed from tumors resected from pediatric patients with one negative for INI1 protein. Molecular analysis of the various models will be presented, which may help inform selection of specific models for future experiments and drug testing.