Summaries of 2015 OMSF-Funded Research Support Grants

Stephen B. Milam Research Support Grant

Overexpression of Notch3 by MSCs in the Pathogenesis of Jaw Bone Giant Cell Tumors
Principal Investigator: Qilin Xu, MD, PhD
Co-PI: Lee Carrasco, DDS, MD PhD
University of Pennsylvania

Giant cell tumor of bone (GCT) comprises approximately 6% of all primary bone tumors and is characterized by significant local osteolysis. GCTs are heterogeneous and composed of several cell types. Although the presence of numerous multinucleated giant cells is the hallmark of GCT, the spindle-like stromal cells or mesenchymal stem cells (MSCs) are actually the neoplastic component of the tumor, owing to their ability to readily proliferate in culture and their capacity to form tumors in mice. High recurrence rates following surgical removal of the tumor may result from residual stromal cells that are capable of re-forming the tumor. The objective of this proposal is to investigate whether stromal cells/MSCs derived from GCT are capable of developing GCT and the potential mechanisms. GCT MSCs play an essential role in the recruitment of the tumor-associated myeloid lineage cells and formation of the osteoclast-like giant cells by secreting a panel of cytokines and growth factors, such as M-CSF and RANKL that play a critical role in osteoclast differentiation and activation. Based on these findings, pharmacological approach using antiresorptive agents have been proposed as adjuvant therapy for management of GCT. However, the lack of deep understanding of the cellular and molecular mechanisms of the stromal cells/MSCs in driving GCT formation has heralded new therapeutic options for GCT patients. The team recently isolated a subpopulation of MSCs from human jawbone giant cell tumor (GCT-MSCs), which not only expressed higher levels of stem cell-related genes such as SSEA-4, Oct-4, Nanog and Notch3, but also exhibited enhanced multipotent differentiation capabilities as compared to MSCs derived from normal jawbone (nJB- MSCs). GCT-MSCs also expressed higher levels of IL-34, M-CSF, and RANKL than nJB-MSCs. Knockdown of Notch3 expression in GCT-MSCs downregulated Oct4 and Nanog expression as well as the expression of IL-34 and M-CSF. Conversely, stimulation with M-CSF significantly increased Notch3 expression in GCT-MSCs. In the present study, they will delineate the key role of Notch3 signaling in the regulation of stem cell properties and sustained autocrine secretory function of GCT-MSCs that contribute to osteolysis and specifically, osteoclastogenesis in pathogenesis of jawbone GCT. The anticipated findings from this study will substantially expand current knowledge regarding the interactions between GCT-MSCs and osteoclast-like giant cells, and will have potential clinical relevance of targeted disruption of the positive Notch3/M-CSF feedback loop to facilitate the repair or regeneration of bone defects manifested in jawbone GCT.

Interaction between Mu-Opioid Receptor and B2 Adrenergic Pathways in Oral Cancer Pain
Principal Investigator: Chi Viet, DDS, PhD
Co-PI: Brian Schmidt, DDS, MD, PhD
New York University, Bluestone Center for Clinical Research

Oral cancer pain is more severe and has a higher prevalence than all other forms of cancer pain. The etiology of oral cancer pain, and cancer pain in general, is unknown. The current standard of care for cancer pain is opioids. While opioids can be initially effective, these drugs have drawbacks: side effects can be prohibitive (e.g. nausea, respiratory depression and constipation), opioid tolerance develops and clinicians can have difficulty getting the drugs to patients due to regulatory controls. The focus of this translational research program is to understand and reverse the mechanisms responsible for cancer pain. They propose that cancer leads to the upregulation of a splice variant of the μ-opioid receptor (6TM MOR1) on neurons that innervate the cancer microenvironment. Activation of 6TM MOR1 leads to neuronal activation, cancer pain, and opioid tolerance. They further propose that the β2-adrenergic receptor acts in concert with 6TM MOR1. The team proposes a series of in vitro and in vivo studies to understand how expression and activation of these two receptors leads to pain in the setting of cancer. They will also explore whether these receptors mediate opioid tolerance, which is a profound challenge in controlling oral cancer pain.
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**Thrombopoietin and stem cells for cranial regeneration**  
Principal Investigator: Tien-Min Chu, PhD, DDS  
Co-PI: Jeffrey Bennett, DDS, MS  
Indiana University School of Dentistry

When large bone loss in the cranial area occurs, surgeons are faced with the very challenging task of simultaneously restoring functional and esthetic demands for patients. In the past, the tissue engineering approach of using a three-dimensional (3D) scaffold that conforms to the shape of the missing bone, loaded with mesenchymal stem cells (MSCs) and bone morphogenetic protein (BMP) has shown great promise. However, several papers since 2011 have revealed serious health risks associated with the use of BMP-2. Also, the in vivo cranial regeneration from MSCs has not been consistent. Recently this team has shown that thrombopoietin (TPO), the main growth factor of megakaryocytes (MKs), can stimulate osteoblast (OB) proliferation through its indirect action on MKs, as well as promote the osteogenic differentiation of MSCs. In vivo, TPO is known to stimulate the proliferation of MKs, which later mature to release platelets capable of producing multiple factors to promote angiogenesis and bone regeneration. Taken together, they hypothesized that TPO can promote bone regeneration indirectly through MKs. This early hypothesis was confirmed when they showed that TPO alone can induce bridging callus formation in rat femoral critical-size defects, followed by another study where it was shown that romiplostim, a similar thrombopoietic agent, can induce regeneration in rat cranial critical-size defects. They also demonstrated the ability to use 3D scaffold fabricated from dicalcium phosphate dehydrate/polypropylene fumarate (DCPD/PPF) with and without MSCs to facilitate bone formation in rabbit cranial defects. Later, they have investigated the potential of using of a biodegradable thiol-acrylate hydrogel to simultaneously carry protein drug and MSCs to enhance the survival and differentiation of MSCs. With this information, they hypothesize in this project that TPO released from thiol-acrylate hydrogel on a 3D scaffold can promote bone regeneration in critical-size cranial defects, and that the use of stem cells will further enhance regeneration. To test the hypothesis, in Aim 1, they will investigate the effect of hydrogel concentration and cell density on the proliferation and differentiation of MSCs encapsulated in thiol-acrylate hydrogel. In Aim 2, they will decipher the effects of TPO dose and presence of MSCs on cranial regeneration. In Aim 3, they will demonstrate the time-response of TPO/MSC combination on cranial regeneration. The results from this study will provide data for future studies to evaluate the clinical applications and safety of TPO for cranial regeneration.

**Development of “immunoprofile” to direct therapy for OHNSCC**  
Principal Investigator: Bernard Fox, PhD  
Co-PI: Richard Bell, MD, DDS, FACS  
Providence Portland Medical Center

Antibodies (Abs) that target T cell surface proteins and adoptive immunotherapy with chimeric antigen receptor modified T cells have produced complete and partial responses as well as durable stable disease in patients with cancer (Brahmer et al 2012, Grupp et al 2013, Kochenderfer and Rosenberg 2013, Topalian et al 2012). These promising developments have led to immunotherapy being dubbed the “cancer breakthrough of the year” in 2013 (Couzin-Frankel 2013). Despite these successes it is becoming apparent that for many diseases only a fraction of the treated patients respond to immunotherapy and experience clinical benefit. As a consequence, a major effort is underway to identify biomarkers that can predict clinical efficacy of immune therapeutics and direct treatment. Recently, the delineation of tumor-infiltrating immune cells using standard immunohistochemistry (IHC) coupled with digital imaging and computer algorithms, termed “Immunoscore”, has proved to be a powerful prognostic biomarker, providing significantly (p<0.001) more prognostic power than TNM staging in patients with colon cancer (Galon et al 2006, Pages et al 2005). This observation and similar findings in a range of solid tumors documents the important role anti-cancer immunity plays in outcomes of patients regardless of the therapy received (Fridman et al 2012). Oral, head and neck squamous cell carcinomas (OHNSCC) are known for their immune suppressive character, complex molecular landscape, and a poor prognosis that has not significantly improved in the last four decades. This team hypothesizes that characterizing the transcripts in a cohort of OHNSCC that ultimately progress with those of tumors that do not
Development of “immunoprofile” to direct therapy for OHNSCC (continued)

progress will identify a series of genes that can be used to develop a NextGen immunoprofile (immunohistochemical assay) that separates progressors from non-progressors. Further, they expect to identify different progressor phenotypes. This information could be used to evaluate potential markers and develop this NextGen Immunoprofile that could be validated and standardized as a routine medical test. They hypothesize that this test would provide information about the suppressive environment present in a patient’s tumor and could be used to tailor therapies that overcome those specific immune inhibitors and ultimately improve patient outcomes. To develop this NextGen Immunoprofile they will characterize mRNA transcripts for OHNSCC from 40 patients (20 progressors and 20 non-progressors). The NextGen immunoprofile will be generated, validated and standardized using archived tumor tissue of 121 OHNSCC snap-frozen resection specimens that are paired with formalin fixed paraffin embedded tissue. Results of molecular and immunoprofiling will be correlated with clinical outcome.

A Prospective Observational Study to Assess Clinical and Radiological Outcomes of Orthognathic Surgery in Individuals with Temporomandibular Disorders
Principal Investigator: Pushkar Mehra, BDS, DMD
Co-PI: Radhika Chigurupati, DMD, MS
Boston Medical Center Henry M. Goldman School of Dental Medicine

Patients who require surgical correction of dentofacial deformities often present with symptoms of Temporomandibular Disorders (TMD) such as facial pain, limitation of jaw function, and clicking sounds or crepitus in the jaw joint during opening or closing. It is unclear whether these symptoms improve or deteriorate after the orthognathic surgery. To date, there are no controlled prospective studies relating clinical symptoms of TMD with appropriate pre and postoperative radiological findings specifically magnetic resonance imaging (MRI) in patients seeking consultation for orthognathic surgery. The correction of functional disability is an important factor in determining the success or failure of orthognathic surgery, although, aesthetic and psychosocial factors are often the primary motivating factors for most patients with DFD. Therefore, they propose to understand how the risk factor of temporomandibular disorders affects specific functional outcomes of orthognathic surgery in patients with dentofacial deformities (DFD). The aim of this prospective observational study is to evaluate the clinical and radiological outcomes of orthognathic surgery in patients with dentofacial deformities and co-existing risk factor of temporomandibular disorders. The primary clinical outcomes of interest are change in symptoms of facial pain, and jaw function after orthognathic surgery. The outcomes will be measured by validated instruments for diagnosis of TMD- Axis I (symptom questionnaire, clinical examination) and Axis II (Pain drawing, Graded Chronic Pain Scale-GCPS, Patient Health Questionnaire-PHQ, Jaw Functional Limitation Scale–JFLS, and Oral Behaviors Checklist). The radiological outcome of interest is periarticular soft tissues changes including articular disc position. This study is significant as it has the potential to clarify some ambiguities, and, help surgeons to better inform patients with concomitant TMD about the expected outcomes of orthognathic surgery. The investigators have vast experience in the management of patients with TMD and DFD, and, have an excellent facility to carry out the clinical and radiological procedures to complete this feasible and important study.

IR-Labeled Cetuximab for Optical Imaging of Odontogenic Neoplasms
Principal Investigator: Anthony Morlandt, MD, DDS
Co-PI: Mary MacDougald, PhD
University of Alabama at Birmingham

Aggressive odontogenic neoplasms, including ameloblastomas (AB) and keratocystic odontogenic tumors (KOT), demonstrate locally aggressive and destructive behavior, primarily in the posterior mandible. Wide variability in treatment modalities have been advocated for aggressive odontogenic neoplasms leading to residual disease and wide ranges of disease recurrence (3-62%), or the use of over-aggressive resection in other cases. The
ability to accurately assess tumor margins with optical imaging could result in the preservation of healthy tissue and improve long-term local tumor control, reducing the risk of potentially life-threatening recurrence and optimizing reconstructive therapies with minimal morbidity. This team hypothesizes that epidermal growth factor receptor (EGFR) expression in aggressive odontogenic neoplasms may be used to specifically fluorescently label tumor cells, which may be used to assess tumor margins intraoperatively. The aims of this study are designed to demonstrate the specificity and sensitivity of fluorescently labeled anti-EGFR antibody, cetuximab-IRDye800, to aggressive odontogenic tumor cells, particularly ameloblastoma (AB) and keratocystic odontogenic tumor (KCOT), in vitro and in vivo. It has been previously demonstrated that intravenous administration of fluorescently labeled anti-EGFR antibody can successfully identify microscopic tumor fragments in multiple in vivo preclinical models of human head and neck squamous cell carcinomas (HNSCC), melanomas, and breast cancers with limited toxicity. These studies will use unique cell models of aggressive odontogenic tumors to develop a novel animal model and demonstrate the binding of fluorescent anti-EGFR in vitro and in vivo. They believe the research outlined in this proposal will give oral and maxillofacial surgeons technology to more confidently remove aggressive odontogenic neoplasms by accurately assessing tumor margin and preserving normal tissue, to improve long-term local tumor control and reduce recurrence in this patient population.