



The National Institutes of Health's Medical Research Scholars Program

Dental Scholars Abstracts
2015-2017



Jason Berglund, Class of 2017

School: Tufts University School of Dental Medicine

Mentor: Michael T. Collins, M.D., Chief, Section on Skeletal Disorders and Mineral Homeostasis

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: Insights into the Molecular and Cellular Etiology of the Tumors Responsible for Tumor-Induced Osteomalacia

Tumor-induced osteomalacia (TIO) is a rare paraneoplastic condition associated with hypophosphatemia, muscle weakness, bone pain, and pathological fracture. It is caused by secretion of FGF23 by phosphaturic mesenchymal tumors (PMTs) with histological features reminiscent of osteogenic cells. The molecular and cellular origins of these tumors have yet to be clearly elucidated, but the recent identification of a fibronectin-fibroblast growth factor receptor 1 (FN1/FGFR1) translocation in a subset of these tumors suggests FGFR1 signaling as a potential tumorigenic driver.

26 tumors clinically proven to cause TIO were assessed. Analyses included translocation testing with FN1/FGFR1-specific FISH, immunohistochemical and/or immunofluorescent testing of markers of FGF23 and FGFR1 pathway signaling, and osteogenic cell markers. A comparison was made *in vitro* of the response in FGF23 production to FGFR and/or mTOR blockade by BGJ398 and rapamycin, respectively, in separate FN1/FGFR1 translocation positive and negative tumors.

14/26 (54%) tumors were successfully assessed by FISH (tumors arising in bone were generally unanalyzable). Of the 14 samples, 4/14 (29%) were positive for a FN1/FGFR1 translocation. All tumors were FGF23 positive. Tumors were positive for various early and late osteogenic lineage markers (including DMP1, which showed a high level of co-expression with FGF23), the FGF23 UDP-GalNAc transferase, GALNT3, and the FGF23 co-receptor KLOTHO. *In vitro*, addition of the FGFR inhibitor BGJ398, with and without presence of the synergistic mTOR inhibitor rapamycin, decreased FGF23 production by 80% in a FN1/FGFR1 translocation positive tumor, but had a negligible effect on a tumor lacking the translocation.

PMTs express osteogenic cell markers consistent with having differentiated from an inducible skeletal stem cell. Significant proportions of PMTs harbor a FN1/FGFR1 translocation, and may be responsive to FGFR pathway blockade. These data suggest a role for FGFR1 signaling in tumorigenesis and FGF23 production, and identify the FGFR1 pathway as a potential target for treatment.

Full-Length Publications:

- **Berglund JA**, Gafni RI, Wodojo F, Cowen EW, El-Maouche D, Chang R, Chen, CC, Guthrie LC, Molinolo AA, Collins MT. Tumor-induced osteomalacia in association with PTEN-negative Cowden Syndrome. *Osteoporosis International*. [Under review]

Travel to Professional Meetings:

- Endocrine Society, 99th Annual Meeting, Orlando, FL; Apr. 1-4, 2017.
- 8th International Conference on Children's Bone Health. Würzburg, Germany; Jun. 10-13, 2017.

Abstract Publications:

- **Berglund JA**, Gafni RI, Forsberg JA, Molinolo, AA, Fernandez de Castro L, Ten Hagen KG, Tian E, Metwally T, Overjero Crespo D, Chong W, Collins MT. Insight into the molecular and cellular etiology of the tumors responsible for tumor-induced osteomalacia. 99th Annual Meeting of the Endocrine Society. Orlando, FL; Apr. 2017. [Oral presentation]
- **Berglund JA**, Tella S, Kim L, Stanton R, Collins MT, Boyce AM. Scoliosis in fibrous dysplasia/McCune-Albright Syndrome. 8th International Conference on Children's Bone Health. Würzburg, Germany; June 2017. [Oral presentation]



John M. Le, Class of 2017

School: University of Michigan School of Dentistry

Mentor: Janice S. Lee, D.D.S., M.D., M.S., Clinical Director and Chief, Craniofacial Anomalies and Regeneration Section

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: *In Vivo* Analysis of Stromal Cell Osteogenic Capacity in a Murine Model

Bone regenerative capacity decreases with age, and the aging microenvironment may alter the differentiation pathway of multipotent bone marrow stromal cells (BMSCs) that reside in the marrow niche. As a result, the ability for bone to heal and regenerate in an aged individual is impaired. The purpose of our study was to define the role of aging on the osteogenic potential of human BMSCs (hBMSCs) coupled with an osteoconductive scaffold within a variable age murine model.

Six hBMSC cell lines were used for the *in vivo* transplantation assay. The hBMSCs were from skeletally growing and skeletally mature subjects. Mean age of young and old donors was 16 (range 12-19) and 46 (range 38-55) years. Primary cell lines were expanded and at 80% confluence, hBMSCs were combined with hydroxyapatite and tricalcium phosphate particles and loaded into either a 12-well Teflon combinatorial cassette (n=4 per cell line) or directly transplanted into subcutaneous dorsal pockets in young (6-10 weeks) and old (32-36 weeks) immunocompromised mice (n=4 transplants per cell line). Transplants were harvested at 8 weeks for analysis of bone formation.

Four cell lines are analyzed to date, consisting of 2 transplants per cell line (n=8) derived from young and old donor mice. Utilizing BIOQUANT® Osteo software, transplants retrieved from younger animal recipients demonstrated a mean (\pm SD) percentage of bone formation volume to total transplant volume of $9.63 \pm 7.65\%$, vs $2.54 \pm 1.62\%$ in transplants harvested from older animals, regardless of the age of the donor cells. Results are pending from the remaining transplants and the 12-well combinatorial cassette transplants. RNA was extracted from the transplants for genomic sequencing.

These preliminary findings demonstrate that the marrow microenvironment contributes to the osteogenic capacity of hBMSCs, regardless of the age of the donor cells. Our results should contribute to the development of bone tissue engineering modalities for restoration and reconstruction of the skeletal complex in the aging niche.



David Kirby, Class of 2016

School: The Johns Hopkins University School of Medicine

Mentor: Marian F. Young, Ph.D., Senior Investigator; Chief, Molecular Biology of Bones and Teeth Section, Craniofacial and Skeletal Diseases Branch

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: Understanding the Role of Biglycan in Fracture-Associated Angiogenesis

Approximately 6 million new fractures occur each year in the U.S., with 5-10% of fractures complicated by non-union/delayed-union, or the failure/delay of bone to heal. The production of new blood vessels within the fracture callus, a process called angiogenesis, is essential for the fracture to heal. Impairment of fracture site vascularity has been identified as a major risk factor for non-union/delayed-union development.

Our lab has previously shown that the proteoglycan biglycan (bgn) plays a key role in the process of bone formation, but it was recently suggested to affect blood vessel formation within the fracture callus.

Our project goal was to determine if bgn has a role in fracture-associated angiogenesis, and attempt to identify the mechanism underlying this effect.

Micro-computed tomography angiography demonstrated mice deficient in bgn had significantly decreased vessel size and volume in the fracture callus at 7 days post-fracture compared to wild-type mice. Real-time RT-PCR confirmed decreased expression of PECAM-1, a marker for endothelial cells, in the fracture callus. Immunofluorescence and immunohistochemistry showed co-localized expression of bgn and endostatin, a potent inhibitor of angiogenesis, in the fracture callus. In an endothelial cell tubule formation assay, bgn was able to significantly inhibit the antiangiogenic effect of endostatin. RNA sequencing data revealed that bgn-deficient mice have differential expression of integrins important in angiogenesis relative to wild-type mice.

These results demonstrate that when bgn is absent, the angiogenic response required for a fracture to heal is mitigated. Additionally, bgn expression colocalizes with endostatin expression in regions of bone formation, and bgn is able to inhibit the effect of endostatin in functional assays. This provides a mechanism by which bgn's effect is mediated. Interestingly, next generation sequencing indicates that bgn has a role with integrins in angiogenesis, and it is through inhibition of integrins that endostatin mediates its antiangiogenic effect.

Full-Length Publications:

- Myren M, Kirby D, Noonan M, Maeda A, Owens R, Ricard-Blum S, Kram V, Kilts T, Young M. Biglycan potentially regulates angiogenesis during fracture repair by altering expression and function of endostatin. *Matrix Biology*. May-Jul;52-54:141-50, 2016.



Tarek N. Metwally, Class of 2016

School: University of Michigan School of Dentistry

Mentor: Michael T. Collins, M.D., Chief, Section on Skeletal Disorders and Mineral Homeostasis, Craniofacial and Skeletal Diseases Branch

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: Molecular and Cellular Characteristics of FGF23-Secreting Phosphaturic Mesenchymal Tumors

Phosphaturic mesenchymal tumors (PMTs) are rare FGF23-secreting tumors associated with the paraneoplastic syndrome, tumor-induced osteomalacia (TIO). There is limited information regarding the etiology or cellular origin of PMTs.

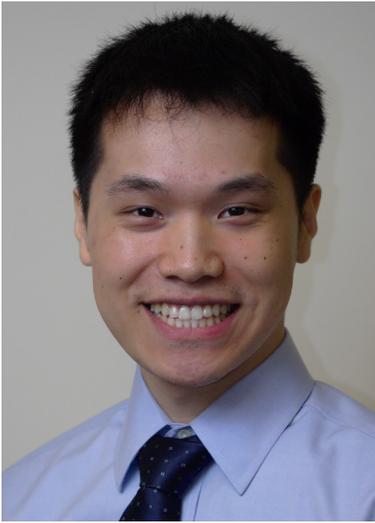
FN1-FGFR1 Translocation in TIO: A recent report of a novel FN1-FGFR1 gene fusion in a set of PMTs suggests that this translocation and the resultant activation of FGFR1 signaling pathway may be important in PMT tumorigenesis and/or FGF23 production by PMTs. To test the role of the FN1-FGFR1 translocation in PMT tumorigenesis, we performed Fluorescence In Situ

Hybridization (FISH) on the NIH collection of PMTs. Of 24 total tumors, 22 have been assessed to date. 45% were FISH positive for the fusion (9/20), 1/20 was deemed intermediately positive for having more positive signals than negative, but did not meet criteria for being fusion positive, 6/20 were fusion negative, and 6/20 were inconclusive. All six inconclusive tumors were from bone, which suggests the typical method used for decalcification, which is known to damage macromolecules, prevented a positive or negative FISH signal.

Cell/Tissue Origin of PMTs: The dual observations of: 1) bone-related mesenchymal tissues in PMTs (chondroid matrix, osteoclasts, and bone) and 2) the fact that bone cells are the physiologic source of FGF23, led us to hypothesize that PMTs are transduced from skeletal stem cells. To test this hypothesis, we performed co-localization studies with bone- and tumor-related markers by immunofluorescence and confocal microscopy. Bone markers included alkaline phosphatase (Alk Phos) and RANKL, both mid-stage bone cell lineage markers, and DMP1, a late-stage bone cell lineage marker; tumor-specific markers included FGF23 and GALNT3 (a galactosylaminotransferase responsible for glycosylating FGF23 and protection from enzymatic cleavage). We found that FGF23-secreting tumor cells co-localize with Alk Phos, RANKL, and DMP1. The presence of these bone cell lineage markers provides support for the hypothesis that PMTs arise from transduced skeletal stem cells. There is also co-localization with GALNT3 and FGF23-secreting tumor cells, suggesting PMTs not only produce FGF23, but also express the enzymatic machinery necessary to make intact hormone. Further experiments will focus on cells experimentally transduced with the FN1-FGFR1 chimeric gene to allow for more detailed investigation of this protein and FGFR1 signaling in tumorigenesis and FGF23 regulation.

Full-Length Publications:

- **Metwally T**, Burke A, Tsai JY, Collins MT, Boyce AM. Fibrous dysplasia and medication-related osteonecrosis of the jaw. *J Oral Maxillofac Surg*, 2016 Apr 7. doi: j.joms.2016.04.001. [Epub ahead of print]



Peter W. Fung, Class of 2015

School: Harvard School of Dental Medicine

Mentor: Janice S. Lee, D.D.S., M.D., M.S.; Chief, Craniofacial Anomalies and Regeneration Section; Clinical Director; NIDCR

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: Long-term Sequelae of Optic Nerve Encasement with Fibrous Dysplasia

Fibrous dysplasia is a developmental disorder characterized by the formation of benign bone tumors that weaken and distort normal bony architecture. These tumors often develop in the craniofacial region and frequently result in the encasement of the optic canals. Some clinicians assume that radiographic encasement by fibrous dysplasia results in gradual constriction, progressive optic neuropathy, and eventual blindness. This assumption has led some clinicians to recommend prophylactic surgical decompression to relieve constriction of the optic nerve. The validity of this premise is particularly salient for patients with normal vision since decompression surgery carries a risk of iatrogenic blindness. The aim of this study was to establish the long-term outcomes of fibrous dysplasia encasement of the optic nerve.

We performed a retrospective longitudinal study of 69 patients with fibrous dysplasia involving the lesser wing of the sphenoid bone who did not have a history of decompression surgery. All patients underwent comprehensive neuro-ophthalmologic examination and computed tomography of the skull at baseline and, at minimum, one additional evaluation. Four parameters (visual fields, acuity, color vision, and fundoscopic exam) were assessed for changes indicative of optic neuropathy, which was defined as a visual field deficit or abnormal findings in at least two of the other three parameters. This longitudinal study demonstrated that vision for the vast majority of patients remained stable without progression over an extended follow-up period. This analysis supports that prophylactic decompression of the optic nerve based solely on radiographic encasement with fibrous dysplasia is not indicated since it is not correlated with the development of visual disturbances over time.



Molly Hague, Class of 2015

School: University of California, San Francisco School of Dentistry

Mentor: Kenneth Yamada, M.D., Ph.D.; Chief, Laboratory of Cell and Developmental Biology; NIH Distinguished Investigator

Institute: National Institute of Dental and Craniofacial Research (NIDCR)

Research: The Role of Fibronectin and Btbd7 in Oral Squamous Cell Carcinoma

Our laboratory previously established that the extracellular matrix protein fibronectin (FN) and its downstream signaling effector Btbd7 promote transient normal epithelial cell migration during development. Therefore, to help bridge the gap between cell migratory roles during development and invasive migratory behavior during cancer, we investigated the role of FN-binding integrins and Btbd7 in oral squamous cell carcinoma (SCC). Specifically, we investigated whether the FN receptor $\alpha 5\beta 1$ integrin and Btbd7 are required for oral SCC invasion and migration.

We evaluated the invasive capabilities of two oral SCC lines (SCC-9 and SCC-25), both isolated from the tongue, compared with a normal cell line (human oral keratinocytes) using a classical Matrigel-coated transwell invasion assay. The SCC-9 cell line, but not the SCC-25 line, proved significantly more invasive than normal oral keratinocyte controls. To further evaluate the difference between the non-invasive SCC-25 cells and invasive SCC-9 cells, we investigated the role of FN in each of these lines. Immunofluorescence demonstrated that confluent SCC-9 cells assembled large FN bundles, whereas SCC-25 cells did not. Additionally, the SCC-9 cells exhibited co-localization of FN and its receptor, the $\alpha 5\beta 1$ integrin. Inhibition of $\alpha 5\beta 1$ integrin function inhibited not only SCC-9 invasion in the transwell assay, but also SCC-9 migration from spheroids in collagen. Because Btbd7 has been shown to be activated downstream of FN, we tested its role in invasiveness by depleting Btbd7 protein levels in SCC-9 cells with siRNAs. Reduction of Btbd7 levels resulted in a substantial inhibition of cell invasion through the transwells. Together, these results indicate that the FN $\alpha 5\beta 1$ integrin complex and Btbd7 play roles in SCC-9 cell invasion. Future work will seek to determine whether exogenous FN promotes Btbd7 function in these cells.
