

NEWSLETTER

The role of Notch signaling... *in bone regeneration*



Dr. Kurt Hankenson, associate professor, is the Dean W. Richardson Chair for Equine Disease Research in the Department of Clinical Studies--New Bolton Center. He received his D.V.M. from the University of Illinois (1992), an M.S. from Purdue University (1997) and his

Ph.D. from the University of Washington (2001). A former equine veterinarian, he began his independent research career at the University of Michigan in 2002 as a faculty member in the Orthopaedic Research Laboratories. In 2006 he moved to Penn Vet.

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Good News for Summer Research

Dr. Michael Atchison has received word from Merial that support for the Merial Veterinary Summer Program (MVSP) is renewed for **Penn Vet's summer program 2014**. The program is designed to expose students in their first or second year of veterinary school to all phases of biomedical research. Students present their summer research project results at **Student Research Day** on March 11th and at the MVSP-NIH Symposium, July 31-August 3, 2014.



PENN VET FACULTY RECIPIENT of the GLOBAL ENGAGEMENT PILOT FUND

Dr. Dipti Pitta, assistant professor in ruminant nutrition, Department of Clinical Studies--New Bolton Center, has been awarded pilot funding. She received notice of the \$18,000 pilot award from Dr. Ezekiel Emanuel, Vice Provost for Global Initiatives. The pilot funding will support her work on a multi-disciplinary approach



to improve the socioeconomic status of small holder livestock farmers in rural India. Dr. Pitta's research expertise is in the field of large animal nutrition that includes sheep, goats, and dairy cattle. She studies nutrient uptake and utilization, feed additives, and feed supplements using her research tools in metagenomics and next generation throughput sequencing.

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The guiding mission of Dr. Hankenson’s research program is to elucidate cellular and molecular mechanisms regulating bone formation. Bone is a highly dynamic tissue, formed by osteoblasts and resorbed by osteoclasts. Osteoblasts develop from progenitor cells, termed mesenchymal stem cells (MSC), and Dr. Hankenson’s laboratory studies molecular and cellular mechanisms of MSC osteoblast differentiation (osteoblastogenesis).

This research has two long-term translational goals: (1) developing therapies to increase peak bone mass and/or restore lost bone and (2) enhancing bone regeneration in both humans and animals, particularly in populations with poor healing, such as geriatric patients, and those with compromised non-healing fractures.

A group of specialized ECM proteins termed matricellular proteins (MP) are highly expressed in the skeleton by osteoblast lineage cells and for over 18 years Dr. Hankenson has been interested in understanding how MP influence osteoblastogenesis in an autocrine manner (1). His work has used thrombospondin-2 (TSP2), as a model MP molecule for many of these studies, and his work was the first to definitively demonstrate a role for one of the TSPs in regulating osteoblastogenesis (2). TSP2-null mice had

an increase in bone formation, which was further linked to an increase in MSC proliferation and number.

Dr. Hankenson’s research has also pursued the significance of TSP2 in bone regeneration. TSP2 is highly expressed in healing tissues and the impact of TSP2 deficiency is often more profound during injury. Their work was the first to show a significant role for TSPs in bone regeneration (4,5). TSP2-null mice have increased proliferation of undifferentiated mesenchyme in healing bone resulting in formation of larger calluses, a critical element of bone repair. Additionally TSP2-null fractures show an increase in callus MSC developing into osteoblasts rather than chondrocytes. This MSC fate-shift results in enhanced direct bone formation via intramembranous ossification and a reduction in indirect bone formation via endochondral ossification (4). Furthermore, in the most recent study, in collaboration with Dr. Jaimo Ahn, an orthopaedic trauma surgeon at the Perelman School of Medicine, his laboratory has shown that TSP2-null mice heal ischemic

fractures much faster than WT mice (5).

Current work with Dr. Ahn that is being conducted in collaboration with Dr. Danny Kelly, Trinity College - Dublin, is asking whether alterations in TSP2-null fracture callus MSC differentiation occur secondarily to increased vascularization and oxygenation and decreased hypoxia inducible factor 1 (HIF)-alpha in MSC. HIF1-alpha enhances chondrogenic differentiation, and may inhibit osteoblastogenesis. They hypothesize that decreased HIF1-alpha in TSP2-null mice results in undifferentiated MSC becoming osteoblasts rather than chondrocytes and they have recently published on the development of a computational model that supports this hypothesis (6). Additionally, in collaboration with Drs. Jeff Isenberg, University of Pittsburgh, and Ralph Marcucio and Ted Mclau, University of California-San Francisco, they are determining whether TSP2 blockade can be used therapeutically to enhance ischemic bone regeneration, which develops during severe traumatic injuries when vasculature is compromised.

Dr. Hankenson’s work with TSP2 led to a set of new experiments focused on Notch signaling in MSC osteoblastogenesis. Notch signaling is a cell-to-cell



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signaling mechanism whereby a ligand (Delta-like and Jagged gene families) located on an adjacent cell binds to a Notch receptor on the signal receiving cell. Working collaboratively with Dr. Michael Wang from the University of Michigan, they showed that TSP2 is a direct regulator of Notch signaling (7). Mice with alterations in Notch signaling and humans with a deficiency in Jagged-1 have alterations in bone mass. In collaboration with Dr. Kathy Loomes at CHOP, the Hankenson laboratory has studied the role of the Notch ligand Jagged-1 in the osteoblast lineage. Dr. Loomes generated Jagged-1 floxed/floxed mice and these mice have been crossed with osteoblast-lineage expressing Cre mice to examine the significance of Jagged-1 in osteoblastogenesis. This work with the Jagged-1 floxed mouse is under preparation for publication. Finally, most recently, the Hankenson laboratory published on the osteoinductive influences of Jagged-1 on human osteoblastogenesis (8). The publication of this work in early 2013 was the subject of a Penn News Release and was widely covered, including consideration as the “Top Story from Stem Cells” in the Mesenchymal Cell News 5.06. (see Fig 1)

As a translational extension of this work, the Hankenson laboratory has become very interested in the role of Notch signaling in bone regeneration. Notch has been implicated in regulating muscle regeneration. Particularly, decreases in muscle regeneration with aging are associated with decreased Notch signaling. This exploration of Notch has led to four funded grants and in 2011 they published the first comprehensive description of Notch signaling activity in bone regeneration (9). The Hankenson laboratory is now pursuing several lines of investigation to ask about the role of Notch signaling in bone healing. Specifically Jagged-1 floxed/floxed and dominant negative Master-mind Like protein (dnMAML) expressing mice have been fractured and bone healing evaluated to ask about the requirement of canonical Notch signaling and Jagged-1, in regulating bone healing.

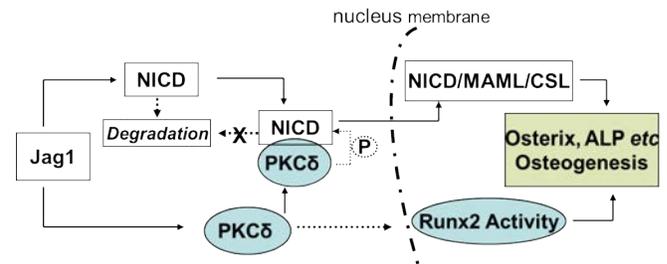


Fig 1. Model of Jagged-1 regulation of hMSC osteogenesis. Dotted lines indicate hypothesized mechanism of activity. In this model Jag1 increases NICD, and increases PKC-delta activity, but not protein levels. NICD and PKC-delta interact, and we hypothesize that NICD is phosphorylated by PKC-delta, which promotes its stability and nuclear translocation. Canonical Notch signaling transcriptional complex (NICD/MAML/CSL) may then directly promote osteoblast gene expression. PKC-delta may also promote Runx2 activity, as has been previously demonstrated.

The study of fracture healing in dnMAML mice has recently been published in PLoS One (10), where it was shown that Notch signaling is required for multiple steps in fracture healing. Analysis of fractures in Jagged-1 deficient mice is currently underway. Dr. Hankenson is also working on the development of Jagged-1 delivery as an osteogenic molecule for bone regeneration and the development of this Jagged-1 coupled biomaterial has led to a patent application and the formation of a company, Skelegen.

Dr. Hankenson’s office is located in the Myrin Building at New Bolton Center.



References:

1. Alford, A. I., and Hankenson, K. D. (2006) Matricellular proteins: extracellular modulators of bone development, remodeling, and regeneration. *Bone* 38, 749-757
2. Hankenson, K. D., Bain, S. D., Kyriakides, T. R., Smith, E. A., Goldstein, S. A., and Bornstein, P. (2000) Increased marrow-derived osteoprogenitor cells and endosteal bone formation in mice lacking thrombospondin 2. *J Bone Miner. Res.* 15, 851-862

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**Trial in Babies begun--
hope based on successful
trials in animal models**



The National Foundation for Ectodermal Dysplasias has announced an exciting research development in a human clinical trial for X-linked hypohidrotic ectodermal dysplasia (XLHED), resulting from the translational research performed by associate professor Margret Casal, Department of Clinical Studies Philadelphia.

X-linked hypohidrotic ectodermal dysplasia (XLHED) is a rare developmental defect characterized by sparse hair, an inability to sweat, decreased tear production (lacrimation), decreased production of saliva, the absence of nasal, tracheal, and bronchial glands, and missing and malformed teeth. As sweating is a major regulator of body temperature, an inability to sweat can lead to life-threatening increases in body temperature (hyperthermia). In addition, the lack of nasal, tracheal, and bronchial glands greatly increases the risk of potentially fatal respiratory tract infections.

XLHED is caused by a genetic defect in EDA, a gene encoding ectodysplasin. Binding of ectodysplasin to the EDA-A1

receptor activates signaling pathways thought to drive normal development of sweat, lacrimal, and bronchial glands, as well as tooth and hair development. Drs. Casal, Haskins and Mauldin have been instrumental in characterizing dogs that suffer from X-linked ectodermal dysplasia, a canine disease that is also driven by genetic defects in EDA. Indeed, their published results clearly demonstrate that the XLHED canine model faithfully recapitulates human disease and serves as a better translational model than do current rodent models. Moreover, the Penn group demonstrated that administration of a recombinant ectodysplasin fusion protein (Fc:EDA1) to newborn XLHED dogs resulted in a pronounced improvement on permanent dentition and long-term resistance to eye and airway infections, the latter due to a partial restoration of tracheal and bronchial glands that restored the impaired mucociliary clearance observed in XLHED. With the exception of hair development, their results demonstrate that short-term neonatal treatment with a recombinant protein can reverse many aspects of this developmental disease, with a consequent reduction in mortality and morbidity. Based on these ground-breaking results, ectodysplasin replacement protein EDI200 (Edimer Pharmaceuticals) is currently being tested for its ability to reverse the devastating effects of EDA deficiency in human XLHED patients.

Mauldin EA, Gaide O, Schneider P, & Casal ML (2009) Neonatal treatment with recombinant ectodysplasin prevents respiratory disease in dogs with X-linked ectodermal dysplasia. *Am. J. Med. Genet. 149A(9): 2045-9. Funding from the National Foundation for Ectodermal Dysplasias*

Calendar

January 23, 2014

Faculty Research Resources Orientation
12 noon in 132 HILL Pavilion

March 11, 2014

Phi Zeta Student Research Day
12 noon - 6 pm Hill Pavilion

Publications



Atanasiu D, Saw WT, Gallagher JR, Hannah BP, Matsuda Z, Whitbeck JC, Cohen, GH and **Eisenberg RJ** (2013) Dual split protein-based fusion assay reveals that mutations to herpes simplex virus (HSV)

glycoprotein gB alter the kinetics of cell-cell fusion induced by HSV entry glycoproteins. *J Virol* 87(21): 11332-45.



H Zheng, V Gupta, J Patterson-Fortin, S Bhattacharya S, K Katlinski, J Wu, B Varghes, Cj Carbone, B Aressy, **SY Fuchs, & RA Greenberg.** (2014) A BRIS-SHMT complex

deubiquitinates IFNAR1 and regulates interferon responses. *Cell Rep* 5(1): 180-93.



D.W. Pitta, S. Kumar, B. Veiccharelli, N. Parmar, B. Reddy, C.G. Joshi (2014) Bacterial diversity associated with feeding dry forage at different dietary

concentrations in the rumen contents of Mehshana buffalo (*Bubalus bubalis*) using 16S pyrotags. *Anaerobe* 25: 31-41.

KURT HANKENSON continued

3. Shitaye, H. S., Terkhorn, S. P., Combs, J. A., and Hankenson, K. D. (2010) Thrombospondin-2 is an endogenous adipocyte inhibitor. *Matrix Biol* **29**, 549-556
4. Taylor, D. K., Meganck, J. A., Terkhorn, S., Rajani, R., Naik, A., O'Keefe, R. J., Goldstein, S. A., and Hankenson, K. D. (2009) Thrombospondin-2 Influences the Proportion of Cartilage and Bone During Fracture Healing. *J Bone Miner Res* **24**, 1043-1054
5. Miedel, E., Dishowitz, M. I., Myers, M. H., Dopkin, D., Yu, Y. Y., Miclau, T. S., Marcucio, R., Ahn, J., and Hankenson, K. D. (2013) Disruption of thrombospondin-2 accelerates ischemic fracture healing. *J Orthop Res* **31**, 935-943
6. Burke, D., Dishowitz, M., Sweetwyne, M., Miedel, E., Hankenson, K. D., and Kelly, D. J. (2013) The role of oxygen as a regulator of stem cell fate during fracture repair in TSP2-null mice. *J Orthop Res* **31**(10): 1585-96.
7. Meng, H., Zhang, X., Hankenson, K. D., and Wang, M. (2009) Thrombospondin2 potentiates notch3/Jagged1 signaling. *J Biol Chem* **284**, 7866-7844
8. Zhu, F., Sweetwyne, M. T., and Hankenson, K. D. (2013) PKCdelta Is Required for Jagged-1 Induction of Human Mesenchymal Stem Cell Osteogenic Differentiation. *Stem Cells* **31**, 1181-1192
9. Dishowitz, M. I., Terkhorn, S. P., Bostic, S. A., and Hankenson, K. D. (2012) Notch signaling components are upregulated during both endochondral and intramembranous bone regeneration. *Journal of Orthopaedic Research* **30**, 296-303
10. Dishowitz, M. I., Mutyaba, P. L., Takacs, J. D., Barr, A. M., Engiles, J. B., Ahn, J., and Hankenson, K. D. (2013) Systemic inhibition of canonical Notch signaling results in sustained callus inflammation and alters multiple phases of fracture healing. *PLoS One* **8**, e68726



CLINICAL RESEARCH PILOT FUNDING by the *Companion Animal Research Fund*

Pilot funding was recently awarded by the **Companion Animal Research Fund** to the following faculty members for their research projects:

Sue Volk and Kim Agnello: “Long term, clinical outcome of patellofemoral osteoarthritis in dogs with naturally occurring unilateral cruciate ligament disease”

Erica Reineke: “Evaluation of a continuous glucose monitoring system for detection of hypoglycemia in ill pediatric dogs & cats”

Mark Oyama: “Pilot study of N-terminal pro-B-type natriuretic peptide (NT-proBNP) guided

therapy for dogs with congestive heart failure due to chronic valve disease”

Meryl Littman and JD Foster: “The use of silymarinin canine protein losing nephropathy”

Mary Beth Callan: “Efficacy of gene transfer using adeno-associated pseudotype 8 viral vector to deliver the genes for canine factors VIII & IX to privately owned dogs with hemophilia A & B respectively”

Mary Beth Callan: “Effect of duration of red blood cell storage on transfusion associated inflammation in dogs with immune mediated hemolytic anemia”

Dorothy Brown: “A phase 2 trial of oral toceranib phosphate & amputation in dogs with naturally occurring appendicular osteosarcoma”

Cynthia Otto: “A novel therapeutic strategy for treatment of canine sepsis”.



RESEARCH STIMULATED IN AQUATIC ANIMAL MEDICINE



Research fellow Toni Erkinbarju

2014 AQUAVET® I & II & III

The University of Pennsylvania and Cornell University are pleased to announce the 2014 AQUAVET® I, II & III Programs. They are aquatic veterinary medicine education programs (introductory and advanced) that will be presented at various venues in 2014. Veterinary students can receive credits for the course and graduate veterinarians can receive CE credits. Specific information can be found at www.aquavet.info. Applications due by January 15, 2014.

AQUAVET® I: An Introduction to Aquatic Veterinary Medicine is a 4-week course (25 May - 21 June 2014) intended primarily for veterinary students.

AQUAVET® II: Comparative Pathology of Aquatic Animals is a 2-week course (25 May - 7 June 2014) that is oriented toward the pathology of diseases of aquatic invertebrates and fish that are used in biomedical research, encountered in display aquaria and are of importance in commercial aquaculture.

AQUAVET® III: Clinical Aspects of Captive Aquatic Animal Medicine is a 5 week course (following AQUAVET® I) and is limited to a small number of students. The venues include GA Aquarium, U of GA and Dolphinaris, Cancún, México.

FISH IMMUNOLOGY AT PENN VET



Rainbow Trout (a teleost fish)



Professor J. Oriol Sunyer



Fumio Takizawa, Postdoctoral Fellow

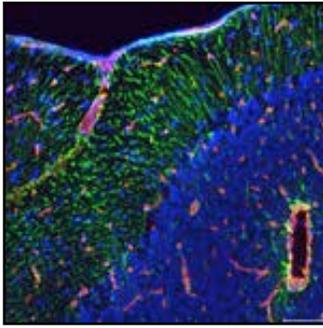


Filtration System



Fish immunologist, **J. Oriol Sunyer**, professor in the Department of Pathobiology, examines his new aquarium system where he conducts studies to elucidate the role of the complement system of Teleost fish in innate and adaptive immunity. An elaborate aquarium is in place with a complex filtration system that filters the incoming city water. Recent research from the Sunyer group has provided a fascinating insight into evolutionary origins of mucosal immune defenses.

RECENT AWARDS



Reactive astrocytes (green) during toxoplasmic encephalitis showing host cell nuclei (blue)

Charles Vite

UPENN Center for Orphan Disease Research and Therapy. "Antemortem 3D EPSI metabolic mapping of the canine MPS I brain".
\$150,000 12/1/13--11/30/14

Gus Aguirre

Poodle Club of North America FDN Genetic test development for optic nerve hypoplasia, micropapillae and juvenile cataracts in poodles
\$149,500 2/15/14--2/14/17

Beth Callan

AKC-Canine Health FDN Effect of duration of red blood cell storage on transfusion-associated inflammation in dogs with immune-mediated hemolytic anemia.
\$56,750 1/1/14--12/31/15

Rebecka Hess

AKC-Canine Health FDN Clinician Scientist fellowship for A. Bertalan
\$12,000 11/1/13--12/31/14

Robert Greenberg

Gates Grand Challenges Explorations Helminth ABC transporters as targets for combination therapy
\$100,000 11/1/13 -- 4/30/15

Carolina López

UPENN Research Foundation Defective viral genomes in human respiratory secretions
\$50,000 8/1/13 -- 7/30/14

Ron Hartly

NIH R21 Innate immune regulation of intracellular pathways involved in Filovirus budding
\$247,500 12/1/13--11/30/15

James Lok

NIH R01 Insulin-like signaling in parasitic nematode development
\$1,235,000 9/20/13--8/31/18

Chris Hunter

NIH R01 IL-27 and Treg Cells
\$1,125,000 12/1/13--11/30/18

Serge Fuchs

NIH P01 (C Koumenis Prime PI) The unfolded protein response in cancer
\$3,671,501 9/13/13--8/31/18

Ellen Puré

Wistar Institute/flow through NIH/NCI (PA2) Mammalian Regeneration, high fat diets and breast cancer: A common link?
\$165,300 08/16/13 – 06/30/14

Marie Eve Fecteau

Commonwealth of PA Comparison of Johne's Disease prevalence on organic and conventional dairy farms in Pennsylvania
\$17,000 1/1/2014-6/30/2014

Kurt Hankenson

Commonwealth of PA Cellular changes in bone remodeling associated with laminitis
\$25,000 1/1/2014-6/30/2014

Billy Smith

Merck Animal Health Effectiveness and safety of Fertagyl injection for use with cloprostenol sodium to synchronize estrous cycles to allow fixed-time artificial insemination in lactating dairy cows compared to a saline control
\$163,633 11/1/2013-4/30/2014

Dieter Schifferli

USDA Allelic variation of Salmonella colonization factors \$350,000 9/1/13--8/31/16



From the Furness Building 1888, Penn Campus

Dr Nicola Mason was recently invited to a charity event organized by Sgt Matty Giuliano of the Monmouth County SPCA in aid of her cancer vaccine research for lymphoma. The event was held at Buttonwood Manor in North Matawan. Sgt. Giuliano's dog Remy was recently diagnosed with lymphoma. The event raised \$5160 for lymphoma vaccine research.



Recent Papers



Defective viral genomes arising in vivo provide critical dangers signals for the triggering of lung antiviral immunity. Tapia K, Kim WK, Sun, Y, Mercado-Lopez X, Dunay E, Wise M, Adu M, & **López CB**. *PLoS Pathog*

9(10):e1003703., 2013 (featured article)

Highly Immunostimulatory RNA Derived from a Sendai Virus Defective Viral Genome. Mercado-López M, Cotter CR, Kim WK, Muñoz L, Tapia K, and **López CB**. *Vaccine* 31(48): 5713-21, 2013

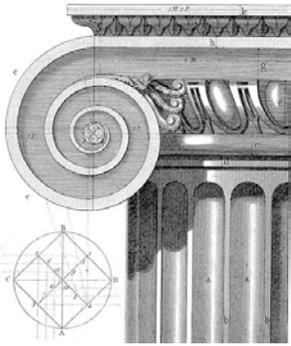


Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis.

Alenghat T, Osborne LC, Saenz SA, Kobuley D, Ziegler CG, Mullican SE, Choi I, Grunberg

S, Sinha R, Wynosky-Dolfi M, Snyder A, Giacomini PR, Joyce KL, Hoang TB, Bewtra M, **Brodsky IE**, Sonnenberg GF, Bushman FD, Won KJ, Lazar MA, **Artis D**. *Nature*. 2013 504(7478): 153-7 [Epub 2013 Nov 3]

NIH news: *individual development plans*



NIH encourages institutions to assist graduate students and postdoctoral researchers to achieve their career goals within the biomedical research workforce through the use of Individual Development Plans (IDPs). **Institutions are encouraged to report on this in all progress reports submitted on/after October 1, 2014**, using the Research Performance Progress Report (RPPR).

Training grant recipients that use the PHS 2590 progress report should include information to document that IDPs are used to help manage the training for graduate student and postdoctoral researchers in the progress report.

A goal of the NIH Director’s working group of the Advisory Committee is to better prepare biomedical graduate students and postdoctoral scientists to successfully participate in an evolving economy.

For more information go to this link: <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-13-093.html>

The Penn Vet Research Newsletter is distributed quarterly. Suggestions, requests, comments, and story ideas should be directed to: resnews@vet.upenn.edu

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seminar.....

“Working dog research--more than playing with puppies”

Thursday, January 30 2014

Dr. Cynthia Otto

4 pm 132 Hill Pavilion