Fourth International Research Conference on Multiple Hereditary Exostoses

Philadelphia, PA
November 1st – 4th, 2012

Sponsored By:
The National Institutes of Health
The MHE Research Foundation
The Children’s Hospital of Philadelphia Research Institute
The Children’s Hospital of Philadelphia, Division of Orthopaedic Surgery
Shriners Hospitals for Children
Fourth International Research Conference on Multiple Hereditary Exostoses

November 1st – November 4th, 2012

The Inn at Penn
Philadelphia, PA

Organizers

Maurizio Pacifici
The Children’s Hospital of Philadelphia

Sarah Ziegler
The MHE Research Foundation

Jennifer Rosa
The Children’s Hospital of Philadelphia

Advisory Group

Jacqui Hecht
University of Texas Medical School at Houston

Hank Kronenberg
Massachusetts General Hospital and Harvard Medical School

Karen Lyons
University of California – Los Angeles

Henry Roehl
The University of Sheffield

Luca Sangiorgi
Rizzoli Orthopaedic Institute

Yu Yamaguchi
Sanford Burnham Medical Research Institute
Saturday, November 2nd

7:00-8:00 Continental Breakfast (Regent Ballroom)

8:00-8:30 Welcome and introduction
Sarah Ziegler, Craig Eaton, Maurizio Pacifici

8:30-10:00 Session 1:
Clinical manifestations of MHE: what can/do surgeons treat?
Chair: Kevin Jones
Discussants: Scott Kozin, Kevin Jones

8:30-9:15 John Dormans
"MHE in Children"

9:15-10:00 Dror Paley
"MHE in Adults"

10:00-10:30 Coffee Break (St. Mark's Foyer)

10:30-12:00 Session 2:
Genetics of MHE: is there a genotype/phenotype correlation?
Chair: Jacqui Hecht
Discussants: Jacqui Hecht, Dan Wells, Matt Warman

10:30-11:15 Wim Wuyts
"Genotype-phenotype studies in MO: current status"

11:15-12:00 Struan Grant
"Genome wide approaches to MHE: tackling the remaining unknown genetic etiology and related severity differences"

12:00-13:00 Lunch (Regent Ballroom)

13:00-16:30 Session 3:
Pathogenic processes during MHE
Chair: Matt Warman

13:00-13:30 Matthew Hilton
"An uneven heparan sulfate gradient within skeletal elements is the likely cause for MHE-related phenotypes"

13:30-14:00 Kevin Jones
"Polarity in peripheral chondrosarcoma"

14:00-14:30 Henry Roehl
"Development of a new tool for the analysis of the PCP pathway's involvement in exostosis formation."

14:30-15:00 Carlos de Andrea
"Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT"

15:00-15:30 Coffee Break (St. Mark's Foyer)
Yu Yamaguchi  
“Involvement of the osteoblastic lineage in MHE”

Andrea Vortkamp  
“Growth factor signaling in osteochondroma development”

Session 4: Presentations by Young Investigators  
Chair: Cathy Merry

Speakers:
Margot Bowen  
“Ptpn11 positively regulates chondrocyte maturation”

Julianne Huegel  
“Homeostasis and degradation of heparan sulfate: Involvement in normal and pathological skeletogenesis”

Overview of the day and general discussion  
Discussion leaders: Jacqui Hecht and Maurizio Pacifici

Saturday, November 3rd

7:00-8:00 Continental Breakfast (Regent Ballroom)

8:00-10:00 Session 5: Related skeletal diseases: what do they tell us about MHE?  
Chair: Hank Kronenberg

Speakers:
Fred Kaplan  
“Osteochondromas: A BuMP in the FOP road”

Wentian Yang  
“Shp2 deficiency in perichondrial groove of Ranvier cells causes metachondromatosis”

Ben Alman  
“Hedgehog signaling, isocitrate dehydrogenase, and tumor initiating cells in the growth plate, enchondromas, and chondrosarcomas”

Marion Kusche-Gullberg  
“Fibroblast EXT1-levels influence tumor cell proliferation and migration in composite spheroids”

10:30-12:30 Session 6: Biology of heparan sulfate I: lessons from multiple systems  
Chair: Marion Kusche-Gullberg

Speakers:
Robert Linhardt  
“Heparan sulfate biosynthesis, synthesis and analysis”

Xinhua Lin  
“Molecular mechanisms of heparan sulfate proteoglycans in developmental signaling pathways in Drosophila”
11:30-12:00  
**Jeremy Turnbull**  
“Chemical biology of heparan sulfates: From insights to applications”

12:00-12:30  
**Jeffrey Esko**  
“Drug discovery for Multiple Hereditary Exostoses”

12:30-13:30  
Lunch (Regent Ballroom)

13:30-15:30  
**Session 7:**  
**Skeletal developmental biology I: insights on MHE**  
Chair: Matthew Hilton

13:30-14:00  
**Stina Schipani**  
“Dual role of VHL in limb bud mesenchyme”

14:00-14:30  
**Michael Underhill**  
“Skeletogenesis and the retinoic acid signaling pathway”

14:30-15:00  
**Karen Lyons**  
“BMP and TGFβ signaling pathways in chondrogenesis”

15:00-15:30  
**Hank Kronenberg**  
“How PTHrP regulates chondrocyte differentiation”

15:30-16:00  
Coffee Break (St. Mark’s Foyer)

16:00-16:30  
**Special Lecture**

Speaker:  
**Matt Warman**  
“Applying massively parallel sequencing and reversible animal models to the study of skeletal disease”

16:30-18:00  
**Session 8:**  
**Skeletal developmental biology II: insights on MHE**  
Chair: Karen Lyons

16:30-17:00  
**David Ornitz**  
“Regulation of skeletal development through osteoblast FGF signaling”

17:00-17:30  
**Bjorn Olsen**  
“Vascular endothelial growth factor in bone formation”

17:30-18:00  
**Linda Sandell**  
“Role of Site-1 Protease in Skeletal Development”

18:00-18:30  
**Overview of the day and general discussion**  
Leaders: David Ornitz, Jeff Esko and Henry Roehl

19:30-22:30  
**Offsite Dinner and Celebration**  
*Pod*  
3636 Sansom Street

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**Sunday, November 4th**

7:30-8:30  
Continental Breakfast (Regent Ballroom)
8:30-9:00  **Special Lecture**

*Luca Sangiorgi*

"MicroRNAs profiling of multiple osteochondromas: identification of disease-specific and normal cartilage signatures."

9:00-10:30  **Session 9: Biology of heparan sulfate II: lessons from multiple systems**

Chair: Jerry Turnbull

Speakers:

9:00-9:30  **Cathy Merry**

"Stem cell differentiation as a model to understand heparan sulphate-mediated control of signalling pathways."

9:30-10:00  **Joseph Yost**

"Are there distinct glycocodes in heparan sulfate fine structure that regulates biological functions?"

10:00-10:30  **Rahul Warrior**

"Heparan sulfate proteoglycan synthesis is translationally regulated in Drosophila"

10:30-11:00  Coffee Break (St. Mark's Foyer)

11:00-12:30  **Future directions and goals for MHE research**

Discussion leaders: Advisory group members
Yu Yamaguchi, Hank Kronenberg, Jeff Esko, Luca Sangiorgi, Karen Lyons, Henry Roehl and Maurizio Pacifici.

12:30  Adjourn
Session 1

Clinical manifestations of MHE: what can/do surgeons treat?

Friday, November 2, 2012

8:30-10:00
Clinical Manifestations of MHE: What Can / Do Surgeons Treat?

“MHE in Children”

John P. Dormans, M.D. 1  Department of Orthopaedic Surgery 1, The Children’s Hospital of Philadelphia, Philadelphia, PA.  dormans@email.chop.edu

Multiple Hereditary Exostoses (MHE) is a debilitating autosomal dominant disorder (with 96% penetrance) that affects approximately 1:50,000. MHE causes cartilage capped prominences to develop from epiphyses of long bones, usually as a result of a mutation in genes EXT1 or EXT2. The EXT1 and 2 proteins function in the biosynthesis of heparin sulfate proteoglycans, which in turn, are involved in several signaling pathways within normal epiphyseal cartilage. Patients with MHE usually suffer pain, limited ROM, numbness and/or tingling (from nerve compression).

The main concerns of an orthopaedic surgeon for MHE patients include limb deformities and/or length discrepancies, impingement or pressure upon surrounding muscles, blood vessels, tendons, ligaments, nerves, or even spinal cord; fractured exostoses, or malignant degeneration (0.5-5% of cases). Depending on the severity of symptoms, and the number of exostoses and their locations, orthopaedic surgeons may treat patients with observation, simple excision, or more extensive surgery that may require bone reconstruction.

Multiple Hereditary Exostoses can affect many members of families and can cause pain and other symptoms, as well as require extensive orthopaedic care. Treatment of MHE is usually unique to each patient, depending on the severity of their symptoms.
Session 2

Genetics of MHE: is there a genotype/phenotype correlation?

Friday, November 2, 2012

10:30-12:00
In approximately 90% of patients affected with Multiple Osteochondromas (MO)/Hereditary Multiple Exostosis (HME) an EXT1 or EXT2 mutation is detected by standard sequencing/deletion/duplication analysis of these two genes. 65% of the mutation positive patients show an EXT1 mutation while the remaining 35% harbour an EXT2 mutation.

Almost all mutation negative patients have no familial history for MO. We therefore performed deep resequencing of both EXT1 and EXT2 exons and detected (low) mosaic mutations in lymphocytes of these patients. This further confirms the hypothesis that also in these patients EXT1 or EXT2 are involved in the development of osteochondromas.

MO is characterized by great clinical variability, both inter and intra familial. Several EXT genotype/phenotype studies have been performed in the past an attempt to explain this. The study design and outcome of these studies will be discussed.

As the causal EXT mutation cannot explain the intra familial clinical variation, additional studies including promoter and whole genome association studies have been performed in MO patients. This has identified potential modifiers of the MO phenotype.
Hereditary Multiple Exostoses (HME) or Multiple Osteochondromas (MO) is a heterozygous-dominant and relatively common orthopedic condition characterized by development of numerous bony protuberances (exostoses) on long bones and other sites.

Although the majority of the genetic etiology of HME has been resolved, through the uncovering of \textit{EXT1} and \textit{EXT2} mutations, there is still a need to characterize the remaining genetic contributors to the disease. In addition, despite individuals harboring the same given mutation, marked severity differences between subjects have been observed. These remaining genetic challenges in the HME field lend themselves well to the application of new high throughput genomic technologies.

The Children’s Hospital of Philadelphia (CHOP) established a Center for Applied Genomics that uses genome wide single nucleotide polymorphism (SNP) genotyping platforms. To date, the center has genome wide genotyped in excess of 100,000 subjects, allowing one to investigate the genetic component of various pediatric traits based on anonymized medical record information. In addition, CHOP is equipped with high throughput sequencing technology to facilitate further research into the genetic basis to pediatric disease pathogenesis.

As such, CHOP is well placed to address the genetic challenges that remain in the HME field with respect to the unsolved genetic component and the underlying genetic contributions to differences in disease severity.
Session 3

Pathogenic processes during MHE

Friday, November 2, 2012

13:00-16:30
An uneven heparan sulfate gradient within skeletal elements is the likely cause for MHE-related phenotypes

Alana M. Osinski¹, Yu Yamaguchi², Raymond Boot-Handford³, Israel Vlodavsky⁴, and Matthew J. Hilton¹
¹Department of Orthopaedics and Rehabilitation, Center for Musculoskeletal Research, University of Rochester Medical Center, Rochester, NY, USA. ²Sanford Children’s Health Research Center, Sanford Burnham Medical Research Institute, La Jolla, CA, USA. ³Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom. ⁴Cancer and Vascular Biology Research Center, Bruce Rappaport Faculty of Medicine and Research Institute, Technion, Haifa, Israel. Matthew_Hilton@URMC.Rochester.edu

Multiple Hereditary Exostoses (MHE) is an autosomal dominant bone disorder characterized by the development of cartilage-capped bone growths projecting from the metaphyses of long bones, bowing of bones, and short stature. MHE affected individuals are haploinsufficient for either the EXT1 or EXT2 genes, although the pathogenic mechanisms underlying MHE remain unclear. EXT proteins control heparan sulfate (HS) chain elongation on cell surface heparan sulfate proteoglycans (HSPGs), which are known to regulate cell signaling within cartilage. It has been proposed that MHE phenotypes require a stochastic loss-of-heterozygosity (LOH) for either EXT allele in cartilage that would create an uneven HS and signaling gradient impairing normal chondocyte-to-chondrocyte and chondrocyte-to-osteoblast communication. To establish an appropriate mouse model of MHE and formally prove this hypothesis, we developed several conditional Ext1 mutant mice. The first model creates a homozygous Ext1 deletion in all chondrocytes (Matrilin1Cre;Ext1f/f)(Ext1Mat1). Consistent with the stochastic LOH hypothesis, histological analyses demonstrate that Ext1Mat1 mutant mice do not develop exostoses-like phenotypes, although exhibit delays in chondrocyte maturation and bowing of some bones during development. The second genetic model, Col2a1CreTM;Ext1f/f (Ext1Col2TM), results in a stochastic, homozygous deletion of Ext1 alleles primarily within chondrocytes. Histological analyses demonstrate that Ext1Col2TM mutant mice develop signs of exostosis formation, hypertrophic cartilage changes, and bowing of bones during development. It is likely that Ext1Col2TM mutants and MHE affected individuals have uneven HS gradients within cartilage resulting in impaired signaling and exostosis development. To rescue exostosis-like development observed in Ext1Col2TM mutants, we generated Ext1Col2TM mice carrying a heparanase allele (Ext1Col2TM;tg-Hpa) to reduce HS chains on HSPGs evenly throughout all tissues and restore even signaling gradients within the cartilage, albeit reduced in HS. Surprisingly, the addition of heparanase to Ext1Col2TM mutants rescues not only exostosis-like development, but also the hypertrophic cartilage changes and bowing of bones.
Polarity in Peripheral Chondrosarcoma

Kevin B. Jones, Carlos E. de Andrea, Judith V. M. G. Bovee, Mario R. Capecchi
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One of the most frightening complications of multiple hereditary exostoses (MHE) is malignant degeneration of an osteochondroma into a peripheral chondrosarcoma. Peripheral chondrosarcoma is a malignancy for which no treatments other than radical surgery have proven effective. These cancers frequently demonstrate grossly invasive lobular growth patterns despite generally low-grade histology. One possible explanation is that peripheral chondrosarcoma cells have lost their sense of direction during transformation. We report not only misorientation, but a decrease in the presence of primary cilia in the osteochondromas that form in our previously published mouse genetic model of MHE, achieved by Ext1 disruption in chondrocytes. We also report the first mouse genetic model of peripheral chondrosarcoma, achieved via additional conditional gene disruptions in chondrocytes. The model is completely penetrant. Evaluation of polarity in these mouse peripheral chondrosarcomas and their human counterparts demonstrates much more profound loss of orientation and cilia, offering insights into cartilage transformation.
Development of a new tool for the analysis of the PCP pathway's involvement in exostosis formation.

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Our research using zebrafish EXT mutants suggest the primary role of heparan sulphate during chondrification is to facilitate chondrocyte polarity via the PCP signalling pathway. This model is in contrast to models proposed by murine researchers which postulate that interference in chondrocyte differentiation is key to tumour formation. However, definitive proof of the PCP pathway's interaction with HS has been difficult to obtain because of the absence of transgenic read-outs of the PCP pathway in cartilage. Recently we have been developing a novel approach using multilox technology (ie Brainbow) which should allow us to express different fluorescently tagged components of the PCP pathway in a mosaic fashion. This new tool will potentially allow us to analyse PCP signalling in developing wildtype and mutant cartilage at the cellular level. I will discuss how we are setting up the system in zebrafish and its potential applications beyond the PCP pathway.
Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT

de Andrea CE, Reijnders CM, Kroon HM, de Jong D, Hogendoorn PC, Szuhai K, Bovée JV.
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Secondary peripheral chondrosarcoma is the result of malignant transformation of a pre-existing osteochondroma. Osteochondromas are caused by genetic alterations in EXT1 or EXT2: homozygous deletion of EXT1 characterizes sporadic osteochondromas, and germline mutations in EXT1 or EXT2 combined with loss of heterozygosity define hereditary multiple osteochondromas. Using FISH with an EXT1 probe, we demonstrated intratumor heterogeneity in sporadic osteochondromas by revealing different genetic alterations (retention or complete loss of EXT1) within a tumor in different areas. Additionally, cells from sporadic secondary peripheral chondrosarcomas predominantly retained one (hemizygous deleted loci) or both copies (wild-type) of the EXT1 locus. Using a targeted-tiling-resolution oligo-array-CGH, we confirmed that homozygous deletions of EXT1 or EXT2 are rare events (2/17, 12%) in sporadic secondary peripheral chondrosarcomas. Immunohistochemistry was used to confirm the presence of cells with functional and dysfunctional EXT1 in these tumors. Our data therefore point to a model of oncogenesis in which osteochondroma creates a niche in which wild-type cells with functional EXT are predisposed to acquire other genetic alterations giving rise to secondary peripheral chondrosarcoma.
Involvement of the osteoblastic lineage in MHE

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Individuals with MHE carry heterozygous loss-of-function mutations of Ext1 or Ext2, which together encode an enzyme essential for heparan sulfate (HS) synthesis. We have previously demonstrated that stochastic, biallelic knockout of Ext1 using Col2a1-based Cre leads to the formation of multiple osteochondromas and other skeletal phenotypes characteristic for MHE in mice (Matsumoto et al., PNAS. 107: 10932-10937, 2010). To further elucidate the pathogenic mechanisms of MHE, we have generated additional Ext1 conditional knockout mouse models and dissected the role of the osteoblastic lineage in the development of osteochondromas and other phenotypes of MHE.

One of these conditional knockout models is Fsp1-Cre;Ext1flox/flox. Fsp1 encodes an intracellular calcium-binding protein expressed in precursor cells of the mesenchymal lineage. Its expression is inherently upstream of terminal differentiation of various mesenchymal cell types. Although the expression of Fsp1 is not limited to skeletal tissues, its expression in developing long bone is restricted to the perichondrium and periosteum. The other model is osteocalcin (OC)-Cre;Ext1flox/flox. Among several OC-Cre transgenic lines, we used the one created in Dr. Tom Clemens’ laboratory, which is more specific for osteoblasts than the other osteocalcin-based Cre lines. Interestingly, it was found that both Fsp1-Cre;Ext1flox/flox and OC-Cre;Ext1flox/flox mice develop multiple osteochondroma-like lesions in various bones, suggesting the potential contribution of osteoblasts and their precursors in the development of MHE.

The OC-Cre;Ext1flox/flox model has also provided a novel insight into the role of HS in bone mass regulation. These mice display severe osteoporosis, which becomes detectable around at 1 month of age and progresses thereafter. Interestingly, the number of osteoblasts and the dynamic bone formation rate were normal, suggesting that ablation of HS in osteoblasts has little cell autonomous effects on osteoblastogenesis and osteoblast function. Rather, the osteoporotic phenotype in these mice is apparently due to increased osteoclastogenesis. The number of osteoclasts is increased more than 2-fold in mutant bones, and biochemical analysis of urine revealed a significant increase in bone degradation products in mutant mice. These results suggest that HS plays a key role in mediating osteoblast-osteoclast crosstalk that is known to be critical for the maintenance of bone mass. Our recent studies point to a defective function of osteoprotegerin in the absence of HS on osteoblasts. These observations are interesting in that there have been sporadic clinical reports as well as anecdotal evidence for the association of low bone mass conditions with MHE. Our collaborative study with Gifu University in Japan indicates that bone mass in Japanese MHE patients is lower than general populations. These results suggest that MHE can indeed be associated with low bone mass conditions.
Growth factor signaling in osteochondroma development

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Multiple osteochondromas is an autosomal inherited human disorder characterized by short stature and the development of benign bone tumors during childhood and adolescence. It is based on heterozygous mutations in EXT1 or EXT2, two glycosyltransferases required for the synthesis of heparansulfate (HS). To investigate the molecular basis of osteochondroma differentiation we have previously established a mouse model in which clonal inactivation of Ext1 in chondrocytes can be induced by Doxycycline treatment postnatally. Using this model we demonstrated that osteochondromas originate in an early chondrocyte subtype, which carries a homozygous deletion of Ext1, indicating that loss of heterozygosity and not haplo-insufficiency is the likely cause for tumor development in human patients.

To understand the role of HS in tumor development we have introduced loss and gain of function mutations of the Ihh and Fgf signaling systems and analyzed the frequency, differentiation and size of the osteochondromas. We found that Fgf signaling seems to influence the severity of the disease, whereas increased Ihh signaling might induce differentiation into chondrosarcomas.
Session 4

Presentation by Young Investigators

Friday, November 2, 2012

16:30-17:30


**Ptpn11 positively regulates chondrocyte maturation**

Margot E. Bowen¹, Wentian Yang² & Matthew L. Warman¹

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Patients with the rare autosomal dominant genetic disorder, Metachondromatosis, develop enchondromas and exostoses during childhood. Recently, we and others have found that loss-of-function mutations in the gene *PTPN11* are a frequent cause of Metachondromatosis (Bowen et al 2011, Sobreira et al 2010). Conditional inactivation of *Ptpn11* in mice leads to multiple skeletal defects including ectopic cartilage formation, similar to human exostoses and enchondromas (Bauler et al 2011 and Wentian Yang, unpublished). The *Ptpn11* protein product, Shp2, is a protein tyrosine phosphatase known to play a role in many signaling pathways. To elucidate the role of Shp2 in chondrocytes, we performed gene expression profiling (RNA-seq) on primary chondrocyte pellet cultures from mice carrying a conditional *Ptpn11* allele and expressing a tamoxifen-inducible Cre recombinase. In comparison to untreated pellets, we found that pellets treated with 4-hydroxy-tamoxifen, to inactivate *Ptpn11*, had decreased expression of many genes associated with chondrocyte maturation (eg *Mmp13* and *Mmp9*) and had increased expression of genes associated with proliferative or pre-hypertrophic chondrocytes (eg. *Ihh, Col2a1, Fgfr3*). These data suggest that *Ptpn11* is a positive regulator of chondrocyte maturation. Further studies will be needed to determine which signaling pathways are regulated by *Ptpn11* to control chondrocyte maturation.
Homeostasis and Degradation of Heparan Sulfate: Involvement in Normal and Pathological Skeletogenesis

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Limb skeletogenesis involves the formation of mesenchymal cell condensations that undergo endochondral ossification. This process is regulated by growth factors that bind to heparan sulfate (HS) chains. Present in the ECM and at the cell surface, the HS chains influence the distribution and bioavailability of these factors. Mutations in HS polymerizing enzymes EXT1 or EXT2 cause Hereditary Multiple Exostoses (HME), a disorder characterized by ectopic cartilaginous outgrowths typically found in long bones. We previously showed that exostosis formation is inversely related to Ext expression, accounting in part for variations in disease severity in patients. Upregulation of heparanase was also suggested to contribute to such variation. However, the exact pathogenic mechanisms of HME remain unclear.

To study these mechanisms, we modeled HME in limb bud mesenchymal micromass cultures. Because HME involves HS deficiency, we mimicked this by treating cultures with the HS antagonist Surfen. The treatment provoked a significant increase in chondrogenic differentiation and cartilage nodule formation. Additionally, when we cultured Ext1fl/fl neonatal forelimb elements in medium with Adeno-Cre, we observed ectopic cartilage formation within the perichondrium. We hypothesized that these changes could be due to enhanced activity of HS-binding pro-chondrogenic factors such as bone morphogenetic proteins (BMPs). Indeed, phosphorylated-Smad1/5/8 protein levels as well as BMP ligand and receptor expression were higher in micromass cultures treated with Surfen than controls. Surfen treatment also stimulated the responsiveness of a BMP reporter plasmid to endogenous and exogenous BMPs. Similarly, nuclear accumulation of pSmad was increased in the perichondrium in Ext1-deficient long bones in vivo and in explant culture. We also tested whether this enhanced activity was due to atypical ligand diffusion. Biochemical assays did indicate that Surfen blocked BMP/heparin binding, suggesting that BMP diffusion is enhanced in HS-deficient tissue. Lastly, we investigated heparanase expression and activity in cells and organ cultures with diminished HS function, finding increases in both. Our data reveal that interference with HS function increases cell response to BMP2, stimulating Smad phosphorylation and chondrogenic differentiation. We propose aberrant BMP signaling as a candidate pathway in HME, and suggest that this may be further enhanced by increases in heparanase activity.
Session 5

Related skeletal diseases: what do they tell us about MHE?

Saturday, November 3, 2012

8:00-10:00
Fibrodysplasia ossificans progressiva (FOP; MIM#135100) is a debilitating autosomal dominant genetic disorder of dysregulated cellular differentiation characterized by malformation of the great toes during embryonic skeletal development and by progressive heterotopic endochondral ossification postnatally. Patients with these classic clinical features of FOP have the identical heterozygous single nucleotide substitution (c.617 G>A; R206H) in the gene encoding ACVR1/ALK2, a bone morphogenetic protein (BMP) type I receptor.

Among the least explored functions of the FOP metamorphogene is its ability to stimulate osteochondromas. A recent study showed that 90 per cent of FOP patients had osteochondromas of the proximal medial tibia, and nearly 100 per cent had one or more asymptomatic osteochondromas at other sites.

Importantly, a recently described Acvr1 R206H knock-in mouse model that develops classic fibrodysplasia ossificans progressiva formed osteochondromas in the same patterns as patients with FOP. The presence of widespread osteochondromas in the FOP knock-in mouse underscores that cells of the perichondrium are highly sensitive to the direct effects of BMP signaling and its interacting pathways.

Osteochondromas are therefore a common feature of FOP in mice and humans, a finding that expands the recognized consequences of recurrent activating mutations in ACVR1/ALK2 to include not only congenital skeletal malformations and heterotopic ossification but also osteochondromas.
Shp2 Deficiency in Perichondrial Groove of Ranvier Cells Causes Metachondromatosis

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Cartilage tumors, accounting for 22% of skeletal system tumors, are characterized by the formation of exostoses, enchondroma(s) or both, and cause significant morbidity and mortality. The molecular mechanism underlying the development and progression of these cartilaginous lesions remains incompletely understood. Shp2, encoded by the Ptpn11 gene, is one of two Src homology 2 domain-containing protein-tyrosine phosphatases, and is required for most, if not all, receptor tyrosine kinase (RTK), cytokine, and integrin signaling pathways. Global deletion of Shp2 in mice results in early embryonic lethality, whereas postnatal Shp2 deficiency in various tissues/cells has diverse effects on their development and function. Several human malignancies, most notably childhood myeloproliferative disorders, are associated with Ptpn11 gain-of-function (GOF) mutations. Several lines of evidence indicate that Shp2 plays an important role in skeletal development and homoeostasis; however, little is known about its role in vivo. Recently Ptpn11 truncated mutations (presumably protein null) are reported to cause human metachondromatosis, a benign cartilage tumor syndrome with malignant potentials. By taking a tissue-specific gene knockout approach, we report here that Shp2 loss-of-function mutation in the cathepsin K-expressing perichondrial groove of Ranvier cells, in contrast to its GOF mutants in other tissues/cells, causes cartilaginous exostosis and enchondromas, strongly suggesting that Shp2 has a tissue specific-tumor suppressor function.
Hedgehog Signaling, Isocitrate Dehydrogenase, and Tumor Initiating Cells in the Growth Plate, Enchondromas, and Chondrosarcomas

Makoto Hirata, Peter Dixon, Qigxia Wei, Jay Wunder, and Benjamin Alman

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Enchondromas are benign cartilage tumors, located adjacent to the growth plate. They can occur as isolated lesions or as part of multifocal disease, and can progress to malignant chondrosarcomas. Hedgehog signaling can maintain growth plate chondrocytes in a proliferative state, preventing terminal differentiation. Human enchondromas, and human malignant chondrosarcomas exhibit constitutive activation of hedgehog signaling. Overexpression of the hedgehog activated transcription factor, Gli2, causes enchondroma like lesions in mice, thus showing a critical role for hedgehog signaling in chondrocyte differentiation.

Mutations in the isocitrate dehydrogenase (IDH) genes were recently identified in the majority of human enchondromas. How these mutations influence Hedgehog signaling, and how Hedgehog signaling regulates chondrocyte differentiation is unclear. Thus, we examined the role of IDH, and the oncometabolite, 2-hydroxyglutarate, produced by mutant IDH proteins, in the regulation of Hedgehog signaling. Mice lacking Idh1 were generated, and these did not show a growth plate phenotype, nor a change in hedgehog mediated signaling in chondrocytes. In contrast, treatment of fetal limb explants with 2-hydroxyglutarate activated hedgehog signaling and prevented chondrocyte differentiation. The changes in chondrocyte differentiation is inhibited by treatment with cyclopamine. Thus, the oncogeneic effect of the Idh mutations are likely related to the generation of the oncometabolite, and we are currently generating a knock-in mouse to determine if this is the case, and the effect of 2-hydroxyglutarate is at least partly mediated by hedgehog signaling.

Chondrosarcomas are composed of a heterogeneous population of cells, and include a small fraction of the side population (SP) cells with stem-like tumor-initiating potential. Here, we studied gene expression profiles in SP cells from chondrosarcomas. This profiling identified that Wnt signaling was activated in the SP population. To determine if Wnt signaling pays a functional role in chondrosarcoma, we established chondrosarcoma xenografts in Nod-Scid mice. The mice were treated with Wnt activators and inhibitors. Inhibiting Wnt signaling slowed tumor growth and inhibited chondrosarcoma self-renewal, as illustrated by the inability of xenograft tumors to be serially transplanted to new hosts.

Our findings show that hedgehog signaling mediate the effect of 2-hydroxyglutarate on chondrocyte differentiation. They also suggest that pharmacologic inhibition of Wnt signaling could be used to treat chondrosarcomas.
Heparan sulfate proteoglycans are ubiquitously expressed in all tissues where they function as adhesion molecules and co-receptors. Thus, they modulate cell/matrix interactions and growth factor signaling. Hereditary multiple exostoses results from mutations in either \textit{EXT1} or \textit{EXT2}, genes that encode glycosyltransferases carrying out heparan sulfate chain elongation. We have employed embryonic fibroblasts from mice with a gene trap mutation in \textit{Ext1}. The mutation results in low expression of \textit{Ext1}, and as a consequence heparan sulfate chain length is substantially reduced (1).

Using \textit{Ext1} mutant and wild-type mouse embryonic fibroblasts we have shown that the response to the growth factor stimulation is markedly decreased in the \textit{Ext1} mutant fibroblasts and that \textit{Ext1} mutants display reduced ability to attach to collagen I and to contract collagen lattices (2).

Stromal fibroblasts are important determinants of tumor cell behavior. They act to condition the tumor microenvironment, influence tumor growth, support tumor angiogenesis and affect tumor metastasis. To investigate the role of \textit{Ext1} expressed by stromal fibroblasts in modulating the growth of tumor cells and in controlling the interstitial fluid pressure we generated spheroids composed of fibroblasts alone, or composite spheroids, composed of fibroblasts and tumor cells. Our results show that stromal \textit{Ext1}-levels modulate tumor-cell migration and proliferation and affect the interstitial fluid pressure (3).

Session 6

Biology of heparan sulfate I: lessons from multiple systems

Saturday, November 3, 2012

10:30-12:30
Heparan sulfate biosynthesis, synthesis and analysis

Robert J. Linhardt, Ph.D. Biocatalysis and Metabolic Engineering Rensselaer Polytechnic Institute, Troy, NY
Email: linhar@rpi.edu

Our laboratory is focusing on developing new methods for the synthesis and analysis of heparin/heparan sulfate. Metabolic engineering of CHO cells and mastocytoma cells is underway and has afforded an improved understanding of biosynthetic control of heparin/heparan sulfate structure in the Golgi. In vitro biochemical studies on the heparin/heparan sulfate biosynthetic enzymes has also provided important information on enzyme specificity. These biosynthetic enzymes are also being used in the chemoenzymatic synthesis of heparin/heparan sulfate that are being tested in cell-based growth factor assays. In parallel with the development of new synthetic methods, our group is also examining new analytical methods for the sequencing of heparin/heparan sulfate. Robust methods for glycosaminoglycan isolation, along with their ultra-sensitive analysis, are making it possible to study the structure of heparin/heparan sulfate and other glycosaminoglycans coming from very small tissue samples requiring as little as a few hundred cells.
Molecular mechanisms of Heparan Sulfate Proteoglycans in Developmental signaling pathways in Drosophila

Yan Zhang1, Jia You2, Wenyan Ran1, Tatyana Y. Belenkaya2, Xinhua Lin1,2

1. State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
2. Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229, USA
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Heparan sulfate proteoglycans (HSPGs) are cell-surface and extracellular matrix macromolecules that are composed of a core protein decorated with covalently linked glycosaminoglycan (GAG) chains. In Drosophila, the biosynthesis of heparan sulfate is carried out by three members of EXT family proteins including tout-velu (ttv), brother of ttv (botv) and sister of ttv (sotv), which are the homologs of vertebrate EXT1, EXTL3 and EXT2, respectively. We and others have previously demonstrated that HSPGs are required for Wnt, Hh, BMP and FGF signaling pathways in variously developmental contacts. Here, we show that HSPGs are essential for Jak/Stat signaling pathway in several developmental contacts in Drosophila. We demonstrated that in the absence of Botv, Jak/Stat signaling activity is strikingly reduced in Drosophila eye and wing imaginal discs during development. We further show that cell surface levels of Unpaired, the Drosophila ligand for Drosophila Jak/Stat signaling pathway is dramatically reduced. Our analyses also indicate that Dally, a Drosophila glypican member, is essential for Jak/Stat signaling. Together, our data argue that HSPG plays essential roles in Jak/Stat signaling pathway via modulating the cell surface levels of its ligand during development.
Chemical Biology of Heparan Sulfates: from Insights to Applications

J Turnbull

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Email: j.turnbull@liverpool.ac.uk

Heparan sulfates (HSs) have diverse biological functions, underpinned by structurally diverse patterns of backbone chain modification, especially sulfate groups. These variant structures represent a molecular code, the ‘heparanome’, that confers the ability to interact selectively with a wide interactome of proteins, the “HS-interactome”. This network of proteins influences a network of cellular events relevant to many biological processes which are important in the development, regulation and repair of tissues. HS are thus strategic node molecules in cell regulation pathways. Understanding the chemical biology of these enigmatic molecules is now becoming possible through a variety of tools, reagents and approaches including saccharide libraries (for exploitation in protein interaction methods and bioassays), microarray methods (for mapping interaction and functional specificity) novel sequencing approaches (using ES mass spectroscopy) and synthetic chemistry to produce targeted libraries for activity screening. These approaches can be integrated with other ‘-omics’ methods and bioinformatics, to permit systems biology studies to decode the molecular basis of the functional diversity of HS. New insights into this code and biomedical applications will be discussed.
Identification of Multiple Hereditary Exostoses (MHE) is an autosomal dominant disorder characterized by formation of ectopic cartilage-capped growth plate-like exostoses next to long bones and other skeletal elements. MHE results from mutations in the genes Ext1 or Ext2, which diminishes the capacity of cells in the growth plate and the surrounding perichondrium to make heparan sulfate. The mechanism by which a change in heparan sulfate content causes ectopic osteochondromas is unknown, but evidence suggests that the decrease in heparan sulfate affects one or more signaling pathways through which growth factors regulate the organization of chondrocytes in the growth plate. Regardless of the mechanism, restoring heparan sulfate levels might diminish the frequency of exostoses. Thus, the objective of this project is to find small, drug-like agents to alter the metabolism of heparan sulfate. All cells make heparan sulfate through a common mechanism. Thus, we have used mutant Chinese hamster ovary (CHO) cell lines that express about 10-30% of the normal level of heparan sulfate due to mutations in Ext1 in a primary cell-based screen to find potential drug candidates that augment heparan sulfate expression. Secondary assays will test positive hits for their impact on heparan sulfate content and structure. Tertiary assays will measure if the hits modulate heparan sulfate expression in mouse chondrocytes and perichondrial cells and in human chondrocytes. Agents that enhance heparan sulfate synthesis will be evaluated for their capacity to reduce exostoses in Ext1+/-;Ext2+/- mice. The central hypothesis is that altering key enzymes involved in heparan sulfate metabolism might result in restoration of functionally normal levels of heparan sulfate and growth factor signaling. Such compounds might reduce exostoses in mice, which serves as a proof-of-principle for pharmacological manipulation of exostosis formation in MHE patients.
Session 7

Skeletal developmental biology I: insights on MHE

Saturday, November 3, 2012

13:30-15:30
Dual role of VHL in limb bud mesenchyme

Ernestina Schipani
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Von Hippel Lindau (VHL) is an E3 ubiquitin ligase, and Hypoxia-Inducible Factors (HIFs) are among its main targets. We have previously reported that deficiency of VHL in fetal chondrocytes causes severe dwarfism. To expand our understanding of VHL in the biology of cells of mesenchymal origin, we genetically deleted VHL in whole limb bud mesenchyme, i.e. both in mesenchymal cells that differentiate into the avascular cartilage and in mesenchymal cells that give origin to the vascularized soft tissue surrounding the avascular cartilaginous primordia. Deficiency of VHL in chondrocytes caused a severe shortening and thinning of the avascular cartilaginous elements, which were markedly deformed and hypocellular, and eventually collapsed as a result of cellular growth arrest, impaired terminal differentiation and death. Conversely, VHL deficiency in the vascularized soft tissue surrounding the growth plates led to massive synovial fibrosis, foci of ectopic cartilage, periosteal osteochondromas and fibrosarcoma-like masses, which invaded and replaced the collapsed cartilaginous structures. We are currently investigating whether this phenotype is mediated by HIFs.
Skeletogenesis and the Retinoic Acid Signaling Pathway

Underhill, T.M., Sampaio, A.V., Dranse, H.D., Scott, R.W.
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Skeletal development and homeostasis involve the orchestrated actions of numerous signals to regulate the commitment, expansion and differentiation of skeletal progenitors. Genetic manipulation of components of the retinoic acid (RA) signaling pathway has been shown to severely impact these processes, and this is especially apparent in mice deficient for CYP26B1, an RA-metabolizing enzyme. Cyp26b1 is expressed in the developing limb bud and Cyp26b1-/− mice present with severe limb defects. In recent studies, we have examined the role of endogenous retinoid signaling in skeletogenesis using Cyp26b1-/− mice and transgenic mice in which Cyp26b1 is conditionally deleted under control of the Prx1 promoter beginning at ~E9.5 (Prx1Cre+/Cyp26b1fl/fl). In these two models, the defect in the severity of the limb phenotype was reduced in the conditionally deleted line and this could be partly attributed to a change in RA signaling. We examined the role of endogenous RA signaling in chondrogenesis and found that Cyp26b1-/− cells and limb mesenchymal cells treated with a CYP inhibitor, are maintained in a pre-chondrogenic state and exhibit reduced chondroblast differentiation. Furthermore, Cyp26b1-/− mesenchyme exhibited an increase in expression of genes in a closely related tendogenic lineage, indicating that retinoid signals in the limb interfere with differentiation and maintains progenitor status. Activation of retinoid signaling in other mesenchymal lineages also has a similar consequence. Together, these studies indicate that RA plays an important role in regulating the behavior of mesenchymal progenitors and their subsequent differentiation.
BMPs are essential for chondrogenesis. Through characterization of mice lacking BMP receptors or the downstream transducers R-Smads, we have found that most of the effects of BMPs are transduced through R-Smads. Unexpectedly, Smad4, the co-Smad that is thought to be required for complex formation in the canonical BMP signaling pathway, has a much more limited role in chondrogenesis. Therefore, R-Smad-dependent but Smad4-independent mechanisms are more important in cartilage than previously recognized. Our current studies are directed towards elucidating these mechanisms. We are also testing whether similar R-Smad-dependent, Smad4-independent mechanisms operate in the maintenance of articular cartilage in adults. Finally, we are investigating the functions of TGFβ R-Smads and receptors in the growth plate. These latter studies suggest that TGFβ mediates the majority of its effects through non-canonical pathways. We are currently investigating the identity of these non-canonical pathways and their cross-talk with and regulation by canonical pathways.
Parathyroid hormone-related protein is secreted from cells at the end of the growth plate and acts on receptors on proliferating and prehypertrophic chondrocytes. This action delays their differentiation into chondrocytes that express Indian hedgehog (Ihh). Ihh, in turn, diffuses to the end of the growth plate to stimulate PTHrP production. Thus, both PTHrP and Ihh must negotiate paths through the extracellular matrix to reach their target cells.

How PTHrP accomplishes its actions has been incompletely understood. Previous studies showed that PTHrP stimulates the PTH/PTHrP receptor to activate Gs, the heterotrimeric G protein that activates adenylate cyclase. Genetic studies showed that Gs is the major mediator of the action of PTHrP on chondrocyte differentiation. PTHrP affects differentiation partly by suppressing expression of Runx2, a major driver of chondrocyte hypertrophy. But how this suppression is accomplished has only recently become clear. Lassar’s group used a chondrocyte cell line and primary chondrocytes to show that protein kinase A activates protein phosphatase 2A, which then dephosphorylates histone deacetylase 4 (HDAC4), driving HDAC4 into the nucleus. There, HDAC4 blocks the actions of MEF2c to stimulate expression of Runx2 and to further activate the hypertrophic program. Here we use in vivo models to demonstrate that HDAC4 is an essential mediator of the action of PTHrP and that, in vivo, PTHrP action drives HDAC4 into the nuclei of chondrocytes. Most strikingly, mice that over-express PTHrP in chondrocytes cannot stop chondrocyte differentiation if HDAC4 is knocked out. Thus, HDAC4 is a crucial mediator of the actions of PTHrP.
Session 8

Skeletal development biology II: insights on MHE

Saturday, November 3, 2012

16:30-18:00
Regulation of skeletal development through osteoblast FGF signaling

Kai Yu and David M. Ornitz
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During skeletal maturation, osteoblasts become less proliferative but grow in size to actively engage in the synthesis and secretion of bone matrix proteins, such as collagen. Although FGF signaling is essential for skeletal development, the roles of FGF signaling during osteoblast maturation could not be investigated due to developmental defects in multiple skeletal lineages. Here, we use Osterix-Cre, which is specifically activated in committed osteoprogenitor cells, to conditionally disrupt both Fgfr1 and Fgfr2 in the osteoblast lineage during skeletal development. The double condition knockout (DCKO) mice showed normal skeletal patterning and underwent normal endochondral ossification during early embryonic development. However, at late embryonic stages, DCKO mice showed reduced intramembranous ossification in calvarial bones. In the long bones, trabecular bone was significantly reduced and the newly formed bone marrow cavity was filled with undegraded cartilage matrix. Additionally, the growth plate hypertrophic zone was expanded. During early postnatal development, DCKO mice showed reduced body weight, longitudinal bone growth, and bone density. Calcein double-labeling studies indicated that DCKO mice had significantly reduced bone formation and BrdU labeling identified decreased chondrocyte proliferation. These results indicated that FGF signaling is required during osteoblast maturation to regulate osteoblast anabolic function and through an indirect mechanism growth plate function.
Vascular endothelial growth factor in bone formation

Bjorn R. Olsen, MD, PhD Hersey Professor of Cell Biology, Harvard Medical School Professor of Developmental Biology and Dean for Research Harvard School of Dental Medicine
Email: bjorn_olsen@hms.harvard.edu

Vascular endothelial growth factor A (VEGF) is critical for normal bone development, growth and homeostasis. It is a survival factor for chondrocytes in endochondral bone formation, its angiogenic activity is essential for endochondral ossification and membranous bone development, it stimulates both osteoblast and osteoclast differentiation, it controls osteoblast and adipocyte fates of mesenchymal stem cells and it inhibits BMP-induced conversion of vascular endothelial cells to mesenchymal stem-like cells (EndMT). Many of these activities, including inhibition of EndMT, involve paracrine signaling while others are the result of intracellular VEGF function. Activation of the VEGFR2 receptor in paracrine signaling is modulated by VEGF interactions with matrix components, including the decoy receptor VEGFR1. In contrast, intracellular control of differentiation of mesenchymal stem cells is either requiring both VEGFR1 and VEGFR2 (in the case of osteoblast differentiation) or is independent of these two receptors (in the case of adipocyte differentiation).
Special Lecture:
Luca Sangiorgi

Sunday, November 4, 2012

8:30-9:00
MicroRNAs profiling of Multiple Osteochondromas: identification of disease-specific and normal cartilage signatures.

Elena Pedrini¹, Alessandro Parra¹, Marco Salvatore², Armando Magrelli², and Luca Sangiorgi¹

¹. Department of Medical Genetics, Rizzoli Orthopaedic Institute, Bologna, Italy  ². National Centre for Rare Diseases, Istituto Superiore di Sanità, Roma, Italy
luca.sangiorgi@ior.it

Multiple Osteochondroma (MO) is a genetic disorder characterized by a large spectrum of related EXT1/2 mutations and a wide clinical heterogeneity. Quite little is known about the pathogenesis of the syndrome, what triggers OC formation, why MO severity varies between individuals and even family members, and how it could be treated. Similarly, it is yet not clear the molecular mechanisms involved in peripheral chondrosarcoma degeneration.

Recent evidences on microRNA expression in normal and pathologic articular cartilage are available in the literature, while no information on growth plate cartilage were reported. To evaluate whether they could play a role in determining currently unexplained features of the disease, we investigated microRNAs expression in osteochondromas and normal cartilage tissues.

Cartilage samples were obtained from 19 MO patients undergoing surgery at Rizzoli Orthopaedic Institute (Bologna, Italy), along with four normal cartilage controls (one from articular cartilage and three from healthy growth plate tissues). Microarray profiling was performed on a custom array by a service provider (LC Sciences, Houston, TX) for 5 osteochondroma samples and 2 controls; results were then validated in a larger group of patients by TaqMan MicroRNA assay kits (Applied Biosystems). DIANA-microT 3.0 and DIANA-mirPath algorithms were used in order to predict putative targets for selected microRNAs.

To identify the differentially expressed microRNA that cause clustering of patients versus control tissues, 45 microRNAs were filtered out; validating their expression by qRT-PCR on a larger cohort of patients, we identified a signature of 8 microRNAs able to properly divide MO-samples from normal controls. Gene putatively targeted by these microRNAs were found to be mainly involved in WNT and Hedgehog pathways, TGF-β, NOTCH and mTOR signaling, Heparan Sulfate and O-Glycan Biosynthesis.

MiRNA microarray analysis performed on MO samples and normal controls evidenced the presence of differential expression profiles of articular cartilage compared to growth plate cartilage and derived osteochondroma tissues. After validation by qRT-PCR, we identified a signature of 8 microRNAs able to further distinguish healthy growth plate control from MO patients. Key biological pathways that may be affected by differentially regulated microRNAs, as predicted by in silico analysis, include important pathways involved in endochondral ossification. Although these results need to be further validated in a larger group of patients, this is the first evidence of microRNAs expression in osteochondroma tissues and growth cartilage, supporting their role as gene expression regulators during MO pathogenesis.
Session 9

Biology of heparan sulfate II: lessons from multiple systems

Sunday, November 4, 2012

9:00-10:30
Stem cell differentiation as a model to understand heparan sulphate-mediated control of signalling pathways.

Claire Pickford, Rebecca Holley, Kate Meade, Donna Tillotson, Alex Smith, Marissa Maciej and Cathy Merry. Stem Cell Glycobiology Group, School of Materials, The University of Manchester, UK
Email: Catherine.Merry@manchester.ac.uk

For a number of years, our group has used mouse embryonic stem (mES) cells as a model system with which to investigate the role of heparan sulphate (HS) in various signalling events. We were able to demonstrate that, in the absence of endogenous HS (EXT1−/−) mES cells were unable to commit to differentiation, remaining halted in a 'primed' state. Interestingly, mES cells lacking a single copy of EXT1 displayed short, highly sulphated HS chains. These cells (EXT1+/-) had a phenotype quite unlike that of either the wild type or fully-HS deficient cells, with an altered morphology and a pro-differentiative phenotype. We have used directed differentiation towards specific lineages to better understand the role of HS patterning in the response of cells to key signalling events. Neural differentiation from ES cells is known to require a positive response to members of the FGF signalling family. In contrast, formation of haemangioblast requires signalling primarily via BMP and VEGF. Using HS deficient cells in a carefully controlled, GAG-free media system we can therefore assay for the specific activity of introduced saccharides and are using this to delineate the activity of specific sulphation patterns. Importantly, this assay system allows us to gain a read-out which is biologically relevant, involving multiple coordinating signalling events. Using model systems such as embryonic and adult stem cells we aim to better understand not just stem cell behaviour but also the fundamental regulation of multiple signalling pathways by HS.
Are there distinct Glycocodes in Heparan Sulfate Fine Structure that regulates biological functions?

Presented by H. Joseph Yost, with contributions from Judith M. Neugebauer, Adam B. Cadwallader, Sheila C. Samson and Brent W. Bisgrove
Molecular Medicine Program, Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT 84112
Email: jyost@genetics.utah.edu

Heparan Sulfate Proteoglycans (HSPGs), which are composed of a core protein and glycosaminoglycan (GAG) sugar chains, function in a wide variety of biological processes. However, it remains unclear whether specific modifications on GAG chains control specific processes. 3-O-sulfation is a rare GAG modification, catalyzed by a large family of 3-O-sulfotransferases (3-OSTs). Using zebrafish as a model system, we find that each member of the 3-OST family has distinct roles in development. Different members of the 3-OST family are critical for regulation of distinct cell signaling pathways, including FGF, BMP, and other pathways. Downstream responses to altered 3-OST functions include changes in transcriptional regulation, sarcomere function, cilia formation and cilia motility. We propose that individual 3-OST family members create distinct modified domains or “glycocodes” on cell surface proteoglycans, which in turn regulate diverse cell-signaling pathways.
Pure White Wings

All children are born as pure as small white doves with spirits eager to soar. With nurture and care, over time they spread their wings as they grow. With the warm summer breeze, parents take great joy in watching their kids learn to fly. Flying around the clear blue sky with endless amounts of energy. Darting gracefully between cotton cloud dreams as these little doves fly after one another while playing and chasing their dreams.

You see our pure white doves were born with the same eager spirit, but also with BROKEN WINGS. Wings that are lame, bumpy and crooked, this hampers their ability to fly. Many times they simply watch through a window as others fly to follow their dreams. They can only imagine the feeling of boundless energies of freedom. Imagining what it would be like to dart at will between their cotton cloud dreams, during the warm summer breeze.

While their wings may be broken, their SPIRITS SOAR, giving these kids the unmatched strength, courage and determination to overcome, and wisdom far beyond their years. It is their SPIRIT! Guiding them to find other adventures in life and spotting joy that others over look.

Their energies are saved for times when it's needed for them to be able to overcome the challenges they face day in and day out. Their strength is used to endure the many surgeries and pain they face, trying to repair broken wings. Hoping that this surgery will be their last.

The WINGS OF HOPE lead the way as we REACH for the CURE! in order to fix our children’s broken wings once and for all. To put an end to a life time of sitting on the side lines, watching others through the window. So they may also one day truly feel all the scenes of freedom others enjoy.

WHO ARE THESE WINGS OF HOPE? YOU ASK!

THEY ARE EVERYONE OF US!!

For you see, we are the care takers of the future, so others may one day be able to fly amongst clear warm blue skies of cotton cloud dreams. Just the way life should be. The MHE / MO / HME RESEARCH being conducted today around the world will open the WINDOW in the future and allow our children not only to FLY LIKE THE PURE WHITE DOVES THEY ARE!

BUT SOAR LIKE EAGLES!!!.

The MHE Research Foundation would like to THANK every one of you for becoming one of our WINGS OF HOPE!!!!
The MHE Research Foundation is a nonprofit organization for the support of researchers, families and physicians dealing with (MHE) Multiple Hereditary Exostoses / (MO) Multiple Osteochondroma a rare genetic bone disease. The MHE Research Foundation five point mission is to REACH, advance & support the following.

RESEARCH, to help fund research programs, so one day a treatment / cure for MHE can be found.
EDUCATION, to provide clinical information, guides to help benefit both families and physicians.
ADVOCACY, to bring awareness about this disease in all areas throughout the world.
CLINICAL, to help provide informational resources to families enabling them to find the medical care they need.
HOPE, is that the research being conducted on MHE, and the information and resources will bring a better quality of life to the families affected by this disease.

Multiple Hereditary Exostoses (“MHE”) is a genetic bone disorder in which benign cartilage-capped tumors (exostoses or osteochondromas) grow from the growth plates of long bones or from the surface of flat bones throughout the body. These exostoses can cause numerous problems, such as: compression of peripheral nerves or blood vessels; irritation of tendons and muscles resulting in pain and loss of motion; skeletal deformity; short stature; limb length discrepancy; chronic pain and fatigue; mobility issues; early onset arthritis; and an increased risk of developing chondrosarcoma. MHE is an autosomal dominant genetic disease and patients have a 50% chance of passing this disorder on to their children. It is not uncommon for MHE patients to undergo numerous surgical procedures throughout their lives to remove painful or deforming exostoses, to correct limb length discrepancies or to improve range of motion. Surgery, physical therapy and pain management are currently the only options available to MHE patients, but their success varies from patient to patient and many struggle with pain, fatigue and mobility problems throughout their lives.

All Proceeds go to help The MHE Research Foundation in its efforts to further the understanding of MHE through research and education.
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