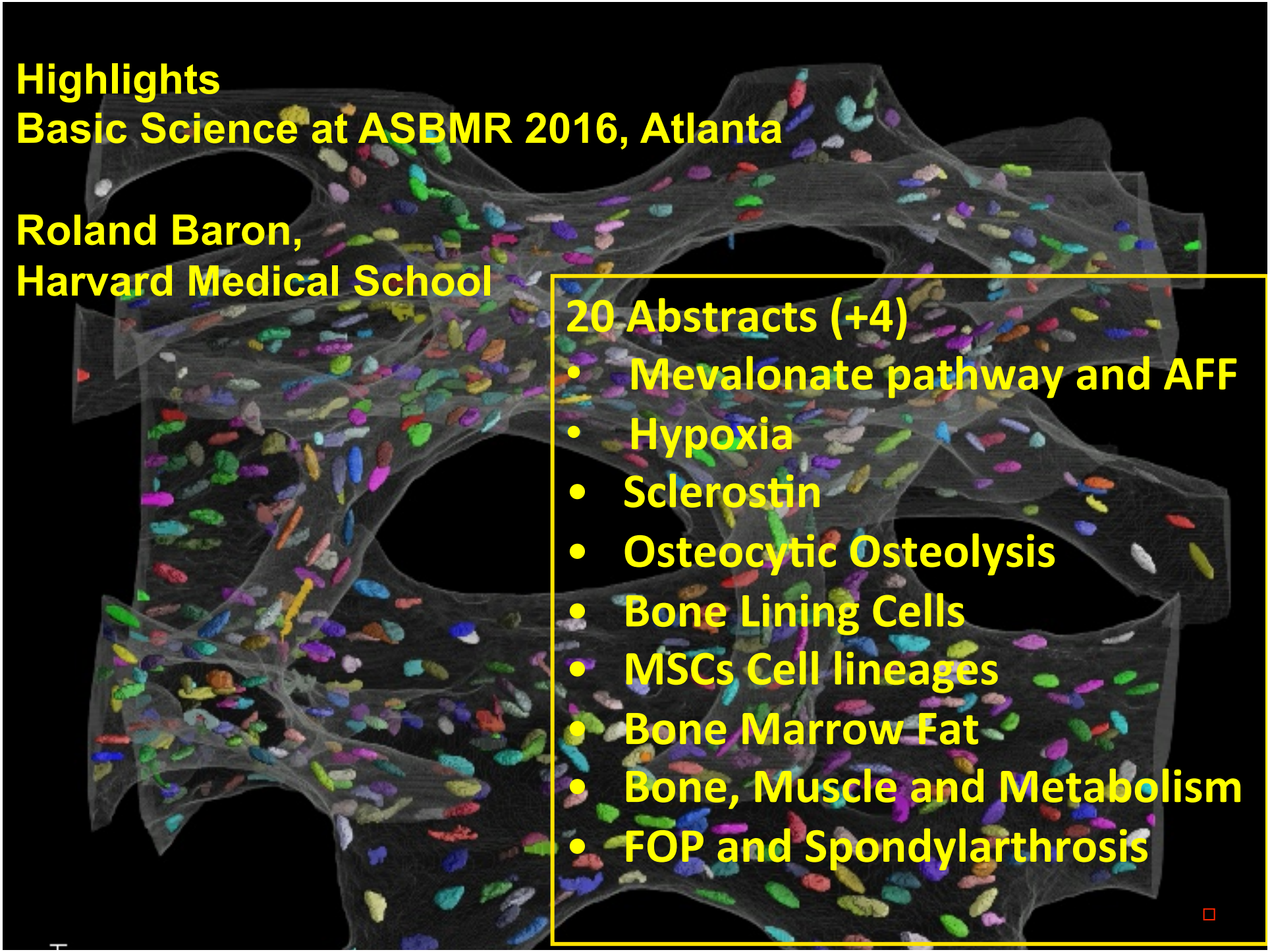

Highlights
Basic Science at ASBMR 2016, Atlanta
Roland Baron,
Harvard Medical School
20 Abstracts (+4)
• Mevalonate pathway and AFF
• Hypoxia
• Sclerostin
• Osteocytic Osteolysis
• Bone Lining Cells
• MSCs Cell lineages
• Bone Marrow Fat
• Bone, Muscle and Metabolism
• FOP and Spondylarthrosis

How Selected?

- **Selection is made from the pool of abstracts blindly selected for oral presentation by the Program Committee, composed of over 100 physicians and scientists among ASBMR members**

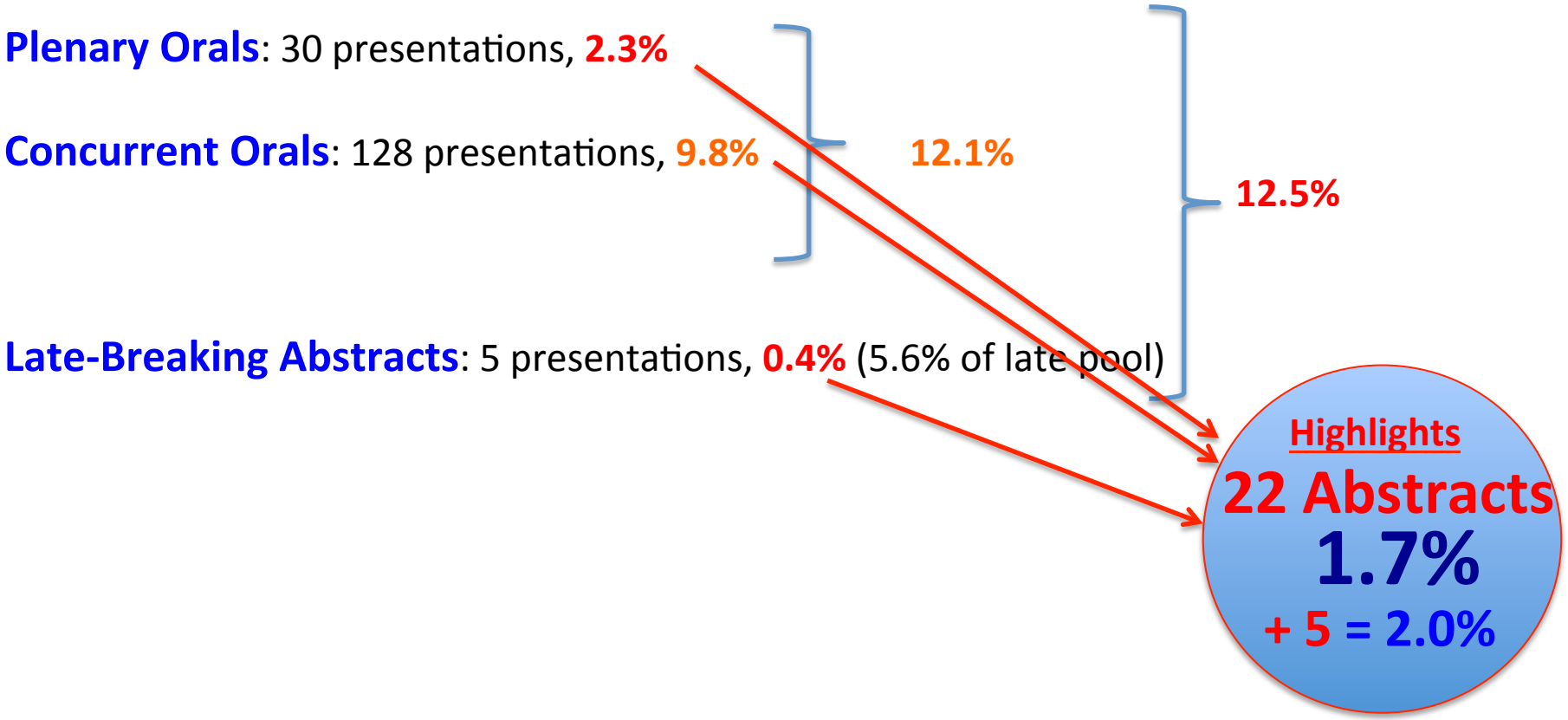
  - **Restricted by limited time for presentation**

- **Thus…if you are not an oral, you could not be selected here, but this does not mean you work is not exciting!**

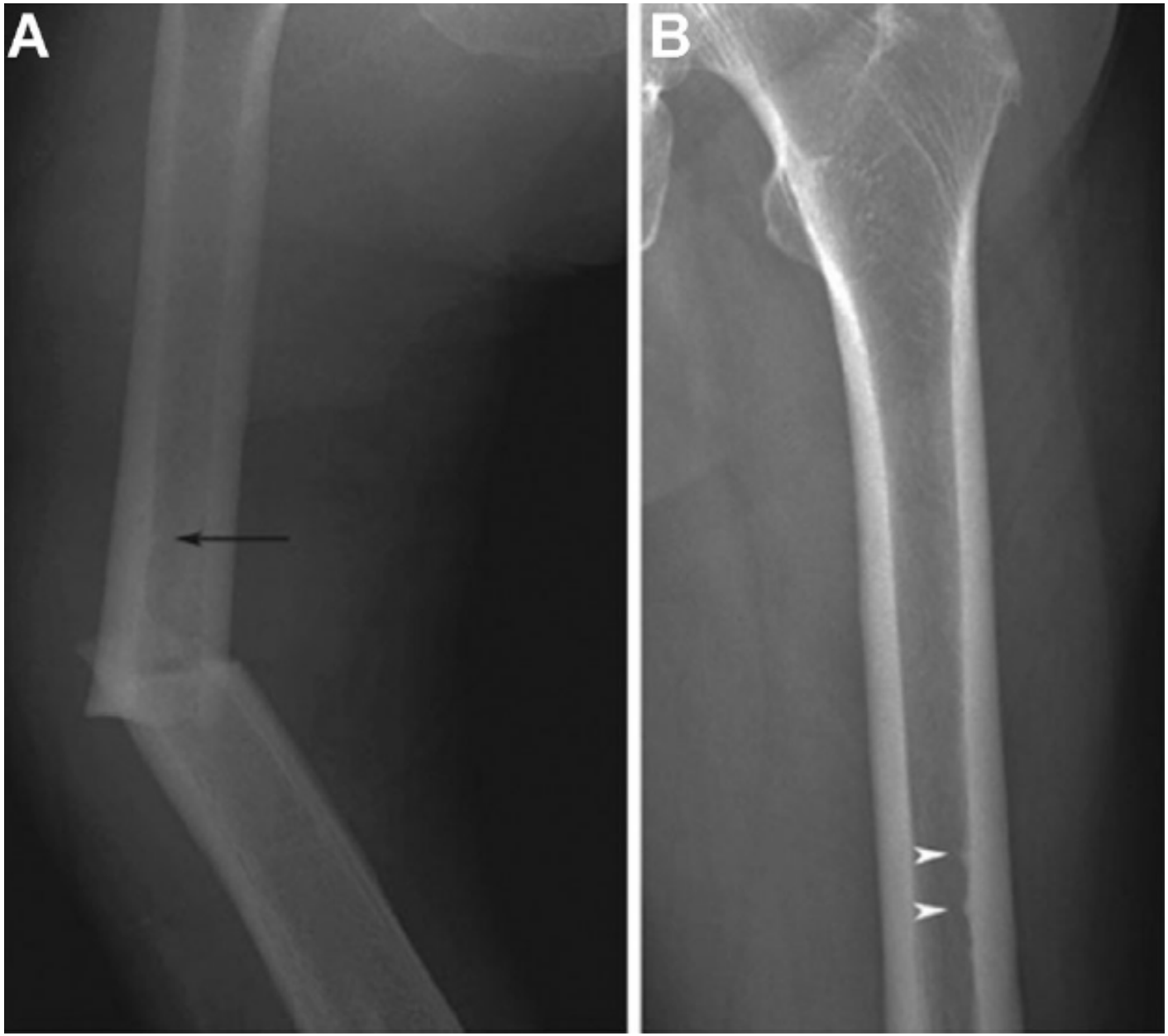
- **If you are an oral and were not selected here for presentation, this clearly means your work is exciting…**
  …but I missed it!

How Selective ?

- **Total Abstracts 1211+ 89 Late = 1300**



Atypical Femoral Fracture

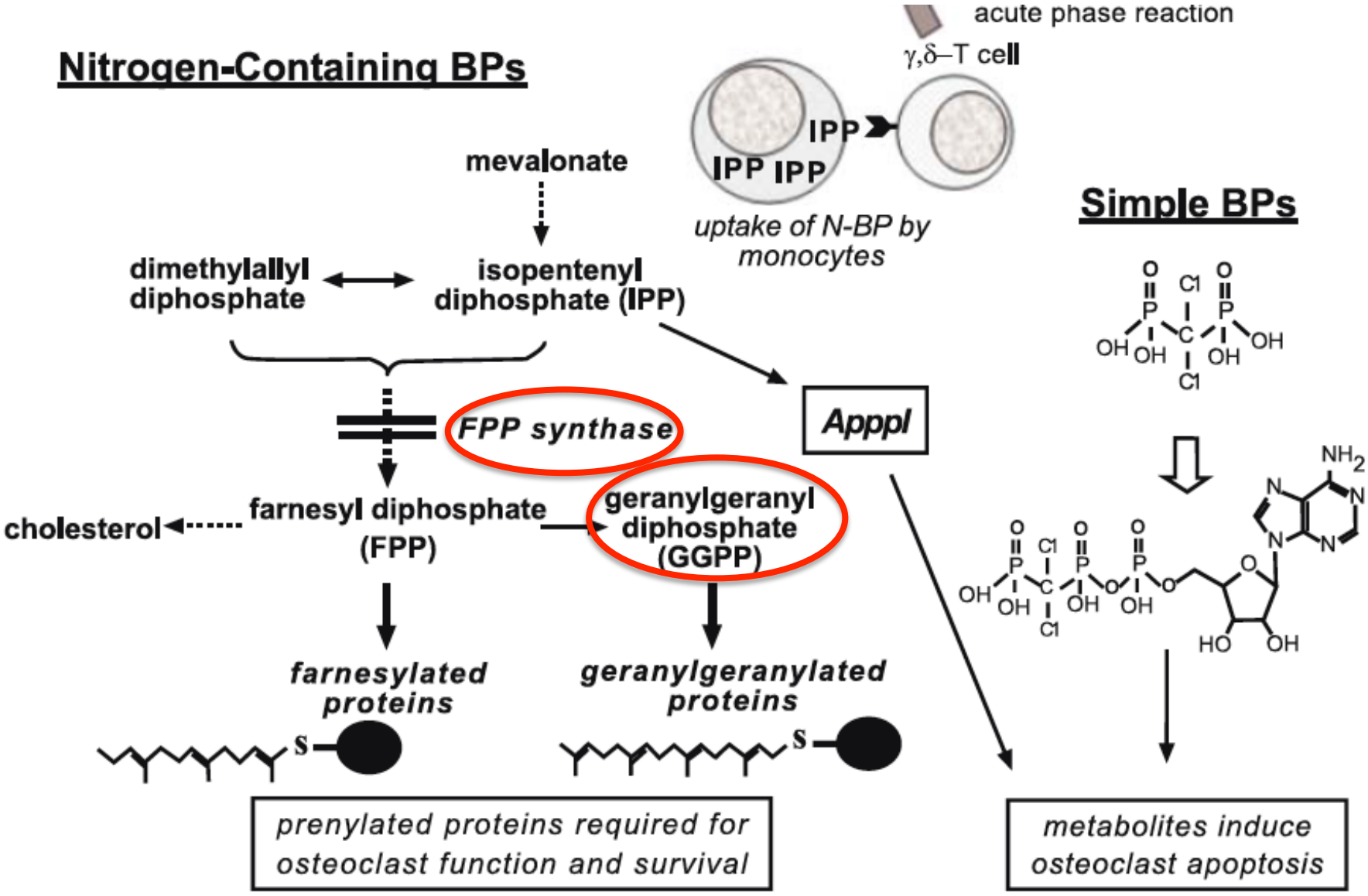


Shane et al., JBMR 2014

ASBMR Task force on AFFs

The epidemiological evidence for a relationship between BP use and atypical subtrochanteric and femoral shaft fractures has become more compelling. AFFs appear to be more common in patients who have been exposed to long-term BPs, usually for more than 3 years (median treatment 7 years), but every series includes patients who have not been treated with BPs, suggesting that the "background rate" of AFF in osteoporosis patients is not zero. Moreover, the risk for AFFs may decline after BPs are stopped. The majority of studies have found a significant association with GC use or duration. Although the relative risks of AFFs are very high in patients on BPs, ranging from 2.1 to 128, their absolute risk is extremely low, ranging from 3.2 to 50 cases per 100,000 person-years. Thus, these fractures are rare,

Shane et al., JBMR 2014



Russell et al., Osteoporosis Intl, 2008

LB-1159

Mutations in Geranylgeranyl Diphosphate Synthase (GGPS1) Identified by Whole-Exome Sequencing in Three Sisters who Sustained Atypical Femoral Fractures during Treatment with Bisphosphonates

Presenter: Neus Roca-Ayats, Adolfo Diez-Perez, Universitat de Barcelona, IBUB Spain

There are **no tests that would assist clinical decision-making by identifying those at high risk of AFF**.

Question: Are there rare genetic causes that might interact with BPs to trigger AFF?

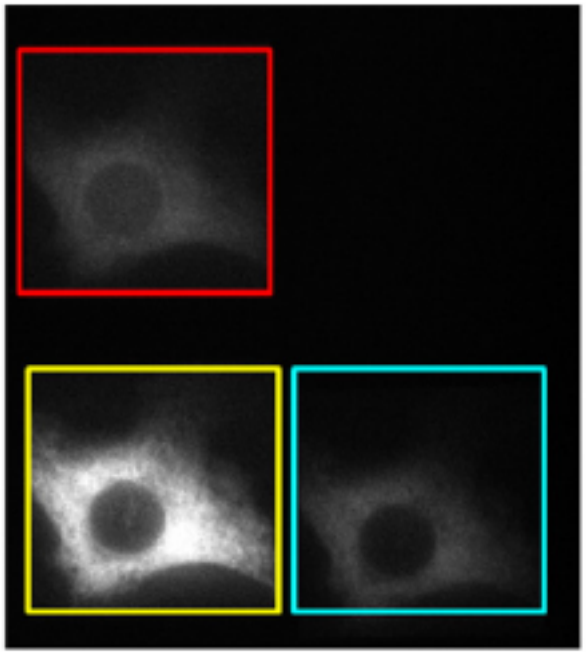
- **Exome sequencing of 3 sisters with AFF detected 37 rare mutations shared by the 3 sisters,** including **in geranylgeranyl pyrophosphate synthase** (GGPPS), **critical to osteoclast function, and inhibited by BPs**.
- **This mutation is predicted to impair function**, so it may interfere with osteoclast function, **with or without additional interaction with BPs**.
- T**he CYP1A1** gene**, involved in steroid metabolism, was also mutated in all 3 sisters and in one unrelated patient**, suggesting another **potential susceptibility gene for AFF**.
- Pathway analysis showed enrichment of the **isoprenoid biosynthetic pathway**.
- Other identified variants include **fibronectin (FN1) and SYDE2 and NGEF, two regulators of small GTPases**.

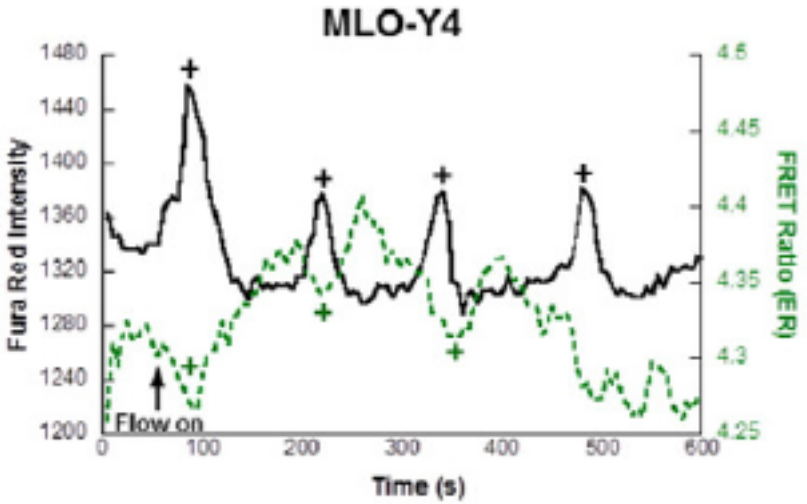
Thus, **an accumulation of susceptibility** genes constitutes a **genetic component of AFF**, and **may lead to novel risk assessment tools** to improve patient **decision-making**.

Conclusion, a possible genetic background for AFF has been identified.

WD 9
Courtesy J. Feng

Fluid Shear Stress Induces Calcium Oscillations in Osteocytes





Brown et al., Bone 2016

MOST OUTSTANDING BASIC ABSTRACT

Mechanically-Induced Calcium Oscillations in Osteocytes Facilitate Release of RANKL, OPG, and Sclerostin Through Extracellular Vesicles and Mediate Skeletal Adaptation

Presenter: Genevieve Brown (Edward Guo) Columbia University;

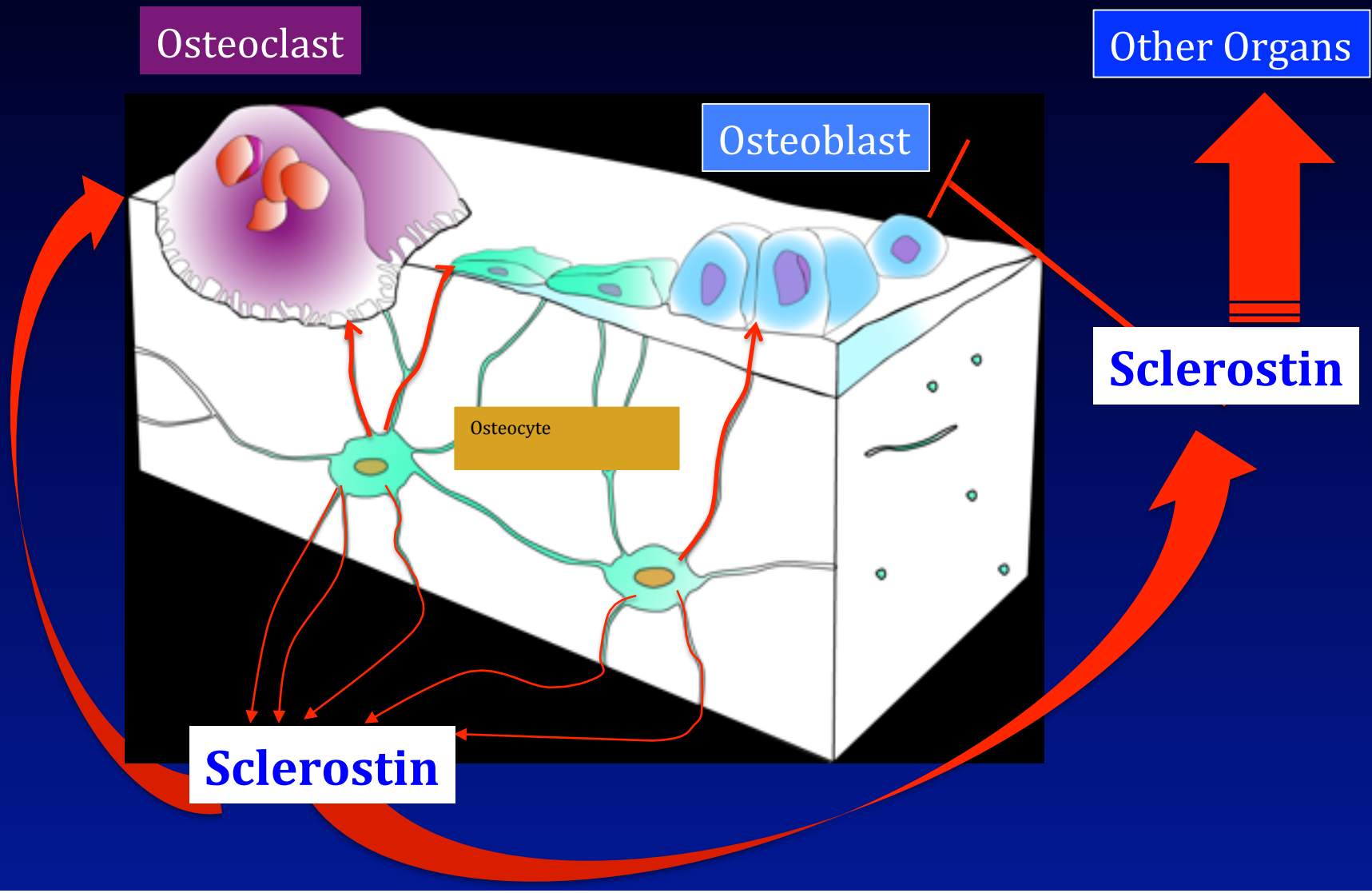
Calcium oscillations are observed in osteocytes in response to mechanical loading and mechanical loading regulates osteocyte protein expression.

Question: What is the connection between Ca2+-mediated mechanosensitivity and protein expression in osteocytes? and are extracellular vesicles (Evs) involved?

- MLO-Y4 cells exposed **to fluid flow increased expression of the secretory vesicle marker LAMP1**. **Neomycin inhibition of Ca2+ oscillations diminished this response.**
- **Mechanical stimulation released EVs into the culture medium, which was blunted by neomycin**.
- **EV lysates identified LAMP1, sclerostin, RANKL, and OPG** among the proteins contained within these EVs.
- **Neomycin-injected mice showed a decreased number of Ca2+ peaks and a reduced increase in bone in response to loading.**

Conclusion: Ca2+ oscillations regulate osteocyte protein expression and subsequent bone adaptation through the production and release of EVs to transport critical proteins through bone.

Local, Regional and Systemic Distribution of Sclerostin



YOUNG INVESTIGATOR AWARD

Sclerostin: a Local Rather Than Systemic Regulator of Bone Mass

Presenter: Rishikesh N. Kulkarni (Paul A. Baldock) Garvan Institute, Sydney, Australia;

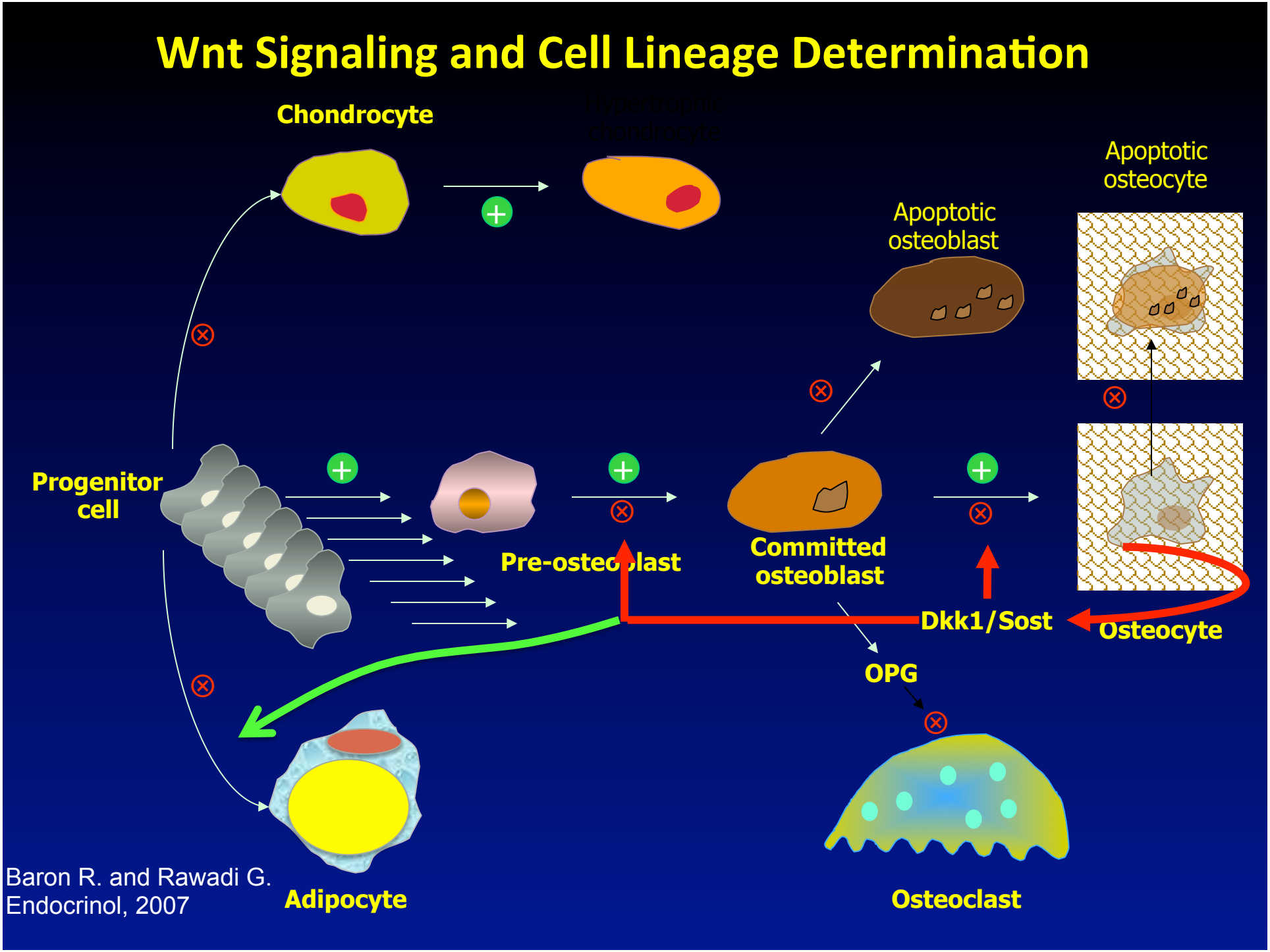
Sclerostin regulates bone homeostasis and mechanotransduction. **Osteocytes inhibit the local production sclerostin in response to mechanical loading**. **Sclerostin travels via the osteocyte canalicular network to act locally upon bone-forming osteoblast, but is also found in the serum**.

Question: Does serum sclerostin alter bone mass in an endocrine manner?

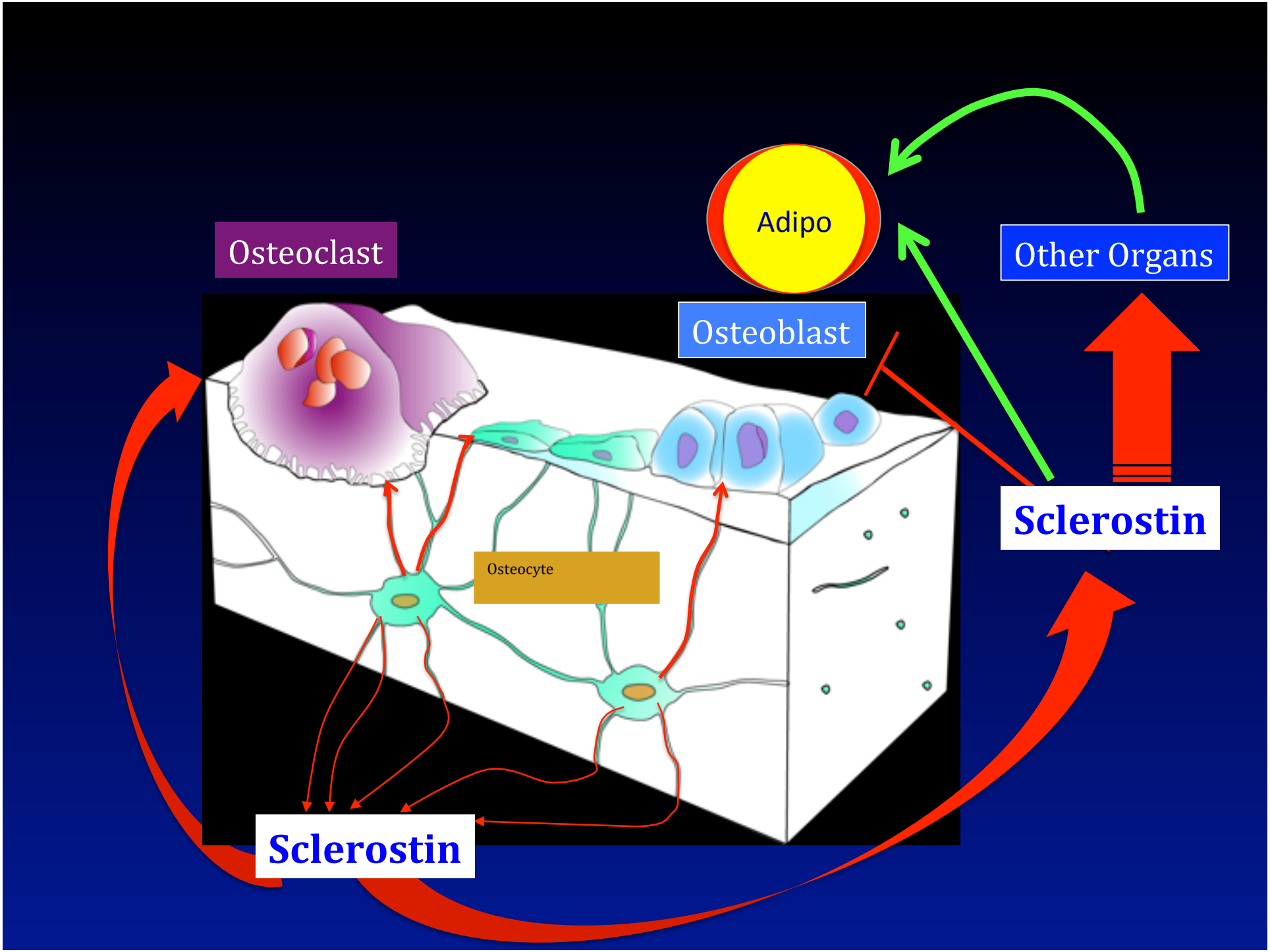
- Generated **limb-specific sclerostin null mice** (Prrx1-Cre Sostf/f) with sclerostin ablated only in the appendicular skeleton but not in the axial skeleton and **compared to wild type and to Sost-/- mice** with sclerostin ablated in the whole skeleton.
- At 16 weeks **serum sclerostin was reduced 2X** in Prrx1-Cre Sostf/f and undetectable in Sost-/- mice. DXA showed **greater BMD and BMC only in the limb but not in the spine** of Prrx1-Cre Sostf/f mice whereas Sost-/- mice showed greater BMD and BMCin both.
- **Cancellous and cortical bone mass in vertebra did not differ between Prrx1-Cre Sostf/f and control mice.**

Conclusion: **sclerostin does not alter bone mass in an endocrine manner** but rather locally and **serum sclerostin might not predict changes in whole body bone mass**.

Wnt Signaling and Cell Lineage Determination



Baron R. and Rawadi G. Endocrinol, 2007


Adipo
Other Organs
Osteoclast
Osteoblast
Osteocyte
Sclerostin
Sclerostin

1034

Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes.

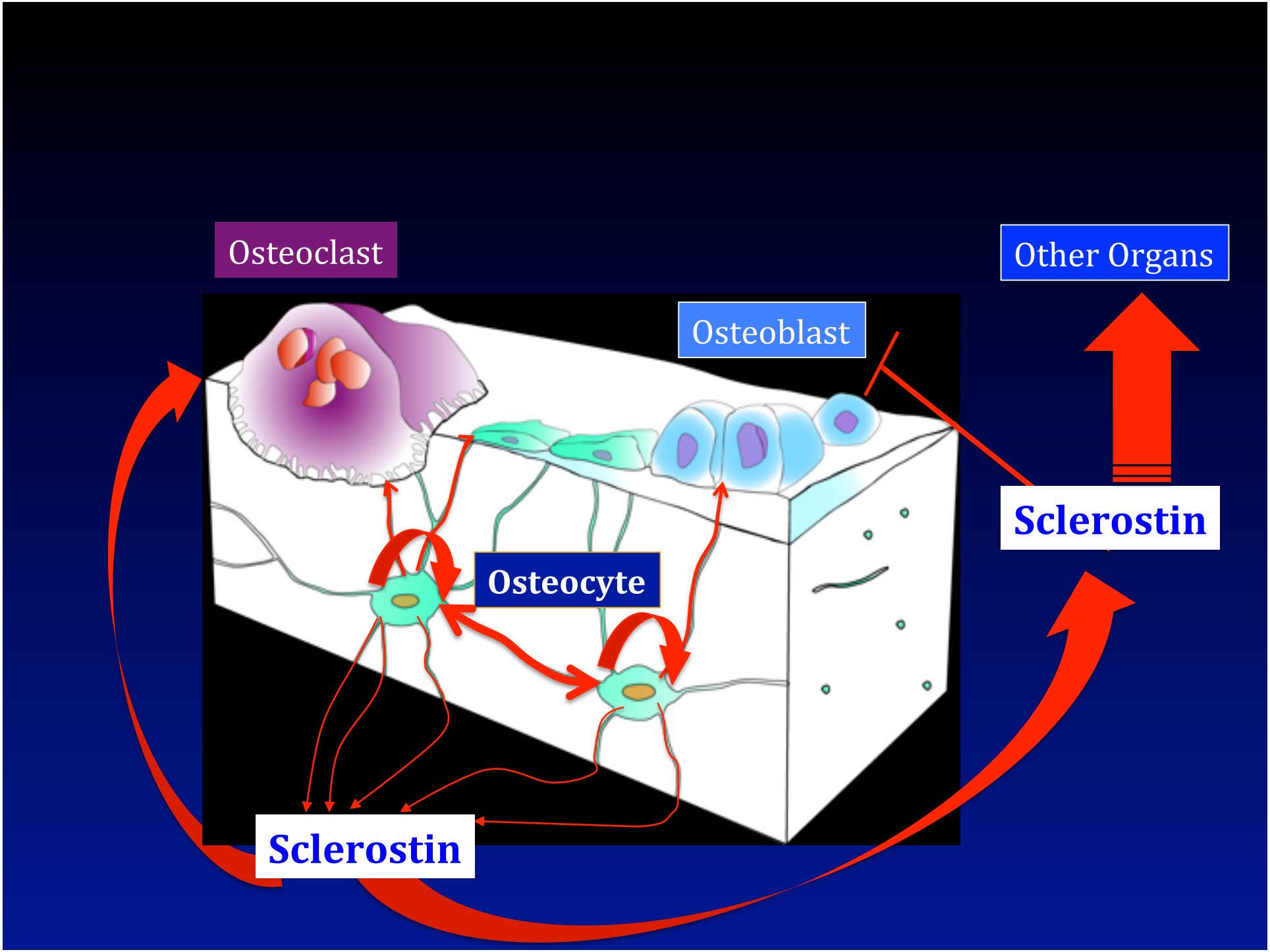
Presenter: Julie Frey (Ryan Riddle) Johns Hopkins University;

Impairment in several metabolic indices have been linked with increases in serum sclerostin levels and increased sclerostin was shown in mouse models of type 2 diabetes,

Question: What are the extra-skeletal functions of sclerostin and does it influence metabolism?

- Whole-body **fat mass, fat pad weightsand adipocyte size are reduced in Sost-/- mice, with improved glucose tolerance and enhanced insulin sensitivity** in WAT.
- **Sost-/- mice are resistant to obesogenic diet-induced disturbances** in energy balance.
- AAV **overproduction of sclerostin** induced the **opposite metabolic phenotype**, with **accumulation of adipose tissue and impairments in glucose handling.**
- **Slerostin's effects are the result of cell non-autonomous enhancements in Wnt signaling in white adipocytes** and a shift towards anabolic metabolism in this tissue.
- **WAT from Sost-/- mice has enhanced ability to oxidize fatty acids** and reduction in de novo fatty acid synthesis.
- **Glucose uptake (18F.FDG) was increased** in the inguinal fat pads of Sost-/- mice.
- **Sclerostin directly regulates adipocyte differentiation** and lipid accumulation **in vitro**.
- **Levels of under-carboxylated osteocalcin are not affected by sclerostin deficiency**

Conclusion: **Sclerostin has an endocrine function that facilitates communication between the skeleton and adipose tissue.**


Osteoclast
Other Organs
Osteoblast
Sclerostin
Osteocyte
Sclerostin

1015

Evidence for Autocrine Effects of Sclerostin on Osteocytes: Sclerostin Antibody Treatment Prevents Spaceflight-induced Osteocytic Osteolysis and Skeletal Bone Loss in Mice

Presenter: Yoshihito Ishihara (Roland Baron, Mary Bouxsein) Harvard Medical School

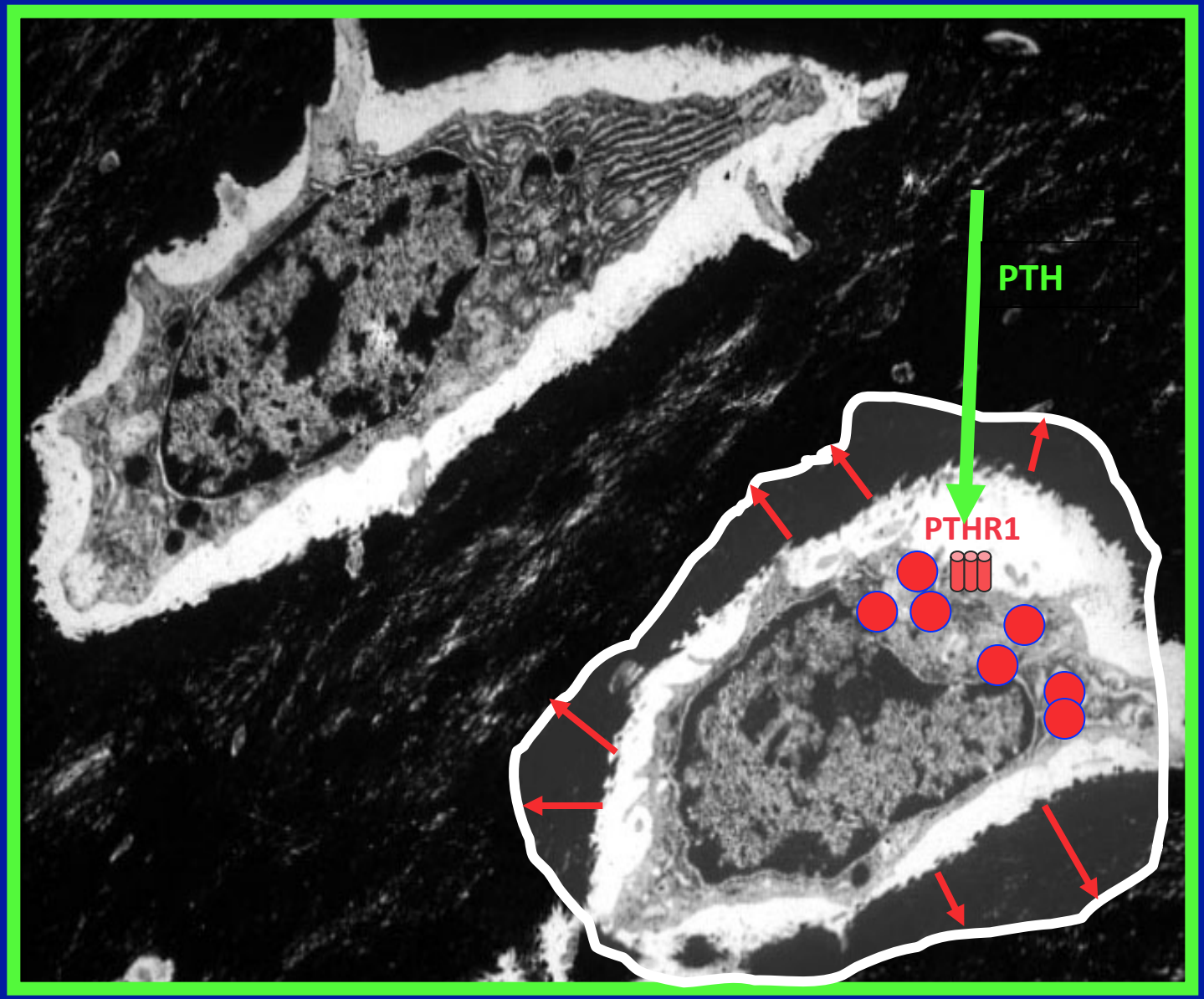
Astronauts lose bone 10X faster than postmenopausal women and sclerostin expression increases during unloading.

Question: Changes in Sost expression affect BFR and BR, does it affect also osteocytes themselves, and osteocytic osteolysis?

- 9-week-old female mice were exposed to **13 days of microgravity on the Space Shuttle** Atlantis (STS-135) **or kept on the ground** and **injected with Scl-Ab or vehicle 1 day prior launch**. Ground-controls were kept in identical housing as the flight mice.
- 13-days **spaceflight enlarged Ocyte lacunar area by 20% with varying degrees of perilacunar demineralization**.
- Pre-flight administration of **Scl-Ab prevented these changes**.
- Spaceflight **decreased trabecular bone due to inhibition of BFR and a 3X increase in osteoclasts. Scl-Ab also improved these parameters** resulting in a **significant increase in trabecular bone regardless of loading condition.**

Conclusion: spaceflight induces osteocytic osteolysis and this is modulated by sclerostin, most likely **in an autocrine manner since osteocytes make Wnts and have the receptors**. **Scl-Ab also counteracts the effects of spaceflight on osteoclast-mediated bone resorption**, and osteoblast-mediated bone formation.

Osteocytic Osteolysis and PTH/PTHrP



Blockade of the Activity of the Osteocytic PTH Receptor Target Gene MMP14: a Therapeutic Tool to Prevent Bone Loss and Potentiate Bone Gain Induced by PTH

Presenter: Jesus Delgado-Calle (Teresita Bellido) Indiana University School of Medicine;

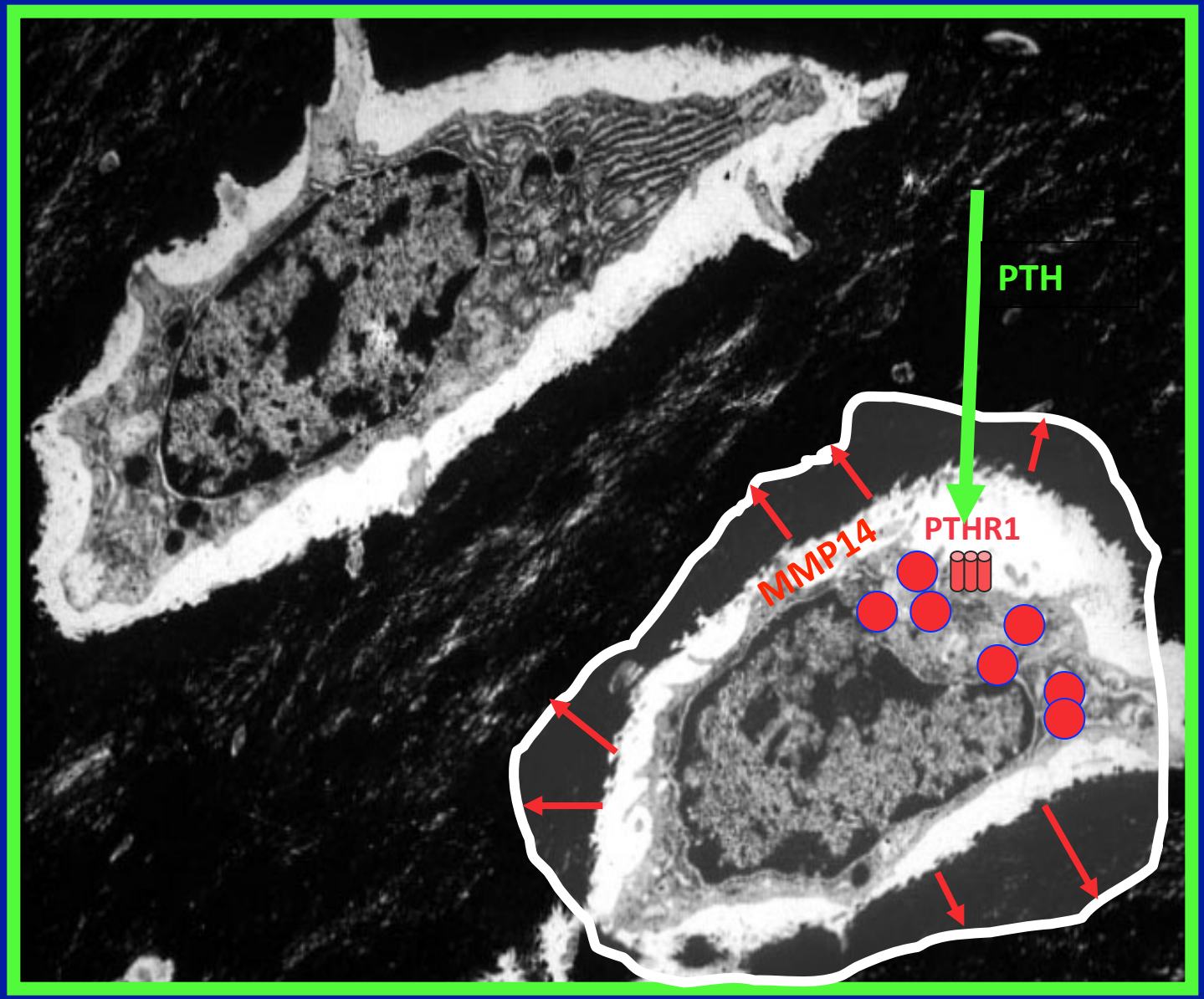
MMP14 is a PTH target and its inhibition with the KD014 antibody decreases bone resorption and remodeling in mice with constitutive activation of the PTH receptor in osteocytes (caPTHR1).

Question: Does inhibition of MMP14 alter PTH-induced bone catabolism and/or anabolism?.

- **KD014 prevented by 50% the decrease in spinal BMD and the increased CTX and P1NP induced by low Ca.**
- **iPTH increased BMD and KD014 potentiated this increase** in the spine but not in the femur.
- iPTH alone did not alter serum CTX or osteoclasts; **KD014 reduced serum CTX and osteoclasts.**
- **The increase in circulating P1NP, MAR and BFR induced by iPTH remained unchanged by KD014.**
- Alone, KD014 increased spinal but not femoral BMD, cancellous BV/TV, and decreased CTX.
- **RANKL and CTX were increased in culture media from ex vivo organ cultures established from caPTHR1 mice bones, and KD014 decreased both**.

Conclusion: pharmacological inhibition of MMP-14 prevents the catabolic and potentiates the anabolic skeletal actions of PTH.

Osteocytic Osteolysis and PTH/PTHrP



Cathepsin K is Directly Involved in Osteocyte Lacunae Remodeling and in the Osteocyte-dependent Skeletal Responses to Mechanical Loading and Unloading

Presenter: Yoshihito Ishihara (Roland Baron) Harvard Medical School

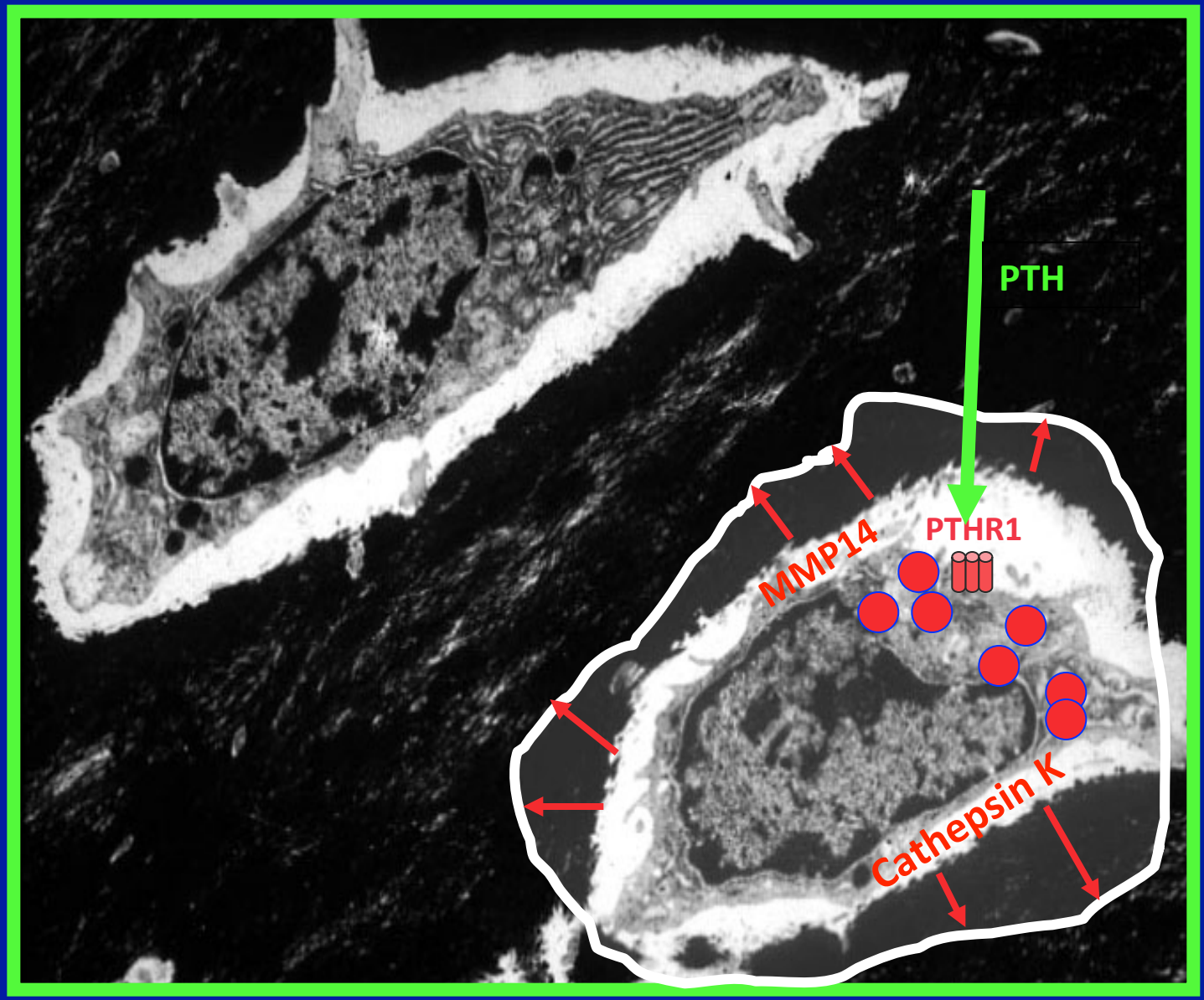
Cathepsin K (Ctsk) expression in osteocytes (Ocytes) contributes to the increased remodeling, bone loss and deterioration of mechanical properties observed in mice during lactation.

Question: What is the role of Ocyte Ctsk in the responses to mechanical loading (ML) and unloading (UL)?

- **DMP1-Cre;Ctskfl/fl mice to delete Ctsk in Ocytes (cKO).**
- **Loading decreased the Ocyte lacunar area in cKO mice but not in their WT littermates.**
- **Loading increased Tb.BV/TV in cKO mice vs. WT mice at 11 weeks of age**. The anabolic response **was lower in the older (15w) WT mice but this age-related decline was prevented in cKO mice where an increased Tb.BV/TV was still observed**.
- For unloading, **10 days of hindlimb suspension did not change Ocyte lacunar area**
- **Unloading decreased trabecular parameters in both cKO and WT, but cortical thickness and area decreased only in WT mice**.
- **cKO prevented trabecular bone loss in 13 wk old mice**, but there were no differences in cortical bone.

Conclusion: Osteocyte-specific deletion of cathepsin K protects against the reduced skeletal mechanosensitivity with aging and partially attenuates the unloading-induced bone loss in mice.

Osteocytic Osteolysis and PTH/PTHrP



1119

Novel Roles of RANK in Osteocytes During Bone Remodeling 8

Presenter: Min Jin (Jianquan Feng); Texas A&M Baylor College of Dentistry;

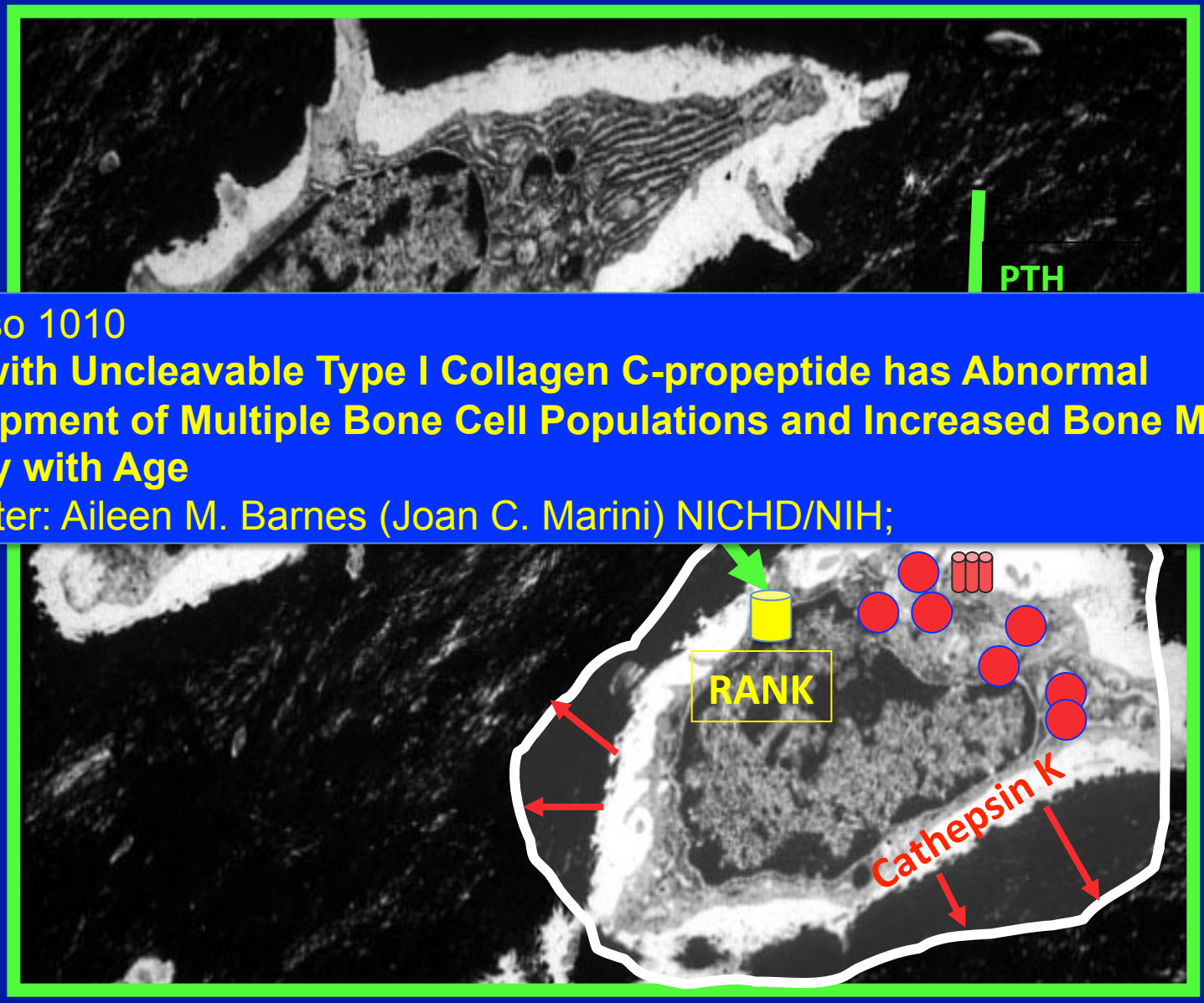
RANK, which is activated by RANKL released from osteoblasts/osteocytes, plays a key role in osteoclast formation. **RANK is also expressed in dog osteoblasts/osteocytes**

Question: Does RANK in osteocytes contribute to bone formation or remodeling?

- **Demonstrate Rank expression in osteoblasts and osteocytes using RT-PCR.**
- Conditionally knockout **(cKO) Rank in late osteoblasts and osteocytes (DMP1 Cre).**
- cKO mice display **no change in bone formation or bone resorption in 1-month-old mice**.
- **4-month-old cKO mice exhibited a reduction in BFR with no change in osteoclast** number, leading to a **reduction in TBV** (4X) and in cortical bone.
- SEM revealed a **change in osteocyte morphologies** with **increases in TRAP+ osteocyte numbers (10X)** in the cKO mice.
- RT-PCR showed **a 6X increase in Trap and an 8X increase in cathepsin K** with no apparent change in Rankl in the cKO bone.
- Immunohistochemistry showed **reductions in osterix, BSP and DMP**1 in the cKO bone.
- In the tail suspension model, no protective role of deleting Rank.

Conclusion: RANK expression in osteocytes contributes to bone remodeling, independent of its mechanosensor role or osteoclast function.

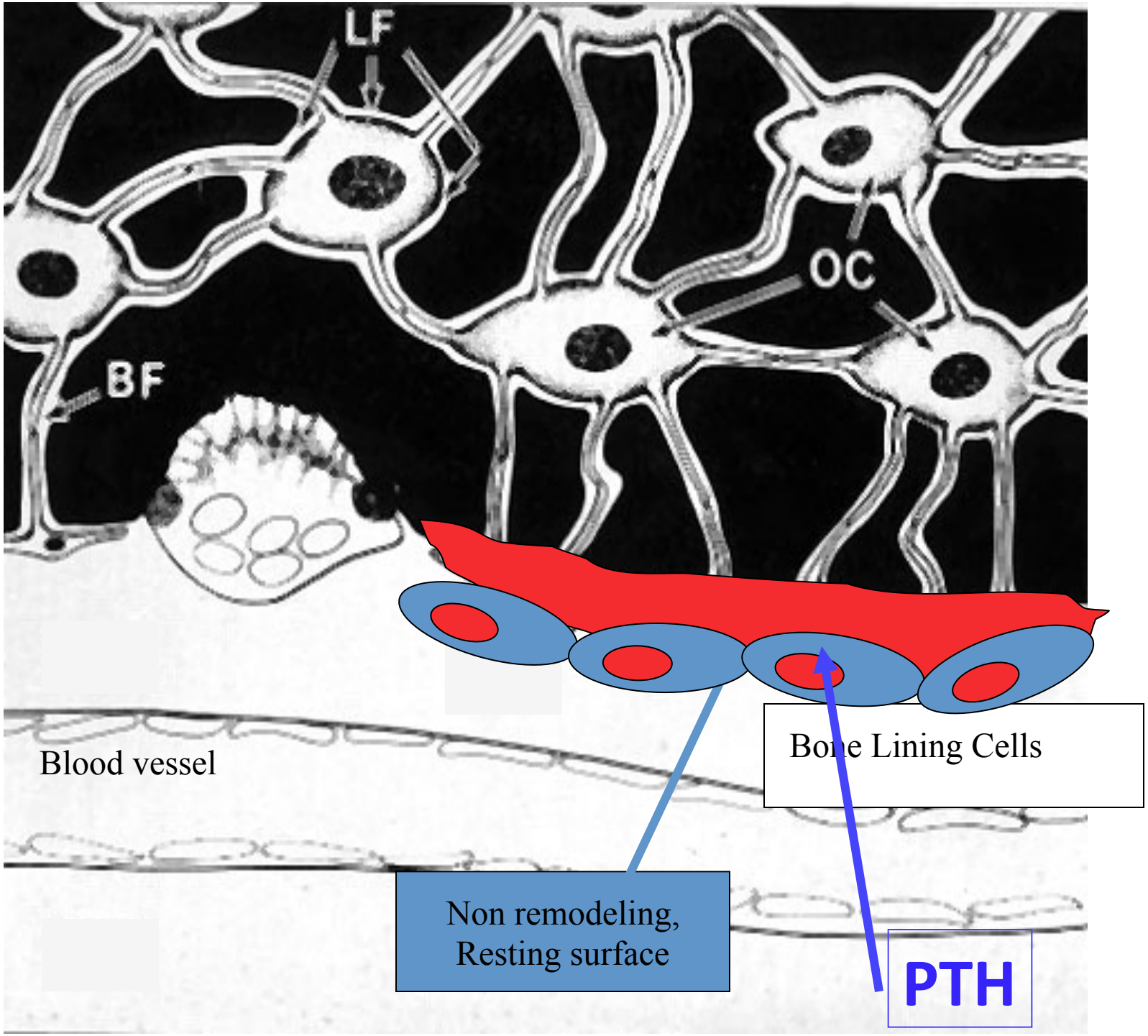
Osteocytic Osteolysis and PTH/PTHrP



See Also 1010

Bone with Uncleavable Type I Collagen C-propeptide has Abnormal Development of Multiple Bone Cell Populations and Increased Bone Mineral Density with Age

Presenter: Aileen M. Barnes (Joan C. Marini) NICHD/NIH;


LF
OC
BF
Blood vessel
Bone Lining Cells
Non remodeling,
Resting surface
PTH

1087

Sclerostin antibody administration converts bone lining cells into active osteoblasts

Presenter: Marc Wein (Henry Kronenberg) Massachusetts General Hospital;

Sclerostin antibody (Scl-Ab) increases osteoblast activity, in part through increasing modeling-based bone formation on quiescent surfaces. Intermittent parathyroid hormone converts quiescent bone lining cells (BLCs) into active osteoblasts.

Question: Does Scl-Ab, like iPTH, promote the conversion of lining cells into osteoblasts ?.

- **To label BLCs, tamoxifen-dependent Osteocalcin-Cre/ER mice were crossed with a Cre-dependent tdTomato allele.**
- In the **osteocalcin-Cre/ER mice, Scl-Ab led to a 74% increase in serum P1NP** and **an increase in the average thickness of tdTomato-positive cells on bone surfaces**.
- **A positive linear correlation was noted between serum P1NP and average cell thickness (R2=0.615, p=0.00012).**
- **Scl-Ab did not induce proliferation of tdTomato-labelled cells, as assessed by EdU staining.**
- **Scl-Ab did not affect apoptosis** of tdTomato-labelled cells.

Conclusion: **reactivation of quiescent bone lining cells contributes to the acute increase in bone formation following Scl-Ab treatment in mice.**

1150

Bone lining cells are an alternative to MSCs as a source of osteoblasts in adult bone

Presenter: Brya G Matthews (Ivo Kalajzic) UConn Health Center;

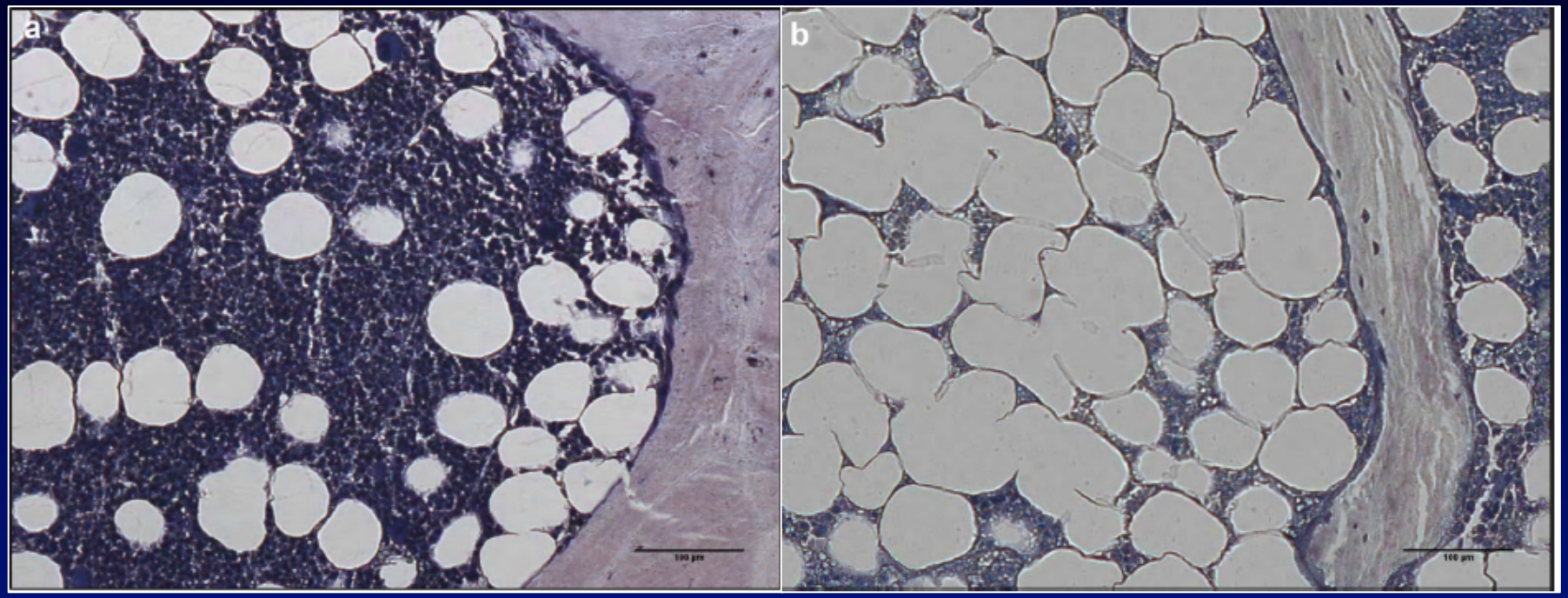
Mesenchymal stem cells (MSCs) provide a continuous supply of mature osteoblasts during bone remodeling. However, **bone lining cells (BLCs) can activate into matrix-producing osteoblasts**.

Question: Can we identify BLCs and understand their role?

- **Tamoxifen in DMP1 Cre directed Tomato expression label osteoblasts, BLCs and osteocytes and some osteoblasts remain 6 months after labeling**.
- **Crossed the Col2.3Delta;TK suicide gene into these mice. Following ganciclovir treatment, osteoblasts were absent, while flat BLCs were labeled.**
- **After 21 days new areas of active bone formation are covered by Tomato expressing osteoblasts, indicating activation of BLCs**
- **BLC-derived cells are proliferative.**
- **In contrast, labeled MSCs tracked following Col2.3Delta;TK osteoblast ablation, showed minimal contribution to osteoblasts.**
- **BLCs express MSC markers while osteoblasts do not.**
- **Single cell gene expression show higher expression of osteoclast regulators M-CSF and RANKL in BLCs compared to osteoblasts, as well as Mmp13, and cell cycle checkpoint gene Cdkn1a.**

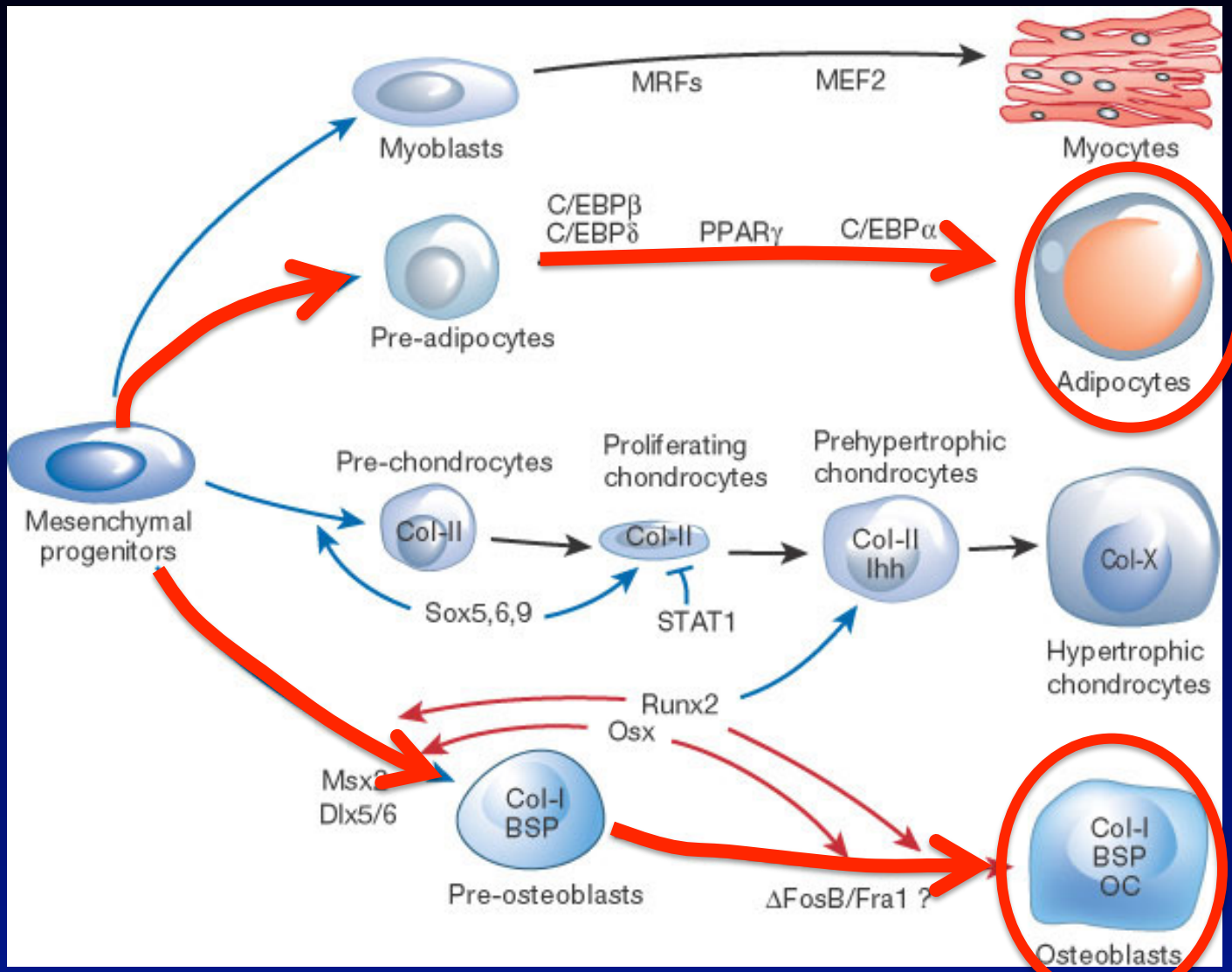
Conclusion: BLCs are a source of proliferating and mature osteoblasts during adult bone remodeling. These cells are **different from osteoblasts** and **may play a role in regulation of bone homeostasis.**

Bone Marrow Adipose Tissue

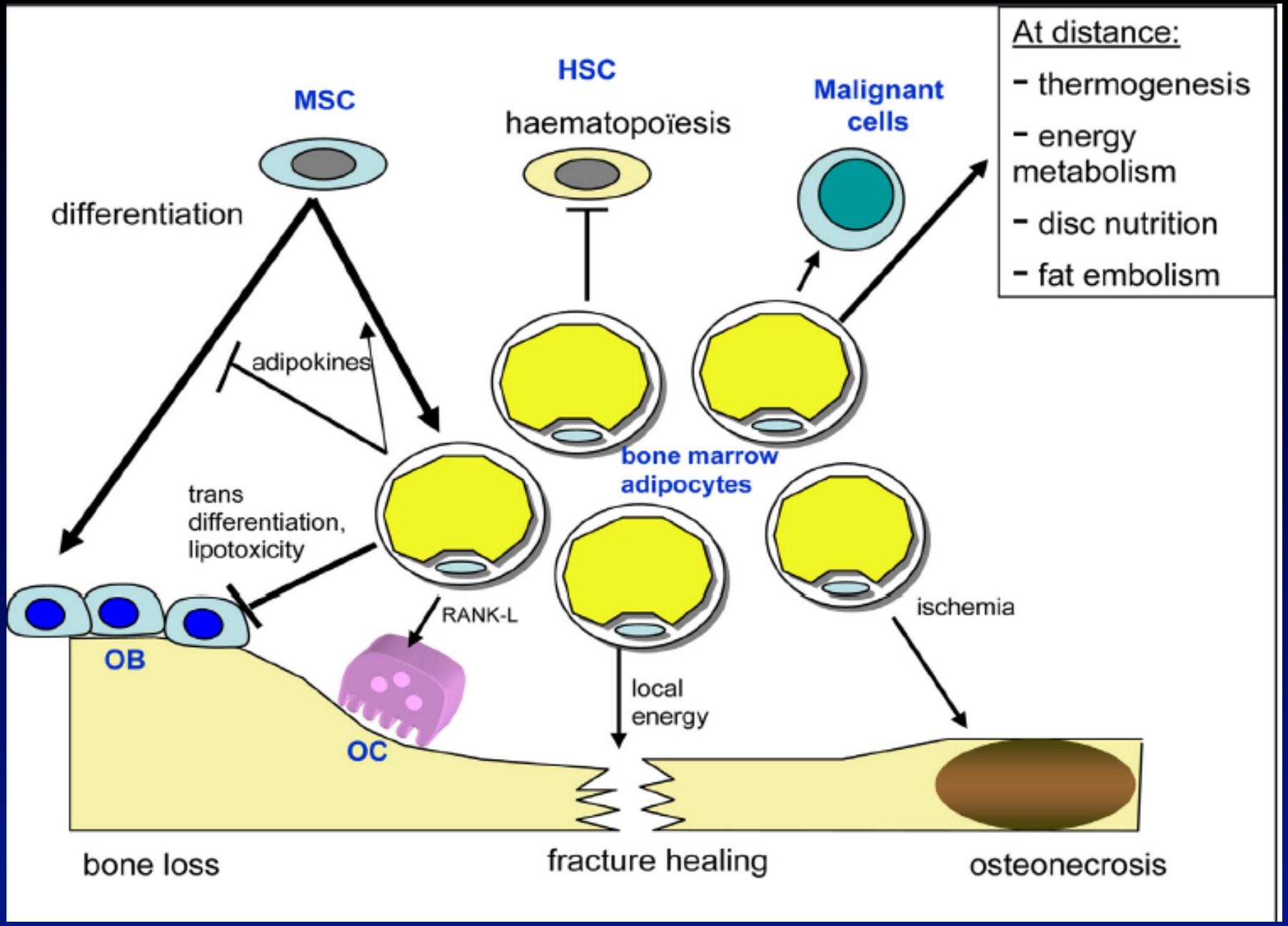


Hardouin et al., Joint Bone Spine, 2014

Mesenchymal Progenitor Cell Lineages



Adapted from Harada and Rodan, *Nature*, 2003



Hardouin et al., Joint Bone Spine, 2014

1046

Critical Function Of PTH1R In Regulation Of Mesenchymal Cell Fate And Bone Resorption

Presenter: Yi Fan (Beate Lanske) Harvard School of Dental Medicine;

PTH1R signaling promotes osteoblastogenesis while suppressing adipogenesis in MSCs and bone marrow **pre-adipocytes produce Rankl** and support differentiation of osteoclasts.

Question: What is the role of PTH1R in mesenchymal cell fate and bone resorption?

- Prx1Cre **deletion of the PTH1R increased marrow adipose tissue (MAT)**.
- Bone marrow stromal cells (**BMSCs) showed enhanced adipogenic differentiation**.
- **PTH(1-34) inhibited adipogenesis** in control mice and BMSCs **but not in PTHR1 null mice or cells ablated for PTH1R.**
- Enhanced adipogenesis was accompanied by **increased bone resorption and low bone mass**
- MAT had **high Rankl mRNA expression and high RANKL protein** levels in serum and marrow supernatant.
- Flow-cytometry of bone marrow cells from mutant mice showed **increased pre-adipocytes (Pref-1) number producing RANKL**.

Conclusion: **Loss of PTH1R from osteoprogenitors favors adipogenic differentiation, RANKL expression and osteoclastogenesis. Thus PTH plays a critical role in determining cell fate in the marrow niche and marrow adipocytes can mediate bone resorption.**

Phosphate Restriction Promotes the Differentiation of Multipotent Marrow Stromal Cells into Marrow Adipose Tissue

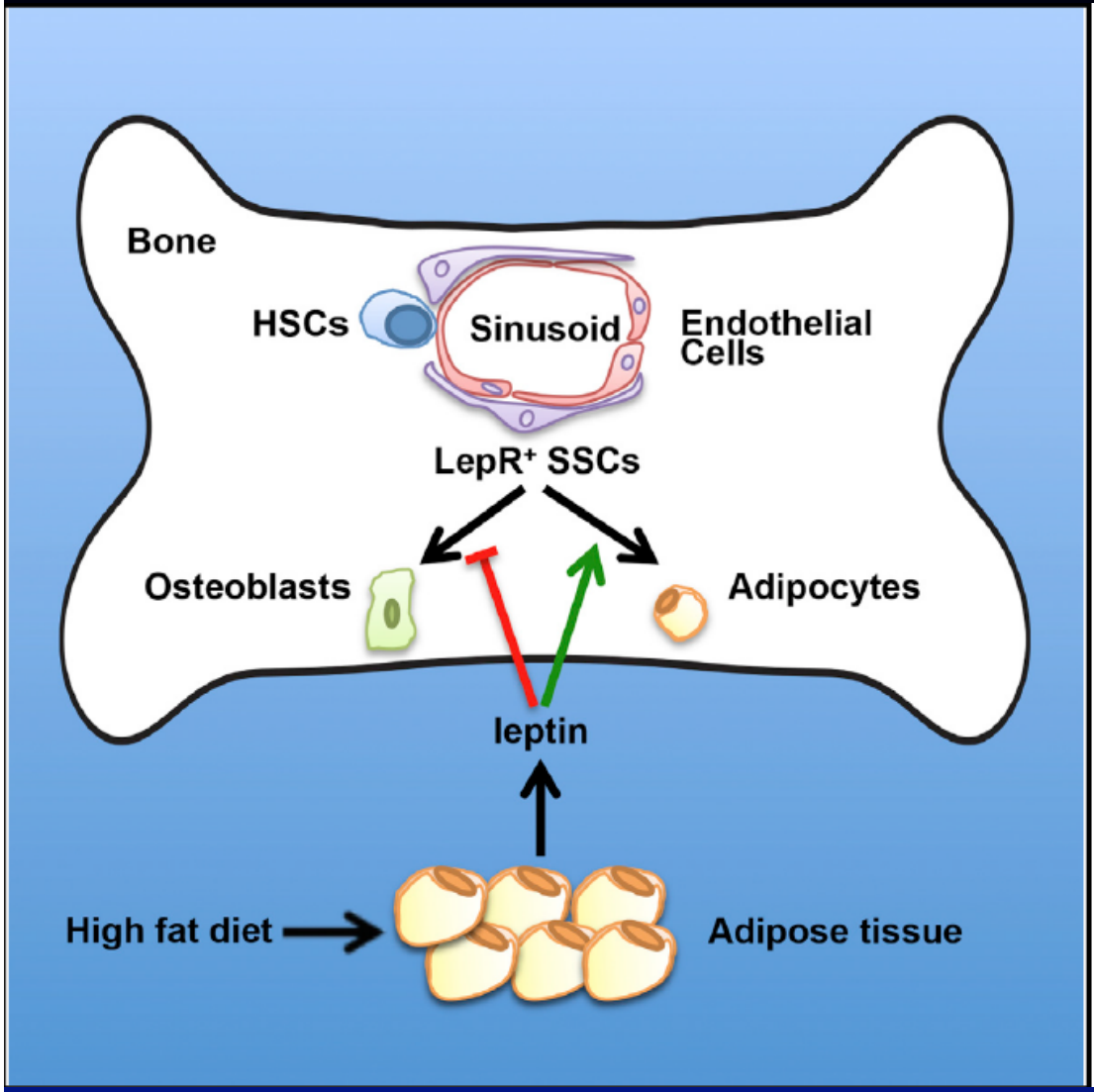
Presenter: Frank Ko (Marie Demay) Massachusetts General Hospital;

Phosphate restriction in growing mice **impairs bone formation, decreases trabecular and cortical bone and increases marrow adipose tissue (MAT) via an arrest in osteoblast differentiation**.

Question: Is the decreased bone due not only to arrested osteoblast differentiation but also to enhanced differentiation of MSCs into the adipogenic lineage?

- **Ablation of canonical Wnt (cWnt) signaling or Gsα in Osterix-Cre expressing cells enhances their differentiation into MAT, but the pathological MAT observed in phosphate restricted mice did not originate from these cells**.
- The **increase in adipocytes in the marrow of phosphate restricted mice originates from LepR expressing CAR** (CXCL12-abundant reticular) **MSCs**.
- **Phosphate restriction led to enhancement of PPARg and CEBPa** in sorted CAR cells, and a **decrease in the expression of the cWnt effector Lef1**.
- **Activation of cWnt signaling with LiCl prevented the increased expression of PPARgamma and CEBPα in bone marrow cells and the block in osteoblast differentiation.**

Conclusion: Acute dietary phosphate restriction of growing mice enhances marrow adipogenesis by interfering with the cWnt signaling pathway.



Rui Yue, Bo O. Zhou, Issei S. Shimada, Zhiyu Zhao, Sean J. Morrison

Correspondence

sean.morrison@utsouthwestern.edu

In Brief

A fundamental question concerns how stem cells are regulated by nutrition and systemic energy homeostasis. Morrison and colleagues demonstrate that leptin receptor acts within skeletal stem cells in the bone marrow as a sensor of systemic energy homeostasis, promoting adipogenesis and inhibiting osteogenesis in response to diet and adiposity.

Cell Stem Cell, 2016

1047

Leptin-induced loss of marrow adipose tissue is mediated by sympathetic and sensory neurotransmission

Presenter: Erica L Scheller Washington University United States

MAT can exert local and systemic effects on metabolic homeostasis and skeletal remodeling. Cold exposure in rodents promotes thermogenesis, through elevation of sympathetic tone, with loss of regulated (rMAT) in the proximal tibia due to decreases in adipocyte cell size and number while constitutive (cMAT) in the distal tibia remains unchanged.

Question: Can differences in sympathetic drive explain site-specific regulation of rMAT, but not cMAT ?

- ICV Leptin caused a 43% decrease in rMAT at the proximal tibia while cMAT in the distal tibia and tail remained unchanged. Inguinal and gonadal WAT mass (iWAT, gWAT) was also decreased, due to a decrease in adipocyte size.
- Pre-treatment with antagonists did not change leptin-mediated decreases in body mass or food intake.
- However, the β3-adrenergic antagonist rescued the loss of rMAT and iWAT/gWAT cell size.
- Unexpectedly, inhibition of sensory neurotransmission also blocked loss of rMAT, with partial rescue of iWAT and gWAT adipocyte size.

Conclusion: MAT is regulated site-specifically by the nervous system, including sensory neurotransmission.

Sensory Nerve Signals Mediate Skeletal Adaptation to Mechanical Loads 14
1042

Presenter: Ryan Tomlinson (Thomas Clemens) Johns Hopkins University;

A critical process for endochondral bone formation is the invasion of TrkA sensory nerves guided by retrograde NGF signaling. TrkA sensory nerves innervate periosteal and endosteal surfaces, a privileged location for the perception of mechanical signals.

Question: is NGF-TrkA signaling in sensory nerves required for skeletal adaptation to mechanical loads?.

- In NGF-eGFP mice, osteocalcin+ osteoblasts on the periosteal and endosteal surfaces of bone expressed NGF 1 and 3 hours after loading, which returned to baseline by 24 hours.
- NGF expression was not observed in osteocytes.
- A similar time course of NGF activation was observed after in vitro stretching of osteoblasts.
- Mice harboring TrkA-F592A knockin that render TrkA kinase sensitive to inhibition by 1NMPP1 decreased both periosteal and endosteal load-induced bone formation, by decreasing mineralizing surfaces.
- NGF increased periosteal and endosteal load-induced bone formation and mineralizing
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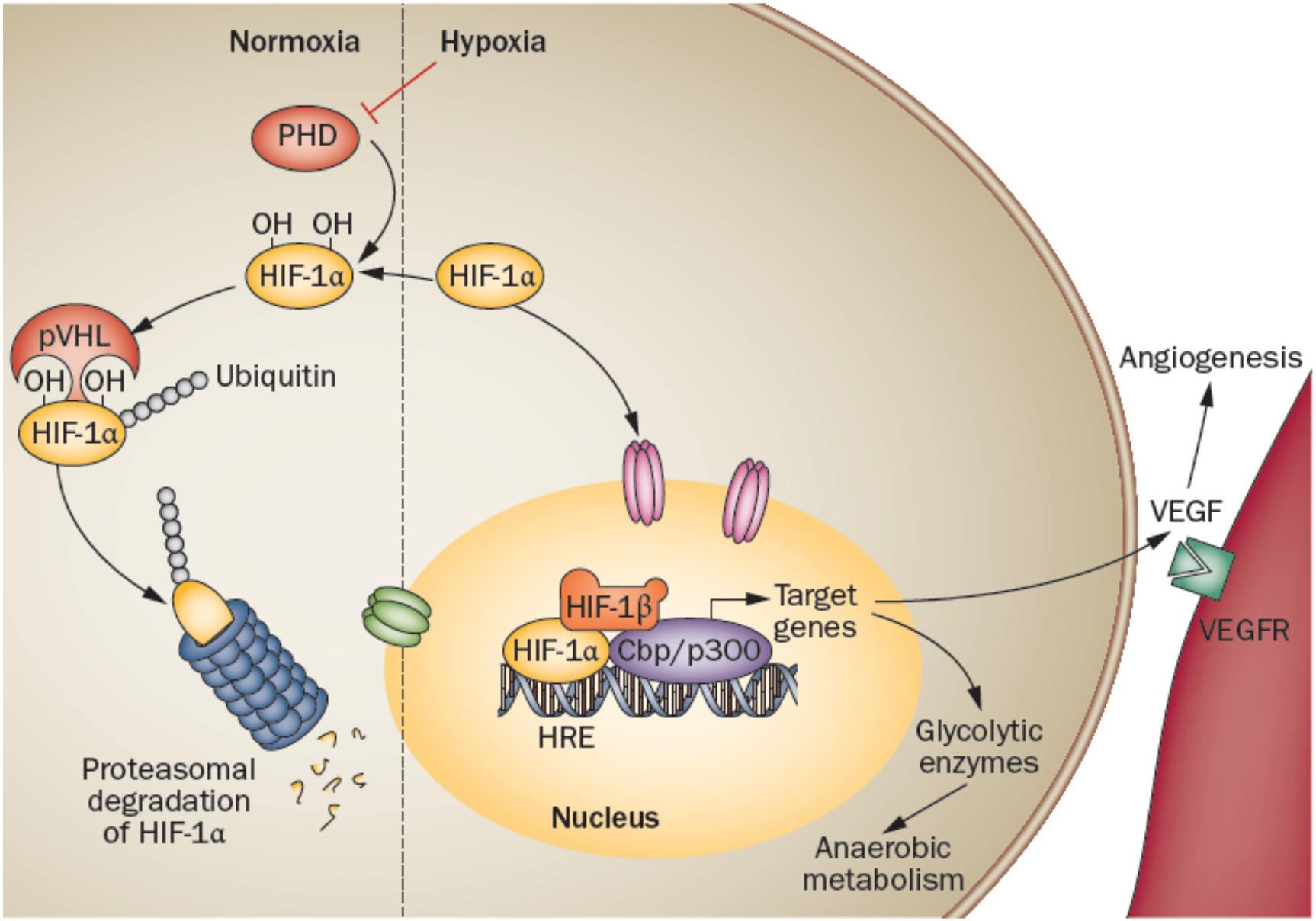
See Also 1124
YOUNG INVESTIGATOR AWARD
Osteocytes Mediate Bone Pain Through Cell-Cell Communication with Sensory Neurons via Connexin 43
Masahiro Hiasa (Toshi Yoneda), Indiana University

Hypoxia



Maes, Carmeliet, Schipani, Nat.Rev. Rheumatology 2012

1138

YOUNG INVESTIGATOR AWARD

HYPOXIA-INDUCIBLE FACTOR 2a IS A NEGATIVE REGULATOR OF OSTEOBLASTOGENESIS

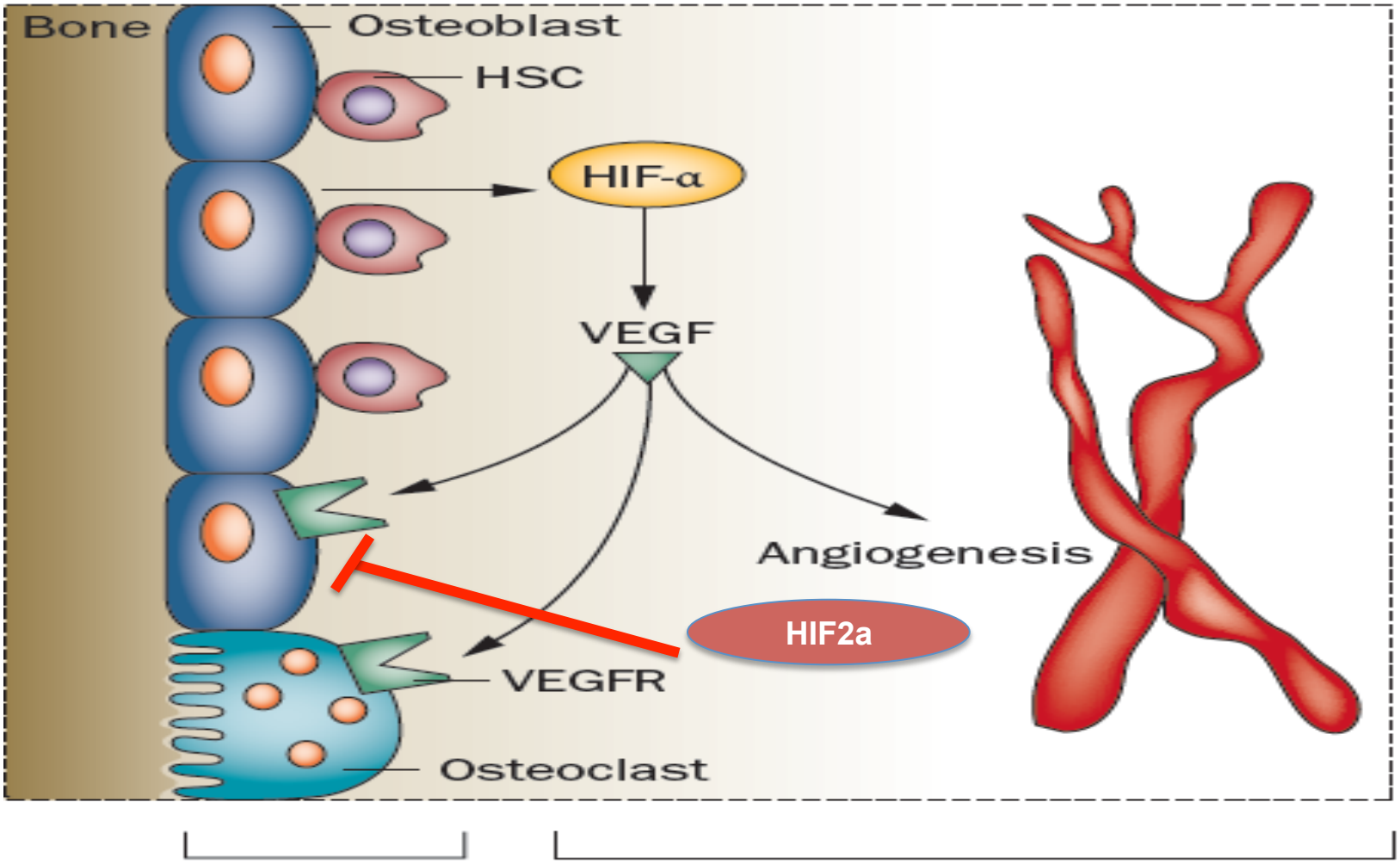
Presenter: Kavitha Ranganathan (Ernestina Schipani) University of Michigan

The hypoxia-signaling pathway regulates bone mass. While the role of HIF-1a in bone is actively investigated, the contribution of HIF-2a to the control of bone mass is still poorly understood.

- generated mice overexpressing a **mutant HIF-2a protein (HIF-2adPA)** that is **constitutively stabilized** regardless of oxygen tension, using PRX1Cre mice.
- long bones from **PRX1Cre;HIF-2adPA mice had high trabecular bone volume** and bone **resorption was impaired**.
- The numbers of osteoblasts in vivo, **bone formation rates**, osteogenic **differentiation** of bone marrow stromal cells in vitro, and expression of **Osterix, were all reduced**.
- Conversely, there was an **increased number of bone marrow stromal cells.**
- Consistent with this, **VEGF was augmented in mutant osteoblasts**.
- Augmenting the number of bone marrow stromal cells, and thus of osteoblast precursors, could in some way counterbalance the HIF-2a inhibitory effect on osteoblast differentiation.
- In summary, 1) overexpression of HIF-2a in cells of the osteoblast lineage results in a net increase of trabecular bone, which is accompanied by an impairment of both formation and resorption; 2) overexpression of HIF-2 expands the pool of osteoblast precursors, but, at the same time, impairs their osteogenic differentiation.

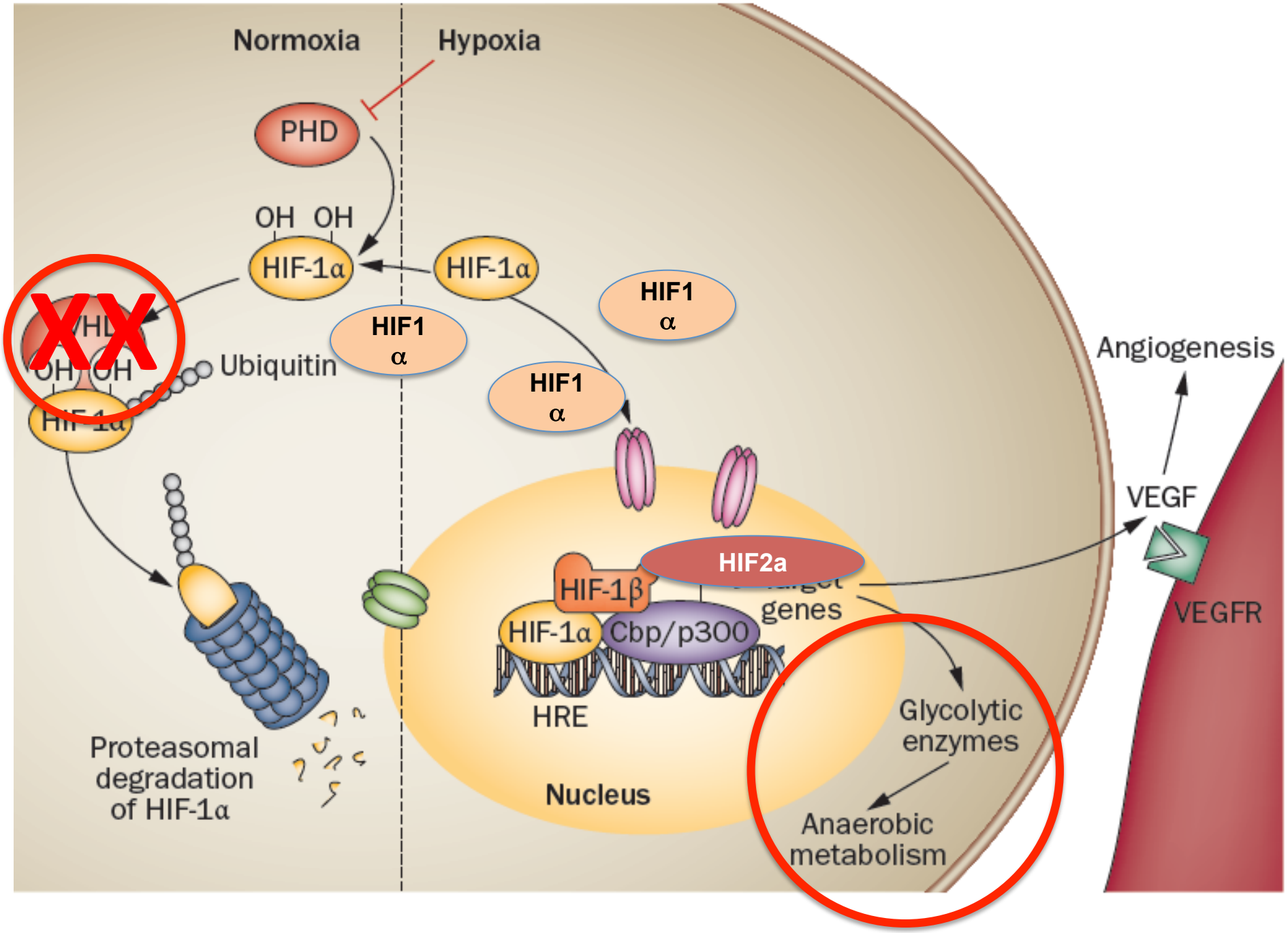
Conclusion: HIF-2a is a negative regulator of osteoblastogenesis, a paradigm shift as activation of the hypoxia signaling pathway has been associated with stimulation of bone formation.

HIF1α Favors but HIF2α Inhibits Bone Formation



See Also 1013
Osteocyte-Specific HIF-1α Activity Increases Bone Mass through Sirtuin 1-Dependent Decrease of Sclerostin
Presenter: Steve Stegen (Geert Carmeliet) KU Leuven, Belgium

Maes, Carmeliet, Schipani, Nat.Rev. Rheumatology 2012



YOUNG INVESTIGATOR AWARD

Hypoxia Signaling-Induced Glycolytic Metabolism in Osteoblasts can Affect Systemic Glucose Homeostasis by Increasing Glucose Utilization by the Skeleton

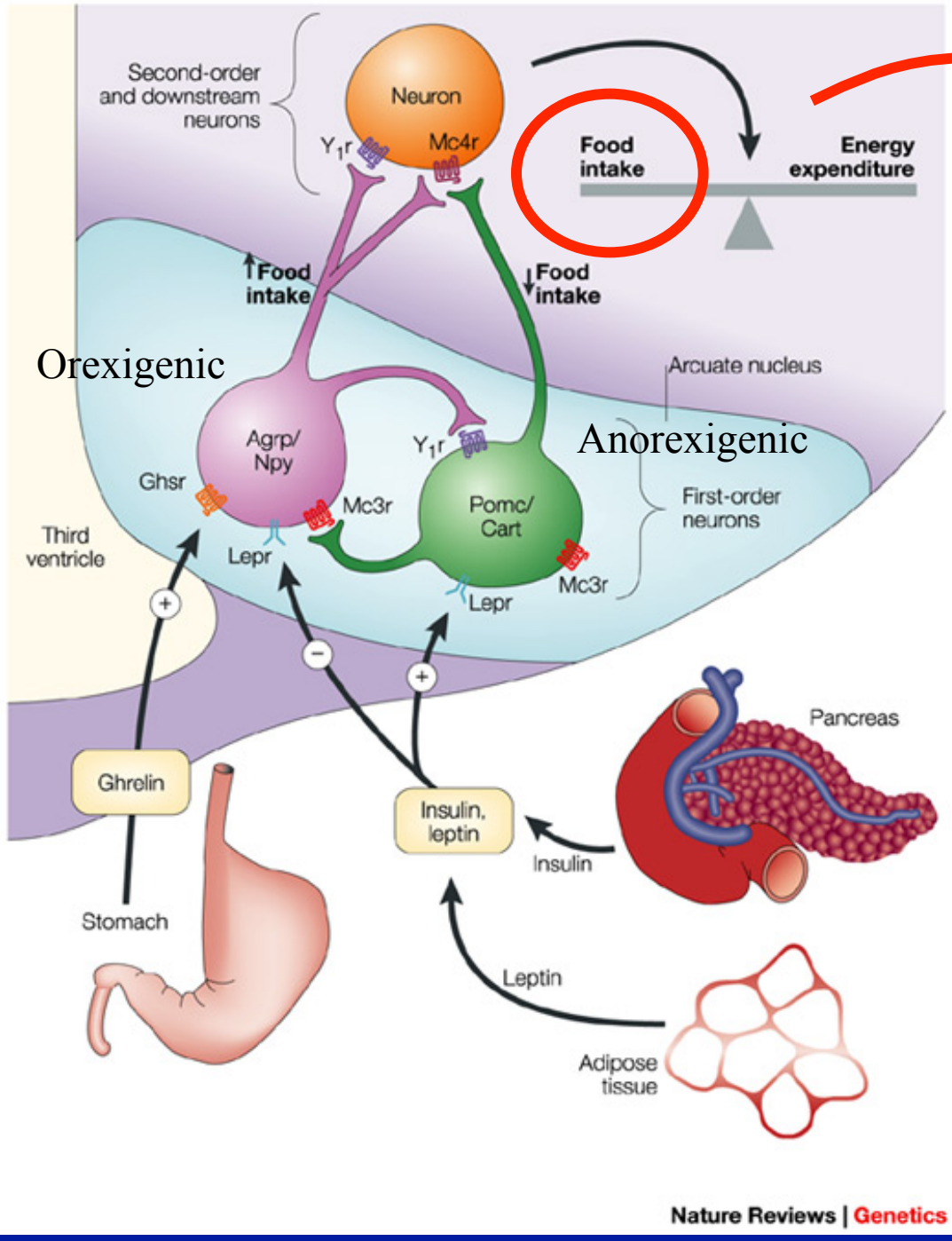
Presenter: Naomi Dirckx (Christa Maes) KU Leuven, Belgium

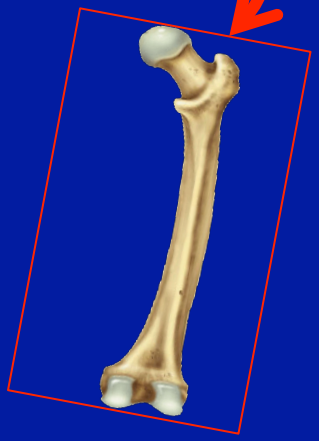
The skeleton regulates systemic energy metabolism and glucose homeostasis.

Question: Does Hypoxia, a known regulator of cellular metabolism, in bone affect systemic metabolism?

- **Deleted Vhl,** a negative regulator of HIF, in osteoblast lineage cells by the **Osx-Cre**:GFP.
- **Vhl-deficient Obs increased glucose uptake and lactate production**, indicating **increased glycolysis at the expense of glucose oxidation, compensated by high glucose uptake**.
- Intriguingly, Vhl cKO **mice were lean and hypoglycemic with increased glucose tolerance**, which **could not be explained by alterations in insulin production or sensitivity, nor by increased osteocalcin**, as serum osteocalcin was 80% reduced in Vhl cKO mice.
- PET scans using 18F-FDG revealed that **the skeleton takes up a high portion of injected glucose (13% in control mice)** relative to soft tissues (e.g. liver 5%, heart 9.7%, brain 6.5%).
- the uptake of 18F-FDG was **increased in bones of Vhl cKO mice**, **with upregulation of glycolytic markers (Pgk1, Pdk1)** and a 3-fold increased Glut1 expression.
- the Pdk1-inhibitor dichloroacetate (DCA) prevented the development of the metabolic phenotype in postnatal induced Vhl cKO mice.

Conclusion: There is a link between cellular metabolism in bone and whole-body glucose homeostasis, controlled by local hypoxia-signaling in the skeleton.


Second-order and downstream neurons
Neuron
Y1r
Mc4r
Food intake
Energy expenditure
↑Food intake
↓Food intake
Orexigenic
Arcuate nucleus
Anorexigenic
Agrp/ Npy
Y1r
Ghsr
Mc3r
Pomc/ Cart
First-order neurons
Third ventricle
Lepr
Mc3r
Lepr
+
−
+
Pancreas
Ghrelin
Insulin, leptin
Insulin
Stomach
Leptin
Adipose tissue
Nature Reviews | Genetics



1035

Regulation of Appetite by the Skeleton

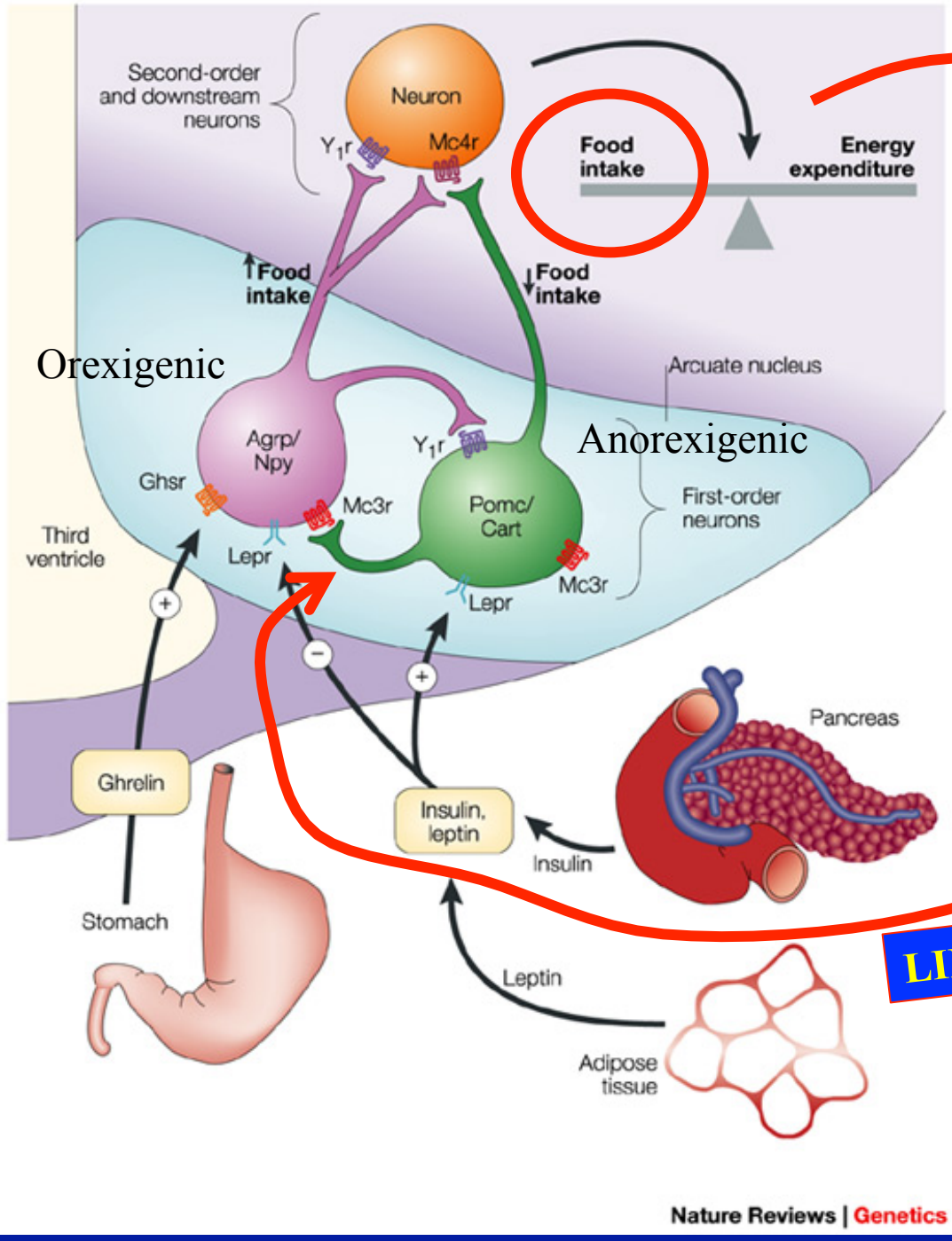
Presenter: Ioanna Mosialou (Stavroula Kousteni) Columbia University;

Bone is a pleiotropic endocrine organ that secretes at least two hormones, FGF23 and osteocalcin, to regulate kidney function and glucose homeostasis.

Question: Is there other bone-derived hormones and what is their potential functions?

- Studies in mice lacking osteoblasts indicated the existence of hormone(s) that regulate appetite, a property not affected by osteocalcin.
- Lipocalin 2 (LCN2), an “adipokine”, is expressed10X more in osteoblasts than adipocytes.
- Inactivation of Lcn2 in osteoblasts (Lcn2osb-/- mice) increased blood glucose, fat mass and body weight, decreased serum insulin and led to glucose intolerance and insulin resistance.
- These effects resulted mainly from a 24% increase in food intake.
- In contrast, inactivation of Lcn2 in adipocytes had no effect on any metabolic parameters.
- Lcn2osb-/- mice showed no changes in any hormone known to affect food intake.
- LCN2 is not expressed in the hypothalamus or brain stem, two areas regulating appetite.
- Peripherally administered r LCN2 accumulated in the hypothalamus of mice with ubiquitous inactivation of Lcn2 (Lcn2-/- mice) at levels equivalent to those observed in wild type.
- Lcn2-/- mice increase food intake and have a metabolic phenotype similar to Lcn2osb-/- mice.
- LCN2 delivered via ICV infusions corrected the increased appetite seen in Lcn2-/- mice.

Conclusion: LCN2 is as an osteoblast-derived anorexigenic hormone and the regulation of appetite is an endocrine function of bone.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Energy expenditure
↑Food intake
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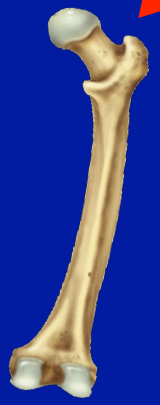
Orexigenic
Arcuate nucleus
Anorexigenic
Agrp/ Npy
Y1r
Ghsr
Mc3r
Pomc/ Cart
First-order neurons
Third ventricle
Lepr
Mc3r
Lepr
+
−
+
Pancreas
Ghrelin
Insulin, leptin
Insulin
Stomach
Leptin
Adipose tissue
Nature Reviews | Genetics



LIPOCALIN

1146 2 years ago

Osteocalcin regulates muscle function and mass

Plenary Orals: Basic Bone Biology II

Presenter: Paula Mera, (Karsenty's lab) Columbia University, USA

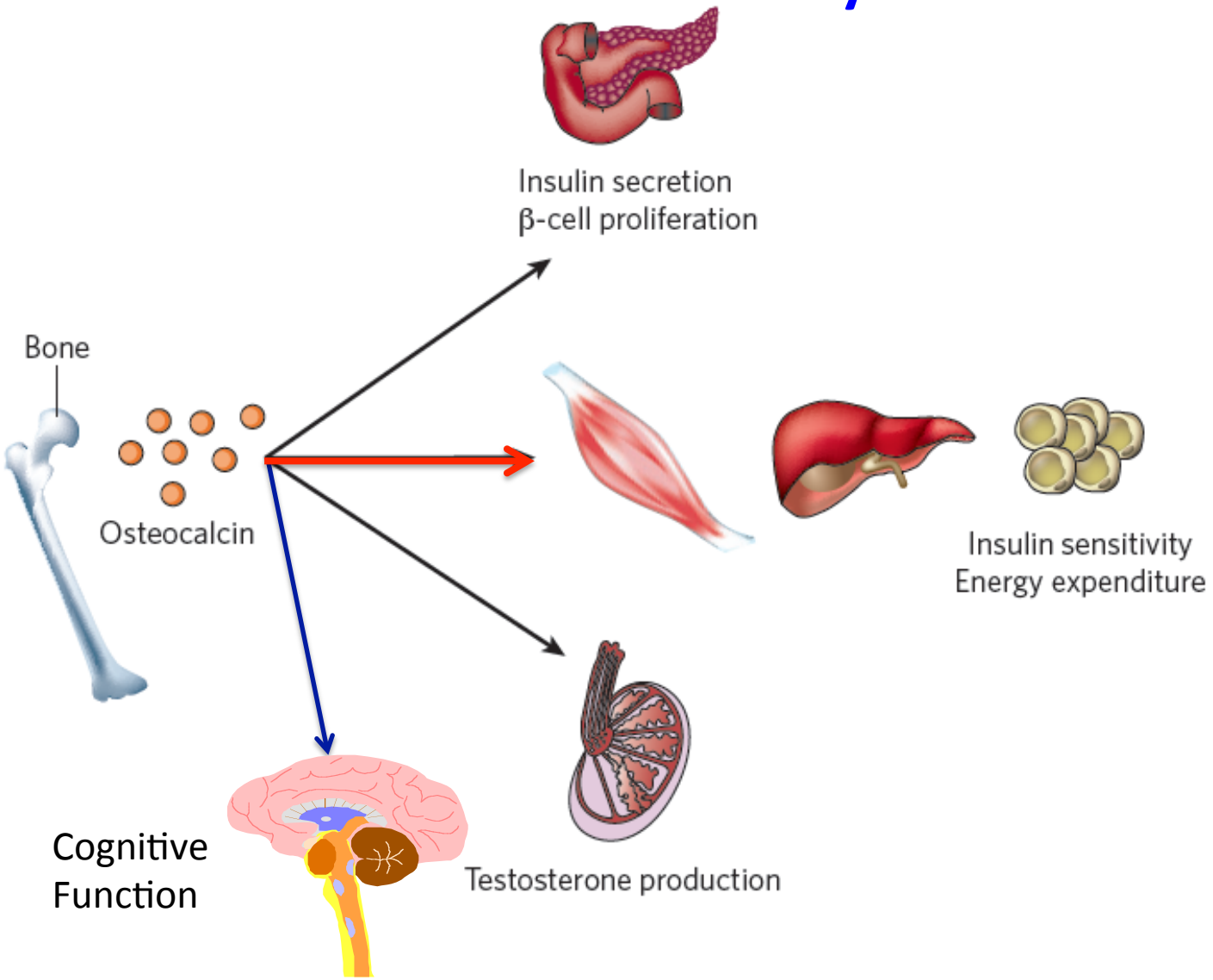
Muscle function and mass decrease with aging at the same time bone mass decreases. **GPRC6A**, a receptor for OCN, **is expressed in muscle fibers and satellite cells**.

Question: **Does osteocalcin regulate muscle function and mass in the mouse?**

- **Ocn-/- mice exhibit a decrease in muscle function and mass.**
- **Converse is true** in Esp-/- mice, a model of **OCN gain-of-function**.
- **mice lacking GPRC6A,** in all cells or **in myocytes display the same muscle phenotype**.
- As in β cells and in Leydig cells of the testis, **OCN activates the cAMP/PKA pathway in myotubes** and this is **dependent on GPRC6A**.
- **OCN** also in a GPRC6A-dependent manner, **inhibits the energy sensor AMPK in myotubes**.
- Concomitantly, OCN promotes tyrosine incorporation into cellular proteins in WT, but not in Gprc6a-/- myotubes.

Conclusion: **Osteocalcin is a regulator of muscle function and mass through GPRC6A-dependent inhibition of AMPK in muscle**

Bone as an Endocrine Organ via Osteocalcin Secretion and Decarboxylation



Adapted from Karsenty and Ferron, Nature 2012

A crosstalk between bone and muscle endocrine functions favors adaptation to exercise
1131
Presenter: Paula Mera (Gerard Karsenty) Columbia University;

Osteocalcin increases muscle function during exercise in mice by increasing the uptake and utilization of glucose and fatty acids in myofibers.

Question: By what mechanism?

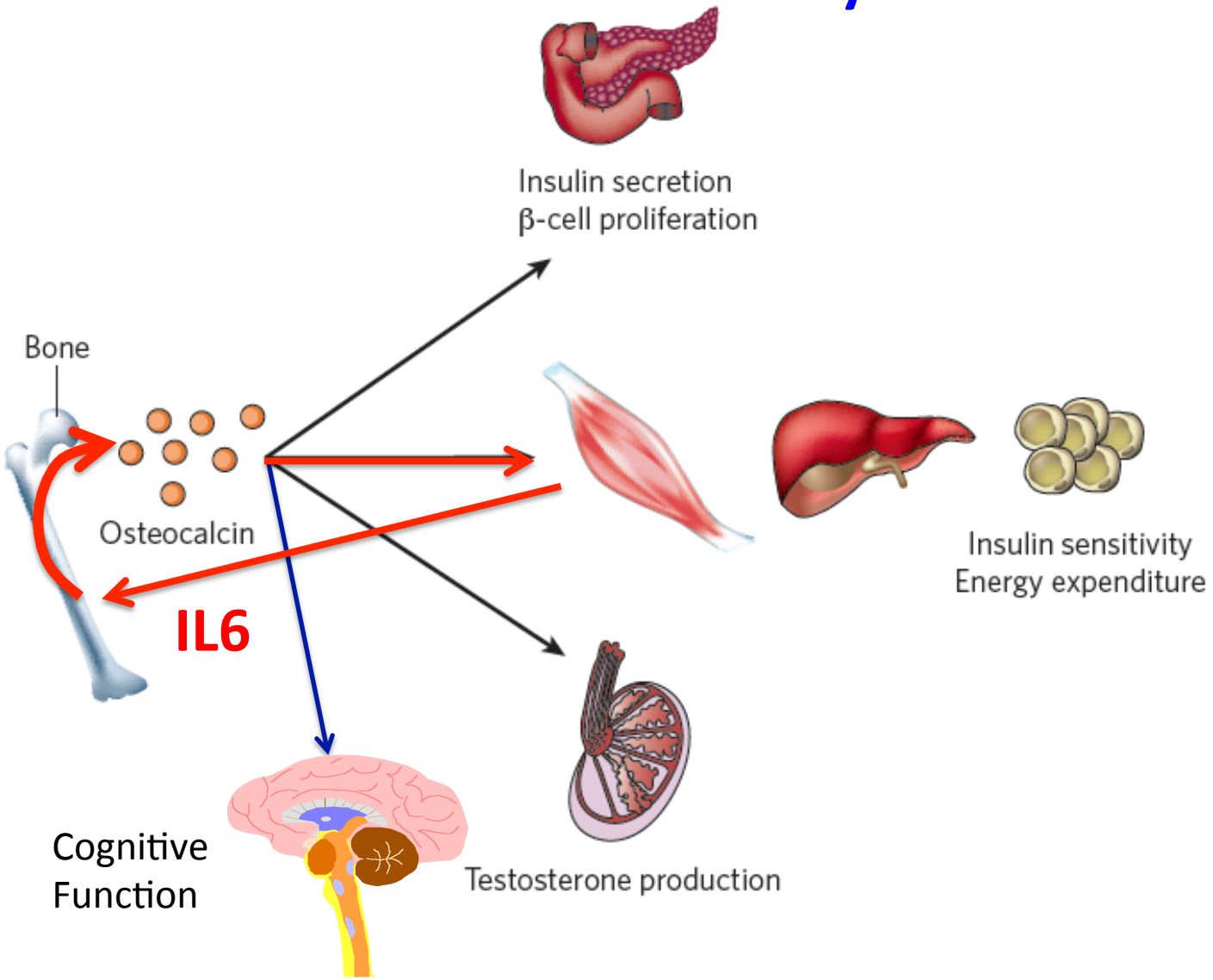
- performed a transcriptomic analysis after exercise in muscles of control mice and mice lacking osteocalcin signaling in muscle (Gprc6aMck-/-). The expression of IL-6 and its receptor, was decreased 5X in muscle of Gprc6Mck-/-.
- IL-6 is a myokine and circulating levels are increased during exercise, when it favors adaptation to exercise through an increase in the production of glucose and fatty acids, the uptake of which is enhanced by osteocalcin signaling in myofibers.
- The increase in circulating IL-6 levels during exercise is smaller in Osteocalcin-/- and Gprc6aMck-/-, revealing that osteocalcin regulates IL6 expression in muscle and that muscle is the main source of IL-6 during exercise.
- Conversely, exogenous osteocalcin to 15 month-old WT mice corrects their inability to

See Also 1037
Osteocalcin Signaling in Myofibers Favors Adaptation to Exercise by Increasing Uptake and Utilization of Nutrients in Adult Mice

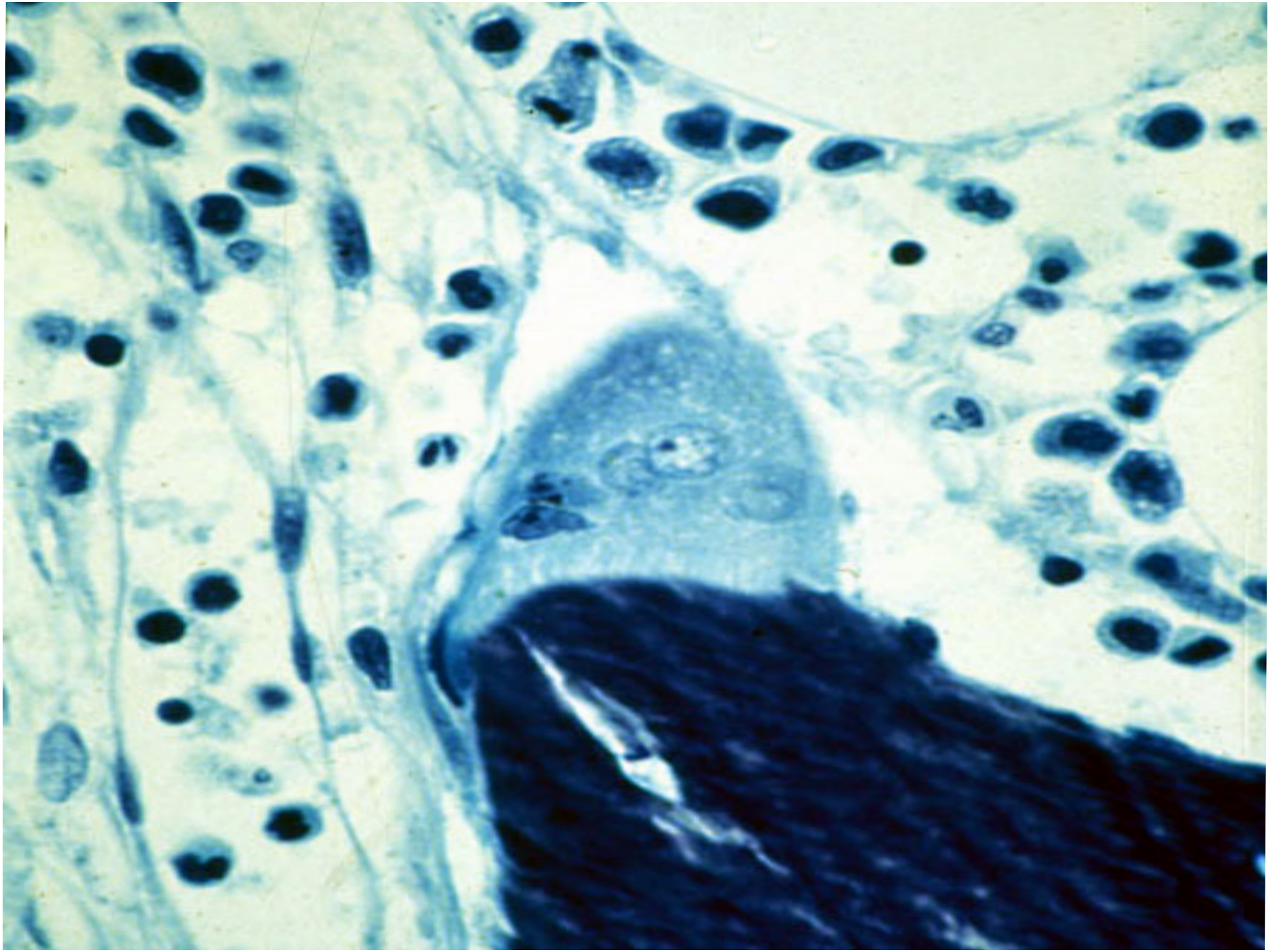
Presenter: Paula Mera (Gerard Karsenty) Columbia University;

Conclusion: There is a positive crosstalk between bone via osteocalcin, and muscle via IL-6, that is necessary and sufficient to allow adaptation to exercise in the mouse.

Bone as an Endocrine Organ via Osteocalcin Secretion and Decarboxylation



Adapted from Karsenty and Ferron, Nature 2012



1055

YOUNG INVESTIGATOR AWARD

Osteoclast Precursor Cells That Form Osteoclasts In Vivo Under Homeostatic Conditions Express CX3CR1, Form Osteoclasts Within 5 Days And Rarely Derive From Circulating Cells.

Presenter: Emilie Roeder (Joseph Lorenzo) UConn Health

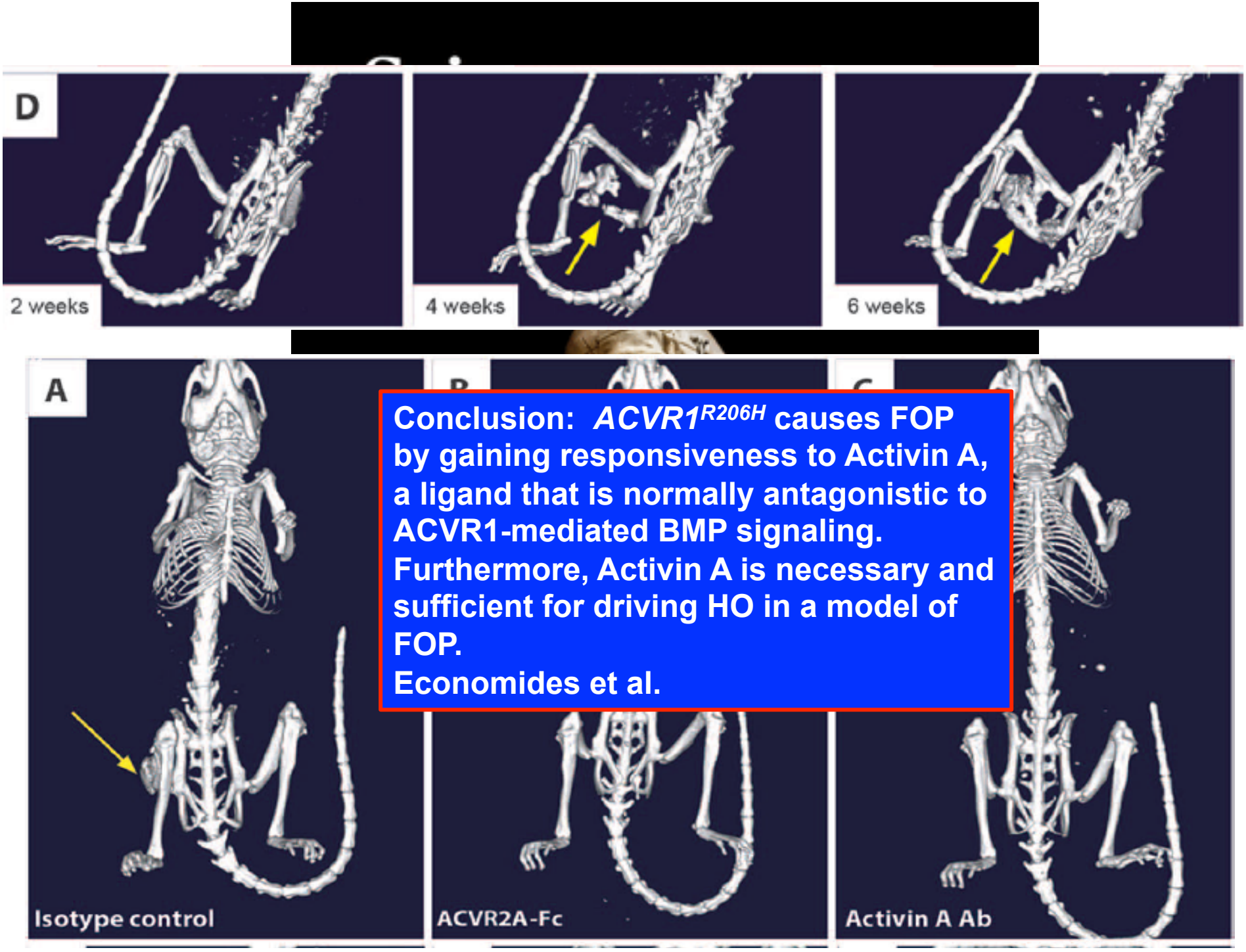
Question: In vivo, What is the nature of the osteoclast (OC) precursor (OCP) ?.

- Mice with EGFP knocked into the CX3CR1 coding locus show that > 99% of OCP populations expressed EGFP (i.e. were positive for CX3CR1) but mature OC did not.
- Mice with Cre-ERT2 knocked into the CX3CR1 coding locus were crossed with tdTomato showed strong tdTomato expression at day 5 in many (TRAP) + OC adjacent to bone.
- Since CX3CR1 is not expressed in mature OC, the fluorescent OC must have originated from CX3CR1-expressing OCP.
- Parabiosed TRAP-tdTomato mice (CD45.2) with wild type (WT) mice (CD45.1) demonstrated abundant tdTomato + OC in femurs of the TRAP-tdTomato mice but these were undetectable in WT mice.

See Also 1060
YOUNG INVESTIGATOR AWARD

Absence of the VDR in Osteoclasts Results in Increased Bone Resorption and Osteoclast Survival

Presenter: Yolandi Starczak (Paul H. Anderson) University of South Australia;



Conclusion: *ACVR1*^{R206H} causes FOP by gaining responsiveness to Activin A, a ligand that is normally antagonistic to ACVR1-mediated BMP signaling. Furthermore, Activin A is necessary and sufficient for driving HO in a model of FOP.
Economides et al.

Dose-response Relationship of Palovarotene in the ALK2 (Q207D) Cre-Inducible Transgenic Mouse Model of HO Under Mild and Severe Injury Conditions

1088
Presenter: Isabelle Lemire (Michael Harvey) Clementia Pharmaceuticals Inc.;

Fibrodysplasia Ossificans Progressiva (FOP) is a rare, genetic disease of skeletal malformations and progressive heterotopic ossification (HO) for which there is no approved treatment.

Palovarotene (PVO) is an orally bioavailable RARγ selective agonist shown to prevent HO formation in injury-induced and genetic mouse models of FOP.

- Mice homozygous for the ALK2 (Q207D) transgene were injected intramuscularly into the posterior left femoral muscle region with different concentrations of Adeno-Cre, to activate the transgene and cardiotoxin (CTX) to initiate the inflammatory triggering event leading to HO.
- MicroCT revealed the presence of HO in vehicle controls. The degree to which PVO prevented the formation of HO depended on the dose and the experimental conditions.
- The dose-response curve showed a shift to the right when severe HO formation was induced in vehicle-treated animals with greater % HO inhibition in animals administered PVO under mild injury conditions than under severe injury conditions.
- PVO is being investigated in Phase 2 clinical trials as a potential therapy for FOP.

Conclusion: higher doses of PVO resulted in greater inhibition of HO in this injury-based Q207D mouse model of FOP.

1089

YOUNG INVESTIGATOR AWARD

The Role of Canonical Wnt Signaling in the Development of Spondyloarthritis

Presenter: Wanqing Xie (Di Chen) Rush University Medical Center;

Up-regulation of canonical Wnt signaling has been reported in patients with Spondyloarthritis (SpA), an autoimmune disease affecting spine and joint.

Question: is activation of β-catenin signaling the key event leading to SpA?

- To mimic β-catenin activation, we bred β-catenin(ex3)flox/floxmice withCol2-CreERT2, Agc1-CreERT2, and Gli1-CreERT2 transgenic mice and observed:
- 1) Severe osteophyte formation in the discs of entire spine.
- 2) Severe destruction of disc tissues, including loss of growth plate (GP), cartilage and disorganized annulus fibrosus (AF) and nucleus pulposus (NP)
- 3) Mineralization of ligaments in the spine.
- 4) Severe loss of proteoglycan and defects in cartilage structure in the facet joint
- 5) Immortalized rat AF and NP cell lines treated with BIO (GSK-3β inhibitor), stimulated Mmp13(8 and 11 folds), NGF (5 and 2 flods) and Ccl2(6 and 11 folds) expression, important mediators for pain, in AF and NP cells.
- 6) increased pain sensitivity, sustained throughout the entire experimental period (4-9 month).
- 7) Deletion of Mmp13 reversed defects observed in disc tissue and reduced pain sensitivity
- . 8) Increased mineralization of bone was observed in sacroiliac joints.

Conclusion: Since all changes observed in β-catenin activation mice are found in patients with SpA, β-catenin may be a key mediator in SpA development.

1091

MOST OUTSTANDING TRANSLATIONAL AWARD

Genetic Sost Deletion or Pharmacological Inhibition of Sclerostin Prevents Bone Loss and Decreases Osteolytic Lesions in Immunodeficient and Immunocompetent Preclinical Models of Multiple Myeloma

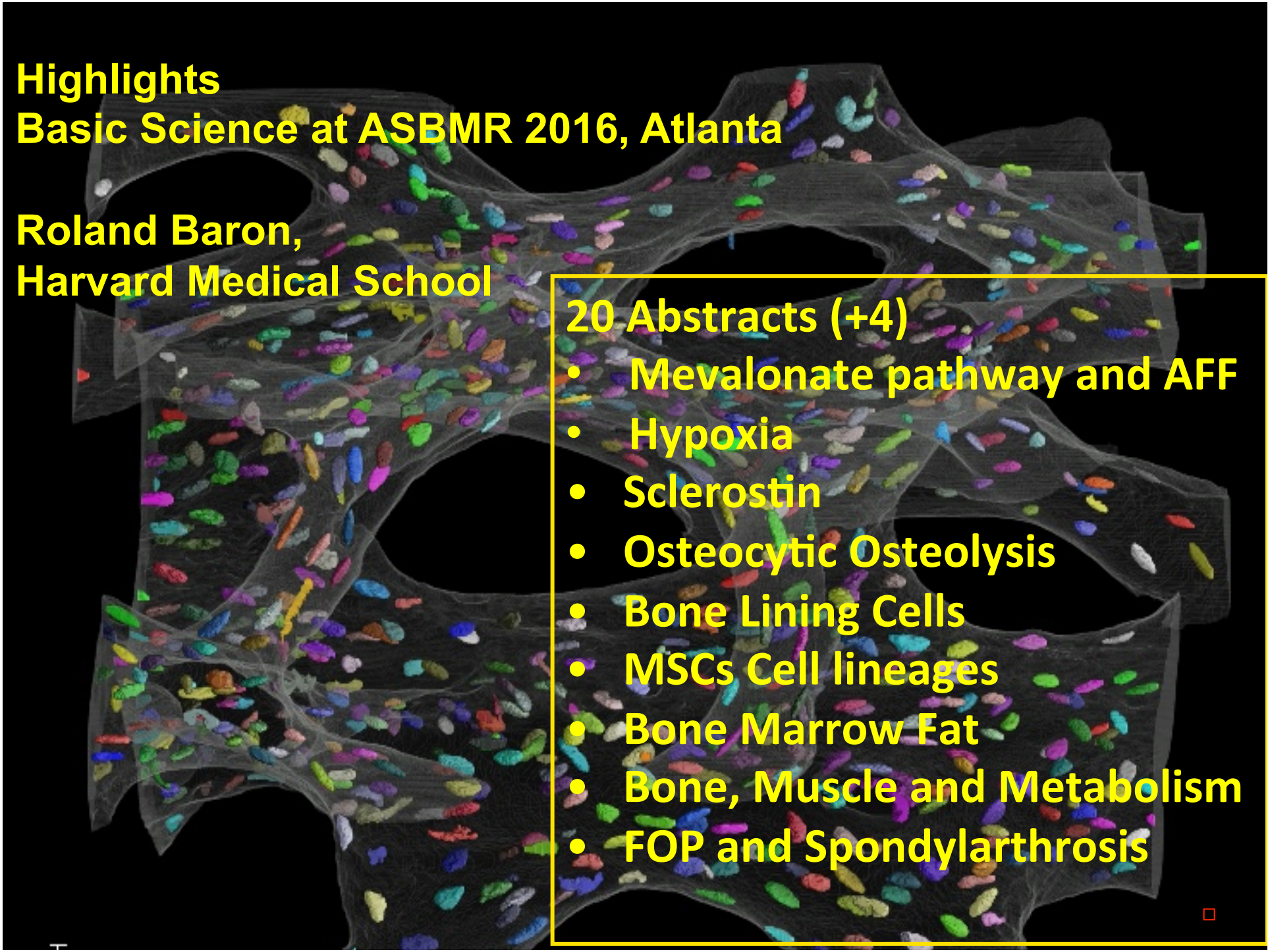
Presenter: Jesus Delgado-Calle (Teresita Bellido, David Roodman) Indiana University

Multiple myeloma (MM) cells induce lytic lesions due to increased bone resorption and suppressed bone formation. Osteocytes in mice bearing MM tumors express elevated Sclerostin (Scl); and serum Scl is increased in MM patients.

Question: What is the impact of Sost/Scl on tumor growth and MM-induced bone disease?

- KO SCID mice displayed the expected high bone mass and hMM-injected KO and WT mice had equivalent tumor engraftment
- hMM-injected WT mice exhibited ~50% decreased tibia BV/TV but hMM-injected KO mice displayed no changes in BV/TV.
- The number and area of osteolytic lesions was reduced in KO mice
- inhibition of Scl in an immunocompetent model of MM showed that mMM-injected mice had decreased BV/TV but Slc-Ab displayed increased trabecular BV/TV and decreased number of osteolytic lesions.
- mMM-injected mice treated with IgG or Scl-Ab showed similar increased serum CTX but mice treated with Scl-Ab had a smaller decrease in serum P1NP

Conclusion: Osteocytic Scl contributes to MM-induced bone loss, without affecting tumor growth. These findings provide the rationale for combining Scl-Ab with anti-tumor drugs to treat MM.


Highlights
Basic Science at ASBMR 2016, Atlanta
Roland Baron,
Harvard Medical School
20 Abstracts (+4)
• Mevalonate pathway and AFF
• Hypoxia
• Sclerostin
• Osteocytic Osteolysis
• Bone Lining Cells
• MSCs Cell lineages
• Bone Marrow Fat
• Bone, Muscle and Metabolism
• FOP and Spondylarthrosis