Bone Homeostasis and Pathology

Instructor: Roman Eliseev
Outline:

- Bone anatomy and composition
- Bone remodeling
- Factors regulating bone homeostasis
- Disorders of bone homeostasis:
  - Bone loss
  - Abnormal bone acquisition
- Methods and Mouse Models
Adult Skeleton

Axial Skeleton

Appendicular Skeleton

206 bones
Adult Bone Architecture

- Cortical bone
- Marrow cavity
- Diaphysis
- Metaphysis
- Epiphysis
- Trabecular bone
Bone Histology

- **Subchondral bone**
- **Trabecular bone**
- **Marrow fat**
- **Bone marrow**
- **Cortical bone**
Bone Composition

Bone is a mineralized organic matrix composed of:

- Type I collagen and non-collagenous proteins (osteoid)
- Hydroxyapatite crystals ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$)

Bone-forming cells are osteoblasts (OB) that produce collagen I and deposit HA
Bone is Formed by Osteoblasts

OBs originate from Bone Marrow Stromal (a.k.a. Mesenchymal Stem) Cells (BMSC) and terminally differentiate into osteocytes (OT).

Wagner et al., PPAR Res., 2010
Bone is Resorbed by Osteoclasts
Osteoclasts (OC) are bone resorbing multinucleated cells that originate from hematopoietic cells (monocyte/macrophage).
Homeostasis = equilibrium (Greek: ὠμοίως + στάσις)

Bone Formation vs Resorption = Dynamic Equilibrium (~10% of adult human skeleton is replaced annually)
Intact Bone

BMSC – bone marrow stromal (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte

Apoptosis (~70%)

TGFb, IGF1, OCN
Collagen I
Remodeling: Initial Phase

BMSC – bone marrow stromal (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte
HSC – hematopoietic stem cell
OCP – osteoclast precursor
Remodeling: Resorption Pit

BMSC – bone marrow stromal (a.k.a. mesenchymal stem) cell
OB – osteoblast
OT – osteocyte
OC – osteoclast

BMS0C
Blood vessel

OB

OC

OC

pH≤4

OT

BONE
Remodeling: Recruitment/Activation of BMSCs into Osteogenic Lineage

- BMSCs
- Blood vessel
- TGFβ, IGF1
- pH ≤ 4
- OC
- OB
- BONE

Remodeling involves the recruitment and activation of BMSCs into the osteogenic lineage. TGFβ and IGF1 are key growth factors that promote this process. The pH level is crucial, with pH ≤ 4 being optimal for osteogenic differentiation.
Remodeling: New Bone Formation

BMSC

Blood vessel

OB

New bone

BONE

OT
Histology of Bone Remodeling

[Image of histological section with labeled cell types: Osteoblast, Lining cell, Osteoclast, Osteocyte, TRAP Staining]

Image – courtesy of Dr. J. Jonason
Phases of Remodeling

- Osteoclast (OC) precursors
- Osteoblast (OB) precursors
- Lining cells
- OCs

**Bone Marrow**

**Bone**

- Resting surface
- Initial excavation
- Reversal
- Osteoid synthesis
- Completed osteon

**Resorption phase**

~2-4 weeks

**Formation phase**

~4-6 months
Factors Regulating Bone Homeostasis

Disorders of Bone Homeostasis

Bone formation by osteoblasts

Bone resorption by osteoclasts

BONE LOSS

BONE GAIN

Bone formation by osteoblasts

Bone resorption by osteoclasts
Disorders of Bone Homeostasis

- **Osteogenesis Imperfecta (aka, Brittle Bone Disease)**
  - Most commonly inherited bone disorder
  - Autosomal dominant mutations (over 800 identified) in the genes encoding the \( \alpha_1 \) and \( \alpha_2 \) chains of type I collagen
  - Clinical features include: skeletal fragility with increased fracture risk, blue sclera, hearing loss, dental imperfections, joint laxity

- **Osteopetrosis (aka, Marble Bone Disease)**

- **Osteoporosis**

- **Paget Disease**

- **Hyperparathyroidism**

*Courtesy of Dr. J. Jonason*
Disorders of Bone Homeostasis

• Osteogenesis Imperfecta \textit{(aka, Brittle Bone Disease)}

• Osteopetrosis \textit{(aka, Marble Bone Disease)}
  • Rare genetic disease characterized by reduced bone resorption
  • Autosomal dominant or recessive mutations affecting osteoclast numbers or function
  • Clinical features include: frequent fractures, scoliosis, cranial nerve deficits, anemia
  • First genetic disease treated with a bone marrow transplant

• Osteoporosis

• Paget Disease

• Hyperparathyroidism

\textit{Courtesy of Dr. J. Jonason}
Disorders of Bone Homeostasis

- Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)

- Osteopetrosis (*aka*, Marble Bone Disease)

- Osteoporosis
  - Common disease associated with decreased bone mass and increased fracture risk
  - Caused by both genetic and environmental factors
  - High societal burden with ~2 million fractures in the US annually due to osteoporosis, at a cost of around 22 billion dollars to treat

- Paget Disease

- Hyperparathyroidism

*Courtesy of Dr. J. Jonason*
Disorders of Bone Homeostasis

- Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)

- Osteopetrosis (*aka*, Marble Bone Disease)

- Osteoporosis

- Paget’s Disease
  - Uncontrolled bone remodeling leading, ultimately, to areas of high bone mass that are architecturally unsound
  - Onset in late adulthood with causes unknown (likely to be a combination of genetic and environmental factors)
  - Most prominent clinical feature is bone pain, but can also lead to fracture, bone deformities, and nerve compression

- Hyperparathyroidism

*Courtesy of Dr. J. Jonason*
Disorders of Bone Homeostasis

- Osteogenesis Imperfecta (*aka*, Brittle Bone Disease)
- Osteopetrosis (*aka*, Marble Bone Disease)
- Osteoporosis
- Paget’s Disease
- Hyperparathyroidism
  - Excess parathyroid hormone (PTH) in the bloodstream due to over-activity of the parathyroid glands
  - Can be *primary* (due to hyperplasia or tumor of the glands) or *secondary* (due to hypocalcemia and compensatory increases in PTH secretion)
  - Results in increased bone resorption leading to increased fracture risk
Osteoporosis

- Fracture
- Delayed repair

Images from Bionews Texas
Epidemiology of Osteoporosis

- Osteoporosis is found in 70% of the elderly population.

- Post-menopausal women are most affected (25 million American women with Osteoporosis).

- 1.9 million fractures annually due to low bone mass.

- 1 of 3 women will develop a vertebral fracture by age 65, and 1 of 3 women will develop a hip fracture by age 85.

- $22 billion dollars per year is spent on treating Osteoporosis.
Epidemiology of Osteoporosis
C57Bl/6J mice:
**Diagnosis of Osteoporosis**

**DEXA**

**Dual Energy X-ray Absorptiometry**

T-score = comparison to healthy young bone

Z-score = comparison to a reference group of the same age, race, and gender as the patient

*Courtesy of Dr. J. Jonason*
# Treatment of Osteoporosis

## Anti-resorptive drugs: Bisphosphonates

<table>
<thead>
<tr>
<th>Drug</th>
<th>Postmenopausal osteoporosis</th>
<th>Glucocorticoid-induced osteoporosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevention</td>
<td>Treatment</td>
</tr>
<tr>
<td>Alementronate (Fosamax)</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Risedronate (Actonel)</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Ibandronate (Boniva)</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Zoledronate (Reclast)</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Treatment of Osteoporosis

**SERMS**

**SERMS**: Selective Estrogen Receptor Modulators

**Action**: Drugs that act as estrogen agonists, activating the estrogen receptor in order to reduce osteoclast activity and bone resorption.

**Drug**: Raloxifene (Evista)

**Dose**: 60mg/day for many years

**Notes**: Not as effective as bisphosphonates. Only appears to protect spine and not hips from fracture.
Treatment of Osteoporosis

Bone anabolic agents

**PTH:** rPTH(1-34); Forteo. Under clinical investigation for the use in fracture non-union repair, treatment of osteoporosis.

**BMPs:** rBMP2 and rBMP7. Under clinical investigation and use for the repair of fracture non-union (InFuse). Recent complications in spinal fusion.

Neutralizing antibodies for Wnt inhibitors: anti-Dkk1 and anti-SOST. Under research and pre-clinical investigation for treatment of OA and fracture repair.
Lab Methods to Assess Bone Homeostasis

DEXA
Lab Methods to Assess Bone Homeostasis

micro-CT

Shum et al., PLoS One, 2016
Lab Methods to Assess Bone Homeostasis

**Histology/Histomorphometry**

- Bone Area (B.Ar.) vs Total Area (T.Ar.)
- Surface, i.e., Bone Surface (BS)
- Cell Number per Surface/Area
  - OB.N.
  - OT.N.

*Primary Measurements:*

*OsteoMeasure or Visiopharm*

*Shum et al., PLoS One, 2016*
Lab Methods to Assess Bone Homeostasis

Histology/Histomorphometry

Primary Measurements:
- Surface: Osteoclast Surface per Bone Surface (OC.S./BS)
- Cell Number: Osteoclast Number per Bone Surface (N.OC/BS)

Shum et al., PLoS One, 2016
Lab Methods to Assess Bone Homeostasis

Dynamic Bone Labeling (Bone Formation)

Mice are injected with Alizarin Red 10d before sacrifice & with Calcein 5d before sacrifice. Bone are frozen and sectioned. Labeled bone is visualized using fluorescence microscopy.

Derived Indices:
- Mineralizing Surface (MS/BS)
- Mineral Apposition Rate (MAR)
- Bone Formation Rate (BFR)

Shum et al., PLoS One, 2016
Lab Methods to Assess Bone Homeostasis

Bone Turnover Serum Markers

Formation: P1NP

Resorption: CTX

Per recommendation of the International Osteoporosis Foundation (IOF) and the International Federation for Clinical Chemistry (IFCC)
Useful Mouse Models

Osteolineage-specific Cre Models:

- **BMSC**: Prx1, Nestin, LepR, aSMA, Osx
- **OB**: Osx (early), Col1 3.2kb (early), Col1 2.3kb (late), OCN (late)
- **OT**: DMP1
Useful Mouse Models

Osteoclast-specific Cre Models:
• CD11b
• Lyzm
• Trap
Bone homeostasis is a dynamic equilibrium due to opposing actions and coupling of bone-forming OBs and bone-resorbing OCs.