Pathways Regulating Bone Resorption

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Tooth eruption
Low power section of developing jaw

2

Tooth eruption
Remodeling of jaw bone to accommodate developing tooth

3

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4. Osteoclastic bone resorption

Adult human bone
Few osteoclasts present

5. Adult human bone

Periosteal remodelling
Phalanx

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Osteoclast in resorption cavity

Normal bone architecture
3rd lumbar vertebra, 30 year old woman
Marrow and other cells removed to reveal thick, interconnected plates of bone

Osteoporotic bone architecture
3rd lumbar vertebra, 71 year old woman
Marrow and other cells removed to reveal eroded, fragile rods of bone
Trabecular bone element eroded by osteoclasts

Trabecular bone element perforated by osteoclast action

Formation and activation of osteoclasts

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Rat osteoclast  
Motility

How do osteoclasts resorb bone?

Osteoclast regulation  
Extracellular pathways

Calcium regulating hormones
- Parathyroid hormone (PTH)
  - ↑ OC recruitment, activity; ↑ plasma Ca²⁺
  - ↑ Direct / Indirect effect?
- 1,25-dihydroxyvitamin D
  - ↑ OC recruitment, activity; ↑ gut Ca²⁺ uptake, plasma Ca²⁺
  - ↑ Direct / Indirect effect?
- Calcitomin
  - ↓ OC recruitment, activity; ↑ plasma Ca²⁺ in young / hypercalcaemic individuals
  - “Emergency” hormone, not much effect in normal adults
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Rat osteoclast
Rapid inhibition of motility by salmon calcitonin (sCT)

sCT
50 pg/ml
- 15 min
- 5 min
+ 5 min
+ 15 min

Osteoclast regulation (2)

• Glucocorticoids
  Complex direct / indirect effects:
  – ↑ OC formation & function; also cause OC apoptosis

• Thyroid hormone
  – ↑ OC formation & function

• Sex steroids (oestrogens & androgens)
  Important direct / indirect effects:
  – ↓ OC recruitment, activity
  – Deficiency → ↑ bone turnover, bone loss

Osteoclast regulation (3)

Prostaglandins
• Normal connective tissue cell product; ↑ and ↓ OC activity
• Mediate some actions of growth factors + cytokines & responses to mechanical stimuli

Growth factors
• Mitogens e.g., transforming growth factor β, bone morphogenetic proteins, insulin-like growth factors I & II, platelet-derived growth factor, fibroblast growth factors.
  Normal OB products, deposited in bone matrix. Mainly ↑ OC recruitment, activity
• Wnt proteins cysteine-rich glycoprotein signalling molecules (blocked by sclerostin); ↓ OC function indirectly

Cytokines
• Normal products of immune cells & also bone cells
• Many ↑ OC recruitment, activity – e.g., interleukins-1,6,11,17,18; tumour necrosis factor-α
• RANK ligand & M-CSF1 (major factors required for OC formation):
  ▼ cytokines ↓ OC function – e.g., interleukins- 4,10,13
Osteoclast formation requires M-CSF and RANK ligand

RANK Ligand is an essential mediator of osteoclast formation, function and survival

Osteoprotegerin (OPG) is a ‘decoy receptor’ that prevents RANKL binding to RANK
Inhibits osteoclast formation, function & survival

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RANK Ligand : OPG ratio
Is critical for the skeleton

- Increases bone loss
- Prevents bone loss

Osteocytes
Potential direct / indirect regulators of osteoclasts

- Comprise 90% of cells in bone
- Recent evidence shows that osteocytes have an important endocrine / paracrine role
- Main source of at least 3 key regulators of bone & mineral metabolism

Osteoclast regulation (4)

Contact with bone
- Contact with organic matrix (collagen + other matrix proteins), mediated via integrins \( \uparrow \) OC differentiation
- Contact with mineral (calcium phosphate / carbonate) \( \uparrow \) OC differentiation and activation

Inorganic agents
- Protons (low pH; acidity) \( \uparrow \) OC activity; \( \downarrow \) mineralisation
- Hypoxia (low oxygen tension) \( \uparrow \) OC recruitment; \( \downarrow \) bone formation
- Phosphate \( \downarrow \) OC recruitment, activity
- Calcium \( \downarrow \) OC recruitment, activity (small effect)

Mechanical loading
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Osteoclast regulation
Intracellular pathways


Extracellular pH & osteoclasts

Osteoclast activation
→ Resorption pit formation – acidification required
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**Acid activation of osteoclasts**

- **Rat**
  - pH vs. No. of pits per osteoclast

- **Human**
  - pH vs. Area resorbed / osteoclast (µm²)

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**Acid-activation is required for osteoclast stimulation by PTH**

- PTH acts directly on human osteoclasts derived from peripheral blood mononuclear cells

- **Expression of PTH receptor**

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**Acid-activation is required for osteoclast stimulation by RANK Ligand**

- **Osteoclast activation is a 2-step process**

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Continuous resorption by activated osteoclasts
(1-2 days)

Osteoclasts work fast
• Bone resorption is much more rapid than bone formation

Calvarial bone control

Resorption of calvarial bone stimulated by acidosis (pH 7.0)
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Stimulation of Ca\(^{2+}\) release from mouse calvaria by HCO\(_3^-\) acidosis

<table>
<thead>
<tr>
<th>pH</th>
<th>Ca(^{2+}) Release (nmol/l)</th>
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<tbody>
<tr>
<td>6.8</td>
<td>0.2</td>
</tr>
<tr>
<td>6.9</td>
<td>0.4</td>
</tr>
<tr>
<td>7.0</td>
<td>0.8</td>
</tr>
<tr>
<td>7.1</td>
<td>1.2</td>
</tr>
<tr>
<td>7.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

H\(^+\) added (meq/l)

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pH also controls osteoclast formation

High (physiological) pH
- Fusion

Low pH
- Stops fusion and activates

7 days @ pH 7.4
5 days @ pH 7.4
+ 2 days @ pH 6.9

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Osteoclasts formed from mouse marrow with M-CSF & RANKL

Low pH halts osteoclast fusion

Results in:
- Activation
- \(\downarrow\) OC size
- \(\uparrow\) OC number

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Formation of authentic osteoclasts requires contact with a natural mineralised substrate
(Bone, Ivory…)

- Cultured on BONE / IVORY
  - Authentic osteoclast morphology
- Cultured on PLASTIC
  - Pancake cells

Resorption requires contact with mineral
Unmineralised collagenous matrix not resorbed

Reciprocal control of bone cell function by extracellular pH

- pH 6.8
- pH 7.4
- These responses may represent a primitive ‘failsafe’ to correct systemic acidosis by releasing alkaline bone mineral

Blood supply & hypoxia
Bone is highly vascular

- Bone gets about 7% of cardiac output
- Blood supply allows greater cellularity than in cartilage
- Enables remodeling and repair

Endochondral ossification
Formation of bones from cartilage model - vascularisation

Hypoxia and bone

Hypoxia occurs when blood supply to tissues is reduced / disrupted

- e.g.: Inflamed / infected tissue
- Tumours
- Fracture sites; implant sites
- Yellow fatty bone marrow (ageing)
- Obesity / diabetes...

<table>
<thead>
<tr>
<th>Condition</th>
<th>PO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric air</td>
<td>21%</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>12%</td>
</tr>
<tr>
<td>Venous blood</td>
<td>5%</td>
</tr>
<tr>
<td>Normal tissues</td>
<td>5-12%</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>≤ 2%</td>
</tr>
</tbody>
</table>
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Normal bone can be hypoxic
Adult mouse calvaria

Cells sense oxygen via $O_2$-dependent hydroxylases (PHDs & FIH) and hypoxia-inducible factors (HIFs)

Osteoclast formation from human peripheral blood mononuclear cells
Hypoxia stimulates osteoclast formation

Transmitted light

Reflected light

20% O₂

2% O₂

Hypoxia stimulates human osteoclast formation and thus resorption

Hypoxia causes maximal stimulation of osteoclast-mediated Ca²⁺ release from cultured mouse calvaria

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Reciprocal control of bone cell function by oxygen tension

Formation and activation of osteoclasts

Osteoclasts
- Adapted to function in harsh environments (unlike osteoblasts)
- Reflects white blood cell origin (cf - macrophages and neutrophils)
Hypothermia stimulates osteoclast formation
(and blocks bone formation)

Blood flow, oxygen tension
and bone cell function

Hypoxia & acidosis

- Critical role of vasculature in bone
  - Many osteotropic factors regulate blood supply
- Potential relevance to the bone loss associated
  with a wide range of pathological states:
  - Inflammation, infection
  - Tumours
  - Fractures
  - Renal disease
  - Severe anaemias
  - Diabetic ischaemia
  - Chronic obstructive airway diseases
  - Ageing, menopause

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Tumour metastases
‘Vicious cycle’ of bone resorption

Hypoxia
osteoclastogenesis
IL-6
IL-8
RANK
RANKL
H+

Bone matrix

TGFβ

Thank you!