Bone and Cancer

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Learning Objectives

To Develop Understanding of:

a. The types of tumours that grow in bone.

b. Cellular and molecular mechanisms that regulate:
   i. Osteolytic disease - multiple myeloma / breast metastasis.
   ii. Osteosclerotic disease - prostate metastasis.

c. Key molecular mechanisms:
   i. RANKL system
   ii. Wnt system

d. The dependence of tumours on bone - the ‘vicious cycle’.
Overview

• The nature of cancers that grow in bone

• Regulating osteoclastic activity in cancer -
  - The RANKL system

• Regulating bone formation in cancer:
  - The Wnt system

• The interdependence between myeloma and bone
Bone Cancers - ‘Primary Cancers’

- Tumours of Cells of Bone
  - Osteosarcoma
  - Chondrosarcoma

- Tumours of Marrow Cells
  - Multiple Myeloma
Multiple Myeloma

Characteristics
- Osteolytic Bone Lesions
- Osteoporosis
- Bone Pain
- Fracture
Bone Cancers - ‘Secondary Cancers’

Breast  65%–75%
Prostate  65%–75%
Lung  30%–40%
Renal  20%–25%
Bladder  40%
Melanoma  14%–45%
Thyroid  60%
Which Bones Are Affected?

- Why bone?
- Why these bones?
- Are these bones special?
The ‘Seed and Soil’ Hypothesis

“When a plant goes to seed, its seeds are carried in all directions; but they can only grow in congenial soil”

Stephen Paget 1889
Lancet 1:571-572
Breast Cancer - Osteolytic Disease

Breast Cancer

Tumour Cells

Osteoclasts

Bone
Prostate Cancer - Osteosclerotic Disease
Bone Cancers - The Consequences

- Hypercalcaemia
- Fractures
- Spinal Cord Compression
- Osteoporosis
- Bone Pain
- Disease specific
- Mortality
Bone Destruction in Multiple Myeloma

**Mechanism**

- Increased osteoclast formation and activity
- Decreased bone formation
- Uncoupling leading to rapid bone destruction
Osteolytic Bone Disease in Breast Cancer Bone Metastasis

Mechanism

- Increased osteoclast formation and activity
- Decreased bone formation

Ottewell et al CABS 2008
Cellular Mechanisms: Osteolytic Disease

**Osteoclast:**
- Increase osteoclast formation.
- Increased osteoclast activity

**Osteoblast:**
- Early increase in bone formation.
- Later suppression of bone formation

Diagram:
- CFU-GM
- Pre-osteoclast
- Tumour Cells
- Mesenchymal Stem Cells
- Osteoclast
- Osteoblasts
- Bone
Cellular Mechanisms:
Osteolytic Disease

Osteoclast:
- Increase osteoclast formation.
- Increased osteoclast activity

Osteoblast:
- Early increase in bone formation.
- Later suppression of bone formation
Bone Resorption is Increased in Osteoblastic metastasis


Cellular Mechanisms: Osteosclerotic Disease

Osteoclast:
- Increase osteoclast formation.
- Increased osteoclast activity?

Osteoblast:
- Increase in bone formation.

Bone

Mesenchymal Stem Cells

+ +

Tumour Cells

CFU-GM

Pre-osteoclast

Osteoclast

Osteoprogenitor
Summary 1

- Tumours develop in bone or can metastasise to bone.
- Develop osteolytic, osteosclerotic and mixed bone lesions.
- Increased bone resorption is found in the majority of tumours.
- Abnormalities in bone formation play a key role and may determine outcome.
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Molecular Mechanisms Regulating Osteoclastic Resorption in Cancer

Molecular Mediators
- Lymphotoxin
- IL-1β
- TNFα
- PTHrP
- IL-8
- HGF
- MIP1α / β
- RANKL / OPG
- SDF1
- Adhesion molecules
Osteoclastic Bone Resorption Is Dependent on RANK Ligand

RANK Ligand Is Essential for Osteoclast Formation, Function, and Survival

Osteoprotegerin (OPG) Inhibits the Effects of RANK Ligand and Blocks Bone Resorption

Osteoclast Formation, Function and Survival Inhibited by OPG

Growth Factors, Hormones, Cytokines

OPG
RANKL
RANK

CFU-M
Pre-Fusion Osteoclast
Multinucleated Osteoclast
Mature Osteoclast

Targeting RANKL \textit{In Vivo}

Stimulating Resorption

\begin{align*}
\text{Osteoblast} & \quad \text{RANKL} \\
\text{Osteoclast Precursor} & \quad \text{RANK} \\
\text{Oc Formation / Function} & \quad _{-}
\end{align*}

Inhibiting Resorption - Physiological

\begin{align*}
\text{Osteoblast} & \quad \text{RANKL} \\
\text{OPG} & \quad \text{RANK} \\
\text{Oc Formation / Function} & \quad _{-}
\end{align*}

Inhibiting Resorption - Pharmacological

\begin{align*}
\text{Osteoblast} & \quad \text{RANKL} \\
\text{OPG.Fc} & \quad \text{OPG Mim} \\
\text{RANK.Fc} & \quad \text{RANKL Ab} \\
\text{Oc Formation / Function} & \quad _{-}
\end{align*}
Fc.OPG Prevents the Development of Myeloma Bone Disease

Group 1 = naive
Group 2 = 5T2MM + vehicle
Group 3 = 5T2MM + Fc.OPG (30mg/kg, 3/week)

Crougher et al Blood 2001
Blocking RANK Ligand Inhibits Tumour-Induced Osteolysis in a Breast Cancer Model

MDA-231 Intracardiac Model
Control + Fc.OPG

Number of Lesions Per Mouse

![Graph showing the number of lesions per mouse with different OPG doses.]

Tumour Area

![Graph showing the tumour area with different OPG doses.]

Morony et al. Cancer Res 2001
Blocking RANK Ligand Inhibits the Development of Prostate Cancer Osteoblastic Bone Lesions

Prostate Cancer LuCaP 35 Hu/SCID Model, Therapy Initiated at 6 Weeks

Myeloma and Prostate Cancer Cells, but not Breast Cancer Cells Express RANKL

- CD38+++/CD45+ cells express RANKL - FACS/RTPCR (Farrugia et al 2003)

Tumour Cells Regulate RANKL & OPG Expression in the Environment: A Key Role in Osteolysis

- Human myeloma cells upregulate RANKL expression in stromal cells *in vitro* (Pearse et al 2001; Guiliani et al 2001)

- Myeloma cells decrease OPG expression in stromal cells-mediated by $\alpha_4\beta_1$ (Guiliani et al 2001)

![Graph showing OPG expression](image)
RANK Ligand Is a Key Mediator of Tumour-Induced Bone Destruction

Cytokines and Growth Factors (PTHrP, IL-6, IL-8, IL-1 \( \beta \), TNF-\( \alpha \), CSF-1, MIP1\( \alpha \), SDF-1)

Cancer Cells in Bone (Myeloma, Breast, Prostate)

Osteoclast

Bone

Summary 2

- Local regulators of osteoclastic resorption have been determined.
- Implicated in regulating osteoclast formation, activity and survival.
- Likely to be tumour type specific
- RANKL is likely to be the critical effector molecule
- Regulators may converge on RANKL to mediate increased resorption or have additional activities
Overview

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• The interdependence between myeloma and bone
Critical role in bone formation

Does increased expression of soluble antagonists inhibit bone formation in osteolytic disease?

Does suppression of wnt antagonists contribute to osteosclerotic disease?

Wnt Signalling and Bone Formation

A. Wnt Signalling

B. Inhibition of Wnt Signalling

+ve Formation

-ve Formation
Tumour regulated suppression of osteoblast differentiation

- IL-6 signaling promotes recruitment
- Wnt antagonists are candidates
- Direct modulation of osteoblast apoptosis
- Limited functional data
Dkk-1 over-expressed in some myeloma cells and is increased in the bone marrow (Tian et al 2003, Giuliani et al).


Dkk-1 increases RANKL and reduced OPG in MM (Qiang et al 2008).
Promoting Wnt Signalling in Myeloma *In Vivo*

- LiCl, as an inhibitor of glycogen synthase kinase 3β, and activator of wnt signaling prevents bone disease (Edwards et al. 2007).
- Anti-Dkk-1 prevents bone disease in the SCID-rab mice (Yaccoby et al. 2006).
- Anti-Dkk-1 prevents bone disease in the 5T2MM model (Heath et al. CABS 2008)

**Graphs:**
- **Osteoblast Number:**
  - Naïve
  - Veh
  - AntiDkk1
  - 5T2MM

- **Lesion Number:**
  - Naïve
  - Veh
  - AntiDkk1

**Experiment Timeline:**
- 0 weeks
- 8 weeks
- 12 weeks
- Inject 5T2MM cells
- Paraprotein
- Sacrifice
- Calcein (-6/-2 days)

**Groups:**
- Group 1 = naïve
- Group 2 = 5T2MM + vehicle
- Group 3 = 5T2MM + Anti-Dkk1 (10mg/kg, 2/week, i.p.)
• Local regulators of osteoblastic bone formation have been determined.

• Limited functional data available.

• Inhibitors of wnt signalling including Dkk1 play a critical role.

• Possible to bypass tumour derived mediators to prevent bone destruction *in vivo*.

• Targeting bone formation may prove to be effective in treating induced bone disease
Overview

- The nature of cancers that grow in bone
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Tumour Development in Bone

Colonisation  Survival/Dormancy  Development  Growth

Environment Dependent  Time  Environment Independent

Environment Modifying
**Fc.OPG Reduces Tumour Burden and Increases Survival in a Murine Model of Myeloma**

* p<0.05

Vanderkerken et al Cancer Res 2003
The effect of targeting osteoclastic activity on Tumour Burden in the 5TMM Models of Myeloma

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<th>Osteolytic Disease</th>
<th>Myeloma Burden</th>
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<td>Vanderkerken et al 2007</td>
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Tumour Development in Bone

- Effecting initial arrival/colonisation in bone
- Effect initial survival events
- Establishment of tumour colonies
- Growth of established tumour cells
Interdependence Between Tumour Cells and Bone - the ‘vicious cycle’

Summary 4

• Increasing evidence for an interdependence between tumour cells and bone

• Spacial and temoral relationship is poorly defined but likely to be key

• Provides unique opportunities for treatment of bone tumours and bone metastasis.
Conclusions

• Cancers that effect the skeleton are not uncommon.
• Hijack both osteoclasts and osteoblasts.
• RANKL is one of the critical mediators of tumour induced bone disease and a key therapeutic target.
• The osteoblastic response may determine the nature of the disease.
• Wnt pathway antagonists may play a central role.
• Increasing evidence for interdependence between tumour cells and bone.
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