Age-related angiocrine signals in bone homeostasis & disease

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Oxford Centre for Haematology
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GOAL: To elucidate the niche functions & therapeutic potential of vasculature in normal and tumour tissues
Blood Vessel - Tissue Interactions

✓ Oxygen
✓ Nutrients

Neighboring Cell

Secreted Factors

Endothelial Cell

Pericyte

Juxtacrine/Paracrine/Angiocrine Signaling
AIMS

- Unravel novel interactions between vasculature and tissues
- Dissect tissue-specific features of vasculature
- Identify dysregulation occurring during ageing and disease
Niche functions of blood vessels in bone

Hematopoietic Stem Cells

Blood & Immune cells

Mesenchymal Stem Cells

Osteoblasts, Adipocytes, Cartilage
One in two adults report a musculoskeletal condition

This burden will escalate in the next 10-20 years

http://www.boneandjointburden.org/
Chondrocytes

Growth plate

Marrow

VE-Cadherin-CreERT2 X R26-mTmG

VE-Cadherin-GFP

300μm
CD31/Endomucin expression demarcates spatially and structurally heterogeneous vessels
## Endothelial cell heterogeneity in bone

<table>
<thead>
<tr>
<th></th>
<th>Type H</th>
<th>Type L</th>
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</thead>
<tbody>
<tr>
<td><strong>Phenotypic</strong></td>
<td>(\text{CD31}^{\text{hi}}/\text{Endomucin}^{\text{hi}})</td>
<td>(\text{CD31}^{\text{lo}}/\text{Endomucin}^{\text{lo}})</td>
</tr>
<tr>
<td><strong>Spatial</strong></td>
<td>Metaphysis + Endosteal</td>
<td>Diaphysis</td>
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<tr>
<td><strong>Structural</strong></td>
<td>Linear</td>
<td>Branched</td>
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</tbody>
</table>
Type H endothelium and osteoprogenitors

Mesenchymal Progenitors

- Pdgfrβ
- Pdgfra

Early Osteoprogenitors

- Runx2

Late Osteoprogenitors

- Collagen1α
- Osterix
HIF-1 signaling

Endothelial Cell

NORMOXIA

PHD2

\[ \downarrow O_2 \]

Hydroxylation

VHL

OH OH

Ubiquitination

proteasome

Degradation

HYPOXIA

Hif1α

Hif1α • Hif1β

HRE

Transcription

Nucleus

Neo-Angiogenesis
HIF-1 signaling

Endothelial Cell

**NORMOXIA**

- PHD2
- \( \downarrow O_2 \) (Oxygen)
- Hydroxylation
- Hif1α
- Ubiquitination
- Degradation

**HYPOXIA**

- Hif1
- Hif1α
- Hif1β
- HRE (Hypoxia Response Element)
- Transcription
- Nucleus

Neo-Angiogenesis
Control

Vhl \textsuperscript{iΔEC}

Endothelial Hif1 stabilization

Control

Vhl \textsuperscript{iΔEC}

Type H

Type H

Osteoprogenitors

Bone Mass
Decline upon Ageing

Type H blood vessels

Associated with osteoprogenitors

Osteogenic Factors

Bone Formation
Specialized vessels


Bone Vasculature

Age-dependent changes

(Kusumbe et al. Nature 2016)
Regulation of bone metastasis by age-associated angiocrine signals
Bone Metastasis

Bone: Most Frequent Site of Metastasis

Very Poor Prognosis

Survival (in months)
Ageing and bone metastasis

Dormancy

Disseminated Tumor Cell (DTC)

Age-associated Relapse

Reactivation
Age-dependent Changes in the Microenvironment May Regulate DTCs

Seed & Soil Theory

Vascular Microenvironments

Dormancy  Reactivation
Vascular bed in bone is heterogeneous

- Arteries
- Veins
- Sinusoids (Type L capillaries)
- Type H capillaries
Niche Dependent

Young

Niche

Aged

Niche
Impact of the Aged Bone Marrow Microenvironment on Bone Metastasis

Young Niche Cancer Cells

Aged Niche Cancer Cells

Intra Cardiac Injections
Intra Tibial Injections

MDA-MB-231-LUC-GFP
MCF-7-LUC-GFP

Recipients

Young
Aged
The aged bone marrow microenvironment:

Promotes expansion of tumor cells

Inhibits cancer cell quiescence
The Aged Bone Marrow Microenvironment

1. Promotes Expansion
2. Inhibits Quiescence
Enrichment of Cell Proliferation Genes in the Aged Bone Marrow
The aged bone marrow:

proliferation promoting cytokines increase
Decline of quiescence inducing factors in the aged bone marrow microenvironment

Validated by ELISA in bone marrow supernatants
The aged bone marrow secretome stimulates proliferation of cancer cells

Bone Marrow (BM) Secretome (SEC)

Intratibial Injection

Sham
Aged BM SEC

Young Mice

Ki67+ CC (%)

P 0.0003

Sham
Aged BM SEC
Radiation leads to increase in quiescent cancer cells in aged mice.
Down-regulation of cell proliferation genes

Biological Process

- Cell Cycle
- Chromosome
- Mitotic Nuclear Division
- Cell Division
- Kinetochore
- Chromosome Segregation
- Centrosome

DOWN

-log10 (P value)

Control
Irradiation

Biological Process

- DNA Binding
- Cell Cycle
- Regulation of Transcription
- Chromosome
- Transcription
- Chromatin Binding
- Mitotic Nuclear Division
- Cell Division
- Kinetochore
- DNA Replication

DOWN

-log10 (P value)

Control
Chemotherapy

Down-regulation of cell proliferation genes
Radiation & chemotherapy leads to enrichment of quiescence inducing factors.
Pericytes & endothelial cells exhibit high levels of quiescence factors
Expansion of pericytes post-radiation

Control

Radiation
Expansion of pericytes post-radiation in aged mice

DAPI/CD31/Endomucin/PDGFR-β
Bone provides specialized quiescent microenvironments

Expansion of pericytes in response to radiation or chemotherapy is bone-specific
Quiescent cancer cells are peri-arteriolar
Type H Endothelial Cells

Associated with PDGFR-β pericytes

Increase upon Radiation & Chemotherapy

Therapy Resistance

Pdgfb
Decreasing blood flow renders cancer cells susceptible to existing therapies.
Blood flow manipulation renders quiescent cells susceptible to conventional therapies

<table>
<thead>
<tr>
<th>No Treatment</th>
<th>Radiation or Chemotherapy</th>
<th>Radiation + Blood Flow Manipulation</th>
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*pericyte expansion*

- cancer cell
- quiescent cancer cell
- pericyte
- chondrocyte

*Singh et al. JCI Insights 2019*
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