Fibrous dysplasia of bone: review of research and potential future treatments

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October 25th, 2014
Fibrous Dysplasia Foundation

I am a US Government employee, and have no conflicts of interest
Points to be covered:

- what stem cells are.
- development (we have to know how the disease develops in order to know how to treat it).
- the role of skeletal stem cells in disease.
- how skeletal stem cells can be used or manipulated to treat disease.
Properties of stem cells

in the body:

• offspring of a single cell are able to reform an entire tissue

• able to self-renew
Self-renewal

post-natal stem cells

symmetric division

asymmetric division

symmetric division (ES cell or iPS cell)

transient divisions (symmetric), more committed progenitors

differentiated
Development

fertilized egg → blastocyst → gastrulation - three germ layers

- ectoderm
- endoderm
- mesoderm

inner cell mass

pluri-potent (cells of the embryo, not cells of placental membranes)

ES cells

induced pluripotent cells (iPS cells)

factors

multi-potent fetal stem cells, lineage committed

birth

adult stem cells differentiated cells
FD & MAS: mutations of GNAS after fertilization

Riminucci et al., JBMR, 2006
Bone marrow:
home of a dual system of stem cells

fat

bone

blood

blood stem cells

"skeletal" stem cells
Bone marrow stromal cells (BMSCs, aka “MSCs”) reform bone and associated tissues because of skeletal stem cells, SSCs.

A single colony-forming unit-fibroblast (CFU-F) forms a colony in vivo transplant.

Pellet culture reforming units. Gold standard assays with toluidine blue cartilage.

Scaffold in vivo transplant.

~1/5 of CFU-Fs are multipotent, are pericytes.

Skeletal stem cells: the “cause” of disease
Skeletal stem cells: the “unit” of post-natal skeletal disease

- SSCs control bone formation and bone destruction
- therefore:
  - genetic mutation or change in their activity caused by their surroundings

will result in a skeletal disease
- e.g., FD and MAS
- (activating Gsα mutations---> excess cAMP)

SSCs are a target for therapeutic intervention
Abnormalities of FD bone

Riminucci et al., AJP, 1997; Bianco et al., JBMR, 2000
Bianco et al., JCI, 1998

fibrotic tissue

Allk Phos
woven bone

# of CFU-F
(approximates the # of SSCs)

CFU-F/10^5 cells

Normal
Fibrous Dysplasia

*
non-clonal BMSCs in vivo transplantation with carrier single cell suspension of fibrotic marrow

1. non-clonal BMSCs
2. normal clone
3. mutant clone

Proof Of Principle

FD ossicle normal ossicle non-ossicle

FD lesions are a mixture of normal and mutant cells
Variability in fibrous dysplasia

Collins et al., JBMR, 2005

Leet et al., JBMR, 2004
CFU-F & % FD-CFU-F decrease with age

Kuznetsov et al., JBMR, 2008
“Normalization”

10 yr old  21 yr old  41 yr old

poor mineralization  red marrow  yellow marrow
Normalization is due to cell death (apoptosis).
normal and mutant progenitors

↑Gsα
excess cAMP

aging
mutant cell death
“Normalization”

cell retraction, abnormal, under mineralized matrix

accumulation of progenitors (fibrosis)

blood-supportive cells

blood

marrow fat cells

lipid

HP

blood

normal and mutant progenitors
Mutant SSCs do not self-renew and die...

excess cAMP (activating mutations of Gsα mutations,)

mutant stem cell

non-self-renewing division (symmetric)

increase in number of progenitors, limited cell division (symmetric)

abnormal differentiation to bone-forming cells and cell death
…but normal SSCs survive and form bone

normal stem cell

self-renewing division (asymmetric)

committed progenitors, limited cell division (symmetric)

normal differentiation
Skeletal stem cells: potential future treatments (still no immediate miracles!)
## Model systems for testing therapies

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<thead>
<tr>
<th></th>
<th>Pluses</th>
<th>Minuses</th>
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<tbody>
<tr>
<td>FD BMSCs in culture</td>
<td>rapid, detailed analyses</td>
<td>true bone does not form in culture, lacks structure, limited number of cells to work with</td>
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<td>(we appreciate your</td>
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<tr>
<td>biopsies!)</td>
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<td>FD-BMSC transplants</td>
<td>human bone in a small animal for testing</td>
<td>so far, only good for bone</td>
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<td>(also from your biopsies!</td>
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<td>genetic mouse models</td>
<td>more generalized aspects of mutation</td>
<td>mice are not humans, mouse models may not mimic human disease</td>
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Gsα<sup>R201C</sup> mouse

Saggio et al, JBMR, 2014
Cell therapy for treatment of FD

- direct transplantation
- optimized composition, size and shape of vehicle
- percutaneous delivery of cells with an injectable carrier

Normal bone marrow
- ex vivo expansion (maintain normal stem cell)
  - cell sorting (remove mutant cells)
  - cell surface markers needed
  - molecular engineering
Defects that will never heal on their own

skull defect

+ BMSCs
(230 X 10^6/3.0 g HA/TCP)

- BMSCs
(3.0 g HA/TCP)

+BMSCs
-bMSCs
biopsy site
margin

+ BMSCs
bone

Mankani et al., AJP, 2006
Separating cells by proteins on their surface

Sacchetti et al., Cell, 2007

CD146+ cells
normal cells

CD146- cells
mutant cells

bone reconstruction

molecular engineering
(silence the mutant allele)

Piersanti et al., JBMR, 2010
Patient-specific induced pluripotent stem cells (iPSCs)

cell therapy - BMSCs, bone

drug testing - small molecules

model human disease
Molecular engineering: RNA interference
Molecular engineering: RNA interference
activating post-zygotic missense R201 mutations in the intrinsic GTPase site of Gsα

excess cAMP
small molecules:
• block dissociation of mutant αβγ subunit
• block mutant Gαs binding to AC
• activate intrinsic GTPase

Collins et al., in progress
Medicinal treatment of FD

Cell membrane

Toxin

Mutant-specific membrane protein

γ, β

Adenylyl cyclase

Mut-αs

Overactive Mut-Gsα, excess cAMP, increase cell death

Speed up cell death

Mutant cell nucleus
Acknowledgements

Alexander Friedenstein
Sergei Kuznetsov
Mahesh Mankani
Paul Krebsbach
Natasha Cherman
Michael Collins
Arabella Leet

Paolo Bianco
Mara Riminucci
and co-workers
FD/MAS

the clinical triad:

- café au lait skin pigmentation
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- endocrinopathies (e.g., precocious puberty)
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- café au lait skin pigmentation
- endocrinopathies (e.g., precocious puberty)
- fibrous dysplasia of bone
Fibrous dysplasia of bone

- Long bones
- Lytic
- Sclerotic
- Normal bone
- Lamellar bone
- Marrow
- FD bone
- Woven bone
- Fibrous tissue
- Cell retraction
- Sharpey’s fibers
Critical size defects in canines

Mankani et al., AJP, 2006
FD bone
intrinsic mineralization defect - osteomalacia

secreted factor(s)

“phosphatomin”? 

FGF-23 (ADHR)

phosphatemia

Tubulopathy
• phosphate wasting
• proteinuria
• aminoaciduria
• vit. D abnormalities

phosphaturia

Collins, et al., JBMR, 2001
Abnormalities of FD bone

Normal bone
- Lamellar bone
- Blood and fat

FD bone
- Woven bone
- Fibrous tissue
- No blood or fat

Normal bone
- Mineral

FD bone
- Unmineralized matrix

Riminucci et al., AJP, 1997; Bianco et al., JBMR, 2000