Skeleton Development

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Skeleton

• ≥ 200 elements
• Two tissues: cartilage, bone
• Three cell types: chondrocytes, osteoblasts, osteoclasts
  Growth  Formation  Resorption
• Three “environments”: marrow, blood, SNS

Embryonic origin of the skeleton

Cranial neural crest cells → Somitic mesoderm → Lateral plate mesoderm → Monocyte lineage

Craniofacial skeleton → Axial skeleton → Appendicular Skeleton

Chondrocytes & Osteoblasts → Osteoclasts

Skeleton Biology

Patterning → Skeletogenesis → Homeostasis

Development

Location and shape of skeletal elements

Birth

Differentiated cells
Bone structure
Growth/Modeling

Life

Remodeling
Balance between Formation/Resorption

Genetic defects associated with skeleton development

Dysostoses

Dysplasia

Mineralization defects
Degenerative diseases
**Skeleton patterning**

- Condensation of mesenchymal cells to form the scaffold of each future skeletal element
  - Migration
  - Adhesion
  - Proliferation
- Early steps use signaling molecules and pathways generally involved in patterning other tissues (FGFs, Wnts, BMPs)
- Orchestrated by specific set of genes acting as territories organizers
- When not embryonic lethal disorders often localized

**Hox transcription factors**

- First described in Drosophila where they control body plan organization
- Arranged in 4 genomic clusters in mammals
- Expression patterns follow the cluster arrangement

**Homeotic transformations in absence of Hox transcription factors**

**Hox transcription factors control vertebrate limb patterning**

![Graphical representation]

- Site of expression
- *only in Hox cluster

**Mutations in HOXD13 cause synpolydactyly* in humans**

*OMIM 18600, 186300

**Skeletogenesis**

- Cell differentiation
  - Chondrocytes, osteoblasts, osteoclasts
- Bone morphogenesis
  - Formation of growth plate cartilage, bone shaft and marrow cavity
  - Vascular invasion and innervation
- Defects generalized
Two skeletogenetic mechanisms

- **Endochondral ossification**
  - Differentiation of a cartilaginous scaffold (chondrocytes) later replaced by bone (osteoblasts)
  - Most of the skeletal elements

- **Intramembranous ossification**
  - Direct differentiation of the condensed mesenchymal cells into osteoblasts
  - Many bones of the skull, clavicles

**Sox9**

- Transcription factor of the HMG family
- Regulates the expression of chondrocyte-specific genes
- Sox9 haploinsufficiency causes Campomelic dysplasia (OMIM 114290)
- Earliest known regulator of chondrocyte differentiation

**Sox9-deficient cells cannot differentiate into chondrocytes**

**Accelerated chondrocyte hypertrophy in PTHrP-deficient mice**
**PTHrP**

- Ubiquitously expressed growth factor
- Shares the same receptor with PTH
- Mice “knockout” only phenotype is a generalized growth plate cartilage defect
- PTHrP protein signals to its receptor in the prehypertrophic chondrocytes and blocks their hypertrophic differentiation

**Dwarfism in Ihh-deficient mice**

**Indian hedgehog (Ihh)**

- One of 3 members of the Hedgehog family of growth factors
- Widely expressed during development
- Expression positively regulated by the transcription factor Runx2

**Reduced chondrocyte proliferation and delayed chondrocyte hypertrophy in Ihh-deficient mice**

**Chondrocyte maturation is regulated by a PTHrP/Ihh feedback loop**

**Mutations in the PTH/PTHrP receptor cause Jansen and Bloomstrand chondrodysplasia**
HD5 - Skeleton Development

Endochondral ossification

- Patterning → Chondrocyte differentiation
- Sox9, Sox5, 6 → Chondrocyte maturation
- Ihh/PTHrP → Runx2
- Vascular浸透
- Osteoblast differentiation
- Osteoclast differentiation

Hypertrophy

Runx2

- One of three members of the runt family of transcription factors
- Identified as a regulator of the Osteocalcin promoter
- Necessary and sufficient for osteoblast differentiation

Arrest of osteoblast differentiation in Runx2-deficient mice

+/- Runx2 -/-

Otto et al., Cell 89 (1997)

Cleidocranial dysplasia (CCD, OMIM 119600) is caused by Runx2 haploinsufficiency

Mundlos et al., Cell 89 (1997)

Lee et al., Nat Genetics 16 (1997)

Intramembranous ossification

Suture formation

Growth

Closure

Endochondral ossification

- Mesenchyme
- Bone cell
- Calified Cartilage
- Ossification
- Ossification
Disorders of suture fusion

Delay
- Msx2, Runx2 haploinsufficiency

Acceleration = craniosynostosis
- FGFR1, 2, 3 activating mutations
- Msx2 activating mutations
- Twist haploinsufficiency

Osteoblast differentiation

Twist (Saethre-Chotzen Syndrome OMIM 101400)

Runx2

Osteoprogenitor
Pre-osteoblast
Osteoblast

Osteoblast differentiation

ATF4

• Divergent member of the ATF/CREB family of leucine-zipper transcription factors
• Required for amino-acid import
• Identified as a regulator of the Osteocalcin promoter
• Activated by the Rsk2 kinase

Delayed osteogenesis in absence of Atf4

WT
Atf4-/-

E14

E15 E16 P0

Delayed osteogenesis in Atf4-deficient mice

WT
Atf4-/-

Yang et al., Cell 117 (2004)

Yang et al., Cell 117 (2004)

ATF4

• Lack of ATF4 phosphorylation by inactivating mutations in Rsk2 causes the skeletal defects associated with Coffin-Lowry syndrome (OMIM 303600)
• Increased ATF4 phosphorylation by Rsk2 causes the skeletal defects associated with Neurofibromatosis Type I (OMIM 162200)
HD5 - Skeleton Development

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**A high protein diet normalizes bone formation in Atf4-/- and Rsk2-/- mice**

**Structure of a growing long bone**

**Control of osteoclast differentiation and function**

**Osteopenia in OPG-deficient mice**

**A low protein diet normalizes bone formation in a mouse model of Neurofibromatosis type I**


Osteopetrosis in RANK-L deficient mice

Lacey et al., Cell 93 (1998)

Research directions

Development

Patterning

Knowledge

Skeletogenesis

Diseases

Life

Homenstasis