

MESENCHYMAL STEM CELLS AND THEIR PROGENY: DEVELOPMENTAL PARADIGMS GOVERNING OSTEOBLAST DIFFERENTIATION & BONE FORMATION

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INTRODUCTION: Bone formation takes place in the organism not only during embryonic development, growth and remodeling, but throughout life in ongoing bone turnover, fracture repair and induction, e.g., by the implantation of decalcified bone matrix or purified or recombinant members of the bone morphogenetic protein family. This suggests there is a large reservoir of cells in the body capable of osteogenesis through life, but the nature of these cells over the lifetime of the animal, contributions from stem cell versus committed progenitor pools, whether there are different stages of committed progenitors recognizable, and identification of transitional steps from stem cell to committed osteoprogenitor to osteoblast remain important issues¹. We will provide insights into the developmental paradigms governing osteoblast differentiation and bone formation from cellular and molecular analyses of developing bone colonies *in vitro*.

METHODS: Cells were isolated from 21 day Wistar rat calvariae, plated at different densities and cultured for up to 3-4 weeks in differentiation medium (α MEM, with antibiotics, 10% FBS, 50 μ g/ml ascorbic acid, 10 mM sodium β -glycerophosphate, and with or without 10 nM dexamethasone (dex). In some experiments, master colonies and their replicas were prepared on polyester cloth. Osteoblast development and bone formation or adipocyte development and fat formation were determined by morphology, histochemistry (Von Kossa, alkaline phosphatase (ALP) staining, Sudan IV or Oil Red O staining), immunocytochemistry with lineage-specific antibodies, and gene expression profiling by *in situ* hybridization, Northern blots, semi-quantitative Real Time PCR, RT-PCR, or global amplification poly(A) PCR. In some cases, myoblast/muscle and chondrocyte/cartilage development were assessed by semi-quantitative RT-PCR or RT-PCR. For isolation of a side population (SP), verapamil-sensitive exclusion of Hoechst 33342 was quantified and used as a sort parameter on a FACStarPlus (BD Biosciences). Populations were sorted on the basis of co-expression profiles of ALP and PTH1R.

RESULTS: Committed osteoprogenitors, i.e., progenitor cells apparently restricted to osteoblast development, can be identified by functional assays of their proliferation and differentiation capacity *in vitro*, i.e., the bone nodule assay or colony forming unit-osteoblast (CFU-O). In RC populations, they are present at low frequencies ($\sim 1/10^2$ cells), a frequency that can be increased substantially by sorting the SP population or sorting on the basis of ALP and PTH1R expression, and have only a limited self-renewal capacity in culture. At least two distinct populations of osteoprogenitors are present: one appears capable of

constitutive or default differentiation *in vitro*, whereas the other more primitive comprises inducible progenitors that are recruited by stimuli such as dex. Gene expression profiling revealed that multiple and complex transitions, not directly associated with proliferative lifetime of the progenitors, demarcate osteoblast development². Notably, bioinformatics and statistical analysis have suggested that osteoblast development may not be governed by simple deterministic paradigms but that multiple developmental routes may lead to the functional osteoblast endpoint³. Co-expression profiles of osteoblast markers with markers of chondrocytes, myoblasts and adipocytes also suggest that multilineage precursors, possibly stem cells, may preface lineage programs prior to or even after commitment. The data also support a surprising level of osteoblast heterogeneity *in vitro* and *in vivo* that may underlie the growing number of examples of anatomical site-specific osteoblast responses to hormones, growth factors and other regulatory molecules.

DISCUSSION & CONCLUSIONS: Osteoblast development is a complex multi-step process in which osteoprogenitors arise from multilineage precursors whose fate may be governed by both stochastic and deterministic events and in which functional osteoblasts comprise a heterogeneous population that may be subject to different regulatory controls.

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