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MEETING REPORT

Osteoblasts-the saga continues (ASBMR 2012)

Renny T Franceschi

Department of Periodontics and Oral Medicine, The University of Michigan School of Dentistry, 1011N. University Avenue, Ann Arbor, MI, USA.

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Meeting report from the 34th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, MN, USA, 12–15 October 2012.

Introduction

Osteoblast-related studies reported at the 2012 ASBMR meeting followed many of the themes established in previous years with some important new and unexpected developments. Areas to be highlighted in this summary include studies on the osteoblast lineage and its relationship to other cell types including vascular smooth muscle cells and adipocytes, reciprocal control of osteoblastogenesis and adipogenesis, involvement of osteoblasts in central control of energy metabolism and new developments related to the transcriptional control of osteoblast activity.

More on Origins

A continuing challenge in osteoblast biology is to define the lineage of this cell type. What is the putative mesenchymal progenitor, what other cell types hitch their fates to this progenitor and, most importantly, what markers can be used to trace the osteoblast lineage? Presentations at this year's meeting continued themes developed over the past couple of years; osteoprogenitors have a perivascular origin, they can be abnormally induced in vascular disease and may share a common precursor with adipocytes. The plenary lecture by Paulo Bianco (Universita La Sapienza, Italy) on Friday morning provided an overview on bone marrow mesenchymal stem cells (BMSCs), their origin in developing sinusoids and relationship to the vasculature. This theme was picked up in a number of subsequent talks that followed the lineage of these cells through the perichondrium into bone. Previous work identified the intermediate filament protein, nestin, as a marker for BMSCs capable of forming osteogenic, adipogenic and chondrogenic cells. The conversion of nestin+ cells into osteogenic precursors was followed using a combined transgenic approach that incorporated the nestin promoter driving GFP to mark all nestin+ cells and a tamoxifen-inducible Cre under control of the osterix (Osx) promoter to temporally mark osteogenic precursors.[1] Early in development, nestin+ cells were distributed throughout the mouse limb bud and expressed the endothelial/vascular marker, CD31. With the onset of growth cartilage formation, a fraction of nestin+ cells became osterix-positive and were localized to the perichondrium, but excluded from cartilage. A tamoxifen pulse-chase protocol was used to follow Osx+ cells, which were found to differentiate into osteoblasts of the primary spongiosa. At early stages, cells remained nestin-positive, but expression declined as osteoblasts matured. Thus, only a subset of nestin+ cells become Osx+ osteoblasts, consistent with nestin being a general marker for multiple lineages. In a further refinement of this approach, Osx-Cre/GFP marking was used to follow the fates of Osx+ cells.[2] Surprisingly, several marrow cells in addition to osteoblasts were identified including reticular, vascular smooth muscle, adipocyte and perineural cells. These studies indicate that BMSCs are derived from a nestin+ progenitor that gives rise to an Osx+ subpopulation. Cells also contain progenitors for other mesenchymal lineages in addition to osteoblasts. The association of Osx-marked cells with non-bone lineages is notable; Osx was previously considered to be a specific osteoblast lineage marker, because Osx-null mice only exhibited defects in bone formation.[3] Current studies indicate that Osx is expressed at earlier stages in the BMSC lineage before commitment to osteoblasts. Consistent with this concept, deletion of Osx from cartilage using a Col2-Cre was shown to block endochondral bone formation.[4]

A Saturday afternoon symposium on MSC-vascular interactions further explored the impact of the vascular origin of BMSCs. The first talk by Bjorn Olsen (Harvard School of Dental Medicine) examined the role of the angiogenic factor, vascular endothelial growth factor (VEGF), in osteogenesis using an Osx-Cre to delete the *VEGF* gene in Osx+ progenitors. Trabecular bone was dramatically decreased and accompanied by expansion of marrow adipocytes. These changes were accompanied by a decrease in CFU-osteoblast. Interestingly, marrow cells from knockout mice could not be rescued with exogenous VEGF. Instead, VEGF actions were intracellular and did not involve cell surface receptor signaling. Interactions between VEGF and nuclear lamin-A were proposed to stimulate osteogenesis and suppress adipogenesis. In the same session, talks by Dwight Towler (Washington University-St. Louis) and Catherine Shanahan (King's College London) discussed vascular calcification as an abnormal osteogenic process involving many of the same signals associated with normal bone formation including BMPs,

chondrogenic/osteogenic transcription factors such as Sox9 and Runx2, and PTH.

Fat or Bone?

In many conditions such as osteoporosis, estrogen deficiency, anorexia nervosa, treatment with thiazolidinediones (TZDs) and bone unloading/immobilization, the observed decline in trabecular bone is associated with increased marrow adipocytes. Furthermore, reciprocal control of adipogenic and osteoblastic lineages is exerted by the two major transcription factors controlling fat and bone, PPARγ and RUNX2.[5,6] A number of studies further explored this bone–fat connection and possible relationships between circadian rhythms, energy metabolism and bone. Several examples were presented of factors that suppress osteogenesis while stimulating adipogenesis. The FOXO 1, 2 and 3 transcription factors suppress bone formation by antagonizing Wnt/Tcf and knockout of FOXO 1, 2, 3 in Osx + cells increases Wnt-dependent proliferation of osteoprogenitors and bone mass.[7] Also, microRNA suppression of RUNX2 and the chondrogenic transcription factor, TRPS1, redirects MSC differentiation from osteoblasts to adipocytes and myoblasts.[8] A switching mechanism between adipocyte and osteoblast lineages was described that is dependent on MAP kinase phosphorylation of PPARγ and RUNX2. RUNX2 phosphorylation at Ser301/Ser319 activates transcription of osteoblast genes and indirectly stimulates Wnt signaling, whereas S112 phosphorylation of PPARγ suppresses adipogenesis.[9] As MAP kinase pathways are activated by mechanical loading,[10] this control mechanism may explain the observed increase in Wnt signaling and suppression of adipogenesis in BMSCs exposed to mechanical strain.[11] TZDs, which are potent activators of PPARγ, stimulate BMSC differentiation to adipocytes while suppressing osteoblastogenesis.[12] In a regenerating bone defect and distraction osteogenesis, the TZD, rosiglitazone, increased adipogenesis while suppressing neovascularization and bone formation.[13] Last, interesting relationships were observed between nocturnin (Noc), a circadian regulated BMSC-associated deadenylase, energy metabolism and osteoblast/adipocyte differentiation.[2] In rodents, Noc is normally upregulated in the early evening during feeding. This factor, which is induced by TZDs, inhibits osteoblastogenesis and stimulates adipogenesis.[14] Consistent with this activity, Noc-null mice have a high bone mass, are resistant to rosiglitazone-induced bone loss and remain lean even when fed high fat diets. These effects were attributed to the localization of Noc in mitochondria where it can suppress energy metabolism in a circadian manner.

Osteoblasts and Metabolism

The bone field was turned on its head in 2007 when the Karsenty laboratory reported that osteocalcin, the most common non-collagenous protein of bone, functions as a metabolic hormone when in its decarboylated form.[15] Osteocalcin was shown to enhance insulin secretion and sensitivity as well as energy expenditure. Consistent with these functions, osteocalcin is induced by insulin via direct actions of this hormone on osteoblasts and can also be released from bone matrix in its decarboxylated form by osteoclast activity.[16] Two talks provided further evidence for an osteoblast role in metabolic regulation. First, high fat diets were shown to induce an insulin resistance in osteoblasts that was accompanied by reduced bone resorption and release of decarboxlyated osteocalcin.[17] Consistent with these findings, osteoblast-selective reduction of insulin receptors reduced glucose tolerance and insulin sensitivity, while receptor overexpression rendered mice resistant to high fat diet-induced insulin resistance. The importance of osteoclast-mediated bone resorption to the control of glucose homeostasis was directly demonstrated by studies where osteoclast levels were either increased in Opg-null mice or reduced by osteoclast-specific expression of diptheria toxin (DTA).[18] Opg-deficient mice had elevated levels of circulating decarboxylated osteocalcin, were hypoglycemic and exhibited increased insulin sensitivity and reduced fat mass. In contrast, ablation of osteoclasts with DTA led to a decrease in decarboxylated osteocalcin, reduced insulin sensitivity and glucose tolerance.

Transcriptional Controls

Although the major transcription factors controlling osteoblast differentiation have been identified, much still remains to be learned about how they interact. As both RUNX2 and OSX are essential for skeletal mineralization, one might predict that they cooperate during osteoblastogenesis. Evidence was presented that this is, in fact, the case.[19] Co-immunoprecipitation and immunofluorescence experiments showed that these two factors physically interact in the nucleus and co-localize to the same subnuclear regions. Furthermore, dramatic synergistic stimulation of several bone reporter constructs was observed when both factors were co-expressed. This interaction required the DNA-binding domain of RUNX2 and the transactivation domain of OSX. Indirect regulation of RUNX2 activity via silencing of the E3 ubiquitin ligase, c-Cbl, was also described.[20] Receptor tyrosine kinases (RTKs) are major targets of c-Cbl, which stimulates their subsequent degradation. Silencing c-Cbl with siRNA increased RTK activity including PDGF and the IGF1 receptors, MSC proliferation and osteoblast differentiation. Also, c-Cbl interacts with STAT5 and prevents its positive interaction with RUNX2. The hypoxia-inducible transcription factor, HIF-1α, is known to be required for the development of cartilage and bone.[21,22] In contrast, HIF-1α appears to restrict osteoanabolic signaling and PTH responsiveness in the mature skeleton.[23] HIF-1α was induced by PTH and appears to restrict the anabolic actions of this hormone in that PTH-induced trabecular bone formation was greater when HIF-1α was deleted from osteoblasts. Effects of HIF-1α may be mediated by the Wnt pathway as PTH induction of Wnt targets was greater in osteoblasts lacking HIF-1α.

Conflict of Interest

The author declares no conflict of interest.

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