Archives of Biochemistry and Biophysics 561 (2014) 3–12

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Osteoblast and osteocyte: Games without frontiers

Mattia Capulli, Riccardo Paone, Nadia Rucci *

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

ARTICLE INFO

Article history:
Received 28 February 2014
and in revised form 30 April 2014
Available online 14 May 2014

Keywords:
Osteoblast
Osteocyte
Bone remodelling
Mechanosensing
Hormonal regulation

ABSTRACT

The portrait of osteoblasts and osteocytes has been subjected to a revision, since a large body of evidence is attributing these cells amazing roles both inside and outside the bone. The osteoblast, long confined to its bone building function, is actually a very eclectic cell, actively regulating osteoclast formation and function as well as hematopoietic stem cells homeostasis. It is also an endocrine cell, affecting energy metabolism, male fertility and cognition through the release of osteocalcin, a perfect definition-fitting hormone in its uncarboxylated state. As for the osteocytes, many evidence shows that they do not merely represent the final destination of the osteoblasts, but they are instead very active cells that, besides a mechanosensorial function, actively contribute to the bone remodelling by regulating bone formation and resorption. The regulation is exerted by the production of sclerostin (SOST), which in turn inhibits osteoblast differentiation by blocking Wnt/beta-catenin pathway. At the same time, osteocytes influence bone resorption both indirectly, by producing RANKL, which stimulates osteoclastogenesis, and directly by means of a local osteolysis, which is observed especially under pathological conditions. The great versatility of both these cells reflects the complexity of the bone tissue, which has not only a structural role, but influences and is influenced by different organs, taking part in homeostatic and adaptive responses affecting the whole organism.

The osteoblast: beyond a bone building cell

Osteoblasts, accounting for the 4–6% of total resident cells in the bone, are commonly known for their bone building function, thus exerting a crucial role in the achievement and maintenance of a correct bone mass, which is accomplished through a close cross-talk with the other bone cells: osteoclasts and osteocytes. Indeed, more and more emerging findings demonstrate that the osteoblasts also have endocrine functions, since they are able to release long range regulation factors (see next paragraphs). These novel findings portray the bone as a central tissue in whole body's homeostasis.

Ontogeny of the osteoblast

As many other cells of the connective tissues (fibroblasts, chondrocytes, myoblasts and adipocytes), osteoblasts arise from a common pluripotent mesenchymal stem cell (MSC),[1] following timely programmed steps requiring the expression of specific genes, which in turn are under the control of pro-osteogenic pathways (Fig. 1A). Among these, Bone Morphogenetic Proteins (BMPs) and Wnt pathways are crucial, especially for the early steps of osteoblastogenesis, where they promote MSCs commitment towards an osteo/chondroprogenitor [1].

* Corresponding author. Address: Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Via Vetoio – Coppito 2, 67100 L'Aquila, Italy. Fax: +39 0862 433523.
E-mail address: rucci@univaq.it (N. Rucci).

[1] *Abbreviations used:* SOST, sclerostin; MSC, mesenchymal stem cell; BMPs, Bone Morphogenetic Proteins; Runx2, Runt-related transcription factor 2; Dlx5, Distal-less homeobox 5; Osx, Osterix; cbfa1, core binding factor alpha1; osf2, osteoblast specific factor 2; CCD, CleidoCranial Dysplasia; ALP, Alkaline Phosphatase; OCN, osteocalcin; IGF, Insulin-like Growth Factor; Satb, Special AT-rich Binding; KO, Knock Out; BSP, Bone SialoProtein; ATF, Activating Transcription Factor; FRZ, Frizzled; GSK3beta, Glycogen Synthase Kinase 3 beta; C/EBP, CCAAT/Enhancer-Binding-Protein; PPARgamma, Peroxisome Proliferator-Activated Receptor gamma; DKK1, Dickkopf-related protein 1; sFRP, secreted frizzled-related proteins; TGF, Transforming Growth Factor; FGFs, Fibroblast Growth Factors; FGFR2, fibroblast growth factor receptor 2; MiRNAs, Micro RNAs; Cx43, connexin 43; $Ca_3(PO_4)_2$, tricalcium phosphate; M-CSF, Macrophage-Colony Stimulating Factor; RANKL, Receptor Activator of Nuclear kappa B Ligand; NFκB, Nuclear Factor-kappaB; OPG, Osteoprotegerin; PTHrP, ParaThyroid Hormone related Protein; IL, Interleukin; TNF, Tumour Necrosis Factor; ESP, Enterococcal Surface Protein; GABA, Gamma-AminoButyric Acid; SNO, Spindle-shaped N-cadherin+/CD45- Osteoblasts; HSC, Hematopoietic Stem Cell; LT, Long-Term; Ang-1, Angiopoietin-1; SDF, Stromal Cell-Derived Factor; DMP1, dentine matrix protein 1; PC1, PolyCystin 1; FAK, Focal Adhesion Kinase; TRAcP, Tartrate Resistant Acid Phosphatase; FGF23, Fibroblast Growth Factor 23.

http://dx.doi.org/10.1016/j.abb.2014.05.003
0003-9861/

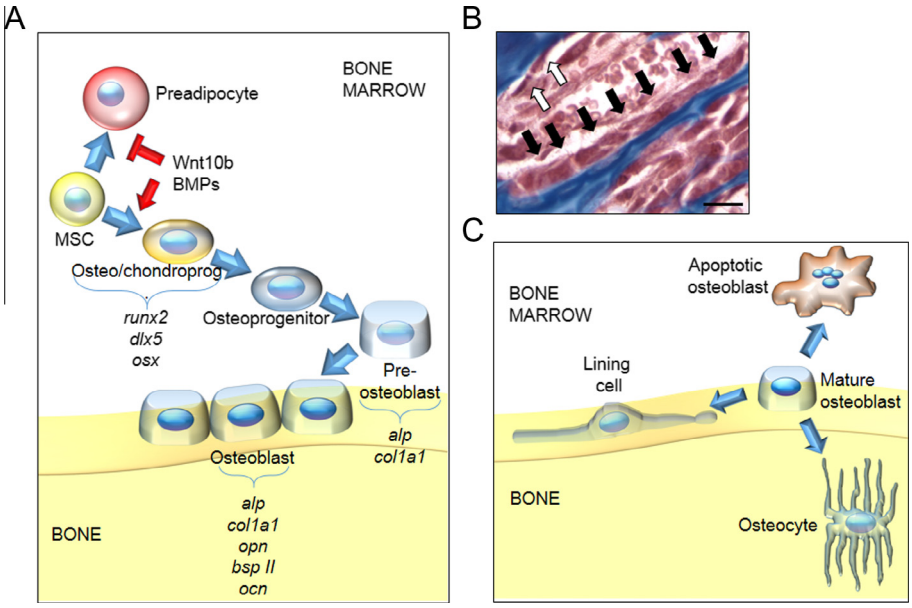

Fig. 1. Osteoblast differentiation, morphology and fate. (A) Schematic representation of the multistep process of osteoblast differentiation (MSC = mesenchymal stem cell). (B) Histological section of a mouse tibia, stained with the Masson's trichrome. Black arrows indicate a row of osteoblasts on a bone trabecula (Blue staining), white arrows indicate bone-lining cells. Bar = 10 μm. (C) Schematic representation of the possible fate of a mature osteoblast.

The minimal criteria of the osteo/chondroprogenitor cell is defined when at least the following osteoblast-specific transcription factors are expressed: Runt-related transcription factor 2 (Runx2), Distal-less homeobox 5 (Dlx5) and Osterix (Osx), the latter being a downstream target of Runx2 (Fig. 1A).

Runx2, *alias* core binding factor alpha1 (cbfa1) and osteoblast specific factor 2 (osf2), belongs to the Runx transcription factors family, which also includes Runx-1 and -3. Runx2 is a master gene of osteoblast differentiation, as demonstrated by the fact that Runx2-null mice are completely deprived of osteoblasts. This leads to bone lack and failure of chondrocytes of cartilage template to undergo hypertrophy [2,3]. Consistently, in humans mutations of Runx2 cause CleidoCranial Dysplasia (CCD), an autosomal-dominant disease with dramatic abnormalities in the bones formed by intramembranous ossification [4]. As far as the specific role of Runx2 is concerned, it has been shown that this transcription factor upregulates osteoblast-related genes (*CollA1*, *Alp*, *BSP*, *BGLAP*), in osteoblasts as well as in non-osteoblastic cells, such as fibroblasts [5]. Moreover, the overexpression of Runx2 in human MSCs isolated from adipose tissue triggers commitment towards osteoblasts, by increasing Alkaline Phosphatase (ALP) activity and osteocalcin (OCN) expression [6]. However, the role of Runx2 seems to be more complex, since other studies demonstrated that Runx2 overexpression in osteoblasts inhibited their maturation and caused osteopenia [7], therefore this transcription factor is for sure pivotal in the early stages of osteoblast development, while it is not critical for the expression of the genes encoding bone matrix proteins in the mature osteoblasts.

Among the several targets downstream of Runx2 there is *Osx*, also known as *Sp7*, whose expression in MSC progenitors is stimulated by BMPs and Insulin-like Growth Factor (IGF)-1 [8]. Although it has been ascertained that Osx is downstream of Runx2, BMP-2-induced activation follows both Runx2-dependent [9] and -independent pathways [10]. Osx in turn promotes transcriptional expression of Satb2 [11]. As far as this latter gene is concerned, it encodes for a transcription factor belonging to the family of Special AT-rich Binding (Satb) proteins that bind to nuclear matrix-attachment regions, thus participating to transcription regulation and chromatin remodelling [12]. Experiments performed with *Satb2* Knock Out (KO) mice revealed its involvement in craniofacial development and osteoblast differentiation [13]. Consistently, haploinsufficiency of *SATB2* causes craniofacial defects in humans [14]. Moreover, osteoblasts in which *Satb2* was knocked out showed reduced expression of Bone SialoProtein (BSP) and OCN. As for the mechanism of action, it has been found that SATB2 interacts with both Runx2 and Activating Transcription Factor (ATF) 4 eventually leading to a positive regulation of osteoblast markers. The importance of the latter molecule in this context has been recently established, since *Atf4* KO mice showed an impairment in collagen I synthesis, thus developing a severe low bone mass phenotype [15].

The last mentioned transcription factor, Dlx5, promotes osteogenesis under the control of BMPs [16]. Dlx5 is highly expressed in the developing skeleton and is also induced during fracture healing [16].

Coming back to the multistep process of osteoblast differentiation (Fig. 1A), it has been observed that once a pool of osteoblast progenitors expressing Runx2 and collagen I has been established, there is a proliferation phase, during which osteoblast progenitors acquire ALP activity. These cells are now pre-osteoblasts, which undergo morphological changes, becoming large, cuboidal cells highly positive for ALP activity and very active in the secretion of bone matrix proteins.

The late stage of osteoblast differentiation is characterized by a higher expression of the bone matrix proteins OCN, BSP I and II and of collagen type I: we have now a mature osteoblast, which will eventually be trapped in the bone matrix just deposed and mineralized (Fig. 1A).

As shown in Fig. 1B, in histological sections mature osteoblasts appear as a single row of cuboidal-shaped cells with a round basal nucleus. These cells can also show cytoplasmic processes towards the bone matrix by which they are in contact with osteocyte processes.

At this point, the aging osteoblasts face three possible destinies: (1) undergo apoptosis, (2) give way to the osteocytes (see the next paragraphs) or (3) become bone-lining cells (Fig. 1B). Bone lining cells are quiescent flat shaped osteoblasts covering the bone surface, functionally representing the resting phase of