TISSUE ENGINEERING OF BONE: THE ROLE OF OSTEOBLASTS IN OSTEOGENESIS AND PERI-IMPLANT BONE HEALING

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Abstract

Osteoblasts are cells of mesenchymal origin, which rebuild resorbed bone by synthesizing bone matrix proteins and by inducing bone matrix mineralization. Osteoblasts play a crucial role in creating and maintenance of healthy bone architecture, bone repair, and peri-implant bone healing (osseointegration). These bone-forming cells are also involved in regulation of osteoclasts function, and hence bone resorption in osteoclastogenesis process. We have presented our own studies on the subsequent stages of differentiation of Human Bone-Derived Cells (HBDCs) that could be a good candidate as an autogenous source for reconstruction and rebuilding of own patient's bone using tissue engineering methods. In this review we discussed the biology of osteoblasts, compared with the HBDCs cultures, under the influence of growth factors (FGF-2, TGF-β, IGF, PDGF) and hormones (PTH, 1,25-dihydroxyvitamin D_3, leptin). Our review is also focused on the participation of intercellular adhesion proteins (cadherins, claudins, connexin, 'OsteoMacs'), transcription factors (Cbfa1, Msx-2, Osx, ATF4), and others molecules (RANKL, OPG, BMP2, lactofferin, PPARγ) in modulating osteoblasts functions on the basis of current reports, throwing new light on the involvement of osteoblasts during osteogenesis and peri-implant bone healing.

Keywords: *bone tissue engineering, osteoblasts, Human Bone-Derived Cells, peri-implant bone healing, osteogenesis, cell culture*

[Engineering of Biomaterials 119 (2013) 2-7]

Introduction

Well vascularized mineralized bone tissue is a major reservoir of calcium ions and serves also as a source of different bone cell types such as osteoprogenitors cells, endothelial cells, periosteum, and bone marrow cells. The particular architecture of bone, its function and metabolism are effects of two opposite processes, i.e. bone formation and bone resorption. Hence bone is a dynamic, highly specialized tissue that undergoes continuous remodeling. This tissue is composed from mineralized extracellular matrix, and three major cell types, i.e. osteoblasts (bone-forming cells), osteoclasts (bone-resorbing cells), and osteocytes (mature osteoblasts entrapped within lacunae), which all are essential for its structural, mechanical and metabolic functions. The structural and metabolic activity of bone is compromised by an imbalance between the formation and the rate of bone resorption, which depend on osteoblasts and osteoclasts, respectively. Therefore, bone mass and homeostasis are controlled by continuous remodeling throughout life. Dysregulation of the process can lead to a variety of defects and bone diseases [1,2].

The osteoblasts are the fundamental cells controlling extracellular matrix (ECM) production and mineralization, as well as receiving and processing mechanical and chemical signals to bone, and most likely also directing osteoclast function [3]. These bone-forming cells are indispensable for bone development, growth, repair, and maintenance of healthy skeletal architecture. This is achieved by the existence of network of interactions between cells and a wide variety of factors or mediators, including intercellular communication with other bone cell types [4]. Active osteoblasts synthesize the extracellular matrix proteins, mainly type I collagen (Col I). Simultaneously, the expression of bone-specific alkaline phosphatase (ALP) increases. With ongoing differentiation cells start to produce a numerous of non-collagenous proteins such as bone sialoprotein (BSP), osteopontin (OP), osteonectin (ON), and osteocalcin (OC). Finally, mineralization of ECM indicates mature osteoblasts [5]. ECM proteins serve also as organic components during osteogenesis in vitro, and are necessary in the course of bone healing after injury. During the osteogenic induction primary human osteoblasts also express collagen and ALP, which is defined as the early marker of osteogenic differentiation in vitro. In the last phase of HBDCs in vitro differentiation, the cells secrete osteocalcin, the late marker of mature osteoblasts. These parameters are routinely used to monitor the phenotype of osteogenic cells during their differentiation in culture because these cells have the potential osteogenic activity and seems to be the best autogenous source for implantation and reconstruction of own patient's bone. Moreover, a dynamic cell seeding in bioreactor and cell culture conditions maintain the proliferative potential and also osteogenic phenotype of HBDCs cultured on the selected three-dimensional polyurethane scaffold [6].

In distance osteogenesis new bone tissue is formed on the surfaces of the existing bone in the peri-implant site. The bone surfaces provide a population of osteogenic cells, which lay down a new matrix encroaching on the implant. An essential observation is that new bone is not formed on the implant, but the latter does become surrounded by the bone. Success in maintaining of dental implant depends largely on the progress of osseointegration. In histology this process is defined as a direct anchorage of an implant by the formation of bone tissue around the implant, without the growth of fibrous tissue at the bone-implant interface [7]. The processes of activation, differentiation, and maturation of osteoblasts lead to the new bone formation and are of particular importance in the phenomenon of peri-implant bone healing. Components accelerating post-implantation healing process also include growth factors and hormones.

In this review, we discussed the biology of osteoblasts, with their role in the regular course of osteogenesis and in the regulation of bone resorption in osteoclastogenesis process, as well as in peri-implant bone healing. Moreover, this report presents the current state of knowledge in the terms of intercellular communication of osteoblasts, with an indication of adhesion molecules and various factors, which promote the process of new bone formation in vivo, and also in in vitro model.

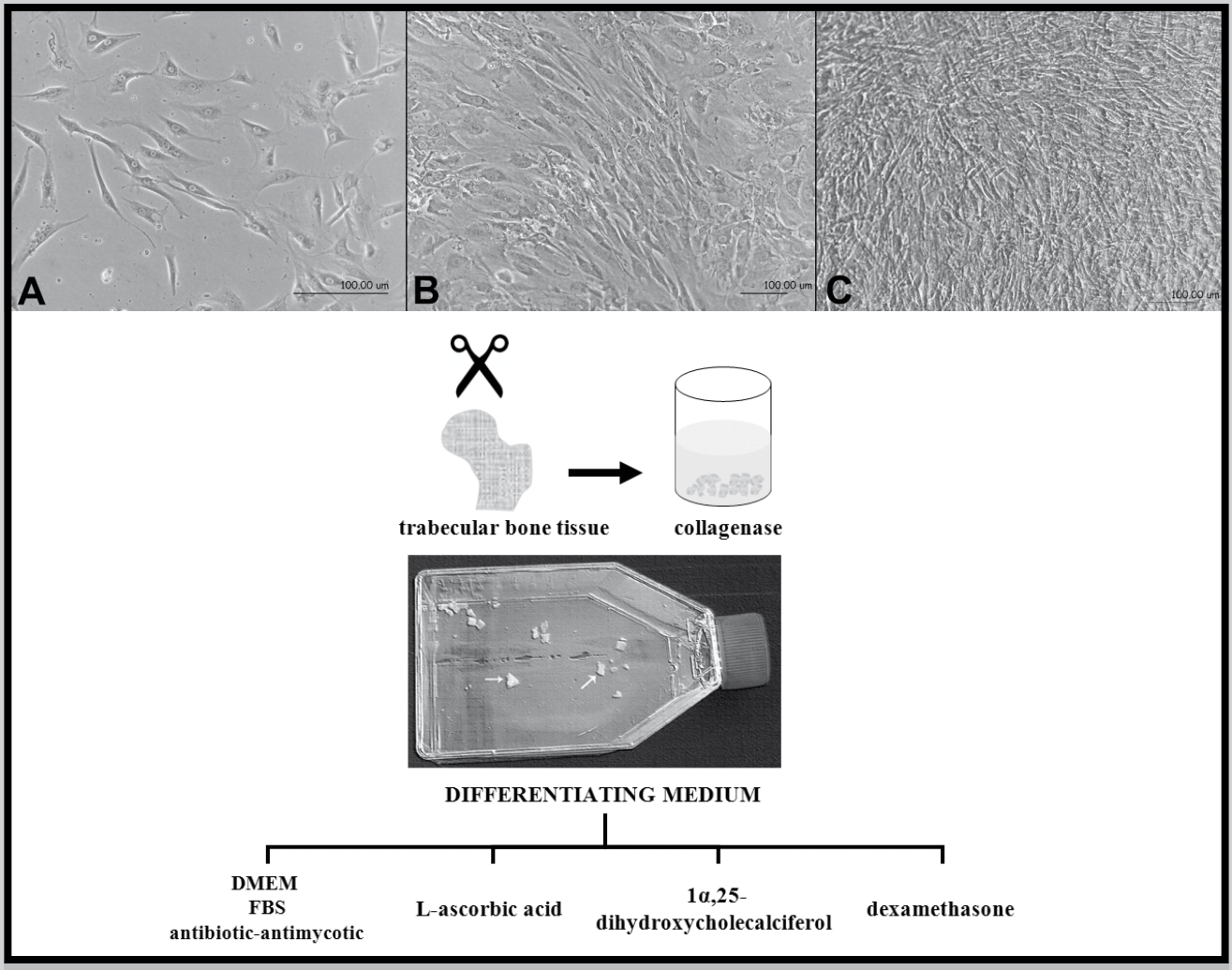

FIG. 1. Morphological changes of primary human osteoblasts during their in vitro differentiation. (A). Adherent osteoblasts – 1st day after cells seeding. (B). Proliferating osteoblasts – 14th day of culture. (C). Differentiated, mature osteoblasts – 28th day of culture.
Human osteoblasts were isolated from the trabecular bone tissue chips, which were harvested from the bottom distal part of the long tight bone during the standard procedure of the knee joint alloplasty. Cells harvested from donor were cultured in DMEM enriched with 10% of heat-inactivated FBS, antibiotic-antimycotic, and L-glutamine. 100 mM L-ascorbic acid 2-phosphate, 10 nM dexamethasone, and 100 nM 1α,25-dihydroxycholecalciferol were used to induce the differentiation of human osteoblasts in vitro. Such culture medium conditions favored also the development of the osteoblasts phenotype. All procedures were performed according to the requirements of the 2nd Local Ethical Committee at the Medical University of Warsaw. The cell culture was observed and analyzed using inverted, phase-contrast microscope (Nikon Eclipse TE2000-u) throughout the experimental period. Morphology of Human-Bone-Derived Cells (HBDCs) changed from a fibroblastic (A) to a cuboidal one (B), and then the cells formed multiple layers (C). Scale bar, 100 μm.

The role of osteoblasts in bone formation

Osteoblasts arise from multipotential mesenchymal stem cells (as well as muscle, adipocytes, chondroblasts, fibroblasts) and under the influence of the appropriate transcription factors, growth factors, and hormones differentiate to preosteoblasts, and then to mature osteoblasts. Molecules mentioned above modulate the proliferation, differentiation, and migration of progenitor cells. In vitro phenotypic osteoblasts heterogeneity is associated with the progress of cell differentiation. In our studies we indicated that the expression of Col I, ALP, and OC demonstrated differed at mRNA level during differentiation of Human-Bone-Derived Cells (HBDCs). We also showed that these factors are fundamental for the developmental expression of the osteoblasts phenotype and formation of the mineralized matrix.

During in vitro HBDCs differentiation three distinct stages were identified (adhesion phase – 24 h after cell seeding; proliferation phase – from day 7 until day 14 of cell culture; maturation and mineralization phase – between 21 and 28 day of cell culture). Each period exhibits unique, stage-specific responsiveness to steroid hormones and growth factors that establish of the primary human osteoblasts phenotype. The process of in vitro differentiation of osteogenic cells is induced by the presence of osteogenic supplements in culture medium, such as dexamethasone, L-ascorbic acid-2-phosphate and 1α,25-dihydroxycholecalciferol (active hormonal form of vitamin D_3). Morphology of primary human osteoblasts during their in vitro differentiation, in the differentiated osteoblasts was analyzed in our laboratory and showed in representative images (FIG. 1). Their differentiated phenotype was verified by demonstrating the induction of osteoblasts-specific indicators, as described above.

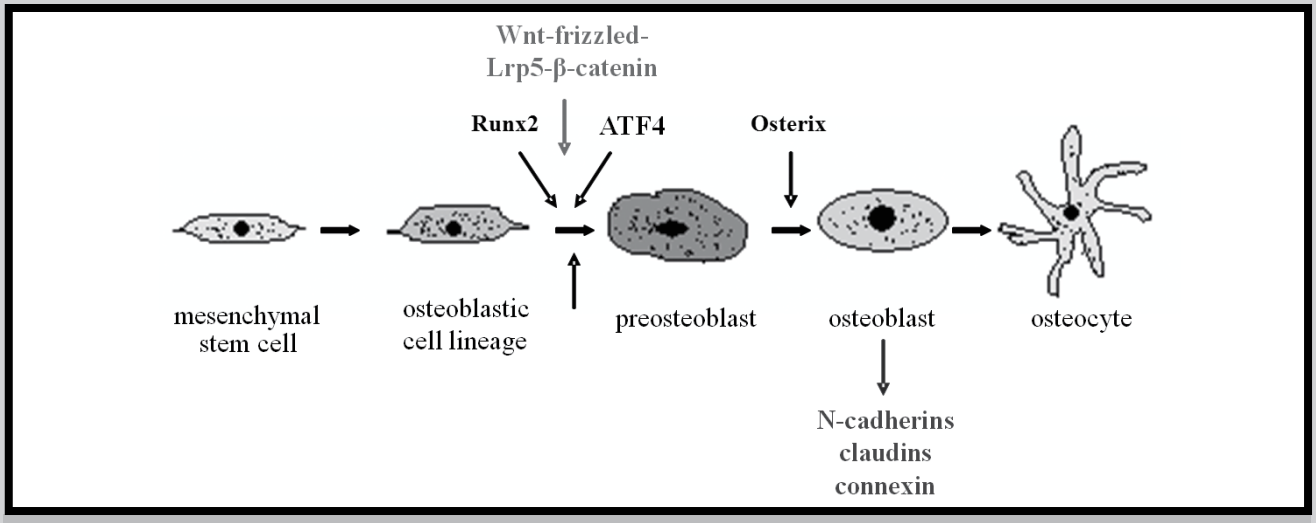

FIG. 2. Osteoblast differentiation pathway involving the major transcription factors and secreted molecules.

Osteoprecursors are found among bone marrow hematopoietic cells. Osteoblasts are usually located on the surface of trabeculae and Haversian canal. When they are active, large Golgi apparatus and abundant rough endoplasmic reticulum are visible, making osteoblasts similar to epithelial cells with a large spherical nucleus and basophilic cytoplasm. These osteoblasts form tight junctions with adjacent osteoblasts and have regions of the plasma membrane specialized in vesicular trafficking and secretion. Ultimately, some osteoblasts become trapped in their own bone matrix giving rise to osteocytes, which gradually stop secreting osteoid [8]. Osteoblasts exhibit also expression of some characteristic genetic markers (Osterix – Osx, Cbfa1 – core-binding factor α1, also known as Runx2) [5,9]. Transcription factors such as Cbfa1, Osx, and ATF4 (activating transcription factor 4) have been identified as the controllers of the osteoblastic lineage [10]. In the absence of Cbfa1 and Osx no osteoblasts are formed. Formation and proliferation of preosteoblasts requires signaling through the Wnt-frizzled-Lrp5-β-catenin signaling pathway (FIG. 2). Deficiencies of Lrp5 (low density lipoprotein receptor 5-related protein) lead to the development of osteoporosis in both mice and humans. The function of mature osteoblasts, including the ability to synthesize ECM proteins requires Lrp5 as well as ATF4 signaling protein [11]. Osteoblasts also play essential role in the regulation of bone resorption through receptor activator of nuclear factor-kB (NF-κB) ligand – RANKL, which links to its receptor (RANK) on the surface of preosteoclast, inducing their differentiation and fusion. In addition, osteoblasts secrete a soluble decoy receptor OPG, which blocks RANK/RANKL interaction by binding to RANKL, and thus prevents osteoclasts differentiation and activation. Therefore, the equilibrium between RANKL and OPG determines the regulation of osteoclastogenesis [8,12]. Lamghari et al. suggested that leptin modulates positively OPG/RANKL balance by inhibiting the expression of RANKL gene [13].

On the other hand, the cellular communication between osteoblasts, osteocytes, and pre-osteoclasts is essential for bone formation as well as for osteoclastogenesis. Osteoblasts induce migration and differentiation of pre-osteoclasts into mature osteoclasts, what initiate bone resorption. After completion of bone resorption, apoptosis of mature osteoclasts and differentiation of osteoblasts are initiated. At this time the osteoblasts do not support osteoclasts differentiation, but promote bone formation. Finally, osteoblasts differentiate into osteocytes in bone or into bone lining cells on the bone surfaces. Direct cell-cell communication occurs between osteocytes and osteoblasts via gap junction channels formed by connexin molecules. In this case osteoblasts may sense osteocyte cell death via gap junctional intercellular communication (GJIC) [14]. Osteoblasts express also adhesion molecules: e.g., cadherins [15,16], claudins [17], connexin [18], and are thought to be polarized cells [19]. Notably, the cell adhesion N-cadherin is an important regulator of chondrogenesis and osteogenesis. The molecular mechanism by which N-cadherin can promote osteoblasts differentiation is by increasing cell-cell adhesion, what results in the activation of gene expression in osteoblasts. Cadherins trigger intercellular signals by interacting with Wnt signaling pathway and with the participation of β-catenin molecules. Recent data provide a new mechanism, where N-cadherin controls the function of osteoblasts. N-cadherin was found to interact with Wnt-co-receptors Lrp5 or Lrp6 in osteoblasts in vitro and in vivo. These findings suggest that N-cadherin is a novel Lrp5 antagonist which negatively regulates Wnt/β-catenin signaling in osteoblasts. This concept is highly relevant to the regulation of osteoblastogenesis since both N-cadherin and Lrp5 are strongly expressed in osteoblasts, and Wnt signaling is a major modulator of osteoblasts function and bone mass [15]. It has been also recently reported that the resident tissue macrophages termed OsteoMacs or cell cycle arrested quiescent osteoclast precursors (QuOPs) are present on osteoblasts. OsteoMacs are an integral component of bone tissues and play a novel role in bone homeostasis through regulating osteoblasts function. These observations implicate OsteoMacs, in addition to osteoclasts and osteoblasts, as principal participants in bone dynamics [20]. However, it is unclear whether OsteoMacs differentiate into osteoclasts or not. The QuOPs may be identical to OsteoMacs, in terms of their localization. These resident osteoclast precursors are one of the sources of osteoclasts, in addition to bone marrow-derived osteoclast precursors [14].

Bone formation, growth, repair, and remodeling, are continuous processes throughout human life. There are three commonly known main mechanisms of bone formation such as intramembranous, endochondral, and appositional. Intramembranous bone is formed by the condensation of mesenchymal cells, what induces the expression of cartilage phenotype [21]. Undifferentiated mesenchymal cells differentiate into osteoblasts. The bone is formed directly within the condensed membranes, made of highly vascularized primitive mesenchyme. At the points of ossification, undifferentiated mesenchymal cells aggregate into membranous layers and differentiate into osteoprogenitor cells.

These cells proliferate within the capillary network and form osteoblasts in contact with primitive bone matrix. They start to produce protein matrix components, which in turn leads to the mineralization, and new bone formation. This is observed in embryonic flat bone formation, bone formation during distraction osteogenesis, and blastema bone in young children after amputations [4]. Endochondral bone is also formed by condensation of mesenchymal cells, but in this case cartilage creates a temporary model of the future bone, a process that induces expression of cartilage phenotype. The cartilage undergoes calcification, and is gradually replaced by bone tissue accumulated on the surface of calcified cartilage matrix compartments. In this way, the primary trabeculae of cartilaginous-bone are formed and covered with osteoprogenitor cells (osteoblasts). These primary trabeculae are removed by the osteoclasts, which penetrate a primary point of ossification, together with vascular buds, mesenchymal cells (the progenitors for bone marrow), and osteoblastic cells. Osteoclasts destroy primary trabeculae, while osteoblasts replace it with secondary trabecular bone, already devoid of cartilage components [21]. Osteoclastic resorption is followed by differentiated osteoblasts producing layer of peripheral bone or periosteal collar and osteoid. Next, it becomes mineralized and extends along the shaft, becoming confluent, and forming a continuous bone lining [4]. The process of appositional ossification observed during long bones formation unfolds within connective tissue. However, after birth trauma-induced activation of periosteal membrane leads to the formation of callus showing the signs of both types of ossification, i.e. endochondral and intramembranous. Appositional ossification involves formation of a new bone by aligned osteoblasts on the surfaces of existing bone, and is observed in periosteal bone enlargement, and general bone formation phases of remodeling [4]. The process of peri-implant bone healing is analogous to intramembranous healing at the fracture site. The early phase of healing proceeds from hematoma formation to woven bone formation, and finally results in bone remodeling [22].

Osteoblasts function in bone healing

Calcification of the hematoma is the first stage of tissue regeneration during peri-implant bone healing. The bone is formed by repetitive revascularization and mineralization. These phenomena occur due to the existence of intercellular communication between different types of bone cells and influence of numerous growth factors, hormones, or mechanical factors.

The success of implant therapy largely depends on the implant osseointegration. Clinically, it is confirmed by the lack of mobility of the implant and the absence of inflammation symptoms. Osseointegration is a direct structural and functional connection between the surfaces of living bone and implant [23]. The mechanisms underlying the process of osseointegration are very similar to those that involved in the repair of fractured bone, and comprise a cascade of cellular and extracellular events. Tissue response to the implant as a foreign body gradually achieves biological stability (osseointegration) in the bone implant system, and results in the formation of new bone tissue on the surface of the implant [24]. Bone healing after injury, which was induced as a result of surgical preparation of the implant bed, consists of several successive phases. The first stage is acute inflammatory reaction, which is the response of the immune system to surgery-related trauma. The factors that activate the healing process include histamine, prostaglandins, bradykinin, leukotrienes, proteins involved in coagulation, cytokines including IL-1, IL-4, IL-8 (interleukin -1, -4, -8), tumor necrosis factor-alpha (TNF-α), growth factors such as BMPs, transforming growth factor-beta (TGF-β), FGF-2, and insulin-like growth factor II (IGF-II). BMPs have osteoinductive properties, which stimulate the undifferentiated stem cells to differentiate toward osteoblasts that are responsible for bone formation. Approximately 2-3 days after implantation the hematoma is formed, and formation of vascularized granulation tissue is started. Undifferentiated osteoprogenitor cells migrate and locate in the network of matrix proteins (type I, V, XIII, XVI collagens and BMPs), and next already differentiated osteoblasts secrete the proteins, which form bone matrix – osteoid. Initially, the bone matrix consists of collagen fibers, but gradually it becomes mineralized [25]. Osteoblast-like cells adhere more readily to rough surfaces. Moreover, rough surfaces induce mature osteoblasts phenotype of these cells with regards to morphology, extracellular matrix secretion, alkaline phosphatase activity, osteocalcin production, and response to systemic hormones such as, 1,25-dihydroxyvitamin D_3. Observations made by Boyan et al., suggest that the roughness-dependent regulation of osteoblasts proliferation, differentiation and local factor production is related to substrate-mediated activation of integrin receptors, thus regulating protein kinase C (PKC) and A (PKA) through phospholipase C (PLC) and A2 (PLA2) pathways [26]. When osteogenesis is completed, the bone remodeling usually begins. High cellular activity at the bone-implant interface initiates the formation of bone trabeculae, which are aligned with the fibers of non-collagenous proteins. In consequence, this leads to the formation of mature bone tissue [27].

Molecules that modulate osteoblast behavior

Locally, bone remodeling is initiated by mechanical stimuli such as fracture, inflammation, and graft implantation. The process of bone remodeling is maintained by the balance between osteoblasts involved in the formation and reconstruction of bone tissue and osteoclasts, which are responsible for degradation and bone resorption [24]. Therefore, the remodeling bone cycle is strictly controlled by systemic hormones as well as local cellular pathways involved in the interaction between osteoblasts and osteoclasts, such as cytokines, growth factors, and intercellular adhesion molecules [13,14,28,29]. Osteoblasts express hormone receptors such as parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3, glucocorticoids, prostaglandins, and estrogens [4]. Stimulation of bone formation in endocortical surfaces is the hallmark of the anabolic effect of PTH, irrespectived of the studied species or experimental model. PTH stimulates osteoblasts to secrete ALP, Col I, OC, and BSP. Intermittent administration of PTH may promote osteoblastogenesis through the recently identified mechanism that involves down-regulation of transcription factor PPARγ (peroxisome proliferators-activated receptor γ) expression [30]. The second crucial hormone for skeletal development and maintenance is 1,25-dihydroxyvitamin D_3, which is involved in the regulation of calcium and phosphorus metabolism and inhibits PTH synthesis. There is clear evidence that effects of 1,25-dihydroxyvitamin D_3 on osteoblasts growth and differentiation are mediated by binding to its nuclear receptor (VDR). This stimulates the synthesis of bone-specific proteins such as ALP and osteocalcin. Moreover, 1,25-dihydroxyvitamin D_3, administered to mature osteoblasts, may promote their further maturation [31]. To compare, in culture model the primary human osteoblasts respond to the presence of ascorbic acid in culture medium by increasing of the level of Col I and ALP mRNAs expression.

Furthermore, 1α,25-dihydroxycholecalciferol and dexamethasone (a synthetic glicocorticosteroid) administered during the proliferation stage of HBDCs result in the inhibition of cell proliferation and up-regulating of osteogenic specific marker expression (Col I, ALP, and OC). As a result the rate of the cells differentiation is augmented.

Bone is also under control of adipose tissue, which modulates its metabolism via different adipocytokines, for example leptin. There are two opposing mechanisms, which could explain leptin's effects on regulation of bone metabolism. Firstly, leptin can act locally to promote the development of osteoprogenitor cells and increase bone formation by activation of osteoblasts. Secondly, leptin may work through the central nervous system via activation of β-2 adrenergic receptors, what results in increasing bone resorption and decreasing osteoblasts activity [32].

Bone formation and bone healing processes depend on the impact of various growth factors directly involved in osteoblasts response. The main growth factors that control thebone formation are FGF-2, FGF receptor-2 (FGFR-2), and BMP-2. BMP-2 is a member of TGF superfamily that stimulates osteoblasts differentiation by signal transduction via three BMP receptors (BMPR-IA, -IB and -II). Increasing of expression level of BMPR-IB in osteoblasts was found to be associated with the significant up-regulation of Cbfa1 and ALP transcripts, as well as ALP enzymatic activity [5]. Since recombinant human BMP-2 (rhBMP-2) became available, many animal studies have been performed examining the induction of bone formation following implantation of rhBMP-2 using of various carriers [33]. Numerous reports from animal studies and cell culture models confirm the special role of FGF-2 and BMP-2, in bone development as well as in proliferation, differentiation, and apoptosis of human osteoblasts [34-36]. The mechanism of action of these compounds is based mainly on the involvement of adhesion molecules (N-cadherin, IL-1) and signaling pathways (via kinases such as Src, PKC) in osteoblasts. Protein kinase C activated by FGF-2 or FGFR-2 causes an increase in N-cadherin expression. As a result of N-cadherin up-regulation, intercellular adhesion is increased and transcription level of specific bone genes formation such as ALP, osteocalcin, and Col I is changed. BMP-2 activates Smad1 signaling pathway, which results in increasing of PKC and Cbfa1/Runx2 expression (critical transcription factor of osteoblastogenesis). In contrast, transcription of another molecules such as caspases 9 and 3 is associated with increased apoptosis of human osteoblasts [37]. Moreover, Spector et al. showed that recombinant human FGF-2 alters osteoblastic expression of BMP-2 and Msx-2 (osteogenic transcription factor) in vitro, to favor the cellular differentiation and osteoinduction. The ERK1/2 intracellular signaling cascade was shown to be necessary for rhFGF-2-mediated BMP-2 transcriptional changes. Furthermore, the cellular production of an intermediate transcriptional modifier was found to be necessary for the rhFGF-2 mediated gene expression changes in BMP-2 and Msx-2 [38]. These findings offer a new insight into the mechanisms, by which FGF-2 modulates osteoblasts behavior. Growth factors are not specific for one type of tissue and they may influence on the several cell types and organs in the body. Due to this fact and the high potency of BMPs (BMP2, BMP7) to form ectopic bone in different tissues, a local and controlled delivery of the growth factor is necessary. To stimulate the healing process in bone the following application methods have been used for local growth factor delivery: (I) in vivo delivery of DNA encoding BMP2, (II) ex vivo gene therapy, (III) various carrier systems for direct protein application which is more frequently used [37]. On the other hand, transcriptome assay experiments revealed that signaling pathways induced by platelet derived growth factor (PDGF), TGF-β, and FGF are important for the differentiation of bone marrow-derived mesenchymal stem cells into osteogenic cell lineages. Furthermore, PDGF and FGF induce osteoblasts proliferation, whereas IGF and TGF-β promote osteoblasts differentiation [39].

The recent study performed by Naot et al., focuses on the mechanisms mediating in the proliferative effects of lactoferrin on osteoblasts. Lactoferrin is an iron-binding glycoprotein present for example in milk, and serves as an osteoblasts survival factor. Reserach conducted by Noat established that lactoferrin acts as a growth factor and has anabolic activity in bone both in vitro and in vivo. Lactoferrin induces proliferation of primary osteoblasts and osteoblastic cell lines, promotes osteoblasts differentiation in vitro and acts as a survival factor, protecting osteoblasts from apoptosis induced by serum withdrawal. Lactoferrin can also inhibit bone resorption by inhibiting osteoclasts development and activity. Local injection of lactoferrin over hemicalvaria of adult mice leads to a significant increase in bone area as well as bone formation rate and mineral apposition rate [40].

The role of osteoblasts in the regulation of osteogenesis is complex, but still partially unknown. The accurate knowledge of growth factors action for these cells or other a little known signaling molecules involved in the process of osteoblasts maturation, requires a more extensive experimental work. It also seems that the interaction and a balance between the various regulatory factors is extremely important for better understanding the overall role and behavior of human osteoblasts in osteogenesis in vitro.

Conclusions

Despite the continuing research on the process of formation, maturation, and functioning of osteoblasts, still little is known about their interactions, relationship, and mechanisms, by which transcriptional factors, growth factors, hormones, and others molecules (in particular the adhesion proteins: e.g., N-cadherin, connexin) regulate the processes of osteogenesis and osseointegration. Understanding the mechanisms regulating bone formation in culture conditions, bone healing and its metabolism would open new opportunities for treating bone diseases and give a real insight into its pathomechanism. From the clinical point of view, especially in cases of bone tissue substitution, understanding the biological interactions between the implantation material used in bone regenerative medicine and the activity of the patient cells is very important. It would also allow controlling of bone metabolism in living cells used to regenerate human bone tissue with tissue engineering methods.

Thus, the behavior of osteoblasts as the bone-forming cells in the osteogenesis and osseointegration still needs to be experimentally elucidated. Further examination of the biological mechanisms underlying bone healing, and the role of the osteoblasts will give not only the possibility of clinical utilization of controlled bone regeneration in the future, but also may give a new hope to orthopaedic patients.

Acknowledgments

This work was supported in part by research grants: No. N N302157037 from the Polish funds for scientific research in 2009-2012 (for Małgorzata Witkowska-Zimny), and grant No. 3 T08A 001 30 from Polish Ministry of Science and Higher Education (for Edyta Wróbel). Special thanks to Professor Jacek Przybylski for his critical reading and helpful comments.

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