REVIEW

Looking for new anabolic treatment from rare diseases of bone formation

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Abstract

Bone remodelling is a complex mechanism regulated by osteoclasts and osteoblasts and perturbation of this process leads to the onset of diseases, which may be characterised by altered bone erosion or formation. In this review, we will describe some bone formation-related disorders as sclerosteosis, van Buchem disease, hypophosphatasia and Camurati–Engelmann disease. In the past decades, the research focused on these rare disorders offered the opportunity to understand important pathways regulating bone formation. Thus, the identification of the molecular defects behind the etiopathology of these diseases will open the way for new therapeutic approaches applicable also to the management of more common bone diseases including osteoporosis.

Key Words

- ▶ bone diseases
- ▶ osteoblast
- ▶ bone formation

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Introduction

In healthy skeleton, the bone structure is maintained by the balanced relationship between osteoblasts and osteoclasts. These cells are responsible for the bone remodelling that begins with the recruitment and activation of bone-resorbing osteoclasts followed by the formation of new calcified tissue by osteoblasts (Del Fattore *et al.* 2012). This activity is well organised and the quality and quantity of bone structures are dependent on fine regulated mechanisms. Defects of bone resorption that cause excessive or impaired bone loss impact skeletal integrity as well as the immoderate, disorganised or reduced bone formation (Del Fattore *et al.* 2012).

Osteoblasts, the bone-making cells, derive from neural ectoderm to form craniofacial bones and from paraxial mesoderm and lateral plate mesoderm to form axial and appendicular skeleton (Tuan 1998). The bone formation process occurs in two distinct processes: intramembranous

or endochondral ossification (Rutkovskiy *et al.* 2016). Bone formation starts with the recruitment of MSC or pericytes of the bone marrow (James & Peault 2019) and it is characterised by four phases: lineage commitment, proliferative expansion, synthesis of extracellular matrix and mineralisation (Rutkovskiy *et al.* 2016) (Fig. 1).

Regarding the lineage commitment phase, osteoblast differentiation from MSC follows a specific programme of gene expression that takes place under the control of both wntless protein (WNT) and bone morphogenetic proteins (BMP). The activation of these pathways and their signal transduction lead to the stabilisation of β -catenin and activation of small mother against decapentaplegic (SMAD) signalling and to the expression of master transcriptional factors involved in osteoblastogenesis such as runt-related transcription factor 2 (Runx2) and transcription factor 7 (Sp7, Osterix) (Wu *et al.* 2016). Following initial lineage

Figure 1

Bone formation phases. Schematic diagram of the osteoblastogenesis and bone matrix production. Osteoblasts arise from a pluripotent mesenchymal stem cell (MSC) which, under proper stimuli, is committed toward an osteoprogenitor, a preosteoblast and an active, mature osteoblast. In this process, the bone matrix is produced and mineralised. At the end of bone formation, some osteoblasts remain embedded in the calcified matrix as osteocytes. Specific markers are indicated for each differentiation stage. Runx2, Runt-related transcription factor 2; ALP, alkaline phosphatase; COL1A, type I collagen α ; OCN, osteocalcin; OPN, osteopontin; BSP, bone sialoprotein; DMP-1, dentin matrix acidic phosphoprotein 1; MEPE, matrix extracellular phosphoglycoprotein; SOST, sclerostin. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

commitment, a phase of intensive proliferative activity of pre-osteoblasts occurs; these cells express Runx2, Osterix, ALP (alkaline phosphatase) and type I collagen ensues, are committed to the osteoblast lineage and represent an intermediate stage. At the end of the proliferation phase, pre-osteoblasts change their morphology in large cuboidal osteoblasts. Osteoblasts express high levels of ALP and generate the not mineralised and flexible bone osteoid (synthesis of extracellular matrix phase), by the secretion of matrix proteins including type I collagen (90%) and small percentage of proteoglycans, fibronectin and specific bone proteins, such as osteopontin and osteocalcin (Rutkovskiy *et al.* 2016). Subsequently, the mineralisation phase occurs and osteoblasts release matrix vesicles (MV) also known as 'calcifying globules' by budding, leading to the formation of hydroxyapatite crystals made of calcium and phosphate ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) (Del Fattore *et al.* 2012). MV contain several enzymes and transporters located on their membrane generating inorganic pyrophosphate (PPi) that is hydrolysed into inorganic phosphate (Pi) by tissue-nonspecific alkaline phosphatase (TNSALP) (Zhou *et al.* 2012). The presence of annexin and phosphatidyl serine into MV leads to the accumulation of calcium ions, which together with Pi promote the hydroxyapatite crystals nucleation and growth. The newly formed hydroxyapatite crystals are released by MV into extracellular matrix where the presence of Ca^{2+} and Pi supports crystals proliferation filling empty spaces between collagen fibrils (Del Fattore *et al.* 2012).

Bone quality is determined by the proportion of organic and mineral matrix that is characterised in healthy adult subjects by 60% mineral, 20% organic and 20% water but may vary in case of diseases and drug therapies. At the

end of bone formation, some osteoblasts die by apoptosis or become lining cells, others remain embedded in the calcified matrix as osteocytes (Rutkovskiy *et al.* 2016).

The number of osteoblasts and their activity are controlled by epigenetic mechanisms and are regulated by local or systemic factors (Sims & Martin 2014).

Molecular dissections of genetic osteoblast disorders allowed to identify the role of proteins involved in osteoblast activity. In this review, we described rare genetic osteoblast-related diseases and the underlying defects of molecular pathways. A deeper analysis of the molecular alterations is relevant for the identification of new therapeutic approaches for these rare bone disorders.

Sclerosteosis and van buchem disease

Sclerosteosis and van Buchem disease (VBD) are two rare autosomal recessive sclerosing disorders, classified as craniotubular hyperostoses and caused by genetic defects of sclerostin gene *SOST*. The first description was reported among the Afrikaners community in South Africa (van Lierop *et al.* 2017). In 1958 Truswell *et al.* defined sclerosteosis for the first time as 'osteopetrosis with syndactyly' (Truswell 1958). Patients are affected by facial distortion due to bossing of the forehead and mandibular overgrowth, hypertelorism and proptosis (van Lierop *et al.* 2017). In the 66% of individuals, syndactyly is noted at birth and it mainly involves the index and third finger. In early childhood, affected patients become tall and heavy due to skeletal overgrowth. Hallmark complication affecting 93% of the patients is facial palsy, due to overgrowth of the skull. This feature is recurrent

and appears early in the first months of life. Because of the bone thickening, blindness, deafness and increased intracranial pressure may be present (van Lierop *et al.* 2017). In the majority of patients, symptoms develop during early childhood and progress until adulthood when they stabilise after the third decade of life (van Lierop *et al.* 2013). Histopathological findings revealed accelerated bone formation with increased trabecular thickness and osteoidosis; the newly formed bone is lamellar and correctly mineralised (Hassler *et al.* 2014). Moreover, bone thickening and cortical hyperostosis are detected by imaging techniques. If not treated, patients have a short life expectancy (van Lierop *et al.* 2013).

Van Buchem disease, first described in 1955 as 'hyperostosis corticalis generalisata familiaris', is a milder type of sclerosteosis (van Buchem *et al.* 1955). It is characterised by the same symptoms of sclerosteosis, except for syndactyly and tendency to tall stature (van Buchem 1976, van Lierop *et al.* 2013). Endosteal hyperostosis produces dense cortical bone with a narrow medullary cavity (Ihde *et al.* 2011). The diagnosis is mainly based on the analysis of skull and mandible abnormalities and on radiological images.

Biochemical analysis of patients affected by both diseases displays increased levels of procollagen type I aminoterminal propeptide (P1NP) and ALP and normal level of calcium, phosphorus and parathyroid hormone (PTH) (van Lierop *et al.* 2013).

The similar features that characterise the diseases are attributable to genetic defects of *SOST* gene, encoding sclerostin. Sclerosteosis is caused by inactivating mutations of *SOST* gene (van Lierop *et al.* 2017) whereas van Buchem's patients harbour a 52 kb homozygous deletion on chromosome 17q12, compromising a downstream enhancer of *SOST* important for gene transcription in adult bone (Balemans *et al.* 2002). Consequently, sclerostin serum levels are undetectable in sclerosteosis, but low levels can be revealed in van Buchem disease (van Lierop *et al.* 2013).

In these two diseases, osteoclast abnormalities have not been reported suggesting that osteoclasts are not involved in the pathological mechanisms responsible for the skeletal changes observed in patients (Stein *et al.* 1983). However, it was demonstrated that sclerostin stimulates osteocyte support of osteoclast activity by a RANKL (receptor activator of nuclear factor kappa-B ligand)-dependent pathway. The catabolic action of sclerostin was further demonstrated; in a rat model of postmenopausal osteoporosis, sclerostin neutralisation protected mice from bone loss induced by ovariectomy due to increased osteoblast surface and reduced osteoclast number (Li *et al.* 2009).

SOST pathway

The genetic analysis of human sclerosteosis and VBD allowed the identification of *SOST* gene (Balemans *et al.* 2002). *SOST* encodes for sclerostin that is a potential new member of the Dan/Cerberus family of BMP (Brunkow *et al.* 2001). Sclerostin mRNA is widely expressed in the organism, especially during embryogenesis; postnatally the protein has been only revealed in osteocytes, chondrocytes and cementocytes (Holdsworth *et al.* 2019). Sclerostin is a monomeric glycoprotein with a cystine knot-like domain containing two N-linked glycosylation sites and many positively charged lysine and arginine residues. The mature structure of sclerostin includes C- and N-terminal arms and three loops around the cystine knot motif (Brunkow *et al.* 2001, Balemans *et al.* 2002). Loop 2 has been identified as the critical region that binds low-density lipoprotein (LRP) 5/6. LRP 5/6 is a co-receptors of the WNT canonical signalling pathway that plays an important role in the regulation of bone formation. The association of sclerostin to the WNT canonical pathway was suggested by the observation of similar clinical traits between patients with high bone mass diseases due to sclerostin absence and those with LRP5 defects (Li *et al.* 2005). Although sclerostin can bind also to LRP4 with structural similarities to LRP5/6, this link is not able to create a co-receptor complex with frizzled that is required to bind WNT proteins (Fig. 2).

In spite of that, LRP4 overexpression enhances the efficacy of sclerostin in the WNT pathway while its silencing reduces sclerostin function (Leupin *et al.* 2011). Interestingly, mutations in LRP4 cause Sclerosteosis type 2, which shows features comparable to sclerosteosis (Leupin *et al.* 2011), suggesting LRP4 as a regulator of sclerostin activity.

Sclerostin plays an essential role in the inhibition of osteoblast differentiation and function, leading to reduced bone formation (Li *et al.* 2008). Indeed, sclerostin limits mesenchymal lineage cell differentiation towards the osteoblast lineage, decreases bone matrix formation by osteoblasts, promotes osteoblast apoptosis, maintains bone lining cells in a quiescent state and inhibits differentiation of late osteoblasts into osteocytes (Holdsworth *et al.* 2019). The production of sclerostin is regulated by mechanical loading, estrogens and PTH and increases with age (van Lierop *et al.* 2017). *Sost* gene deletion in mice causes increased bone mineral density (BMD) in the entire skeleton, while the overexpression leads to osteopenic phenotype (Li *et al.* 2008). In mice, the van Buchem phenotype is created via deletion of the non-coding enhancer ECR5, that reproduces a milder phenotype than *sost*^{-/-} mice (Collette *et al.* 2012).

Treatment

There are no specific treatments for sclerosteosis and VBD. The management of the diseases aims to surgical correction of syndactyly, decompression of cranial nerves and reduction of mandibular overgrowth. In some patients, skull bones are replaced by prosthetic cap or are replaced after bone reduction (Hamersma *et al.* 2003). Life expectancy for sclerosteosis patients is 33 years and the major causes of death are herniation of the brain or perioperative complications to correct intracranial pressure (Hamersma *et al.* 2003); instead for van Buchem affected people life expectancy can reach up to 82 years (van Lierop *et al.* 2017).

Hypophosphatasia

It is important to note that not only osteoblast-specific alterations cause defects to bone mineral density. Indeed, hypophosphatasia (HPP) is characterised by *loss-of-function* mutation of *ALPL* gene, localised on chromosome 1p36-34, that encodes for tissue-nonspecific alkaline phosphatase (TNSALP) protein (Whyte 2017). The disease is classified in seven clinical forms: perinatal, infantile, childhood, adult, odonto-HPP, pseudo-HPP and benign prenatal HPP. The major clinical manifestations are hypomineralisation of bone and teeth, which results in defective ossification, osteomalacia and loss of dentition (Whyte 2017). HPP could already manifest *in utero*, causing profound skeletal hypomineralisation that can lead to death due to asphyxia after few days of life.

Defects of bony fusion of cranial sutures can lead to increased intracranial pressure and cerebral damage. The typical manifestation of childhood form is premature loss of deciduous teeth (Whyte 2016). The deposition of calcium pyrophosphate dihydrate crystals in articular cartilage is the main cause of musculoskeletal pain in HPP adult patients (Shapiro & Lewiecki 2017). In severe forms patients are affected by respiratory failure, short stature, hypotonia, fractures (Fig. 3) and musculoskeletal pain (Shapiro & Lewiecki 2017).

The hypomineralisation is caused by accumulation of inorganic pyrophosphate (PPi) in the extracellular matrix, which inhibits hydroxyapatite crystals' formation and affects the cementum in the outside faces of teeth root (Foster *et al.* 2012). Indeed, osteoblasts from *tnsalp* null mice differentiate normally but are unable to initiate mineralisation *in vitro* while osteoclastogenesis and bone resorption are not altered (Wennberg *et al.* 2000).

Laboratory tests may reveal low levels of ALP activity, increased levels of urinary phosphoethanolamine (PEA), serum PPi and serum pyridoxal 5'-phosphate (PLP), and normal serum values of calcium, ionised calcium, inorganic phosphate, PTH, 25-hydroxy and 1,25-hydroxy vitamin D (Whyte 2017). These parameters may be useful for HPP diagnosis, which is based on genetic analysis, even in prenatal context. Severe HPP could be explained by recessive inheritance whereas milder form could have an autosomal dominant inheritance.

The heterogeneity in clinical manifestations reflects the mutations identified in *ALPL* gene; so far, 409 variants

Figure 2

Sclerostin in bone physiology and in Sclerosteosis/VBD. In osteoblasts, binding of Wnt ligands to the LRP5/6 and frizzled co-receptor stimulates bone formation. Sclerostin also binds to LRP4 and inhibits the Wnt signalling pathway, an action facilitated by LRP4. In physiological condition (*upper panel*), the bone-forming role of Wnt is well balanced with the inhibitory effect of sclerostin on bone formation. In Sclerosteosis or van Buchem disease (*lower panel*), the mutation/deletion in *SOST* gene, that causes a defect of sclerostin production, reduces the levels of sclerostin and determines an increase of bone formation. VBD, van Buchem disease; Wnt, wingless protein; LRP, low-density lipoprotein receptor-related protein. The figure was produced using Servier Medical Art. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

associated with HPP disease have been described (http://www.sesep.uvsq.fr/03_hypo_mutations.php) (Mornet 2018).

ALPL gene

ALPL gene encodes for tissue nonspecific alkaline phosphatase enzyme (TNSALP) that is, with the placental, the intestinal and the placental-like forms, one of the four metalloenzymes ALPs in humans. The *ALPL* gene is located on chromosome 1, at the tip of the short arm, whereas other ALPs genes are found on the long arm of chromosome 2 (2q34–q37) (Mornet 2018). No inherited disorders have been described for tissue-specific ALPs (Millan & Whyte 2016).

Figure 3

X-ray picture from a 46-year-old patient affected by hypophosphatasia. Asterisks indicate multiple vertebral fractures. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

TNSALP is widely expressed in the body, particularly in the liver, bone and kidney. While its role in bone calcification is well known, the role in extra-calcified tissue is still under investigation (Millan & Whyte 2016).

This enzyme is a membrane glycoprotein linked to the membrane by glycosylphosphatidylinositol (GPI). It has five main domains: catalytic site, calcium-binding site, crown domain, homodimer interface and N-terminal alpha helix (Mornet 2018). During the synthesis of the peptide, the N-terminal domain guides the protein to the endoplasmic reticulum (ER) lumen where it folds in the tertiary structure and creates a quaternary structure as homodimer. Moreover, in the ER, the neo-synthesised peptide is modified by N-glycans, disulfide bonds and GPI. In the Golgi the immature 66 kDa form of TNSALP becomes the 80 kDa mature form modifying the high-mannose type N-glycans to complex type N-glycans (Mornet 2018). Subsequently, mature protein is transported to the plasma membrane to localise in the surface via GPI where it dephosphorylates PPI releasing inorganic phosphate (Satou *et al.* 2012). The essential role of TNSALP for skeletal mineralisation came from the study of HPP in the 80s. The accumulation of PPI, PEA and PLP observed in HPP was essential to identify these compounds as natural substrates of TNSALP (Salles 2020). Mutation of *TNSALP* reduces enzyme activity leading to high level of PPI that is an inhibitor of hard tissue mineralisation; this results in the blocking of hydroxyapatite crystal growth (Whyte 2017) (Fig. 4).

Treatment

The hypomineralisation that characterises HPP often caused a misdiagnosis of the disease as osteoporosis and, as a consequence, led to treatment with antiresorptive drugs, such as bisphosphonates. The wrong treatment worsened the prognosis of HPP because bisphosphonates are analogous of PPI and accumulates in HPP (Whyte 2017). The implementation of diagnosis and treatment is still necessary. The therapeutic options were based on treatment of hypercalcemia with fluid hydration, low calcium diet and surgery for fractures. To ameliorate the reduced ALP level, infusion with ALP-rich plasma obtained from Paget's patients was performed (Choida & Bubbear 2019). Whyte *et al.* reported life-treating intravenous infusion in four patients with severe HPP and after 5 weeks partial or complete correction of the deficiency of circulating ALP activity was observed, but PEA and PPI remain elevated and skeletal deformities persisted (Whyte *et al.* 1986).

Bone marrow transplantation (BMT) was also used as therapeutic approach for HPP. In the short-term, BMT was

efficient to improve bone mineralisation; however the return of host hematopoiesis was observed few months post-BMT; restoration of ALP activity was limited, and normal bony architecture could not be achieved (Taketani *et al.* 2015).

A positive effect was produced by bone anabolic drug Teriparatide, a recombinant peptide mimicking PTH, which leads to increased ALP levels and fracture healing (Whyte *et al.* 2007, Righetti *et al.* 2018); however patient osteoblasts still produced a defective enzyme (Subbiah *et al.* 2010).

Due to the poor effects of aforementioned treatments, the new frontier is the development of a recombinant TNASALP enzyme. Asfotase alfa is the human recombinant enzyme created to replace mutated TNSALP; it is a glycoprotein that contains the catalytic domain of TNSALP thus being able to degrade PPI. This enzyme replacement therapy (ERT), approved by FDA in 2015, is administered from perinatal to adulthood via subcutaneous injection (Kitaoka *et al.* 2017).

Studies of affected infants, children, adolescents and adults assessed the efficacy and positive effects on skeletal mineralisation, growth, mobility and survival up to 5 years of therapy (Whyte *et al.* 2016). Despite good results in treating HPP, asfotase alfa requires lifetime treatment administration with frequent injections (3–6 months) and has several adverse effects as injection-site reactions and possible long-term tachyphylaxis. Alternative therapies are still under development including gene therapies that use lentiviral vector (LV) delivery of TNSALP or

transduction of bone marrow cells *ex vivo* with LV-TNSALP and, interestingly, anabolic treatment with anti-sclerostin antibody (Matsumoto *et al.* 2011, Whyte *et al.* 2016, Seefried *et al.* 2017).

Camurati-Engelmann disease

Camurati-Engelmann disease (CED), also known as progressive diaphyseal dysplasia (PDD), is a rare genetic skeletal disorder, first described in 1920 by Cockayne (Cockayne 1920), later by Camurati in 1922 (Camurati 1922) and by Engelmann in 1929 (Engelmann 1929). This is a very rare disease, indeed about 300 cases have been reported to date; it has an incidence of 1:1 million births and displays an autosomal dominant inheritance. It is characterised by limb pain, muscular weakness and atrophy, cortical thickening of long bones diaphyses, cranial hyperostosis that usually appears during childhood. Clinical manifestations and severity of disease are extremely variable within patients for the age of onset, rate of progression and bone involvement, making the diagnosis challenging (van Hul *et al.* 2019). The main radiographic feature of CED is a thickening of the diaphysis due to abnormal periosteal and endosteal apposition of new bone, which sometimes affects metaphyses. The most involved regions are tibiae, femora, skull and pelvis (Spranger 2018) (Fig. 5). Bone scintigraphy identifies accumulation in the affected sites of the skeleton and it is a valuable tool for early stages diagnosis (Clybourn *et al.* 1994). Thickening of skull bone can lead to blindness and/or deafness; minor other orthopaedic defects are reported as kyphosis, scoliosis and flat feet (Yuldashev *et al.* 2017). Muscle weakness, easy fatigability and leg pain may occur especially during childhood, wrongly leading to a diagnosis for muscular disorders. Radiographic manifestations are usually detected before age of 30 (van Hul *et al.* 2019).

In 2000, Janssens *et al.* identified the transforming growth factor $\beta 1$ (*TGF β 1*) gene (chromosome 19q13.2) as the primary causative gene in CED (Janssens *et al.* 2000).

At present, 13 different mutations have been reported in patients, that are located in the latency-associated peptide (LAP) or in the signal peptide of the protein (van Hul *et al.* 2019). Three of them are the most recurrent: R218C, R218H and C225R (Janssens *et al.* 2006). The result of the mutations found in LAP is a reduction of the LAP ability to bind *TGF β* thus leading to increased activity. Regarding the mutations found in the *TGF β* signal peptide, they cause intracellular accumulation of *TGF β* and increased signalling

Figure 4

TNSALP function in physiological condition and in HPP. TNSALP enzyme is responsible for the formation of Pi cleaving PPI (*left panel*). PPI is a potent inhibitor of hydroxyapatite crystals, while Pi is the main actor of crystals formation with calcium. In HPP, mutations in TNSALP cause inactivation of the enzyme and an accumulation of PPI that inhibit bone mineralisation in patients (*right panel*). TNSALP, tissue nonspecific alkaline phosphatase; Pi, inorganic phosphate; PPI, inorganic pyrophosphate. The figure was produced using Servier Medical Art. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

(Janssens *et al.* 2003). CED mutations lead to a net increase of bone mass in some regions of the skeleton. Despite some conflicting findings, most experiments suggest that TGF β promotes proliferation, early differentiation of osteoblast progenitor cells, and matrix production, while inhibiting later differentiation and matrix mineralisation. At the same time, bone erosion is also enhanced in CED as also shown by high levels of bone resorption markers in patients (Hernandez *et al.* 1997). Moreover, *in vitro* experiments

confirmed an increase of osteoclastogenesis and bone resorption in cell cultures from CED patients.

So far it is not completely understood why symptoms are mostly in the skeleton, even though TGF β 1 and its receptors are ubiquitously expressed (Weiss & Attisano 2013).

TGF beta pathway

TGF β 1 is a member of the TGF β family and is a potent stimulator of cell migration, proliferation, differentiation and apoptosis (Del Fattore *et al.* 2012). The protein is produced as a precursor with an N-terminal signal peptide, a latency-associated peptide necessary for folding and dimerisation, and the C-terminal growth factor. In the ER, the precursor forms cystine bridges and the signal peptide is cleaved. After glycosylation in the Golgi, LAP is also cleaved by furin (Nuchel *et al.* 2018). The homodimer with the mature peptide, non-covalently linked to LAP subunits, is secreted and embedded in the bone matrix (Miyazono *et al.* 1988). It could be found in either a latent form composed of a mature peptide homodimer, a LAP homodimer and a latent TGF β binding protein, or in an active form characterised by the mature peptide homodimer. The mature peptide may also form heterodimers with other TGF β family members.

TGF β is the most abundant cytokine in the bone matrix, deposited by osteoblasts, and can have both a positive and negative role in the development and maintenance of the skeleton when released by osteoclast activity during bone resorption (Wu *et al.* 2016); indeed the growth factor is released from the extracellular matrix during bone resorption and the acid pH of the Howship's lacuna breaks the interaction between mature peptide and LAP, causing the activation of TGF β 1. TGF β 1 regulates both osteoblast and osteoclast differentiation and activity. It regulates bone remodelling by the interaction with its constitutively active type II receptor, recruiting also type I receptor to form heteromeric complex (Shi *et al.* 2011). TGF β 1 stimulates osteoblast differentiation in the early stages, and inhibits the later phase of proliferation and mineralisation that are more susceptible to BMP signalling. Moreover, TGF β 1 inhibits further osteoclast differentiation and activation, coupling bone formation with bone resorption. TGF β isoforms and their receptors, type I receptor (TGF β RI) and type II receptor (TGF β RII), play important roles in endochondral and intramembranous ossification (Chen *et al.* 2012).

The binding of TGF β 1 with its receptors modulates the activation of SMAD transcription factors.

Figure 5

X-ray images from a 41-year-old patient affected by Camurati-Engelmann disease. Arrows indicate the thickening and irregularity of endosteal and periosteal sides of long bones including tibia, femur and humerus. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

SMAD2 and SMAD3 (R-SMAD) respond to TGF β receptors, and, competing with SMAD7, they form complexes with SMAD4, one of the common mediator of TGF β pathway (Shi & Massague 2003) (Fig. 6). However other growth factors including interferon gamma (IFN γ) and tumor necrosis factor alpha (TNF α) can modulate SMAD proteins expression with attendant inhibition of TGF β signalling (Fig. 6). In recent papers R-SMAD were reported to be involved in a molecular complex that impacts on TNF receptor-associated factor 6 (TRAF6), confirming the inhibitory effect of the growth factor on the RANKL-induced osteoclastogenesis (Yasui *et al.* 2011).

TGF β 1 has also a non-canonical pathway, via mitogen-activated protein kinase (MAPK), that promotes osteoprogenitor proliferation and differentiation

(Matsunobu *et al.* 2009). Both canonical and non-canonical pathways converge at *RUNX2* gene to control mesenchymal cell osteogenic differentiation (Lee *et al.* 2002).

Treatment

The treatment of CED is not well established and different therapeutic options are based on short case reports. In some cases, corticosteroid-based treatment could solve pain and improve muscle function (Wallace *et al.* 2004). A dose of 1 mg/kg/day of prednisone is often administered to patients with severe symptoms. Histological analyses after steroid therapy have shown an increase of bone resorption and new bone formation but in several studies, bone scintigraphy reported no regression of sclerosis (Bas *et al.*

Figure 6

TGF β 1 in physiological state and in CED. TGF β 1 is responsible of osteoblast differentiation, proliferation and activity. In physiological conditions (*left panel*), its binding to TGF β receptor II activates SMAD proteins via phosphorylation. SMAD2 and SMAD3 trimerise with SMAD4. The trimer enters into the nucleus and activate gene transcription. Smad7 attenuates phosphorylation and nuclear translocation of Smad2/Smad3. IFN γ inhibits the TGF β /Smad signalling pathway by activation of Smad7. TNF α inhibits the pathway by AP-1 that directly interfere with the interaction of the Smad2/3-Smad4 complex with DNA. In CED (*right panel*), the hyperactivation of TGF β 1 leads to the increase of signal transduction and downstream pathway causing an hyperactivation of the genic transcription. TGF β 1, transforming growth factor beta 1; IFN γ , interferon gamma; TNF α , tumor necrosis factor alpha; CED, Camurati-Engelmann disease; SMAD, small mother against decapentaplegic. The figure was produced using Servier Medical Art. A full colour version of this figure is available at <https://doi.org/10.1530/JOE-20-0285>.

1999). An angiotensin II type receptor that downregulates TGF β 1, was used to treat CED. However, conflicting effects are reported (Yuldashev *et al.* 2017). Preclinical studies that target TGF β 1 type I receptor have been reported to treat the high bone turnover disease (Akhurst 2017), but no clinical trials data are still available. Few clinical trials of TGF β inhibitor could open the way to a new therapy for CED.

Discussion

In the physiological process of bone remodelling, bone formation is a tightly regulated process. Signals that influence the differentiation and function of osteoblasts play an important role in the mechanism of action of anabolic agents in the skeleton. So far, the use of Teriparatide, analogue of PTH, has been the only safe, effective and approved anabolic drug treatment for osteoporosis, with limitation in the use and in the time of administration.

The study of rare diseases paved the way to the comprehension of molecular mechanisms involved in the physiology of bone, revealing also possible targets to improve bone formation. In this review, we described four rare diseases characterised by bone formation defects and the molecular pathways involved in the onset of these disorders that suggested or can suggest new targets for anabolic treatments.

Sclerosteosis and van Buchem diseases highlighted the importance of sclerostin in bone physiology and made patients a unique model to study the role of this protein in bone metabolism. The generation of *sost*^{-/-} mice confirmed that the lack of sclerostin only impaired bone tissue, without affecting other organs.

Sclerostin could represent also a target by which nature counteracts the postmenopausal bone loss. Indeed, increased methylation of *SOST* promoter has been revealed in bone biopsies of postmenopausal women. The hypermethylation of the promoter is associated to the reduced expression of *SOST* gene thus leading to reduced inhibition of WNT signalling. This could be a compensatory mechanism to stimulate bone formation that is reduced in postmenopausal condition (Reppe *et al.* 2015).

All these observations indicated sclerostin as a possible novel target for the development of new anabolic therapies for osteoporosis without side effects on other organs.

Indeed, several sclerostin inhibitors have been tested for osteoporosis treatment. In an elegant review, McClung reported the positive effect on the bone formation of Romosozumab, a human MAB against sclerostin (McClung

2018), that was recently approved by Food and Drug Administration for its use in osteoporotic patients with the high risk of fractures. Beside teriparatide, romosozumab is another safe and efficacious bone anabolic treatment.

Although patients with sclerosteosis or van Buchem disease contributed greatly to understand the function of sclerostin in bone and to identify new therapy for osteoporosis, they still not have efficacious treatment for their diseases.

The development of gene therapy or enzyme replace therapy could be a safe way to treat bone formation defects as for hypophosphatasia. The comprehension of TNSALP role in bone led to the production of Asfotase Alfa enzyme replacement therapy to treat HPP. This therapy has a good safe profile (Whyte *et al.* 2012), with evident effects in infants, children and adults disease. Since potential adverse effects such as site injection reaction (in about 75% of patients), anaphylactoid reaction, exacerbation of calcification in vascular and kidney and immune reaction to treatment (Whyte *et al.* 2012) have been observed, further studies to ameliorate these features are needed. Thus, also for this disease innovative and life-saving therapy with long-term efficacy and reduced side effects is still under investigation.

The bone-specificity, that let sclerostin be a good target for bone diseases without side-effects, is not the main property of TGF β that, beyond the important role in all phases of chondrogenesis, mesenchymal condensation, matrix deposition and skeletal homeostasis maintenance, has a broad spectrum of function in other pathologies, such as cancers and vascular diseases (Chen *et al.* 2012).

The pleiotropic effect of TGF β 1 on the skeletal cells, with roles both in osteoclasts and in osteoblasts, makes the growth factor appealing as a target for the treatment of bone diseases (Chen *et al.* 2012). *Tgfb1*^{-/-} mice display reduced bone growth and mineralisation (Geiser *et al.* 2005) while the osteoporotic mice treated with TGF β displayed increased BMD (Gazit *et al.* 1999). Despite its ubiquitous distribution, TGF β is one of the growth factor to be considered as anabolic agent to promote fracture healing or to reverse bone resorption in osteoporosis. The limit of its use is related to the wide distribution of TGF β that could cause adverse effects highlighting the requirement to develop a good carrier that can deliver drugs specifically to bone or, for genetic diseases' treatment, to develop a tissue-specific gene therapy.

The implications of TGF β in several mechanisms reveal that additional studies are required to better identify its role in body physiology and to evaluate a possible use as bone-forming drug.

Advances in the understanding of cellular and molecular signalling contributing to osteoblast differentiation and function, based also on the study of genetic diseases, have indicated new targets to treat either rare or common skeletal pathologies. Further development and study of therapeutic approaches targeting bone formation fulfil a clinical need for treatment option for bone loss to promote bone regeneration despite targeting only bone resorption.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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