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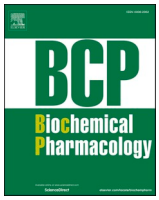

Review

Osteostatin, a peptide for the future treatment of musculoskeletal diseases

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ABSTRACT

Nowadays, the treatment of musculoskeletal diseases represents a major challenge in the developed world. Diseases such as osteoporosis, osteoarthritis and arthritis have a high incidence and prevalence as a consequence of population aging, and they are also associated with a socioeconomic burden. Many efforts have been made to find a treatment for these diseases with various levels of success, but new approaches are still needed to deal with these pathologies. In this context, one peptide derived for the C-terminal extreme of the Parathormone related Peptide (PTHrP) called Osteostatin can be useful to treat musculoskeletal diseases. This pentapeptide (TRSAW) has demonstrated both in different in vitro and in vivo models, its role as a molecule with anti-resorptive, anabolic, anti-inflammatory, and anti-antioxidant properties. Our aim with this work is to review the Osteostatin main features, the knowledge of its mechanisms of action as well as its possible use for the treatment of osteoporosis, bone regeneration and fractures and against arthritis given its anti-inflammatory properties.

1. Introduction

Nowadays, treatment of musculoskeletal diseases represents a major challenge for health systems. Diseases as osteoporosis and osteoarthritis have a high incidence and prevalence as consequence of population aging being associated to an enormous socioeconomic burden [1,2]. Osteoporosis that is suffered by around 6.3 % of men and 21.2 % of women above 50 years old in the world and the fractures resulting from this disease known as fragility fractures (8.9 million annually) are a matter of concern for the health systems [3–5]. Besides, comorbidities such as diabetes mellitus (DM) have been reported to aggravate osteoporosis and delay or impair fracture resolution [6,7]. Thus development of new strategies to manage these diseases has become a priority for researchers and clinician. Most of the new strategies developed to deal with these diseases are based on the accumulated knowledge of how the bone tissue is maintained. Bone homeostasis works through a complex mechanism that is called bone remodeling in which there is an anabolic part carried out by osteoblast and a resorption phase mediated by osteoclast action [8]. As an example of the different ways in which bone remodeling is controlled, osteoblast and osteoclast modulate each other through the secretion of different factors such as Osteoprotegerin (OPG) and Receptor activator of nuclear factor kappa-B ligand (RANKL) and above of them there is a master and commander of bone remodeling which is the osteocyte that as a mechanosensory cells also regulates the bone remodeling affecting osteoblast and osteoclast [9]. In this complex mechanism other local factors that have been names as osteokines such as Sclerostin, Osteocalcin, Fibroblast growth factor-23 (FGF-23), Transforming growth factor-β (TGF- β), Bone morphogenic proteins (BMPs), Insulin like growth factor-1 (IGF-1) and the Parathormone related peptide (PTHrP) plays an important role in the correct function of bone remodeling acting locally in different cells of bone tissue [10]. In the case of PTHrP it has been demonstrated that the N- and C- terminal portions of this polypeptide display different anabolic and even anti-resorptive actions [11].

Regarding osteoporosis treatment, there are three kinds of treatments called anabolic, anti-resorptive therapies respectively and mixed.

Abbreviations: ALP, Alkaline Phosphatase; BV/TV, Bone volume/trabecular volume; BVF, Bone volume fraction; Co. Th., Cortical Thickness; Ct.Po., Cortical Porosity; DA, Degree of anisotropy; DM, Diabetes mellitus; MTA, mineral trioxide aggregate; OPG, Osteoprotegerin; PTHrP, Parathormone related peptide; RANKL, Receptor activator of nuclear factor kappa-B ligand; SMI, structure model index; Tb.N., trabecular number; Tb.Th., Trabecular Thickness; Tb.Sp., Trabecular Separation; Tb.Pf., Trabecular bone pattern factor.

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The first one is represented by the treatment with Teriparatide (PTH) and Abaloparatide [PTHrP (1–36 analogue)]. PTH treatment was the first anabolic treatment available to treat osteoporosis and it's based on the ability of PTH to induce bone accrual when is administered intermittently. Abaloparatide, it is an analogue of PTHrP (1–36) which has been recently approved by USA Food Drug Administration (FDA) for the treatment of postmenopausal osteoporosis. Clinical trials have demonstrated that Abaloparatide produced more bone accrual with less secretion of calcium to blood [12]. PTH and Abaloparatide mechanism of action are related to differential activation of PTH1R receptor [13]. On the other hand, antiresorptive therapy is based on blocking osteoclast activity. In this category are included selective estrogen-regulator modulators (Raloxifene and Bazidoxifene), Calcitonin, and Bisphosphonates (Alendronate, Risedronate, Ibandronate, and zoledronate) [14] and a novel antiresorptive treatment that is the humanized antibody against RANKL ligand called denosumab [15,16]. Regarding mixed treatment that works as well as anabolic and anti-resorptive we must mention Romosozumab (an anti-sclerostin humanized antibody) and Odanacatib (a Catheptsin K inhibitor) [17,18].

Besides, one of the new strategies for healing bone is the use of biomaterials. Different biomaterials are being tested with this aim and some of the more promising are the ones based on Calcium and Silicious because these materials could produce an osteogenic response by themselves. In this regard, the unique properties of mesoporous silica materials – their high surface area, impressive drug loading capacity, and excellent biocompatibility – have made them a focal point in drug delivery research. Notably, the surface can be chemically engineered with various functional groups. These modifications are often employed to enhance targeted delivery or develop stimuli-responsive carriers. Also, it is possible to build different scaffolds or nanoparticles with these types of materials that can be used as a reservoir of osteogenic molecules or even as a vector for introducing cells in cells for bone tissue regeneration [19–21]. In this sense, Osteostatin has been loaded by different methods into different scaffolds and nanoparticles to enhance the osteogenic effect of biomaterials and bone regeneration in different in vitro and in vivo models which are further described in this review.

Here, we review the information available about Osteostatin origin, structure and mechanism of action as well as discuss challenges that Osteostatin application must face to be used as a peptide to treat bone diseases and fractures alone or in combination with different biomaterials.

2. PTHrP structure

PTHrP is a cytokine that was first isolated from human cancers associated with the syndrome of human hypercalcemia of malignancy [22,23]. PTHrP gene is located in humans at chromosome 8 and contains multiple exons (https://www.ncbi.nlm.nih.gov/gene/5744) that through alternative explaining produce up to three isoforms of 3 isoform transcripts of 139, 141 and 173 aa receptively that are present in multiple human cell lines [24]. All fragments contain a prepro sequence or signal peptide of 36 (-36 to −1) amino acids that are split off to form the

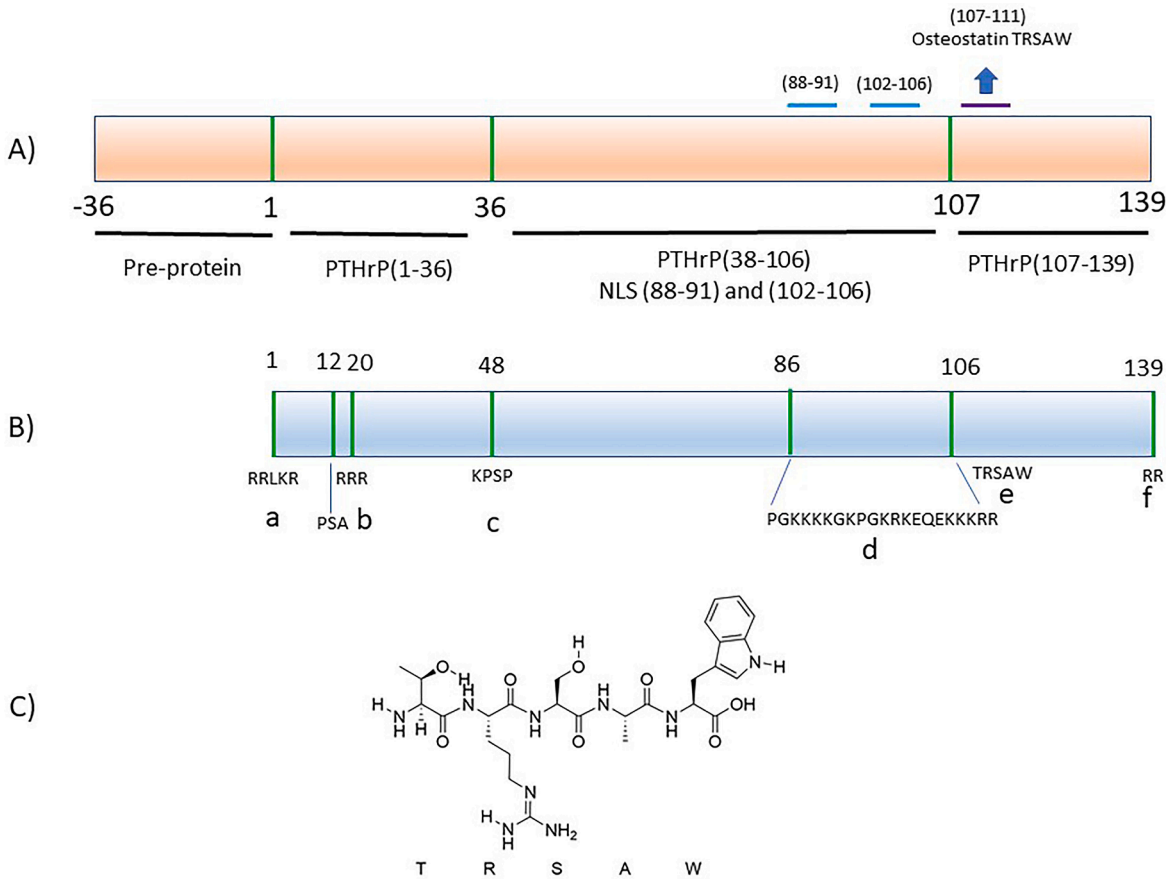

Fig. 1. PTHrP layout of polypeptide −36–139 (A) and potential endopeptidase cut places in this polypeptide (B). A) It has been shown that there are 3 different polyproteins of different sizes 139, 141 and 173 amino acids all including the propreptide that extends from −36 to 1. The image represents the peptide PTHrP 1–36, the nuclear signaling sites (NLS 88–91 and 103–106) and the C-terminal fragment including Osteostatin extending from amino acids 107–111. B) the image represents the main cutoff points for different proteolytic enzymes such as Furin dipeptidyl peptidases and pro-hormone convertases, as well as the sequences on which they act. Here we describe the proteases for each section a) Furin, pro-hormone convertase PACE4, PC1, PC2, PC4 PC5 and pro-hormone convertase PSA, b) pro-hormone convertase PACE, PC1, PC2, PC4 PC5, c) Dipeptidyl peptidase, d) Furin, pro-hormone convertase PACE4, PC1, PC2, PC4 PC5, e) Furin and PC1, Arg-C proteinase, clotrispain, Tripsin, LysC and LysN peptidases and f) Furin, pro-hormone convertase PACE4, PC1, PC2, PC4 PC5. C) Osteostatin molecule (TRSAW).

active cytokine (Fig. 1A). This preprotein sequence is followed by a fragment of 36 aa (1–36) with a high homology to PTH and that exerts its action through the same receptor but follows another mechanism of action that produce different effects such as more bone accrual with less hypercalcemia [11,25]. The middle region of PTHrP is encompassing between 88 and 106 amino acids and contain a nuclear localization signal peptide (NLS) [26–29] (Fig. 1A). It has been described that this region is necessary to enhance the growth and viability of murine osteoblast-like cells (MC3T3-E1) with PTHrP overexpressing [30]. Thanks to this NLS, PTHrP also participates in intracellular signaling and it has been shown in the nucleus of chondrocytes to enhance their survival in serum starvation conditions [31] and increase the proliferation of smooth muscle cells [32] and keratinocytes [33]. Some authors have proposed that an alternative initiation translation site might produce this fragment to be exported to the nucleus [34].

The possible existence of a C-terminal peptide with biological properties that may encompass amino acid from 107 to 139 in PTHrP had been suggested in different reports [35–37]. Thus, Burtis et al in 1990 demonstrated that the peptide from 107 to 139 was present in an elevated manner in patients with cancer and with chronic renal failure [36]. However, the fragment of PTHrP (107–139) was not isolated with an immunoaffinity column loaded with an anti PTHrP antibody raised against 1–36 region, which pointed out to the possibility that a peptide may circulate separately from the rest of the cytokine in the plasma of this patients [36]. Later, Fenton et al described that fragment 107–139 possesses a tremendous antiresorptive capacity [38]. Further work from the same research group reported that the minimum domain request to produce this effect as was the sequence TRSAW that comprises the amino acid 107–111 [39]. Although, there are no reports confirming that Osteostatin might be produced by the cellular machinery, there are scarce evidences that may support this hypothesis. It has been demonstrated that a separate C-terminal fragment has been isolated from lysates and conditioned media from Chinese hamster ovary (CHO) and rat insulinoma (RIN) cell lines that reacts with an antibody raised against region 109–139 [24]. Another argument that supports that 107–139 might suffer proteolytic cleavage is that there is a peptide signal expanded from 102 to 106 aa (KKKRR) that might potentially suffer processing from endopeptidases (Fig. 1B) [40] and this fragment might be cut with Arg-C proteinase, clotrispain, Trypsin, LysC and LysN peptidases. Therefore, further studies are needed to demonstrate whether bone tissue cells could process PTHrP (107–139) to produce Osteostatin.

3. PTHrP mechanism of action

Adding information to the previously explained structure of Osteostatin (Fig. 1A,B,C), the three-dimensional structure of this peptide has been resolved using two-dimensional proton NMR spectroscopy showing that is compatible with a finger like-structure that might be able to bind its potential receptor [41]. Besides, the three-dimensional structure shows that the rest of the amino acids from 112 to 137 form a globular domain that could be used as a carrier for the pentapeptide. Unfortunately, nowadays no receptors have been described for Osteostatin and this is one of the reasons why there has not been more advances in its applications. However, it has been demonstrated that Osteostatin and PTHrP (107–139) signal through a different mechanism than PTHrP (1–36) such as the increase of intracellular calcium, activation of protein kinase C, and the transactivation of vascular endothelial growth factor receptor type 2 (VEGFR2) [42–45]. Intracellular calcium increase was reported in rat osteosarcoma UMR-106 cell line using picomolar concentrations of Osteostatin and PTHrP (107–139) that was abolished by the use of verapamil [45]. PKC activation by Osteostatin has also been demonstrated in osteosarcoma rat cells using picomolar concentration [42], splenic lymphocytes [46] and on skin keratinocytes (BALB/MK-2 murine skin keratinocytes) with the use of Osteostatin and PTHrP (107–139) but without calcium surge or production of cAMP [43]. In the latter study, the administration of both peptides to the cells also modulated in a time and concentration manner the proliferation and DNA synthesis of the keratinocytes [43]. Besides, it has been shown that PTHrP (107–139) could transactivate the VEGFR2 resulting in a partial protection of induced apoptosis of osteoblastic cells in a mechanism that involves the activation of Runx2 transcription factor [47]. Transient treatment of the human osteosarcoma cell line MG-63 and osteoblastic cells from human trabecular bone with PTHrP (107–139) produced anabolic results such as increase of ALP activity together with an increase of the expression of osteocalcin and OPG with a downregulation of RANKL expression. The authors demonstrate that these effects were related to the transactivation of VEGFR2 [48]. VEGFR2 transactivation was later demonstrated using Osteostatin and also PTHrP (107–139) in MC3T3-E1 murine cells and UMR-106. This transactivation was related to the phosphorylation of Src kinase and parallel to the phosphorylation of Erk and Akt and produced a survival effect in the cell cultures [44] In a recent work, it has been tested the effect of Osteostatin in osteogenesis under hypoxic conditions in mesenchymal stem cells which use to trigger angiogenesis through overexpression of VEFG. In this setting, the Osteostatin combination with hypoxia restores the osteogenic capacity of mesenchymal cells enhancing proliferation, migration and the angiogenesis of Type H endothelial cells [49]. Of note, related to PTHrP intracrine actions, it has been shown that PTHrP (107–139) is necessary for the increase of proliferation produced by the translocation of PTHrP into the nucleus of smooth muscle cells [50]. Table 1 and Fig. 2 shows the main molecular pathways activated by PTHrP (107–139) and Osteostatin.

4. PTHrP (107–139) and Osteostatin as peptides for restoring bone function

The anti-resorptive activity of PTHrP C-terminal domain (107–139) and Osteostatin were reported in different works from Fenton and coworkers where they demonstrated that both peptides inhibited bone resorption in a pit-model resorption bone using osteoclast from chicken and that resorption activity [37,38,51]. However, other groups failed to find the same effect in similar models [43,52,53]. Recently, it has been demonstrated that Osteostatin blocks osteoclast differentiation through the decrease in the expression of NFATc1 which plays a key role in osteoclast formation [54]. The authors support the idea that Osteostatin inhibits the formation of osteoclast, but it does not reduce the activity when they are terminally differentiated.

On the other hand, further compelling evidence demonstrated that Osteostatin has also anabolic features [11]. This points out that this peptide could have a dual action depending on the experimental and physiological conditions, which also suggests that in vivo might also have this dual action depending on the metabolism requirement of the bone tissue in a particular moment and thus contribute to bone homeostasis.

Bone metabolism alterations are studied through different in vitro and animal models. Thus, PTHrP (107–139) was administered to mice under glucocorticoid treatment to test whether this peptide could restore bone tissue in a bone defect after bone marrow ablation [55]. In fact, this administration totally restored the number of osteoprogenitor cells and new bone formation and partially restored the cortical vascularization, improving bone microarchitecture parameters such as Cortical Thickness (Ct.Ch), Cortical Porosity (Ct.Po.) and Trabecular Degree of Anisotropy generated by glucocorticoid administration. This

Table 1
Mechanism of actions used by PTHrP (107–139) and Osteostatin.

Reference	Cells	Pathway
[45]	UMR-106 rat osteosarcoma	Intracellular calcium mobilization
[42,43,46]	Keratinocytes, T lymphocytes	PKC activation
[44,47,48]	MC3T3-E1, UMR-106	VEGFR2 transactivation
[71]	Human Dental Pulp cells	Erk Phosphorylation

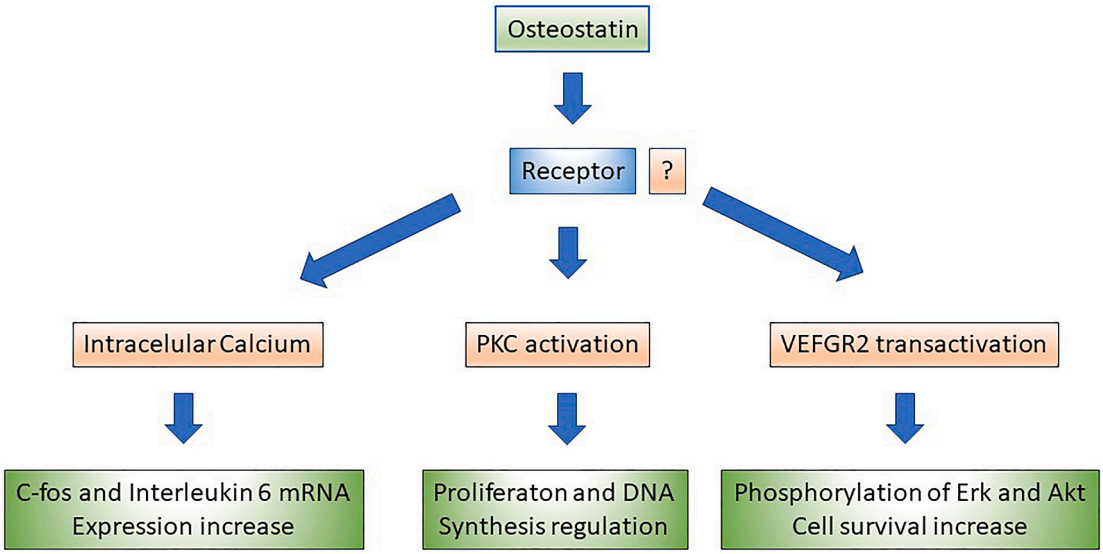

Fig. 2. Osteostatin main molecular mechanisms discovered to date. Osteostatin, through the interaction with one or more receptors, unknown to date, is capable of increasing the intracellular concentration of calcium, the activation of PKC and the transactivation of VEGFR. These actions involve increasing the expression of genes such as Interleukin 6, c-fos, the phosphorylation of Akt or Erk and the regulation of DNA synthesis and cell replication.

beneficial effect for bone recovery of PTHrP (107–139) was also confirmed in an animal model of osteoporosis induced by ovariectomy [56]. In the same work, the authors showed that PTHrP (107–139) restored to the control values the osteogenic potential of osteoprogenitors cells derived from the bone marrow of the former mice and C3H10T1/2 murine cell line under oxidative stress conditions. In another model of osteopenia produced by diabetes induction PTHrP (107–139) treatment restored to the control values bone parameters Bone Surface, Trabecular Bone Volume/Tissue Volume; Degree of Anisotropy, Structure Model Index, Trabecular Number and Trabecular Bone Pattern Factor in the bone of these mice and also this peptide reversed up to control values the changes produced by high glucose treatment in osteoblastic bone cells from the bone marrow of these mice and MC3T3-E1 cell line [57]. Thus, PTHrP (107–139) administration to diabetes mouse models and treatment in cell lines under the influence of high glucose showed efficacy in restoring bone tissue associated with an increase in the activation of Wnt-pathway [58,59]. Osteostatin was also useful in treating the skeletal defects associated with the lack of IGF-1 in bone. Thus, using a mouse model deficient for IGF-1, the treatment of these mice with Osteostatin ameliorate bone structure in trabecular but not in cortical bone associated with the activation of Erk 1/2 and FoxM1 [60].

Of particular interest was the research carried out by Maycas and co-workers where the additive effect of Osteostatin treatment together with mechanical loading was studied [61]. In this work, Osteostatin corrected the effects of DM in combination with mechanical loading avoiding osteocytes dead through activation of Erk pathway and translocation of β-catenin to the nucleus.

5. Osteostatin effects on inflammation and senescence

Among others oxidative stress and inflammation are hallmarks associated with senescence and aging [62]. Regarding oxidative stress, PTHrP (107–139) and Osteostatin have also shown anti-oxidative properties [63]. In this work, the authors demonstrate in primary human osteoblastic cells and in MC3T3-E1 that both peptides decreased oxidative stress produced by H_2O_2 treatment, preventing the increase of malondialdehyde (a marker of lipid peroxidation levels), and restoring the expression of bone anabolic markers such as ALP, Runx-2 and Osteocalcin as well as protect cell viability and rendered up proliferation rate.

Exploring the actions of Osteostatin on the bone under DM and aging conditions, it has been reported that the use of gelatin-glutaraldehyde-coated hydroxyapatite loaded with Osteostatin enhances the fracture healing in a cortical defect produced in the femur of aging rats with DM, compared to gelatin-glutaraldehyde-coated hydroxyapatite control increasing the expression of VEGF and Osteocalcin above the control values, and producing higher amount of bone tissue around the implant vs controls without Osteostatin [64].

Osteostatin has also displayed some anti-senescence, immunomodulatory and anti-inflammatory activities [65–67]. Thus, using human primary bone cells from aging donors incubated in the presence of interleukin 1β (IL-1 β) and with Osteostatin, it was demonstrated the senescence phenotype induce by IL-1β, was reversed by Osteostatin which decreased the levels of p21, p16 and p51 and β-galactosidase activity. Furthermore, Osteostatin also reduced the production of IL-6 and prostaglandin E as well as the downregulation in the expression of *cyclo*-oxigenase 2 [66]. On the other hand, rheumatoid arthritis (RA) is a chronic inflammatory disease in which there is a loss of cartilage and bone tissue. In a model of RA in mice induced by collagen administration, the treatment with Osteostatin reduced the loss of bone and cartilage. This reduction was related to a decrease in join osteoclast area, T lymphocytes activation and the reduction of the levels of IL-1β, IL-2, IL-6, IL7 and tumour necrosis factor alpha [65]. Related to anti-inflammatory properties of Osteostatin a recent report from Catalan and co-workers has demonstrated that this molecule is effective in preventing the consequences of gouty arthritis in a mice model through the upregulation of Nrf2 and inhibition of caspase 1 activation [67] Table 2 resumes the actions of PTHrP (107–139) and Osteostatin in bone and other tissues.

6. Osteostatin actions outside bone and cartilage tissues

The effects of Osteostatin in other tissue different from bone and cartilage have been poorly studied, however, there are a few data on its action on cardiomyocytes, teeth, smooth muscle, and keratinocytes [68–71]. Actions of Osteostatin on smooth muscle and keratinocytes have previously been aforementioned [32,33]. Osteostatin has been described as a peptide that can produce cardiac hypertrophy in cardiomyocytes [68] and when injected into mice with hemodynamic stress can exert a mild cardioprotective action [69] Regarding the odontogenic potential of Osteostatin, this was tested in human dental pulp cells

Table 2
PTHrP (107–139) and Osteostatin actions in bone and other tissues.

Reference	In vitro/in vivo model	Concentration/ Dosage	Main findings
[37,38,51]	In vitro pit-resorption osteoclast model	10^{-11} M	Osteoclast resorption
[54]	In vitro model. Human osteoclast	100–500 nM	Osteostatin inhibits osteoclast differentiation
[55]	Mice osteopororis model induced by glucocortidoids. Bone defect after bone marrow ablation	100 μg/kg/every other day, subcutaneously	Improvement of bone regeneration after treatment
[56]	Osteoporosis induced in females through ovariectomy, C3H10T1/2 cell line and osteoprogenitors	80 μg/kg/every other day, subcutaneously	Improving of bone tissue quality after peptide treatment
[57]	Osteoporosis induced by diabetes mellitus in mice Bone marrow stromal cells and MC3T3-E1 cell line	100 μg/kg/every other day, subcutaneously. 100 nM, every other day	Peptide treatment restore partially bone status
[58,59]	Osteoporosis induced by diabetes mellitus in mice. MC3T3-E1 cell line	100 μg/kg/every other day, subcutaneously.	Activation of Wnt-pathway to ameliorate bone status
[60]	IGF-1 deficient mouse model	100 μg/kg/every other day, subcutaneously.	Peptide treatment compensate bone defects
[61]	Osteoporosis induced by diabetes mellitus in mice treated with mechanical loading and peptide administration	3 daily s.c. injections of vehicle 7 μg/kg	Mechanical loading and peptide treatment restore bone loss
[63]	Human primary bone cells and MCT3-E1 cells	100 nM	Peptide treatment inhibit in part oxidative stress produced by H_2O_2
[64]	Aging Rats with DM. Cortical defect.	100 nM loaded in gel	Peptide treatment enhance bone regeneration
[66]	Osteoblast from Osteoarthritis patients	100 nM	Anti-inflammatory and anti-senescence properties
[65]	Rheumatoid Arthritis in model in mouse	80 or 120 μg/Kg/ every other day subcutaneously	Reduction of bone and cartilage bone loss
[67]	Gouty Arthritis mouse model	80 or120 μg/Kg every other day subcutaneously	Pyroptosis and pro-inflamatoy cytokine reduction

(hDPCs) alone or in combination with mineral trioxide aggregate (MTA) [70]. In this study Osteostatin, stimulates odontogenic differentiation of hDPCs (higher levels of nodule mineralization and expression of odontogenic markers such as sialophosphoprotein, dentin-matrix protein 1 and ALP), increases the phosphorylation of Erk alone or in combination with MTA. Further studies using a rat animal model tried to validate this previous data [71]. Thus, an occlusal cavity was carried in thirty molars from Sprague Dawley rats, the pulp was exposed and then the cavity was filled in with Osteostatin (two concentrations 10 nM and 100 nM) and MTA. After 4 weeks the molars treated with MTA and Osteostatin showed more mineralized dental bridge compared with the controls [71].

7. Osteostatin loaded in biomaterials for bone regeneration

Perhaps, one of the most interesting features of Osteostatin is its potential to be used as a tool for bone regeneration after being loaded in different biomaterials such as silica-based materials, ZnO-mesoporous scaffolds or even titanium [72]. Table 3 summarizes the main findings in this field.

The first attempt to combine Osteostatin with biomaterials to study their delivery kinetics was carried out in silica-based materials (SBA-15) with or without organic functionalization [73]. Osteostatin was loaded into the material by impregnation for 24 h in saline, with a loading efficiency of 82 %. The release mechanism of Osteostatin is assumed to be due to diffusion through the mesopore and could be described by the Noyes–Whitney equation [73], characteristic of mesoporous materials. This Osteostatin release profile will be maintained, with small differences, in all the biomaterials studied where the peptide loading has been done by impregnation or adsorption. In this case [73], kinetics analysis showed that most of the peptide release was achieved within the first 50 h after loading the disk. Then SBA-15 disks loaded with Osteostatin were put in contact with osteoblastic cells (MC3T3-E1 cell line from mouse) and the osteoinductive potential was evaluated. Results from this experiment demonstrated that the peptide released produced an increase in cell growth compared to control values (SBA-15) and the upregulation of the expression of different osteoblastic-related genes such as ALP, Osteocalcin, Collagen type I, Opg (1.5n-fold) and VEGF (2.5n-fold) and down regulate (0.5n-fold) the expression of RANKL [73].

These previous results aimed at the same group of researchers to test the bone-regenerative potential in an in vivo model. Thus, Osteostatin loaded in SBA-15 was used to evaluate the bone regeneration in an osteoporotic rabbit femur cavity defect model (control in this setting were non osteoporotic rabbits with the same cavity defect) [74]. In this model the authors demonstrated after analyzing the bones at 4 and 8 weeks after the implantation of the biomaterials by Micro-CT and calculating the bone volume/trabecular volume index, that the implants created new bone, being this formation stronger in the vicinity of the implant and decreasing as the distance from the implant increases, compared to the SBA-15 control values. Furthermore, the histological analysis of the regenerated tissue showed the expression of osteogenic markers such as Runx2 and Osteocalcin as well as cell proliferative marker PCNA. Of note, there was an absence of RAM11-positive macrophages which are implicated in the inflammation process. Thus, the

Table 3
Main action of Osteostatin loaded in different biomaterials and in models in vitro and in vivo.

Reference	Biomaterials	In vitro/in vivo model	Main findings
[73]	SBA-15	MC3T3-E1	Upregulation of expression of ALP, Oc, VEGF and OPG
[85]	SBA-15	Rabbit cavitary defect	Enhance of bone regeneration
[74]	SBA-15	Osteoporosis rabbit model	Enhance early repair of bone defect
[75]	Si-doped hydroxyapatite	MC3T3-E1 cell line	Enhance of growing, differentiation and mineralization
[77]	Gelatin-glutaraldehyde biopolymer-coated hydroxyapatite	Cavitary defect in rats	Increase bone regeneration (bone volume, trabecular and cortical thickness)
[79]	ZnO-mesoporous glass	MC3T3-E1 cells	Enhance cell growth and differentiation
[78]	ZnO-mesoporous glass	Human mesenchymal cells	Cell growth and differentiation
[81]	Mesoporous silica nanoparticles	Mouse embryonic fibroblast (MEF) cells Mice osteoporosis model induced by OVX	Peptide treatment compensate gene expression bone alterations
[82]	Mesoporous silica nanoparticles	Mice osteoporosis model induced by OVX	Peptide treatment compensate bone defects alterations

authors concluded that the increase in bone regeneration promoted by Osteostatin was associated with a decrease in inflammation, as proven by the absence of RAM-11 macrophages in the vicinity of the implant loaded with Osteostatin.

It has also been tested if Osteostatin may support the regeneration produced in biomaterials Si-doped hydroxyapatite (Si-HA) [75]. Si-HA is a biomaterial with high biocompatibility, bioactivity and osteoconductive properties which made it a good candidate to tissue regeneration. Si-HA loaded with Osteostatin either adsorbed (loading efficiency of 66 %) or covalently bound stimulated the cell growth, differentiation and mineralization of MC3T3-E1 cells, compared to the Si-HA controls [75].

In another experiment Si-HA was also loaded with Fibroblastic Growth factor-2 (FGF-2) which is a cytokine that produces angiogenesis as well as osteoblast proliferation and cell adhesion. Si-HA-FGF-2 was loaded with Osteostatin by adsorption was added to osteoblastic cell cultures. In this new setting, it was also demonstrated that SI-HA-FGF2-Osteostatin increased gene expression of Runx2 (3n-fold), osteocalcin (2.5n-fold), vascular endothelial growth factor (VEGF) and the VEGF receptors 1 and 2 (3.5n-fold) in osteoblastic mouse cells and in primary bone cells compared to the Si-HA controls. The authors also demonstrated that the mechanism by which Osteostatin and FGF-2 promoted these effects depended on the activation of mitogen-activated protein kinases and intracellular calcium signaling [76].

Osteostatin has also been loaded in mesoporous ceramics (SBA) to test its potential for bone regeneration in an osteoporosis animal model in rabbit [74]. Osteoporosis was induced in rabbits through methylprednisolone and a femoral cavitary defect was done, then SBA loaded by adsorption with Osteostatin (loading efficiency of 66 %) was implanted in the cavitary defect. The results showed that just after two weeks the implants were surrounded by a fibrotic layer but without signs of inflammation and with an increase in the levels of Runx2 (25 %), Osteopontin (50 %) and VEGF (15 %), compared to the SBA control values. These data lead to the conclusion of the SBA-Osteostatin implants favors the early repair of bone tissue even in an osteoporotic model.

In another approach to improve the bone regeneration produced by Osteostatin and biomaterials, Osteostatin was loaded by impregnation (loading efficiency of 60 %) in a degradable gelatin-glutaraldehyde biopolymer-coated hydroxyapatite [77]. Scaffolds of this biomaterial implanted in a cavitary defect carried out in the tibial metaphysis of rats produced a bone healing in a time of 4 weeks after the production of the cavitary defect. The authors found an increase in bone volume/tissue volume, the trabecular and cortical thickness (15 %) that was parallel to the downregulation (0.5n-fold) of the expression of *Sost* gene (an inhibitor of Wnt-pathway) compared to the gelatin-glutaraldehyde biopolymer-coated hydroxyapatite control values.

A recent strategy to enhance the osteogenic capacities of Osteostatin was to combine it with ZnO-mesoporous glasses, which have the advantage of great porous volume and high bioactivity. When this biomaterial was loaded with Osteostatin by impregnation (loading efficiency of 70 %) and then was administered to mesenchymal cells, there was an increase of the osteogenic markers produced by these cells such as Runx2 and ALP (2.5n-fold). Moreover, this mesenchymal cell increased their proliferation and osteogenic differentiation in the presence of de biomaterial and in the combination with Osteostatin compared to the ZnO-mesoporous glasses control values [78,79].

In another study, collagen-hydroxiapaptite scaffolds were loaded with Osteostatin by crosslinking treating them with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in combination with N-hydroxysuccinimide) to test osteoregeneration potential in vitro and in vivo. In vitro, this biomaterial produces a significant increase in the production of ALP as well as upregulation in the expression of ostepontin and osteocalcin in MC3T3-E1 cells in contact with the biomaterial. Osteostatin loaded in this biomaterial produced a considerable amount bone formation as shown the results for bone volume/total volume an area of new bone volume in a model of critical-size defect in calvaria of rat when compared with the biomaterial alone or with the controls without any material [80].

Of special interest are the studies of Raimundo-Mora and co-workers [81,82] where Osteostatin was loaded by adsorption onto mesoporous silica nanoparticles (0.5 Âμg of peptide per milligram of material) carrying a silencer of the *Sost* gene [83]. This complex nanosystem was administered locally (femur) or systemically to osteoporotic mice, reversing the deleterious effects of the disease on gene and protein expression of Sost and other osteogenic factors, as well as trabecular parameters (BVF, BV/TV, BS/BV, SMI, Tb. Th., Th. Sp.) measured by Micro-CT. These positive effects on bone parameters resulted in the recovery of bone mineral density and content values, compared to the control mice, which had been decreased by the osteoporosis. This approach represents a new treatment for osteoporosis with better results than the current clinical anabolic treatment (PTH), reducing the negative side effects.

Not only bioceramics biomaterials can be combined with Osteostatin but porous titanium which is used in most of the prothesis replacement in surgery, can also be coated with this peptide for its use in bone regeneration after surgical implant. Thus, in an animal model using Osteostatin porous titanium coated in a critical bone defect, this material increased bone regeneration (66 %) up to 12 weeks after de implant [84].

8. Conclusions and future perspectives

Diseases associated with the musculoskeletal system have a high prevalence rate and represent a challenge when it comes to treating them, requiring new strategies to prevent them or to treat their most extreme consequences, such as fragility fractures. The aging of the population and the comorbidities that accompany these diseases, such as obesity, diabetes or hypertension, will increase the number of patients in the coming years. In this context, Osteostatin appears as a peptide with multiple actions that can remedy, at least in part, the harmful effects of these diseases. In Fig. 3 we show the main features and applications of Osteostatin regarding bone tissue. As we have shown, numerous studies confirm that Osteostatin has anabolic and antiresorptive capacities together with antioxidant and angiogenic properties. In addition, the already demonstrated possibility of coupling it to different biomaterials that can both transport them and control their release when using them as regenerative therapy, makes it an ideal candidate for possible administration in situations of low bone remodeling or fractures that do not unite. However, the road to its application still presents some difficulties. It has been described several molecular mechanisms by which Osteostatin generates the described effects, but it is not yet known specifically which receptor or receptors could mediate Osteostatin actions. On the other hand, studies are still necessary, both in vitro and with animal models in vivo, to verify its longer-term effects. Therefore, new research focused on these fields is necessary to develop all its therapeutic potential. Taking all together, the actions that Osteostatin produces on bone tissue postulated it as a molecule of great interest for its application in the possible treatment of diseases that affect the musculoskeletal system.

CRediT authorship contribution statement

Daniel Lozano: Writing – review & editing, Writing – original draft, Investigation. **Arancha R. Gortazar:** Writing – review & editing, Writing – original draft, Investigation. **Sergio Portal-Núñez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

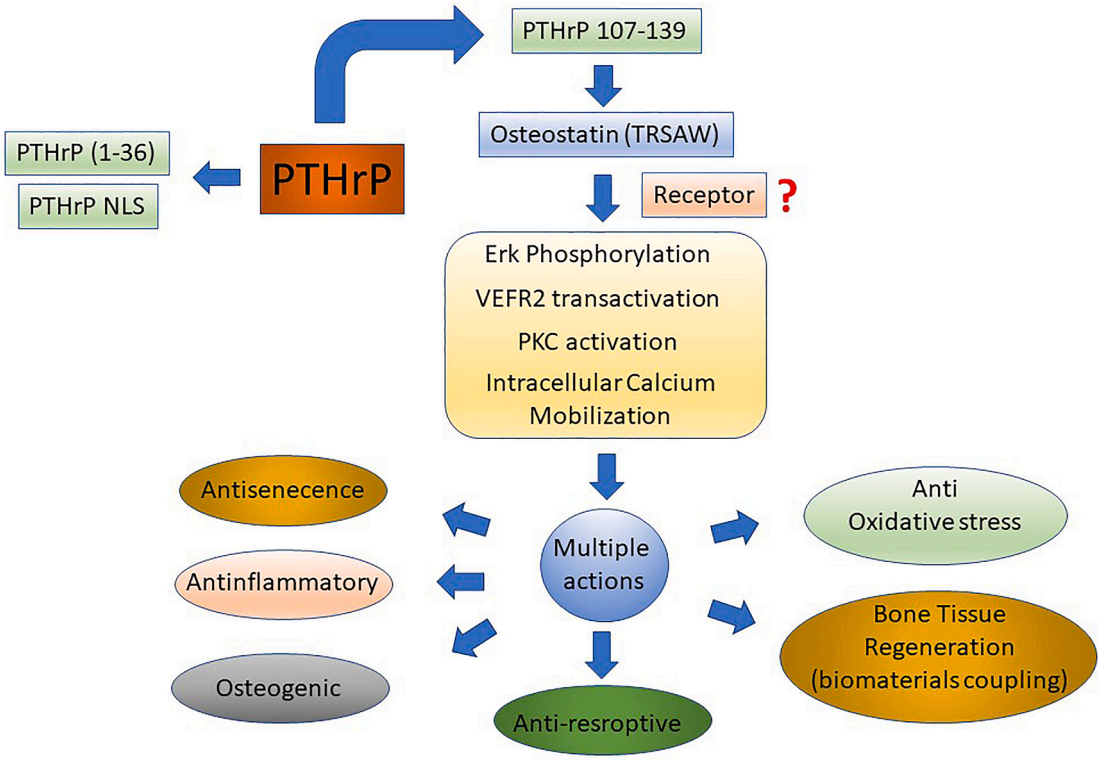

Fig. 3. Summary of main activities of Osteostatin and PTHrP (107–139). The PTHrP polypeptide has been shown to have various biological actions. The N-terminus is similar to PTH actions, the core domains that carry nuclear recognition signals (NLS) generates intracrine actions, and the C-terminus that produces the actions explained in this work. This C-terminal peptide, which expands from aa 107 to 139, has a pentapeptide 107–111 (TRSAW) that seems necessary for its biological activity. This peptide signals through an unknown receptor, activating the PKC pathway, mobilizing intracellular calcium, transactivating VEGFR2 and producing Erk phosphorylation. The activation of these molecular mechanisms produces osteogenic, anti-resorptive, anti-oxidative stress, anti-senescent and anti-inflammatory activities. Moreover, this peptide in combination with different biomaterials has shown to have excellent capacities to enhance bone repair.

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