Interactions among bone, liver, and adipose tissue predisposing to diabesity and fatty liver.

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Giovanni Musso M.D.[1]*, Elena Paschetta M.D.[2]*, Roberto Gambino PhD[2] , Maurizio Cassader PhD[2], Federica Molinaro M.D. [2]

* equal first author

[1] Gradenigo Hospital, University of Turin, Turin, Italy

[2] Dept. of Medical Sciences, San Giovanni Battista Hospital, University of Turin, Turin, Italy

Corresponding author:

Giovanni Musso

Gradenigo Hospital, Turin

C.so R Margherita 8

10132 Turin, Italy

E-mail: giovanni_musso@yahoo.it

Abstract

Growing epidemiological evidence connects obesity and its complications, including metabolic syndrome, diabetes and non-alcoholic fatty liver disease (NAFLD) to reduced bone health and osteoporosis. Parallel to human studies, experimental data disclosed a complex network of interaction between adipose tissue, the liver and the bone, which reciprocally modulate each others function. The main mediators of such cross-talk include hormonal/cytokine signals from the bone (osteopontin, osteocalcin and osteoprotegerin), the liver (fetuin-A) and adipose tissue (leptin, TNF-α, adiponectin). Dysregulation of this network promotes the development of diabesity, NAFLD and osteoporosis. We will review recent advances in understanding the mechanisms of bone-liver-adipose tissue interaction predisposing to obesity, diabetes, NAFLD and osteoporosis and their potential clinical implications

Introduction

Growing epidemiological data link obesity, metabolic syndrome and NAFLD, the hepatic manifestation of metabolic syndrome, to a reduced bone mineral density (BMD) and osteoporosis[1, 2, 3, 4, 5, 6, 7], thereby challenging the conventional belief that excessive body weight prevents bone loss through increased mechanical load to the skeleton and enhanced cortical bone formation[8]. Parallel to human data, cellular and animal models highlighted a complex network of interactions between the bone, adipose tissue and the liver, which mutually modulate their function through the secretion of pro/anti-inflammatory cytokines and hormones[9]. We will review recent mechanicstic insights into the cross-talk among these organs, with focus on potential clinical and therapeutic implications for NAFLD, insulin resistance and osteoporosis.

Osteopontin

Osteopontin(OPN) is a matrix glycoprotein secreted by a variety of cell types including immune cells (activated T-helper 1 cells and macrophages), osteoclasts, endothelial cells, epithelial cells (Table 1)[10]. Human OPN gene consists of seven exons encoding the OPN protein, which contains several highly conserved amino acidic structural sequences for binding integrins, calcium and CD44 receptor (Figure 1). Human OPN gene is subjected to alternative splicing, producing three splice variants (OPN-a, the full-length molecule, OPN-b, lacking exon 5, and OPN-c, lacking exon 4), and the protein undergoes extensive post-translational modifications including serine/threonine phosphorylation, glycosylation, tyrosine sulfation, and proteolytic fragmentation, resulting in molecular isoforms ranging from 25 to 75 kDa[11]. These posttranscriptional and posttranslational modifications generate numerous OPN isoforms, which are cell type specific, depend on physiological and patho-physiological factors, and are likely to affect both OPN binding affinity and biological functions, with potentially relevant diagnostic and therapeutic implications (Table 1; Box 1).

In NAFLD patients, hepatic and plasma OPN expression correlate with the severity of histological steatosis, inflammation and fibrosis[12, 13, 14, 15], and in dietary murine models and cell cultures, OPN expression was required for the development of Nonalcoholic Steatohepatitis (NASH) and insulin resistance, which were prevented or improved by functional or genetic OPN deletion[16, 17, 18, 19, 20]. Furthermore, high (>14.7 ng/mL) serum OPN levels were associated with a 2.96-fold increased risk of osteoporosis in menopausal women, while OPN-deficient mice are resistant to ovariectomy-induced osteoporosis [21, 22, 23].

Numerous mechanisms connecting OPN to liver injury and insulin resistance have been recently described, including enhanced recruitment and activation of circulating monocytes to adipose tissue and liver, activation of hepatic gluconeogenesis through modulation of STAT3 and FOXO1 transcription factors[18, 19](Table 1). Beside recruiting proinflammatory cells to adipose tissue, OPN can also directly bind to its receptors $\alpha v\beta 1$ and $\alpha v\beta 5$ integrins on adipocytes and induce insulin resistance and secretion of pro-nflammatory adipokines, thereby promoting adipose tissue dysfunction, a key pathogenic feature of NASH and metabolic syndrome[17, 24](Table 1).

Recent data connect OPN to oxidative stress-induced hepatic fibrogenesis: OPN works as both a soluble cytokine and an extracellular matrix(ECM)-bound protein that can remain intracellular or is secreted, allowing autocrine and paracrine signalling on hepatic stellate cells(HSC)[25]. As a matricellular phosphoglycoprotein, OPN functions as an adaptor and modulator of cell-matrix interactions, regulating cell migration, ECM invasion and cell adhesion through its binding to integrins

Enhanced hepatic OPN expression, which is induced by the hedgehog signalling pathway activation and by reactive oxygen species in damaged hepatocytes, promotes fibrogenesis through HSC activation and recruitment of ciculating natural killer T(NKT) cells from the blood[15, 20,25], and has been also connected to growth and metastasis of hepatocellular carcinoma (HCC), an emerging complication of NASH[26, 27, 28, 29, 30, 31, 32] (Table 1; Box 1). Therefore, OPN may contribute to the whole spectrum of severity of liver disease in NAFLD.

In the bone, OPN enhances bone resorption by stimulating osteoclast expression of CD44, which is required for cell motility, and by directly mediating osteoclast attachment to bone ECM: osteoclast activation enhances bone resorption, which in turn releases OPN from ECM into surrounding bone and into the circulation, thus perpetuating local and systemic actions of OPN[9]. Furthermore, animal models demonstrated OPN mediates high fat diet-induced differentiation of bone marrow-derived mesenchymal multipotent stromal cells toward an adipogenic phenotype and away from an osteogenic phenotype, thereby reducing bone deposition[16](Table 1).

Collectively, these data suggest that OPN upregulation may play a pivotal role in obesity-associated insulin resistance, NAFLD and osteoporosis. Future research is needed to clarify the biological activity and impact of distinct osteopontin isoforms on tissue inflammation and metabolism, as well as their clinical utility as a biomarker for progressive liver disease or tumor invasiveness in HCC and as a therapeutic target for the treatment of HCC.

Osteocalcin

Osteocalcin is a 49-amino acid bone matrix noncollagen protein secreted by osteoblasts, which is involved in bone deposition and calcium homeostasis (Table 1). Mounting evidence suggests osteocalcin plays also a major role in energy homeostasis and glucose metabolism: in cross-sectional and prospective epidemiological studies circulating osteocalcin levels are inversely associated with the risk of type 2 diabetes[33, 34], metabolic syndrome[35, 36], overall/abdominal adiposity and insulin resistance[37, 38], reduced BMD[39], and with the presence and severity of NAFLD[40, 41, 42, 43]. Gain –of-function and loss-of-function mouse models were consistent with the results of epidemiological studies: genetic osteocalcin deletion induced glucose intolerance, increased fat mass, insulin resistance, decreased expression of insulin target genes in liver and muscle and decreased adiponectin gene expression in adipose tissue, while recombinant osteocalcin

administration improved insulin secretion and sensitivity and prevented high-fat-induced obesity, diabetes and NAFLD[44, 45].

Several mechanisms modulate the secretion and biological activity of osteocalcin (Table 1, Figure 2): in osteoblasts, osteocalcin expression is enhanced by insulin and IGF-1 by relieving the suppression of Runx2 by the transcription factor Twist2[46] and inhibited by the two transcription factors Fox01 and ATF4 and by ER stress response activation[47, 48, 49, 50, 51]. Furthermore, osteocalcin undergoes posttranslational modification whereby three glutamic acid residues undergo a vitamin K-dependent carboxylation to form γ-carboxyglutamic acid residues (Figure 2). Carboxylated osteocalcin has a higher affinity for hydroxyapatite and this is thought to be involved in bone extracellular matrix mineralization, while the undercarboxylated form appears to be more metabolically active: however, loss- and gain-of-function mutations in osteocalcin gene demonstrated that osteocalcin is not required for bone ECM mineralization[52], and data regarding the metabolic activity of carboxylated vs. undercarboxylated osteocalcin in humans are not conclusive [37, 44, 53].

Osteocalcin exerts its actions through binding its receptor G protein-coupled receptor family C group 6 member A (GPRC6A), an amino acid-sensing GPCR, highly expressed in a wide variety of tissues, and with considerable multiligand specificity, including L-amino acids, cations, and anabolic steroids in addition to osteocalcin[54](Table 1).

Upon binding to pancreatic β-cell and enteroendocrine L-cells GPRC6A receptors, osteocalcin increases intracellular cAMP and stimulates β-cell proliferation and insulin secretion both directly and through enhanced glucagon-like peptide-1(GLP1) secretion, restoring a normal β-cell function[55, 56]. Notably, the association with enhanced pancreatic insulin secretion has been demonstrated in experimental and epidemiological studies and is the most consistent metabolic effect of osteocalcin to date.

Beside enhancing pancreatic insulin secretion, experimental models suggest osteocalcin may protect against high fat-induced obesity, insulin resistance and NAFLD[50, 51], although epidemiological

human data are conflicting and require further confirmation[57,58]. Several potential mechanisms underlying the insulin-sensitizing, anti-obesogenic and anti-steatotic properties of osteocalcin have been identified in adipocytes, hepatocytes and skeletal myocytes: enhanced adiponectin gene expression in adipocytes[44], reduced endoplasmic reticulum (ER) stress response and NF-κB-mediated inflammation[51], and increased mitochondria biogenesis and function[45] (Table 1).

In conclusion, osteocalcin is emerging as an important modulator of energy homeostasis and glucose metabolism in various tissues, raising the possibility that this bone-derived hormone may become a novel treatment for obesity-related disorders, a hypothesis currently being tested.

The RANK/RANKL/Osteoprotegerin system

The receptor activator of nuclear factor-κB ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily, is an osteoclast differentiation factor expressed mainly, but not exclusively, by osteoblasts as both a trans-membrane and a secretory protein; RANKL binds to its receptor RANK, expressed by mature osteoclasts and osteoclasts progenitors, leading to osteoclast differentiation and activation and consequent bone resorption[[59]]. The RANKL–RANK interaction is inhibited by osteoprotegerin (OPG) a glycoprotein belonging to the TNF receptor (TNFR) superfamily which is secreted by osteoblasts and acts as a decoy receptor for RANKL: it binds as a homodimer to the homotrimeric RANKL and prevents its binding to RANK, resulting in inhibited osteoclast activation and bone resorption. Studies in vitro confirmed the requirement for OPG dimerization in this process, as the monomeric form has a reduced RANKL-binding affinity [60](Box 2). The relevance of OPG for bone turnover has been demonstrated in vivo, as OPG-deficient mice have decreased bone mineral density[59].

Both OPG and RANKL are expressed in adipocytes and their expression in osteoblasts and adipose cells is modulated by a complex network of interactions involving sex hormones, redox balance, other adipokines, and nuclear transcription factors PPAR-γ, PPARβ/δ, and liver X-receptors (LXRs)[61, 62, 63, 64, 65, 66](Table 1). In particular, different nuclear transcription factors play a

major role in the interaction between adipose tissue and bone, often with clinically divergent results: the PPAR-γ agonists, thiazolidinediones, significantly improve insulin resistance, glucose homeostasis and NASH[67], and PPARβ/δ and LXR agonists are giving encouraging results for the treatment of obesity-related disorders in preclinical and clinical trials[68]. Nevertheless, thiazolidinediones have been associated with bone loss and an increased risk of fractures: while a well-documented mechanism is the reallocation of the fate of bone marrow mesenchymal stem cells to adipocytes rather than to osteoblasts, eventually promoting bone marrow adiposity and bone loss[69], recent evidence suggests that thiazolidinediones increase RANKL and decrease osteoprotegerin expression in osteoblasts, which may also be important for the bone-losing effect of these drugs. Preliminary experimental data suggest if PPARβ/δ and LXR agonists retain the metabolic benefits of thiazolidinediones with no detrimental or even beneficial effects on bone metabolism[65, 66].

Despite promising experimental data, cross-sectional epidemiological studies examining the association between the RANKL/OPG axis and insulin resistance, obesity, bone mineral density and risk of fractures yielded mixed results, finding direct, inverse, or no correlation between with disease states[70, 71, 72, 73, 74], due to several potential reasons: most studies assessed serum RANKL/OPG levels, which may not accurately reflect their tissue levels; commercial ELISA detect all forms of circulating OPG, while only the homodimeric OPG binds RANKL; laboratory measurements of serum RANKL is hampered by the relative instability of this molecule; finally, several factors including age and sex hormonal status may affect RANKL/OPG tissue expression, for example, the interaction between 17β-estradiol and adiponectin in modulating osteoblast RANKL/OPG expression[60, 61].

Recently, reduced serum OPG has been associated with the presence of progressive NASH in NAFLD patients [75]. The anti-inflammatory and antiapoptotic action of OPG may potentially underlie the hepato-protective effects of OPG, furthermore, OPG acts as a decoy receptor for the TNF-related apoptosis-inducing ligand (TRAIL), neutralizing TRAIL-induced apoptosis[59]. As

hepatocyte apoptosis is a key determinant of progression from simple steatosis to NASH, it can be speculated that a defect in OPG production may contribute to liver disease progression in NAFLD [68]. However,, two drawbacks may limit the therapeutic use of RANKL antagonists/OPG agonists, i.e. the potential increased risk of malignancy, due to inhibition of TRAIL, which is a potent apoptotic pathway in various tumour cells, and of infections, due to the inhibition of RANK, which is required for activation of various immune system cells, including T cells, monocytes, and dendritic cells. Accordingly, in a randomized controlled trial with denosumab, a human monoclonal antibody against RANKL, a 2% incidence of neoplasm and a 1% incidence of infection occurred in the denosumab groups, whereas neither problem developed in controls[76].To overcome these limitations, tissue-specific RANKL/OPG modulation is under development, and experimental liver-specific RANKL signalling blockade prevented high fat diet-induced hepatic steatosis, insulin resistance and diabetes in mice[77], with no apparent side effects. Future research will evaluate the feasibility, long-term efficacy and safety of such approach in humans.

Fetuin-A

Fetuin-A (α2-Heremans-Schmid glycoprotein, α2-HS-glycoprotein), first isolated from fetal bovine serum, is synthesized in the liver and has been implicated in several physiological and pathological conditions, including vascular calcification, regulation of bone metabolism and insulin action, protease activity control, keratinocytes migration, breast tumor cell proliferative signaling and neurodegenerative disease[78]. The relevance of fetuin-A for metabolic disease emerged in gain-of-function and loss-of-function studies: fetuin-A knockout mice exhibit increased glucose tolerance and insulin sensitivity and are resistant to diet-induced obesity, NAFLD and age-associated insulin resistance, conditions that are all induced by fetuin-A administration[79, 80]. In humans, fetuin-A gene is localized on chromosome 3q27, which has been identified as a susceptibly locus for type 2 diabetes and metabolic syndrome[81], and higher serum fetuin-A levels are independently associated with metabolic syndrome components and diabetes, while they are

reduced by pioglitazone treatment[82, 83, 84, 85]. Higher serum and hepatic fetuin-A levels were also associated with the presence of NAFLD and with the severity of liver histology in biopsy-proven NAFLD patients, independently of age, sex, BMI, fasting plasma glucose and triglycerides[86,87, 88] with hepatocyte fetuin-A expression correlating with key enzymes in glucose (phosphoenol pyruvate kinase 1, glucose-6-phosphatase) and lipid (sterol regulatory element-binding protein 1c, carnitine palmitoyltransferase 1) metabolism[89]. Mechanisms underlying fetuin-A regulation and action are being unravelled [90]: fetuin-A inhibits insulin receptor tyrosine kinase activity by inhibiting the autophosphorylation of tyrosine kinase and IRS-1 in skeletal muscle and hepatocytes[78], thereby promoting insulin resistance; fetuin-A directly suppresses adiponectin secretion by adipocytes[80]; furthermore, fetuin-A is a major carrier of free fatty acids (FFA) in the circulation and is required for FFA interaction with toll-like protein receptor 4 (TLR4) in adipocytes, thereby triggering proinflammatory adipokine expression and insulin resistance[91].

Beside its metabolic effects, fetuin-A is also a mineral chaperone in plasma and tissues, facilitating transport and clearing of potentially proinflammatory and procalcific cargo[92, 93]. In physiological and pathological conditions, tissue mineralization occurs through the interplay of three key determinants: extracellular matrix suitable for mineralization, extracellular levels of inorganic phosphate and calcium, and systemic or local expression of mineralization inhibitors. Fetuin-A is a prototypic systemic inhibitor protein of mineralization, as it complexes with calcium and phosphate to form stable colloidal mineral-protein spheres called calciprotein particles (CPPs), which are cleared by tissue macrophages and hepatic Kupffer cells through the Scavenger Receptor A[94]. In the bone, fetuin-A regulates matrix mineralization by inhibiting crystallization in the area surrounding the collagen fibril, thereby enabling increased mineralization within the fibril[95].

In vitro, fetuin-A can inhibit or stimulate osteogenesis, depending on its concentrations[96, 97]The importance of fetuin-A for bone and systemic matrix mineralization homeostasis *in vivo* emerged in fetuin-A knock-out mice, which show extensive ectopic soft tissue calcification, increased cortical

bone thickness but impaired growth of their long bones and premature growth plate closure due to insufficient inhibition of excessive mineralization in the growth plate cartilage matrix[98].

In epidemiological studies, serum fetuin-A levels were positively associated with BMD in older women, but not in men, in the Health ABC study[99], while in another study they correlated positively with bone turnover biomarkers in diabetic patients[100].

Further research is needed to determine the exact role of fetuin-A in the pathogenesis of metabolic disease, and to elucidate factors interacting with fetuin-A to explain its metabolic and bone-related effects.

Leptin

Encoded by the "ob" gene, leptin is synthesized by mature adipocytes in response to changes in body fat mass and nutritional status. In obesity, plasma leptin levels are increased proportionally to BMI and acutely decrease in response to fasting or restriction of energy intake to a much larger extent than would be expected for smaller reductions of adiposity, thus signaling a negative energy balance[101]. Adipocyte size and anatomical location (subcutaneous) appear to be the major determinants of leptin mRNA expression and secretion, but other factors modulate leptin secretion[101](Table 1). Beside its anorexigenic action in hypothalamus, leptin is an insulin-sensitizing hormone and reduces muscle and hepatic lipid content by promoting FFA β-oxidation, glycolysis and triglyceride assembly into VLDL particles and by inhibiting gluconeogenesis and *de novo* lipogenesis [101, 102, 103](Table 1). Animals devoid of leptin expression, ob/ob mice (leptin gene mutation), db/db mice and fa/fa rats (leptin receptor gene mutations) are obese, insulin resistant and have NAFLD, alterations reversed by leptin administration[104, 105]. Parallel to mice models, in lipoatrophic human diabetes, characterized by scarce adipose mass, diminished leptin levels, and markedly elevated intrahepatic triglycerides, leptin administration reduces liver enzymes, BMI, hepatic fat content and histological steatohepatitis[106]. However, in obese patients

leptin levels are normal or elevated, rather than reduced, and correlate with liver fat, thus suggesting the presence of resistance to the beneficial anorexigenic, anti-steatotic and insulin-sensitizing actions of leptin[107]. Mechanisms for hypothalamic leptin resistance have been unraveled, including impaired leptin transport across blood brain barrier[108] and defective intracellular signal transduction through the Jak/STAT pathway, caused by increased expression of the cytokine suppressors of cytokine signaling(SOCS)-3 and of protein tyrosine phosphatase(PTP) 1B, and by activation of IkB kinase b (IKK), JNK and protein kinase C(PKC)τ[109] . The "compensatory" increase in leptin expression in obesity may enhance the unwanted effects of inappropriate leptin elevation on cells that maintained a normal leptin responsiveness, promoting hepatic inflammation through enhanced Kupffer cells response to circulating bacterial endotoxin[110] and hepatic fibrogenesis through HSC activation[111, 112], thereby promoting steatosis progression to NASH, in rodent models.

Leptin is also an important regulator of bone mass through direct and indirect mechanisms: leptin increases central sympathetic activity by binding to its receptors on both hypothalamic ventromedial (VHM) nucleus and on serotoninergic brainstem neurons, which in turn project to VHM neurons[113]. From this nucleus, sympathetic fibers transmit stimuli to effector osteoblasts through the β2-adrenergic receptor (β2-AR), inhibiting osteoblast differentiation and undercarboxylatec-osteocalcin production [9] (Figure 2, Table 1).

Consistent with the importance of central sympathetic-mediated regulation of bone formation by leptin, obese leptin-deficient *ob/ob* mice showed an increased bone mass which can be rescued by intracerebroventricular (ICV) infusion of leptin and by isoproterenol administration, and leptin receptor-deficient *db/db* mice have a high bone mass, despite elevated circulating leptin[[114], [115]]. Furthermore, chemical lesion of VHM adrenergic signaling results in high bone mass that is resistant to correction by ICV leptin, and β2-AR disrupted-mice share with *ob/ob* and chemical-injured VHM mice the same bone alteration[116]. Collectively, these experiments, suggest that the predominant effect of leptin on bone is through the central nervous, and are mirrored by the 24–

32% reduction in the risk of fractures experienced by people receiving β-blockers, from several large studies[9].

Notably, none of the aforementioned adrenergic manipulations affect fat or muscle mass[116], suggesting that the leptin/adrenergic pathway for bone mass regulation is dissociated from the leptin pathway controlling adiposity.

Beside sympathetic system modulation, leptin can influence bone metabolism through other pathways(Table 1): leptin receptors have been cloned from osteoblasts and it has been proposed that leptin directly stimulates osteoblast differentiation and bone mineralization[116]; leptin enhances hepatic secretion of insulin-like growth factor binding protein (IGFBP-2), which improves insulin sensitivity and enhances osteoclast differentiation[117, 118]; leptin decreases renal expression of the 25-hydroxyvitamin D3 1α-hydroxylase gene and increases osteoblast secretion of the phosphaturic factor fibroblast growth factor 23 (FGF-23), with consequent reduction in phosphate resorption and in the synthesis of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3] [119].

The relative importance of these often divergent pathways *in vivo*, the effect of leptin interaction with age, sexual hormones and other bone-regulating cytokines, remain to be established and may explain the somewhat controversial epidemiological association of leptin with bone turnover and mineral density[120, 121].

Tumor necrosis factor (TNF)-α

The proinflammatory cytokine tumor necrosis factor (TNF-α) is produced by adipocytes, activated macrophages and Kupffer cells and promotes hepatic and systemic inflammation, liver injury and insulin resistance by interacting with TNF-R1 and TNF-R2: TNF-R1 mediates apoptosis and lipolysis while TNF-R2 induces insulin resistance[122].

In adipocytes, TNF-α reduces secretion of leptin and adiponectin and induces insulin resistance by reducing GLUT-4 expression and lipoprotein lipase (LPL) activity and by increasing expression of hormone sensitive lipase[123]. TNF-α also impairs insulin signaling in adipocytes and hepatocytes

through activation of stress-related protein kinases, such as JNK-1, and of the inhibitor kappa kinase beta (IKKβ)/nuclear factor kappa B (NF-κB) pathway, resulting in a state of chronic low-grade inflammation and increased production of cytokines, including TNF-α and interleukin (IL)-6, that perpetuate hepatic and systemic insulin resistance[122].

Substantial experimental evidence points to TNF-α as a key mediator of liver injury and steatohepatitis: in the methionine-choline deficient (MCD) animal model of NASH, anti-TNF-α antibody administration ameliorated necrosis, inflammation and fibrosis[124]. Furthermore, inhibition of hepatic TNF-α production and TNF-α knockout improved high fat diet-induced steatohepatitis and hepatic insulin resistance[122]. Adipocyte and hepatic TNF-α expression have been strongly associated with the severity of NASH and of insulin resistance in humans, though the correlation between circulating levels of this cytokine and the severity of liver injury was somewhat variable across studies [122, 125].

In the bone, TNF-α promotes osteoclastogenesis while simultaneously inhibiting the activation of osteoblasts from their progenitor cells. TNF-α increases the expression of M-CSF and RANKL which promotes osteoclast differentiation, in several target cells including osteoblasts; TNF-α promotes osteoclast differentiation both directly through binding to TNF-R1 and activating NF-κB pathway activation and indirectly by mediating RANKL stimulation of osteoclast differentiation by an autocrine mechanism[126]; furthermore, TNF-α has also been shown to inhibit osteoclast apoptosis through mammalian target of rapamycin/S6 kinase activation[127].

Through binding to osteoblast TNF-R1, TNF-α inhibits their proliferation and differentiation and increases apoptosis of their progenitors; these effects appear to be mediated primarily via activation of multiple downstream signaling pathways[128,129], including a reduced RUNX2 expression through its ubiquitynation and an inactivation of pro-osteogenic mitogen-activated protein kinase (MAPK)[127]. Furthermore, TNF-α inhibits the expression of genes involved in bone formation, including alkaline phosphatase, vitamin D receptor, parathyroid hormone receptor[130].

Adiponectin

The adipokine adiponectin has been most robustly and inversely associated with the incidence and severity of different metabolic disorders, including diabetes, obesity, metabolic syndrome and NAFLD in experimental and epidemiological studies [131, 132]. Consistently, recombinant adiponectin administration markedly improved metabolic profile and liver histology in animal models of NASH[133], through its potent insulin-sensitizing, anti-lipogenic, anti-oxidative/inflammatory, anti-inflammatory and anti-fibrotic properties (Table 1). Experimental studies showed adiponectin can modulate bone turnover through binding its specific receptors on osteoblasts and osteoclasts: adiponectin promotes osteoblasts proliferation, differentiation and activity and enhances RANKL secretion while inhibiting osteoprotegerin secretion; in osteoclasts, adiponectin inhibits RANKL-mediated osteoclastogenesis through interaction with its adaptor molecule APPL1 *in vitro*[61, 62, 134, 135]. Despite this consistent experimental evidence, epidemiological studies reported conflicting results: most studies reported an inverse relationship between serum adiponectin, BMD or fracture risk and a positive relationship with bone turnover markers[136,137, 138], other studies showed a positive correlation[139], while other studies found no association[140]. Several reasons may underlie the epidemiological discrepancy: all studies evaluated circulating, and not bone adiponectin levels, which may differ considerably; furthermore, adiponectin should not be considered in isolation, but rather adiponectin-related signaling in bone should be considered within the network of hormonal and cytokine signals that influence both adiponectin secretion/action and skeleton biology, including sexual hormones, undercarboxylated osteocalcin and osteoprotegerin/RANKL axis[62, 141].

Concluding remarks

Emerging experimental and epidemiological evidence disclosed a complex cytokine and hormonal cross-talk among bone cells the liver and adipose tissue, which coordinately regulates bone

remodeling, energy metabolism and glucose homeostasis; alterations in this network may contribute to the pathogenesis of obesity and related disorders, including NAFLD and diabetes. Future research will have to elucidate the clinical relevance of such alterations for human disease, and the potential use for screening purposes, i.e. to identify subjects at increased risk of developing such complications, and for therapeutic purposes.

Conflict of interest statement: the author has no present or past conflict of interest to disclose

BOXES

Box 1: Osteopontin and hepatocellular carcinoma (HCC)

HCC cells overexpress OPN, and its expression correlates with tumor invasiveness and metastasis: consequently, plasma OPN levels or OPN gene polymorphisms have been proposed as potential diagnostic and prognostic biomarkers for HCC[28, 29]. OPN isoforms seem to play different activities in HCC growth and metastasis: Chae et al. found that hepatocellular carcinoma cells (HCCs) predominantly expressed OPNa and OPNb splice variants, while normal hepatocytes expressed OPNc *in vivo*; in cell proliferation and invasion assays, OPNa and OPNb induced Hep3B cell migration, while OPNc had no significant effects. By contrast, OPNc suppressed the migratory activity of SK-Hep1 cells[31]. OPN antagonism by blocking antibodies to inhibit iOPN binding to receptors or by directly decreasing tumor OPN expression through small interfering RNA (siRNA) suppressed HCC cells migration, invasion and neoangiogenesis *in vitro,* decreased metastases and improved survival *in vivo*[27, 30].

BOX 2: Intracellular signaling mechanism of RANKL-RANK-OPG system

The RANKL–RANK interaction leads to the recruitment of TNF receptor-associated factors (TRAFs) to the intracellular domain of RANK, among which TRAF6 seems to have a central role

for the osteoclasts resorptive activity. TRAFs recruitment activates many downstream targets, which lead to osteoclast differentation (transcription factor NF-κB, p38, JNK mitogen-activated protein kinases -MAPKs) and survival (PI3K/Akt/mTOR signalling pathway, MAPK extracellular signal-related kinase - ERK). RANKL seems to support osteoclast formation as a membrane-associated factor, while its role as a soluble form is still debatable: activated T cell, which have been found involved in the osteoclastogenesis in rheumatoid arthritis, produce membrane-anchored RANKL that is shed by metalloprotease-disintegrin TNFα convertase (TACE) to form soluble RANKL (sRANKL). OPG produced by osteoblasts exerts osteoprotective effects by inhibiting the RANKL-RANK interaction, binding as a homodimer to the homotrimeric RANKL, thus preventing its binding to RANK and subsequent osteoclast activation.

BOX Outstanding Questions

- what is the biological and clinical impact of distinct osteopontin isoforms on tissue inflammation, cancerogenesis and metabolism?
- can these novel hormones/cytokines (osteocalcin, osteopontin, osteoprotegerin/RANKL, fetuin-A) be used as biomarkers to screen subjects at increased risk for more severe liver-related, metabolic and osteoporotic disease?
- are these novel hormones/cytokines accurate just biomarkers or do the play a causal role for the pathogenesis of human diseases?
- what are the exact biological mechanisms and clinical impact of adiponectin on the pathogenesis of osteoporosis?
- what is the role of oxidative stress in the modulation of the bone-liver-adipose tissue axis and in the pathogenesis of obesity-associated osteoporosis?

GLOSSARY

Alternative splicing: the process whereby identical pre-mRNA molecules are spliced in different ways, eventually leading to splice variants coding distinct proteins with different biological functions. Alternative splicing is important for both normal development and disease processes

Adaptor protein containing pleckstrin homology domain, phosphotyrosine domain, and leucine zipper motif (APPL1): the first identified protein interacting with adiponectin receptors. It is suggested to be an adaptor protein responsible for intracellular mediation of adiponectin signal transduction

Activating transcription factor 4 (ATF4): a member of the cAMP-responsive element-binding protein transcription factor family, that is regulated by sympathetic tone, and regulates osteoblast endocrine functions by inducing Esp and Ocn

Extracellular signal-regulated protein kinase (ERK): ERK1 and ERK2 are related protein-serine/threonine kinases that participate in the Ras-Raf-MEK-ERK signal transduction cascade, involved in the regulation of a large variety of processes including cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription

Forkhead box protein O1 (FOXO1): one of the four FoxO isoforms of Forkhead transcription factors, highly expressed in insulin-responsive tissues, including pancreas, liver, skeletal muscle, adipose tissue, bone. FoxO1 orchestrates the transcriptional cascades regulating glucose metabolism, insulin sensitivity and energy expenditure.

Jak/STAT: The janus kinase (Jak)–signal transducer and activator of transcription (STAT) pathway is a major intracellular signalling pathway activated by leptin.

c-Jun NH2-terminal kinase (JNK): a MAPK family member that mediates cellular responses to several stressing stimuli, including TNF, free fatty acids (FFAs) and reactive oxygen species (ROS). Activated JNK1 and JNK2 isoforms phosphorylate the AP-1 subunit c-Jun, increasing its transcriptional activity.

Mitogen-activated protein kinase (MAPK): The MAPK family includes a set of protein kinases that sequentially activate each other to regulate many processes including cell growth,

differentiation, cell survival, and the immune function in response to a wide range of extracellular stimuli including growth factors, hormones, and cytokines.

Osteoprotegerin (OPG): also known as tumor necrosis factor receptor superfamily member 11B, is a cytokine receptor and a member of the TNF receptor superfamily. Osteoprotegerin is a decoy receptor for RANKL. By binding to RANKL, OPG inhibits NF-kB-mediated activation of osteoclasts.

Receptor activator of nuclear factor k-B ligand (RANKL): a member of the tumor necrosis factor (TNF) cytokine family. RANKL is a ligand for osteoprotegerin and a key factor for osteoclast differentiation and activation, and in T helper cells is involved in dendritic cell maturation.

Runt-related transcription factor 2 (Runx2): a runt domain-containing transcription factor that is a transcriptional activator of osteoblast differentiation and master gene for bone development.

Signal transducer and activator of transcription 3 (STAT3): a members of the STAT transcription factor family, implicated in signal transduction by different cytokines, growth factors and oncogenes

TNF-related apoptosis-inducing ligand (TRAIL): A cytokine produced by immune cells, such as monocytes, in response to interferon α and γ. It activates tumor cell apoptotic signaling pathways through the death receptors DR4 and DR5: OPG binds to TRAIL thus preventing its interaction with the functional death receptors and allowing cells to escape apoptosis.

FIGURE LEGENDS

Figure 1: Osteopontin molecular structure and interactions

Osteopontin binds to integrins, transmembrane and dimeric proteins consisting of α and β subunits. It has several cell interacting domains that facilitate integrin binding in different cell types: an arginine-glycineaspartic acid (RGD) cell binding sequence, which interacts with cell surface integrins αvβ3, αvβ1, αvβ5 and α8β1; a serine-valine-valinetyrosine-glutamate-leucine-arginine (SVVYGLR)-containing domain, located between the RGD sequence and the thrombin cleavage site, which interacts with α9β1, α4β1 and α4β7; A ELVTDFTDLPAT domain is also reported to bind to α4β1; A calcium binding site and 2 heparin binding domains also exist: the heparin binding domains bind CD44 receptor. Furthermore, OPN can be cleaved by at least 2 classes of proteases: thrombin and matrix-metalloproteases (MMPs). *In vitro*, fragments generated by cleavage expose new active domains that may impart new activities.

Figure 2: regulation of osteoblast endocrine activity and osteocalcin secretion

In osteoblasts, undercarboxylated osteocalcin (uc-OCN) undergoes posttranslational modification whereby three glutamic acid residues are carboxylated to form γ-carboxyglutamic acid residues (carboxylated osteocalcin, carboxy-OCN). In animal studies, the undercarboxylated form appeared to mediate the metabolic effects of osteocalcin (i.e. increased β-cell proliferation, insulin secretion, insulin sensitivity and adiponectin and adiponectin expression, while carboxy-OCN has a lower metabolic bioactivity and higher affinity for hydroxyapatite and this is thought to be involved in bone extracellular matrix mineralization[52], although conclusive data in humans are lacking. The *Esp* gene, expressed only in osteoblasts, embryonic stem cells, and Sertoli cells, codes for the protein tyrosine phosphatase osteotesticular protein tyrosine phosphatase (OST-PTP), which induces osteocalcin γ-carboxylation in a vitamin K-dependent reaction, thereby reducing osteocalcin metabolic bioactivity.

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Fox01 inhibits *Ocn* gene expression by interacting with the *Ocn* gene-promoting transcription factor Runx2 and suppressing its binding to its cognate site within the *Ocn* promoter region[48]. Insulin and IGF-1 stimulate osteoblast differentiation and osteocalcin expression by relieving the suppression of Runx2 by Twist2 [46]. Insulin and IGF-1 antagonize Fox01 activity by promoting its phosphorylation through the PI3K/AKT-dependent pathway: Fox01phosphorylation (P-Fox01) results in its nuclear exclusion and inhibition of target gene expression[48]. FoxO1 inactivation favours osteocalcin activity in a dual mode of action. On one hand, it down-regulates expression of *Esp* thereby promoting osteocalcin decarboxylation. On the other hand, it reduces production of the anti-osteoclastogenic factor osteoprotegerin (*Opg*), and promotes osteoclastogenesis and bone resorption[48]. Through these feedback pathways, one at the transcriptional level with FoxO1 and the other at the hormonal level with osteocalcin, the skeleton and pancreas interact to tightly regulate energy metabolism and bone turnover.

Homocysteine is another factor regulating osteoblast endocrine activity: it inhibits osteoprotegerin by inducing FOXO1 loss through protein phosphatase 2A (PP2A) phosphorylation and enhances RANKL expression by activating JNK MAP kinase signalling pathway[63].

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BOXES

Box 1. Osteopontin and hepatocellular carcinoma(HCC).

HCC cells overexpress OPN, and its expression correlates with tumor invasiveness and metastatization: consequently, plasma OPN levels or OPN gene polymorphisms have been proposed as potential diagnostic and prognostic biomarkers for HCC[28, 29].

OPN isoforms seem to play different activities in HCC growth and metastatization: Chae et al. found that hepatocellular carcinoma cells (HCCs) predominantly expressed
OPNa and OPNb splice variants, while normal hepatocytes expressed OPNc *in vivo*; in cell proliferation and invasion assays, OPNa and OPNb induced Hep3B cell migration, while OPNc had no significant effects. By contrast, OPNc suppressed the migratory activity of SK-Hep1 cells[31].

OPN antagonization by blocking antibodies to inhibit iOPN binding to receptors or by directly decreasing tumor OPN expression through small interfering RNA (siRNA) suppressed HCC cells migration, invasion and neoangiogenesis *in vitro*, decreased metastases and improved survival *in vivo*[27, 30].

BOX 2. **Intracellular signaling mechanism of RANKL-RANK-OPG system**

The RANKL–RANK interaction leads to the recruitment of TNF receptor-associated factors (TRAFs) to the intracellular domain of RANK, among which TRAF6 seems to have a central role for the osteoclasts resorptive activity. TRAFs recruitment activates many downstream targets, which lead to osteoclast differentation (transcription factor NF-κB, p38, JNK mitogen-activated protein kinases -MAPKs) and survival (PI3K/Akt/mTOR signalling pathway, MAPK extracellular signal-related kinase - ERK).

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GLOSSARY

Alternative splicing: the process whereby identical pre-mRNA molecules are spliced in different ways, eventually leading to splice variants coding distinct proteins with different biological functions. Alternative splicing is important in both normal development and disease processes

APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine domain, and leucine zipper motif). the first identified protein interacting with adiponectin receptors and is suggested to be an adaptor protein responsible for intracellular mediation of adiponectin signal transduction

ATF4: activating transcription factor 4, belongs to the cAMP-responsive element-binding protein transcription factor family, is regulated by sympathetic tone, and regulates osteoblast endocrine functions by inducing Esp and Ocn

ERK: extracellular signal-regulated protein kinase. ERK1 and ERK2 are related protein-serine/threonine kinases that participate in the Ras-Raf-MEK-ERK signal transduction cascade, involved in the regulation of a large variety of processes including cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription

FOXO1: Forkhead box 01

Jak/STAT: janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway , a major intracellular signalling pathway for leptin. Leptin binds to its receptor OB-Rb and activates the receptor-associated kinase JAK2 via transphosphorylation and phosphorylates three tyrosine residues(Y985, Y1077, and Y1138 in mice).The signals emanating from the LepRTyr985

control hepatic insulin sensitivity. .Leptin stimulates JAK2-dependent phosphorylation and nuclear translocation of the transcription factor signal transducer and activator of STAT3. In the liver, STAT3 regulates glucose homeostasis by suppressing the expression of gluconeogenic genes; in the hypothalamus,.pSTAT3 translocates to the nucleus, where it increases the expression of proopiomelanocortin (POMC) and inhibits that of NPY.

JNK: c-Jun NH2-terminal kinase

MAPK: mitogen-activated protein kinase

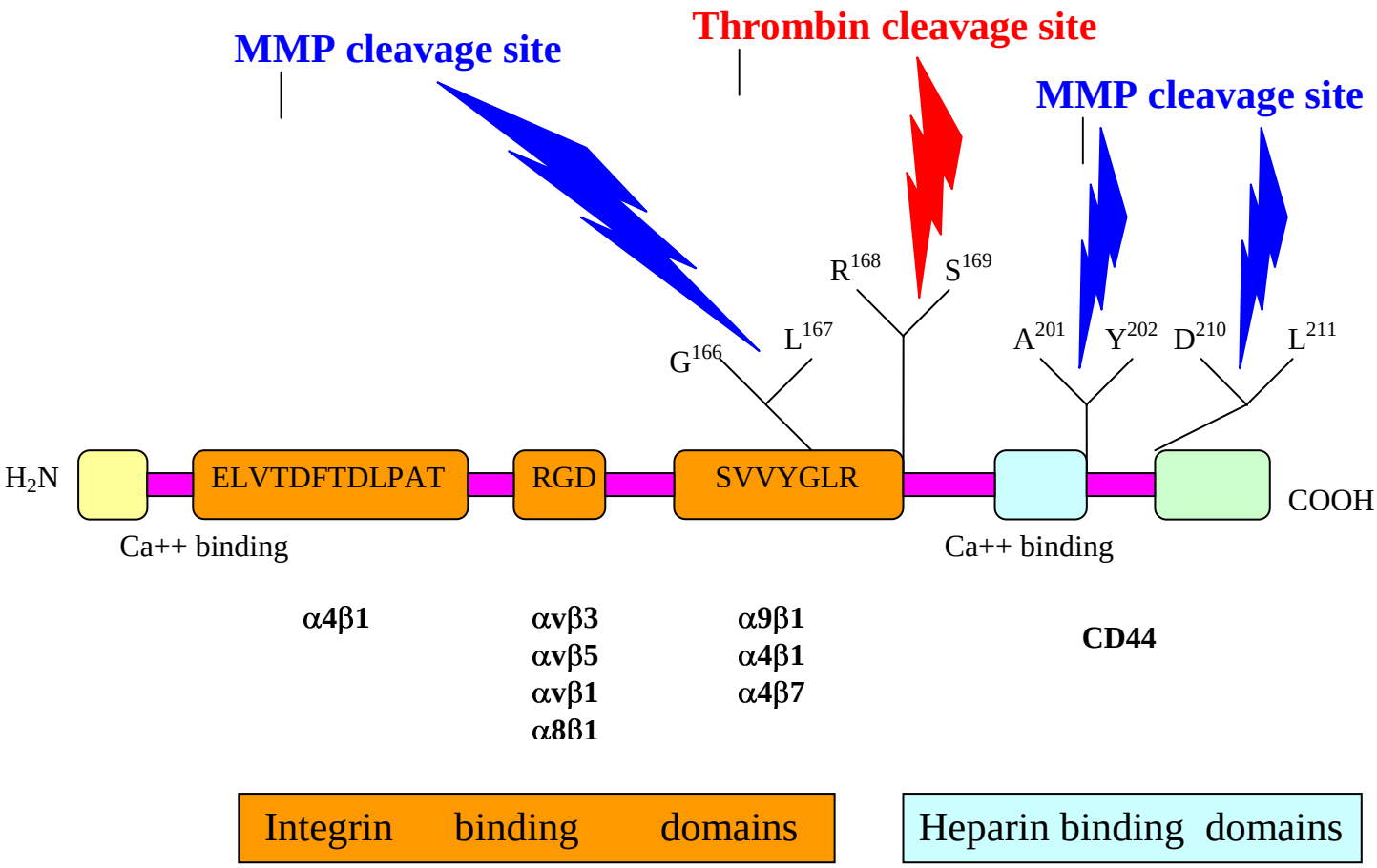
Osteoprotegerin (OPG): also known as tumor necrosis factor receptor superfamily member 11B, is a cytokine receptor and a member of the tumor necrosis factor (TNF) receptor superfamily. Osteoprotegerin is a decoy receptor for RANKL. By binding to RANKL, OPG inhibits NF-kB-mediated activation of osteoclasts.

RANKL(Receptor activator of nuclear factor k-B ligand): a member of the tumor necrosis factor (TNF) cytokine family. RANKL is a ligand for osteoprotegerin and a key factor for osteoclast differentiation and activation, and in T helper cells is involved in dendritic cell maturation.

Runx2: a runt domain-containing transcription factor that is a transcriptional activator of osteoblast differentiation and master gene for bone development.

STAT3: signal transducer and activator of transcription 3

TRAIL(TNF-related apoptosis-inducing ligand). TRAIL is produced by immune cells, such as monocytes, in response to interferon α and γ . This cytokine activates tumor cell apoptotic signalling pathways through the death receptors DR4 and DR5: OPG binds to TRAIL thus preventing its interaction with the functional death receptors and allowing cells to escape apoptosis.



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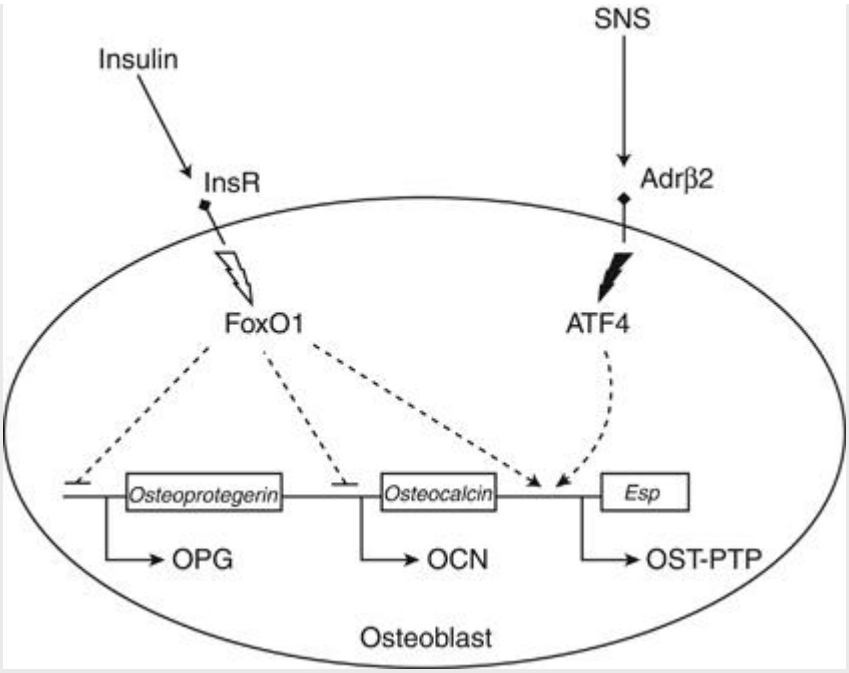

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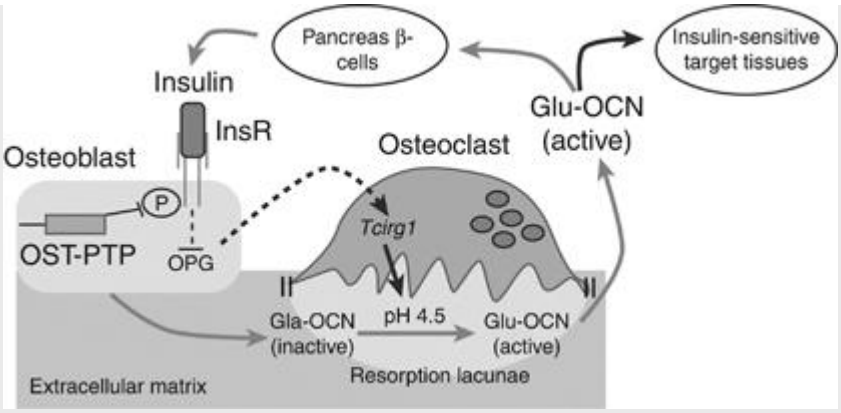

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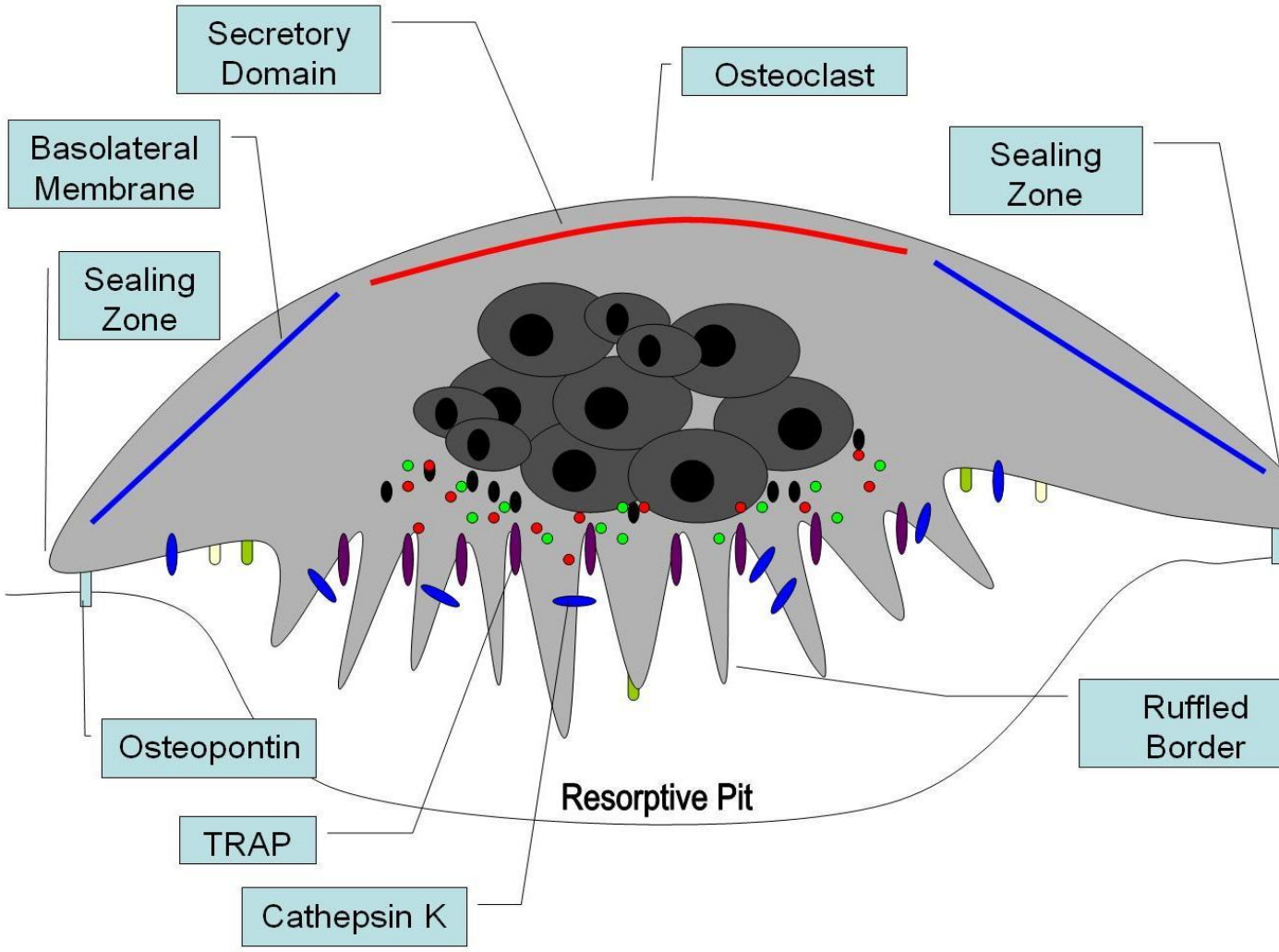

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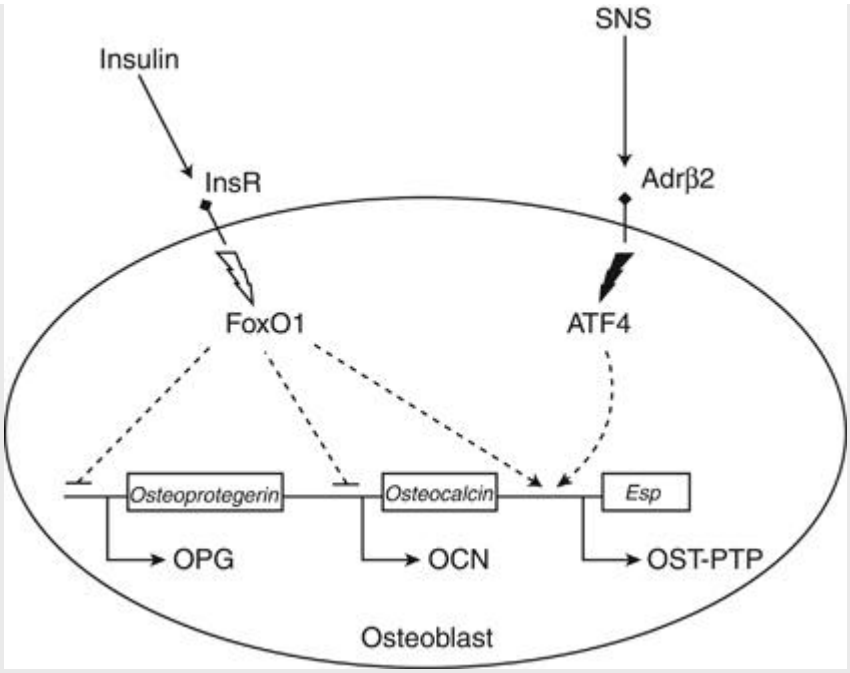

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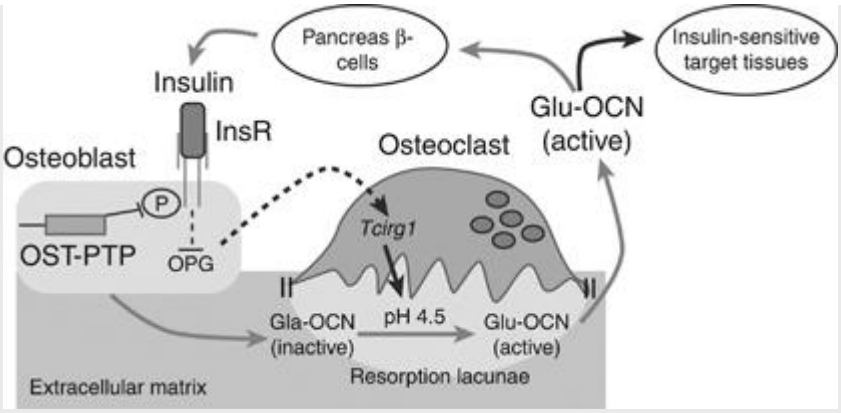

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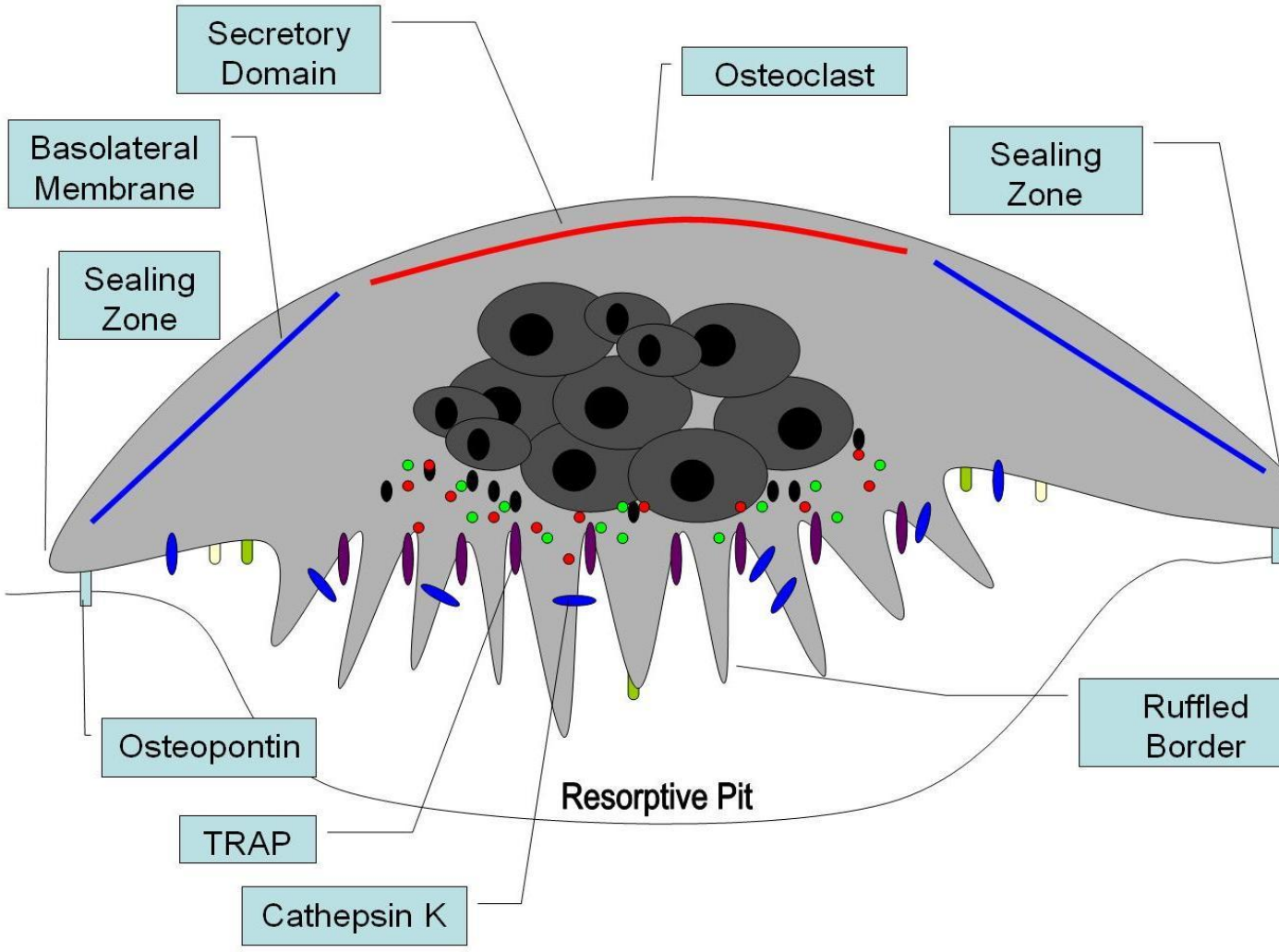

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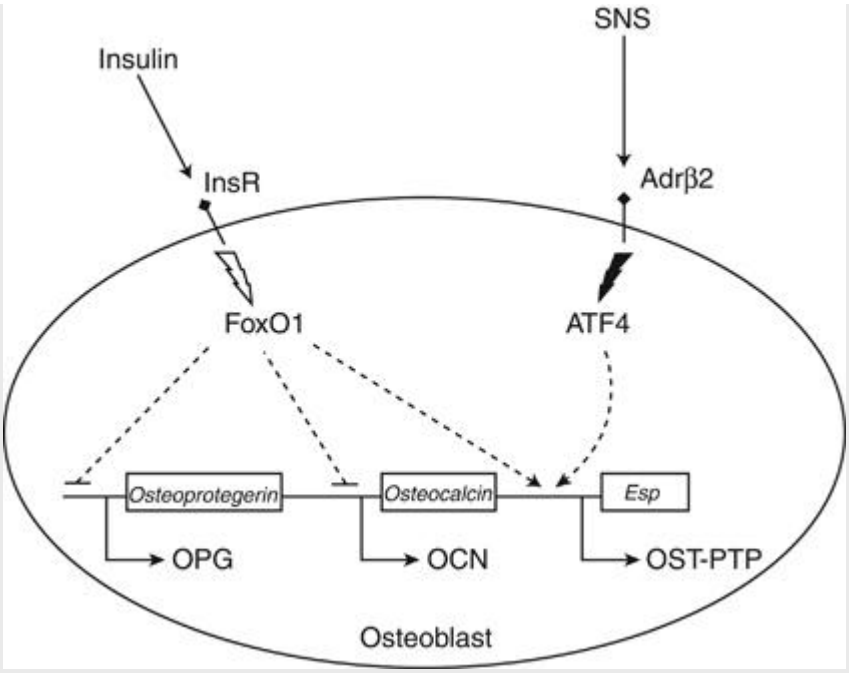

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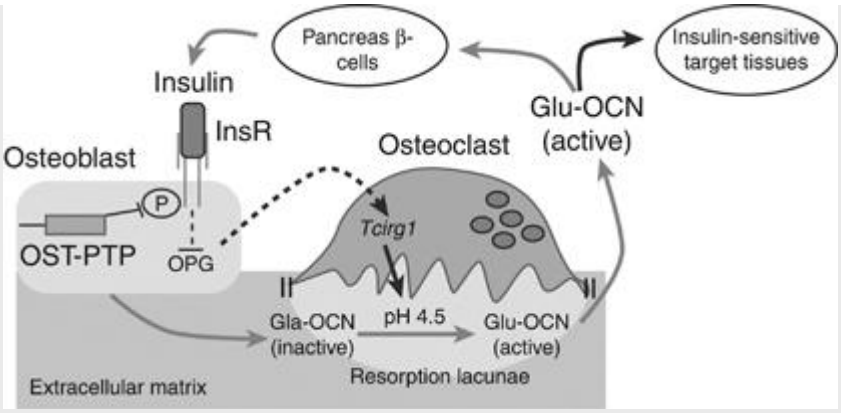

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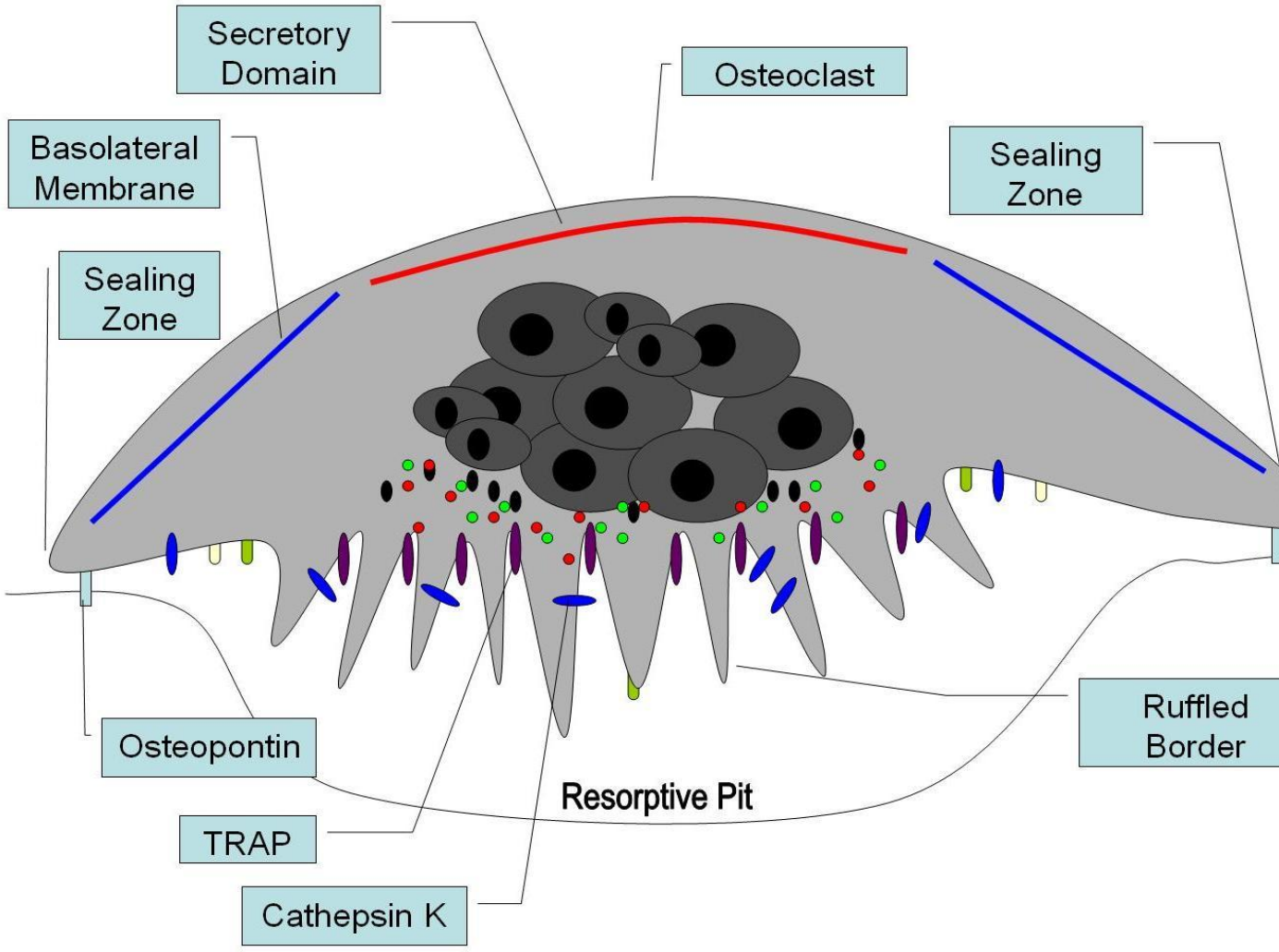

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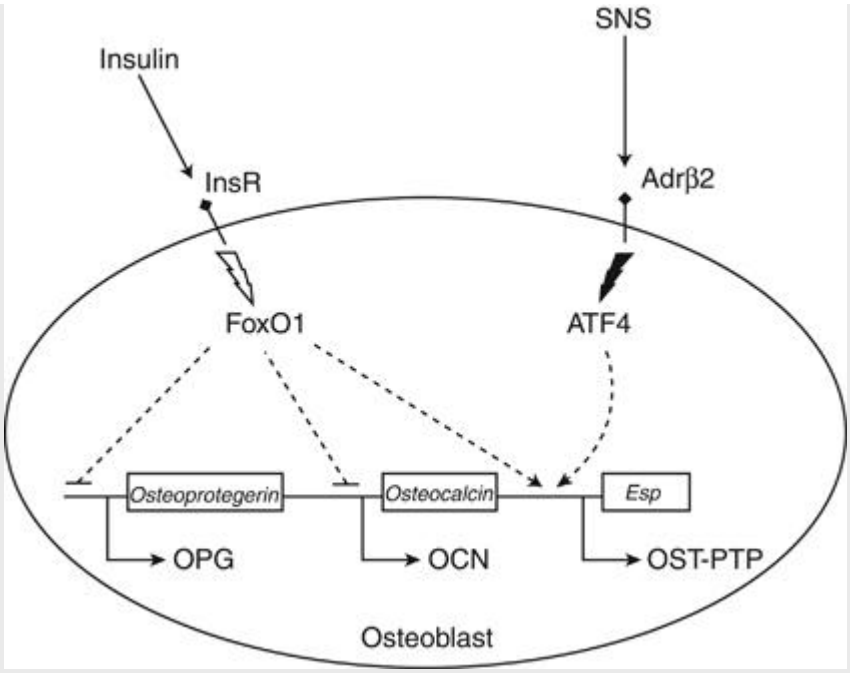

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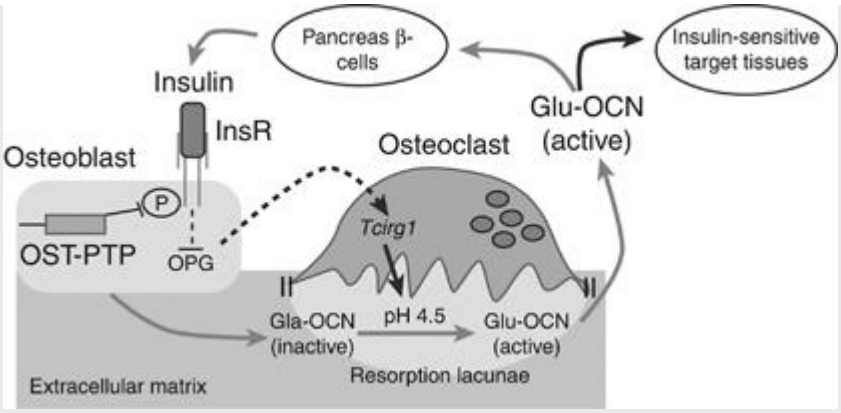

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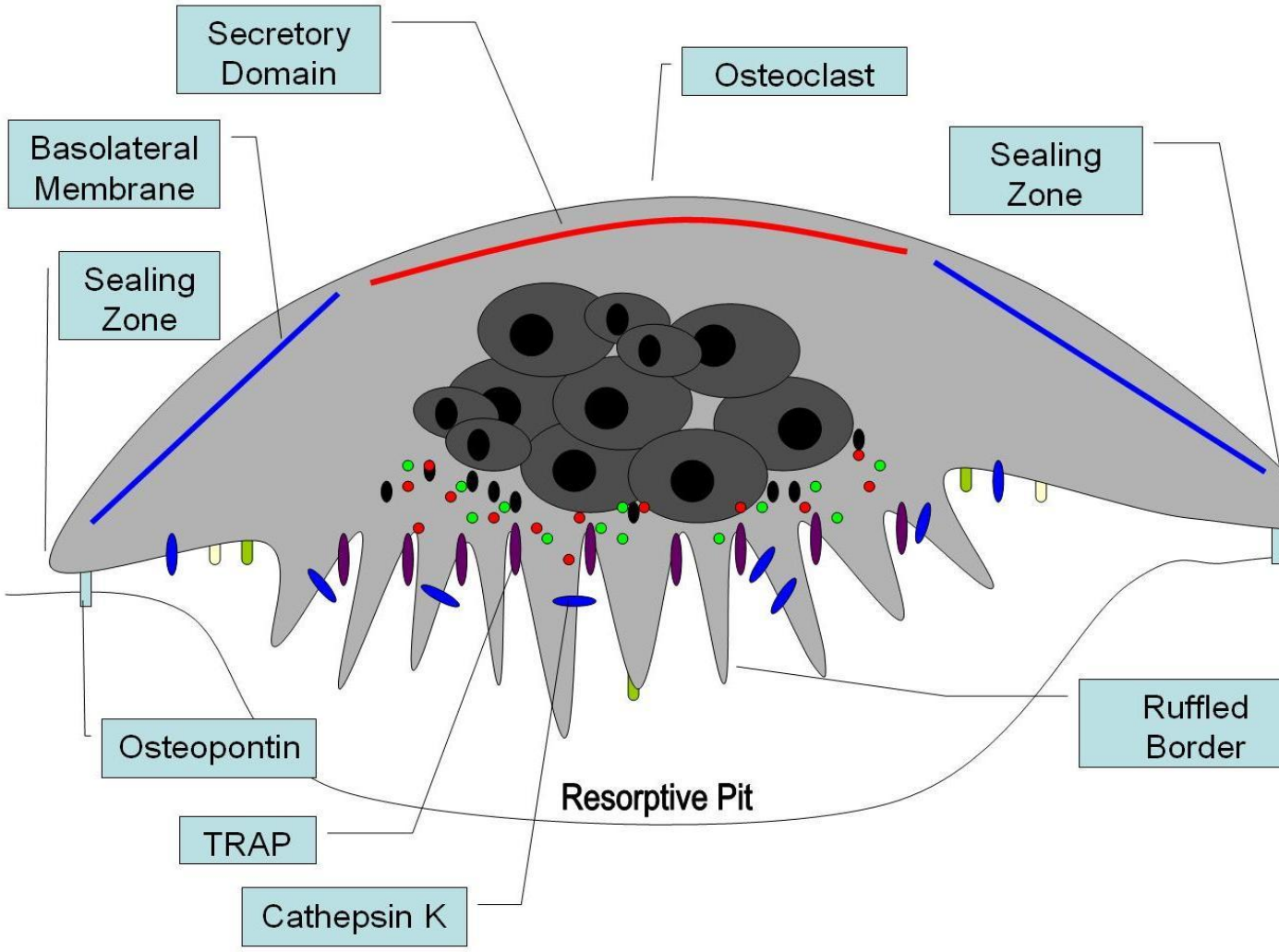

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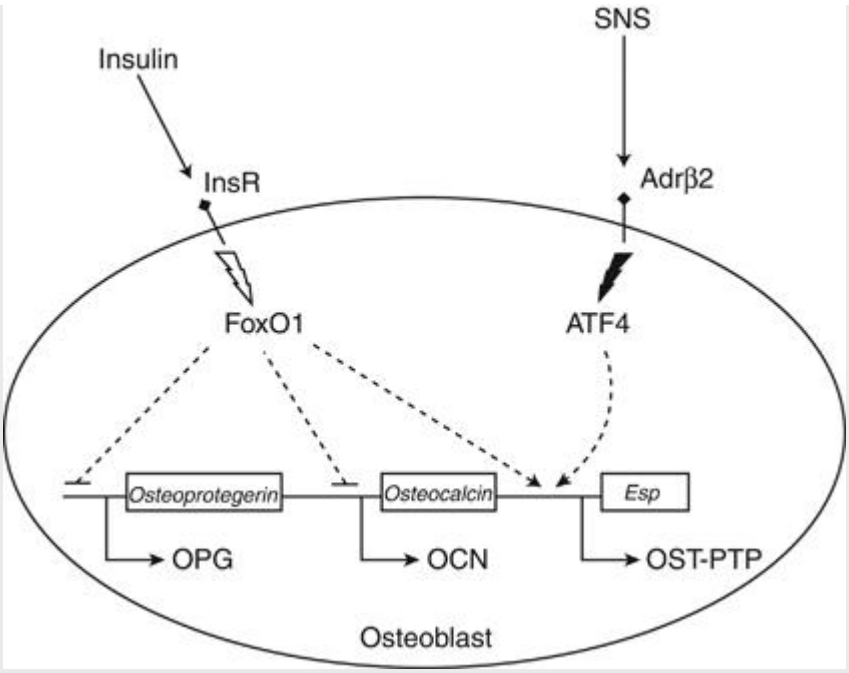

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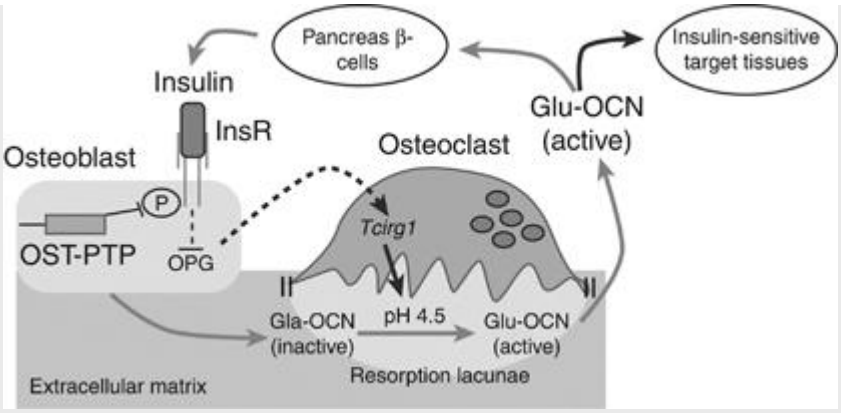

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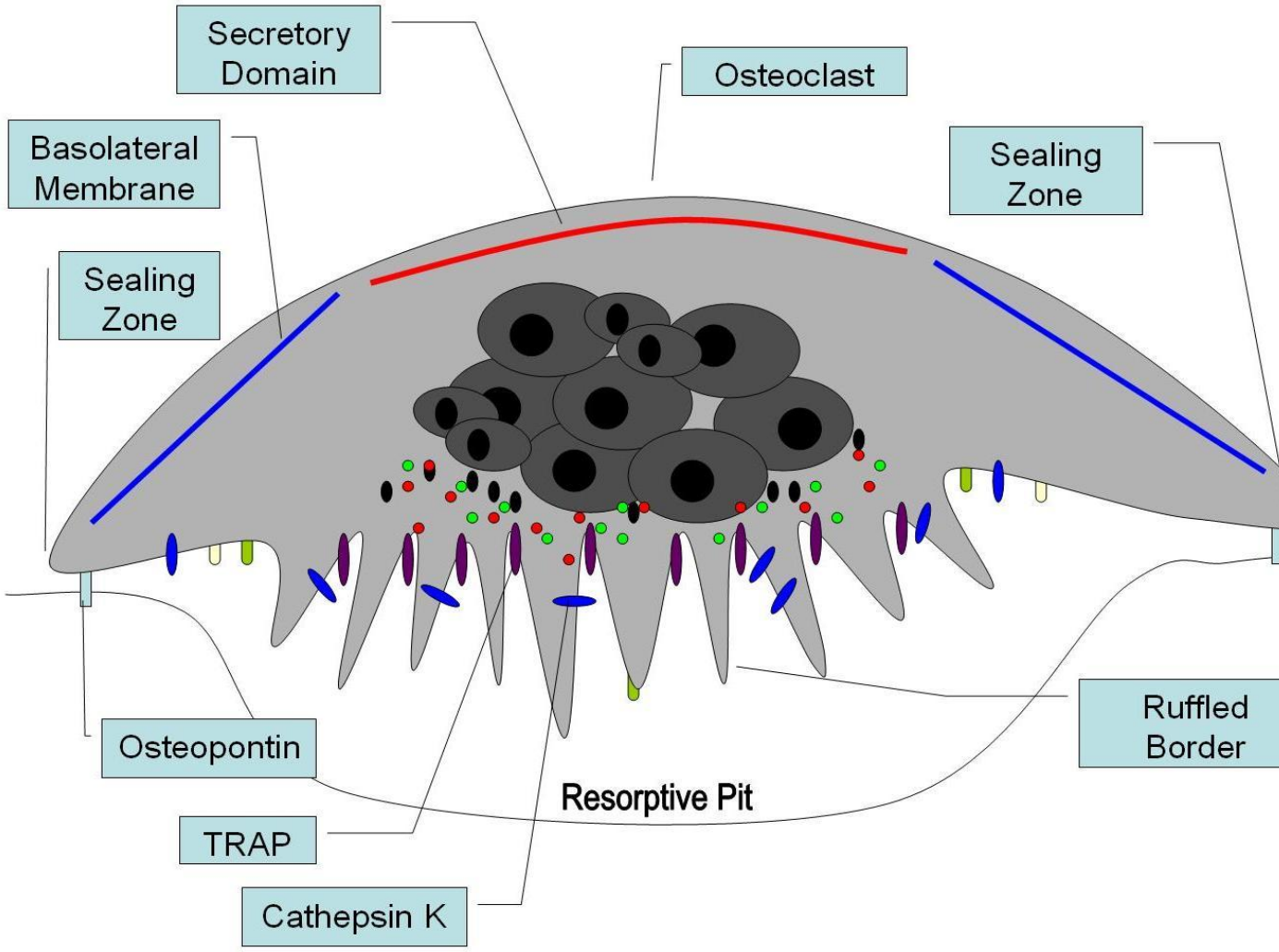

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Figure 2. mechanisms regulating secretion and biological activity of osteocalcin

In osteoblasts, uncarboxylated osteocalcin (uc-OCN) undergoes posttranslational modification whereby three glutamic acid residues are carboxylated to form γ-carboxyglutamic acid residues (carboxylated osteocalcin, carboxy-OCN). In animal studies, the uncarboxylated form appeared to mediate the metabolic effects of osteocalcin (i.e. increased β-cell proliferation, insulin secretion, insulin sensitivity and adiponectin and adiponectin expression, while carboxy-OC has a lower metabolic bioactivity and higher affinity for hydroxyapatite and this is thought to be involved in bone extracellular matrix mineralization, although conclusive data in humans are lacking. The *Esp* gene, expressed only in osteoblasts, embryonic stem cells, and Sertoli cells, codes for the protein tyrosine phosphatase osteotesticular protein tyrosine phosphatase (OST-PTP), which induces osteocalcin γ-carboxylation in a vitamin K-dependent reaction, thereby reducing osteocalcin metabolic bioactivity.

The endocrine function of osteoblasts is regulated by 2 transcription factors, the Activating transcription factor 4 (ATF4) and the Forkhead transcription factor FoxO1. ATF4 belongs to the cAMP-responsive element-binding protein transcription factor family, is upregulated by sympathetic nervous system (SNS) activation and induces Esp and Ocn gene expression, with the eventual effect of promoting glucose intolerance and insulin resistance: consistently, osteoblast-specific ATF4 deletion enhanced glucose tolerance and insulin sensitivity (Kode JBC 2012, 48). The transcription factor FoxO1 synergizes with ATF4 by reducing *Ocn* gene transcription and inducing *Esp* gene transcription, with the eventual result of reducing osteocalcin expression and promoting its carboxylation. Accordingly, osteoblast-specific FoxO1 deletion reduced osteoblast OST-PTP expression, increased osteocalcin and protected against obesity, diabetes and fatty liver (Rached JCI 2010, 46).

Fox01 inhibits *Ocn* gene expression by interacting with the *Ocn* gene-promoting transcription factor Runx2 and suppressing its binding to its cognate site within the *Ocn* promoter region(Yang IBC 2011, 47)

Insulin and IGF-1 stimulate osteoblast differentiation and osteocalcin expression by relieving the suppression of Runx2 by Twist2(46 Fulzele 2010). Insulin and IGF-1 antagonize Fox01 activity by promoting its phosphorylation through the PI3K/AKT-dependent pathway: Fox01phosphorylation (P-Fox01) results in its nuclear exclusion and inhibition of target gene expression Yang IBC 2011, 47). . FoxO1 inactivation favours Osteocalcin activity in a dual mode of action. On one hand, it down-regulates expression of *Esp* thereby promoting Osteocalcin decarboxylation. On the other hand, it reduces production of the anti-osteoclastogenic factor Osteoprotegerin (*Opg*), and promotes osteoclastogenesis and bone resorption Ferron M,. Cell. 2010;48). Through these feedback pathways, one at the transcriptional level with FoxO1 and the other at the hormonal level with osteocalcin, the skeleton and pancreas interact to tightly regulate energy metabolism and bone turnover.

Homocysteine inhibits osteoprotegerin by inducing FOXO1 loss through protein phosphatase 2A (PP2A) phosphorylation and enahnces RANKL expression by activating JNK MAP kinase signalling pathway(62 Vijayan FRBM 2013).

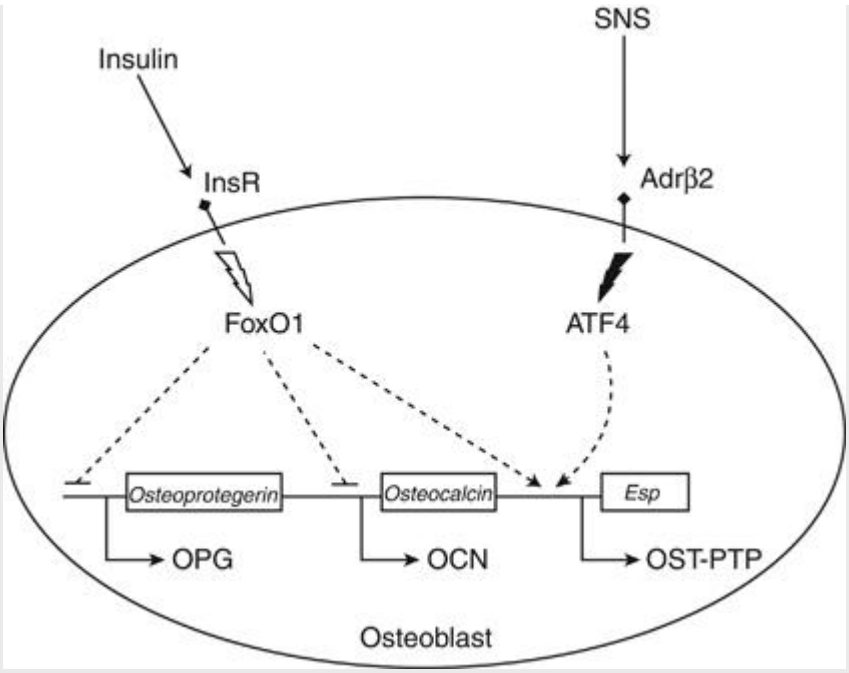
Another important regulator of osteoblast differentiation and osteocalcin secretion is sympathetic nervous system(SNS), which inhibits osteoblast differentiation and ucOC formation. The clinical importance of sympathetic tone, which mediates part of the indirect effects of leptin on osteoblast activity, for bone health is suggested by the 24–32% reductios in the risk of fractures experienced by people receiving β-blockers emerged in several large studies

Figure 2. Endocrine connection between osteoblasts and adipocytes

The transcriptional factor FoxO1 is also expressed in osteoblasts (OB). Osteoblast-specific FoxO1 deficiency is associated with an anabolic metabolism profile due to increased osteocalcin expression and decreased expression of *Esp*. In addition, osteocalcin may stimulate adipose tissue to secrete adiponectin, an insulin-sensitizing factor. Adipocytes (AD) affect bone and energetic metabolism by secreting leptin. In the CNS leptin stimulates the sympathetic nervous system (SNS) thereby activating the β2-adrenergic receptor (Adrβ2) in bone and subsequently

decreasing OB proliferation. Leptin also improves insulin sensitivity and this effect may be, at least in part, mediated by IGFBP2.

Osteocalcin bioactivity can be regulated in a bimodal mechanism of action. In the first mechanism osteocalcin activity is negatively regulated by another gene expressed in osteoblasts, *Esp*. Protein-tyrosine phosphatase (OST-PTP), the product of *Esp*, decreases osteocalcin bioactivity by favoring its carboxylation (2). In the second mechanism bone resorption induces a change in the pH that spontaneously decarboxylates and activates osteocalcin (3). Respective to the latter mechanism, we did not detect any increases in osteoclast function in *FoxO1*osb _/_;*Atf4*_/_ mice that would indicate an increase in bone resorption (Fig. 5*G*).



Osteoblast signaling pathways involved in energy metabolism regulation. Effects of insulin and sympathetic nervous system (SNS) on osteocalcin (OCN), osteotesticular protein tyrosine phosphatase (OST-PTP), and osteoprotegerin (OPG). Adrβ2, β2-adrenergic receptor; ATF4, activating transcription factor 4; InsR, insulin receptor. Adapted from Confavreux.[22]

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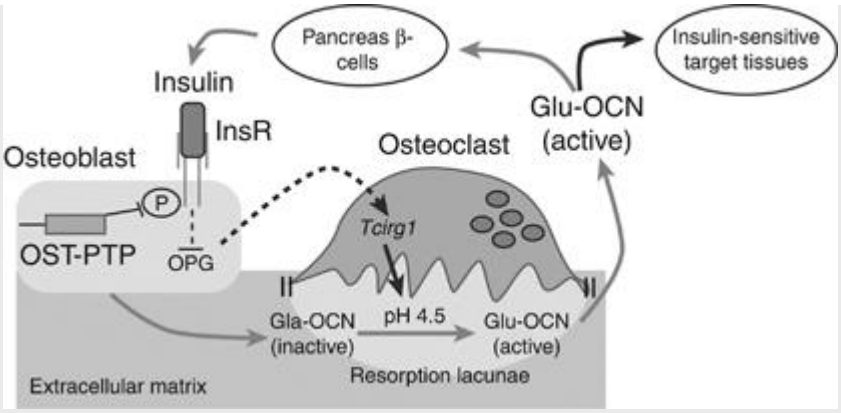

Insulin and bone resorption affect circulating uncarboxylated osteocalcin (Glu-OCN). Insulin binds its receptor (InsR), which reduces osteoprotegerin (OPG) expression, thus enhancing osteoclast formation and activity. During bone resorption, acidification of the extracellular compartment decarboxylates inactive osteocalcin (Gla-OCN) to Glu-OCN. Glu-OCN is then released into the blood stream to affect target tissues. OST-PTP, osteotesticular protein tyrosine phosphatase. Adapted from *Cell*,[27] with permission from Elsevier.

Table 1. Effect of β-blockers on fracture risk[12]

Figures and tables index

Study	Study design	Subjects	Fracture type	Hazard or odds ratio (95% CI)	Reference
SOF	Prospective cohort	8412 (13.1% users)	Hip	0.76 (0.58–0.99)	Reid *et al.*[10]
GPRD	Retrospective case–control	120,819 controls (17.6% users), 30,601 cases (16% users)	Any	0.77 (0.72–0.83)	Schlienger *et al.*[11]
			Hip	0.68 (0.52–0.89)	
Geelong Osteoporosis Study	Population-based case–control	775 controls (14.5% users), 569 cases (10.4% users)	Any	0.69 (0.49–0.96)	Pasco *et al.*[9]

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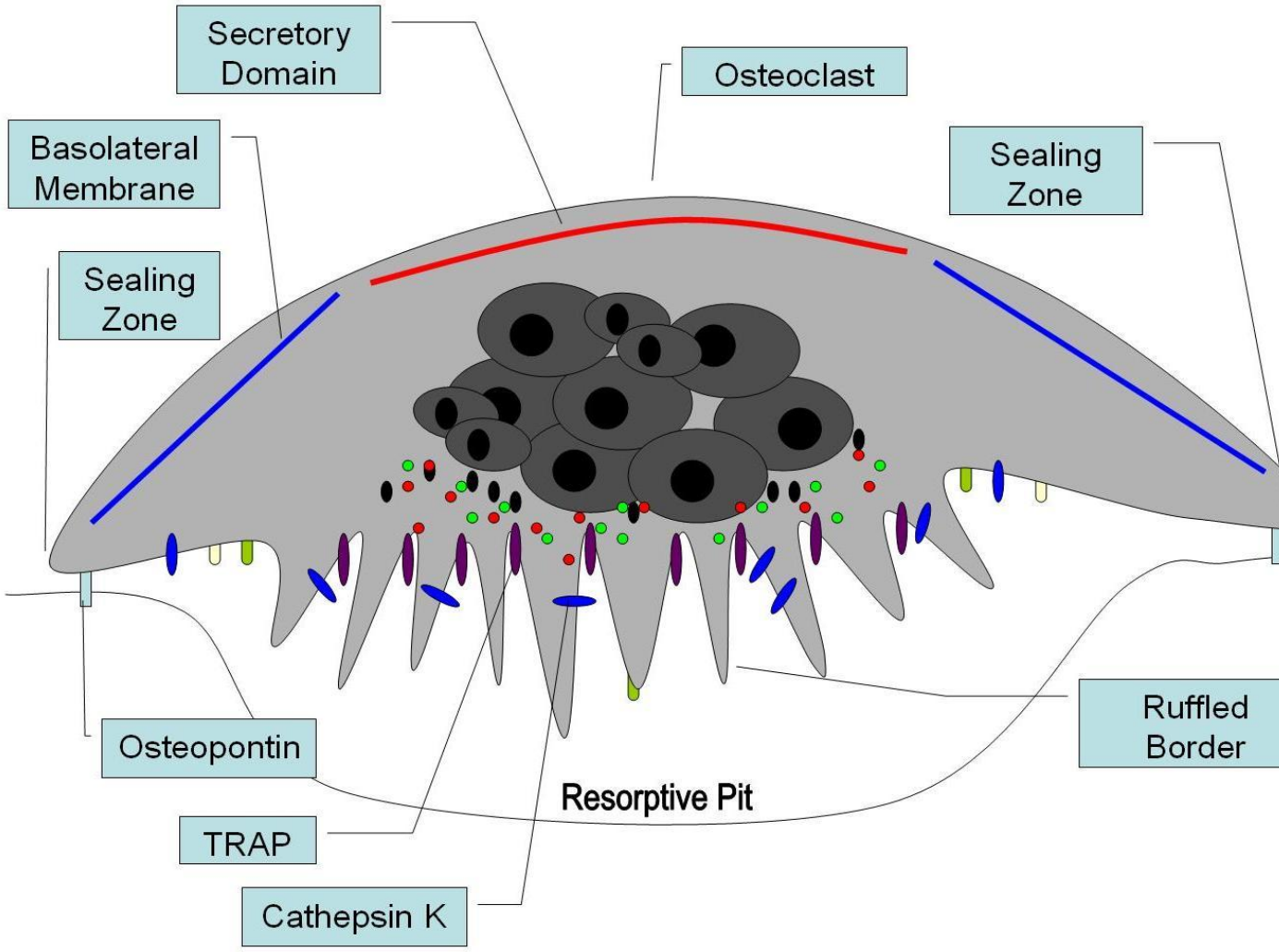

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Table 1. Main mediators of interaction between the liver, adipose tissue and the bone

Osteopontin					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
activated T-helper 1 and macrophages, NKT cells, osteoclast, SMC, endothelial cell, hepatocyte, hepatic stellate cells, adipocytes	**Stimulators** PTH; hedgehog signalling pathway; reactive oxygen species; NR4A2; Wnt/β-catenin signalling; Akt; EGF **Inhibitors** PPAR-γ; miRNA-181a	Hepatocytes	↓ STAT3 activation→↑ GSK-3β expression→ ↑ gluconeogenetic enzymes PEPCK and G6P ↑ FOXO1 expression →↑ gluconeogenesis, ↓ IRS-2 phosphorylation ↓ secretion of anti-inflammatory, insulin-sensitizing cytokine IL-10 ↑ SREBP-1c expression→↑ *de novo* lipogenesis	↑ hepatic insulin resistance and inflammation ↑ hepatic steatosis	[10] [18] [19] [22] [19] [20] [25]
		Circulating NKT cells	↑ recruitment to the liver→↑ activation of fibrogenesis	↑ hepatic fibrosis	[15]
		Hepatic stellate cells	↑ αvβ3 integrin-mediated PI3-K/pAkt/NFκB pathway activation→ ↑ proliferation, collagen I deposition, TGF-β1 receptor expression ↓ extracellular MMP-13 secretion→↓ collagen-I resorption	↑ hepatic fibrosis	[25] [25] [32]
		Circulating monocytes	↑JNK1/2 activation→ ↑monocyte adhesion, migration, differentiation, phagocytosis and secretion of proinflammatory cytokines TNF-α, MCP-1, IL-1, IL-6	↑ adipose tissue and hepatic macrophage infiltration	[12] [16] [17] [18]

		Adipocytes	↑ JNK1/2 and ERK2 activation→↑insulin resistance, ↓ adiponectin secretion, ↑ IL-6 secretion ↔ NF-κB pathway(unaffected)	and inflammation ↑ adipose tissue and hepatic insulin resistance	[19]
		HCC	↑ PI3-K/Akt and HIF-1 pathway activation→↑ NF-κB pathway activation ↑ MMP-2/-7/-9 and Upa expression→↑ ECM degradation ↑ VEGF expression→↑ angiogenesis	pro-,survival, anti-apoptosis and proliferation ↑ tumor invasion and metastasis	[26] [27] [30]
		Bone marrow multipotent stromal cells (BMSC)	OPN mediates high-fat diet-induced differentiation of BMSCs toward adipocyte and away from osteoblast cell line	↓ bone deposition	[16]
		Osteoclast	↑ surface CD44 expression→↑ motility ↑ attachment of osteoclast to bone matrix via the αvβ3 integrin receptor in the sealing zone of resorptive pit	↑ bone resorption	[16]
Osteocalcin					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
Osteoblast	**Stimulators** Insulin	Pancreatic β-cell	↑ intracellular cAMP→↑ ERK activation→ ↑ β-cell viability and function	Preserved insulin	[55]

	Inhibitors			secretion	
	Twist2; Fox01; ATF4; ER stress	Enteroendocrine L-cells	↑ GLP-1 secretion	↑ insulin secretion	[46] [47] [50] [51] [56]
		Adipocyte	↑ adiponectin expression	↑ insulin sensitivity in muscle, liver, adipose tissue ↓ hepatic steatosis and inflammation	[44]
		Adipocyte, hepatocyte, miocyte	↑ PI3-K/Akt pathway activation→↑ NF-κB pathway activation ↓ phosphorylation of PERK, eIF2α, and IRE-1α and the expression of ATF6β→↓ ER stress response. ↑ UCP-1 and PGC-1α →↑ mitochondrial biogenesis and function in adipocytes. ↑ mitochondrial number and mass in miocytes.	↑ energy expenditure→ ↓ fat content in adipose tissue, liver and skeletal muscle ↑ insulin sensitivity	[51]
		Bone matrix	Carboxylated osteocalcin binds hydroxyapatite	Uncertain effects on bone formation	[52]
Receptor activator of nuclear factor-κB ligand (RANKL)					

Secreting cells	Modulators of secretion	Target cell	Cellular mechanism	Biological effect	Ref.
Osteoblast, T cells, adipocyte	**Stimulators** Adiponectin ; PPAR-γ; IL-1β ; TNF-α; Homocysteine ; **Inhibitors** 17β-estradiol; PPARβ/δ; LXRs;	Osteoclast	RANK activation→ ↑ Akt1 activation→ ↑ osteoclast proliferation and activation RANK activation→ ↑TRAF6-mediated NF-κB pathway activation→↑ osteoclast proliferation and activation	↑ bone resorption	[59] [61] [63] [64]
		dendritic cells (DC) , T cells, monocytes/ macrophages	↑ proliferation, activation and survival	↑ immune function and inflammation	[62] [65] [66]
		hepatocytes	↑ NF-κB pathway activation→↑ hepatocyte proinflammatory pathway activation	↑ hepatic insulin resistance and inflammation	[77]

Osteoprotegerin

Secreting cells	Modulators of secretion	Target cell	Cellular mechanism	Biological effect	Ref.
Osteoblast, T cell, adipocyte, endothelial cell	**Stimulators** 17β-estradiol; IL-1β ;	Osteoclast	decoy receptor for RANKL→↓ RANKL binding to osteoclast RANK→↓ osteoclast proliferation and activation	↓ bone resorption	[59]
		dendritic cell (DC) ,	↓ proliferation, activation and survival	↓ immune function and	[61] [62]

	TNF-α; Insulin ; PPARβ/δ; LXRs; **Inhibitors** Adiponectin ; Homocysteine; PPAR-γ	T cell, monocyte/macrophage		inflammation	[64] [65] [66]
		Hepatocyte	interaction with TRAIL→↓ apoptosis activation	↓ liver injury	[63] [64]
Fetuin-A					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
hepatocyte	**Stimulators** ER stress ; ERK1/2	Hepatocyte, miocyte	↓ tyrosine kinase and IRS-1 autophosphorylation→↓ insulin receptor tyrosine kinase activity	↑ muscle and hepatic insulin resistance	[78] [90]
		Adipocyte	↓ adiponectin secretion ↑ interaction of circulating FFA with TLR4→↑ FFA-induced proinflammatory adipokine secretion	↑ adipose tissue insulin resistance and inflammation	[80] [91]
		Bone and extracellular matrix	Complexation with calcium and phosphate to form stable colloidal mineral-protein spheres(calciprotein particles, CPPs).	Modulation of bone mineralization and ectopic calcification of soft tissues	[95] [96] [97]
Leptin					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
Mature adipocyte	**Stimulators** Adipocyte size;	Hypothalamic arcuate nucleus	↑ JAK2/STAT3 pathway→ ↓ neuropeptide Y (NPY) and ↑ POMC synthesis	↓ food intake	[101]

	Feeding; Glucocorticoids; Glucose; Insulin; **Inhibitors** Fasting; Exercise; Cold exposure	Miocyte, hepatocyte	↓ malonyl-CoA synthesis→ ↑ CPT I activity→ mitochondrial FFA oxidation. ↑ AMPK activation→ ↑ FFA β-oxidation and glycolysis. ↓ SREBP-1c expression→ ↓ *de novo* lipogenesis. ↑ hepatic vagal tone→↓ PEPCK and G6P→ ↓ gluconeogenesis. ↑ Tg incorporation into VLDL particles→↑ hepatic Tg secretion. ↓ hepatic lipoprotein lipase activity→↓ hepatic Tg uptake from plasma ↑ insulin-like growth factor binding protein(IGFBP)-2 secretion	↓ muscle and hepatic triglyceride content ↑insulin sensitivity ↑ hepatic insulin sensitivity ↑osteoclast differentiation	[102] [103] [118] [119]
		Kupffer cells, HSC	↑ STAT3 signaling→↑ transforming growth factor (TGF)-β secretion →↑ HSC activation. ↑ STAT3 signaling→↑ CD14 expression→↑ responsivity to low-dose bacterial endotoxin. →↑ endotoxin-induced hepatic inflammation	↑ hepatic inflammation and NASH ↑ hepatic fibrogenesis	[106] [110]
		β-cells	↓ intracellular glucose transport via GLUT-2	↓ pancreatic insulin secretion	[102] [103]

		Hypothalamic ventro-medial nucleus (VHM) Serotoninergic brainstem neurons	↑ β-adrenergic sympathetic tone→↓ osteoblast proliferation, activity and secretion of uc-osteocalcin (β2 receptor-mediated) ↑ serotononergic neuron activity→↑ VHM nucleus sympathetic tone	Bone loss	[9] [113]
		Osteoblast	↑ phosphorylation and inactivation of glycogen synthase kinase (GSK)-3β activity→↑ osteoblast differentiation and bone matrix mineralization ↑ FGF-23 secretion→↓ renal 1,25(OH)2D3 synthesis and phosphate resorption	↑ bone mineralization	[116] [119]
		Renal proximal tubules	↓ 25-OH-D(3)-1α-hydroxylase (CYP27B1) activity→ ↓1,25(OH)2D3 synthesis	↓ bone mineralization	[119]
TNF-α					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
Adipocytes, macrophages, Kupffer cells, hepatocytes,	**Stimulators** Oxidative stress **Inhibitors** adiponectin	Adipocyte	↓ secretion of leptin and adiponectin. ↓ GLUT-4 expression. ↓ lipoprotein lipase (LPL) activity. ↑ hormone-sensitive lipase→↑ lipolysis.	Insulin resistance Adipose tissue and systemic inflammation	[122] [123]

			↑ JNK-1 activation→ phosphorylation of IRS-1→insulin receptor signalling inactivation. ↑ IKKβ/NF-κB pathway activation→↑apoptosis, proinflammatory cytokine secretion		
		Hepatocyte	↑ JNK-1 activation→ phosphorylation of IRS-1→↓ insulin receptor signalling. ↑ JNK-1 and NF-κB pathway activation→↑apoptosis and proinflammatory cytokine secretion	Insulin resistance Hepatic and systemic inflammation NASH	[122]
		Kupffer cell	↑ JNK-1 and NF-κB pathway activation→↑apoptosis, proinflammatory cytokine secretion	Insulin resistance Hepatic and systemic inflammation NASH	[122] [124] [125]
		Osteoblast	↑ubiquitynation of RUNX2→↓ RUNX2 expression→↓ osteoblast differentiation, proliferation and activation. ↓ MAPK pathway activation→↓ osteoblast differentiation, proliferation and activation. ↓ expression of alkaline phosphatase, vitamin D receptor, parathyroid hormone receptor. ↑ RANKL secretion	↓ bone formation	[127] [130]
		Osteoclast	↑ TNF-R1-mediated NF-κB stimulation of osteoclast differentiation.	↑ bone resorption	[126] [127

			↑ RANKL-induced stimulation of osteoclast differentiation . ↑ rapamycin/S6 kinase activation→↓ apoptosis		]
Adiponectin					
Secreting cells	**Modulators of secretion**	**Target cell**	**Cellular mechanism**	**Biological effect**	**Ref.**
Adipocytes	**Stimulators** Weight loss; uc-osteocalcin **Inhibitors** TNF-α; IL-6; resistin; insulin; glucocorticoids	Hepatocyte, adipocyte, muscle	↑ AMPK activation→ ↑mitochondrial fatty acid β-oxidation. ↑ insulin signalling. ↓ SREBP-1c expression→ ↓ *de novo* lipogenesis. ↓ apoptosis. ↓ proinflammatory cytokine secretion.	↓ steatosis ↓ hepatic, muscle and adipose tissue insulin resistance ↓ hepatic, necroinflammation	[44] [131] [132]
		Hepatic stellate cell	↑ apoptosis. ↓ activity and collagen deposition.	↓ fibrogenesis	[131] [132]
		Osteoblast	↑ proliferation, differentiation and activity. ↑ RANKL secretion. ↓ osteoprotegerin secretion.	↑ bone deposition	[61] [62] [134]
		Osteoclast	↓ APPL1-mediated Akt1 activity→ ↓ RANKL-induced osteoclastogenesis ↑ osteoclast apoptosis. ↓ survival/proliferation of osteoclast precursor cells.	↓ bone resorption	[135]

Abbreviations: SMC: smooth muscle cells; HCC: hepatocellular carcinoma; PI3-K : phosphoinositide 3-kinase; Akt: protein kinase B; APPL1: daptor protein containing pleckstrin homology domain, phosphotyrosine domain, and leucine zipper motif) HIF-1: hypoxia-inducible factor-1; NF-κB: nuclear factor-κB; MMP: matrix metalloproteinase; ECM: extracellular matrix; uPA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor ; JNK: c-Jun NH2-terminal kinase; BMD: bone mineral density; PTH: parathyroid hormone; ERK: extracellular signal-regulated protein kinase; TNF: tumor necrosis factor; MCP: monocyte chemoattractant protein; PPAR: peroxisome proliferator-activated; STAT3: signal transducer and activator of transcription 3; GSK-3β: glycogen synthase kinase-3; PEPCK: phosphoenolpyruvate carboxykinase; G6P: glucose 6 phosphatase; SREBF: sterol regulatory binding protein; DGAT: diacylglycerol acyltransferase; FoxO1: Forkhead box 01; NKT: naturalkiller T ; TGF: transforming growth factor; PI3-K: phosphoinositide 3-kinase; pAkt, phosphorylated Akt; NR4A2: nuclear receptor subfamily 4, group A, member 2; miRNA: microRNA; EGF: epidermal growth factor; eIF2α: α-subunit of eukaryotic initiation factor 2; IRE-1α: inositol-requiring enzyme-1α; PERK: protein kinase–like endoplasmic reticulum kinase; UCP: uncoupling protein; PGC-1α: peroxisome proliferator-activated receptor γ (PPAR-γ) coactivator-1α; RANK: receptor activator of nuclear factor-κB; RANKL: receptor activator of nuclear factor-κB ligand; TZD: thiazolidinediones; IL-1β: interleukin 1-β; TRAF6: TNF receptor-associated factor 6;
TRAIL: TNF-related apoptosis-inducing ligand; IRS-1: insulin receptor substrate-1; TLR: toll-like receptor; CPT : carnitine palmitoyltransferase; JAK: janus kinase; GLUT-2: glucose transporter 2; POMC: proopiomelanocortin; TGF: transforming growth factor; AMPK: 5'adenosine monophosphate -activated protein kinase; FGF-23: fibroblast growth factor 23;

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