Review

Animal models for glucocorticoid-induced postmenopausal osteoporosis: An updated review

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\textbf{A B S T R A C T}

Glucocorticoid-induced postmenopausal osteoporosis is a severe osteoporosis, with high risk of major osteoporotic fractures. This severe osteoporosis urges more extensive and deeper basic study, in which suitable animal models are indispensable. However, no relevant review is available introducing this model systematically. Based on the recent studies on GI-PMOP, this brief review introduces the GI-PMOP animal model in terms of its establishment, evaluation of bone mass and discuss its molecular mechanism. Rat, rabbit and sheep with their respective merits were chosen. Both direct and indirect evaluation of bone mass help to understand the bone metabolism under different intervention. The crucial signaling pathways, miRNAs, osteogenic- or adipogenic-related factors and estrogen level may be the predominant contributors to the development of glucocorticoid-induced postmenopausal osteoporosis.

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\textbf{Contents}

1. Introduction .................................................. 439
2. Strengths and limitations of OVX and/or GC treatment .................................................. 439
   2.1. Ovariectomy ........................................ 439
   2.2. Glucocorticoid treatment .................................. 439
   2.3. Union of OVX and GC treatment ......................... 439
3. Creation of GI-PMOP model ........................................ 439
   3.1. Animals ........................................ 439
      3.1.1. Rat ........................................ 439
      3.1.2. Rabbit ...................................... 440
      3.1.3. Sheep ..................................... 440
   3.2. Experimental protocol .................................... 440
   3.3. Methods to evaluate bone mass ........................... 440
      3.3.1. Noninvasive methods .............................. 440
      3.3.2. Invasive methods ................................ 441
4. The underlying mechanism of OP induced by GCs in OVX animals .................................... 442
   4.1. Regulation of vital signaling pathway of bone formation and resorption by estrogen and GC ......... 442

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1. Introduction

Osteoporosis (OP), is a systemic skeletal disorder, characterized by decreased bone mass, microarchitectural deterioration and increased fragility as well as consequent increase in risk of bone fracture, which greatly affects people’s life quality and even gives rise to the increased mortality, arousing extensive concerns among the population. OP is generally classified as the primary OP and secondary OP. Glucocorticoid (GC) intake is the most common cause of secondary OP, while menopause is one of the common cause of primary OP [1]. Epidemiological study showed up to 4.6% of postmenopausal women are taking oral GC [2]. GC intervention and menopause often simultaneously play roles in developing OP. Related study has reported that GCs aggravate the postmenopausal or aged osteoporotic status and impairment of skeletal metabolism [3], and thus increase the morbidity of OP and the risk of OP-related fractures. Therefore, more attention should be paid to the patients with glucocorticoid-induced postmenopausal osteoporosis (GI-PMOP), and more basic research related to GI-PMOP should be devoted to exploring its underlying mechanism and the potential therapeutic target, which requires suitable animal models simulating the population. However, available relevant studies are still inadequate, and how to successfully establish and correctly select an animal model of GI-PMOP are still an outstanding question.

To characterize GI-PMOP well, GCs were administered to ovariecctomized animals. Ovariectomy (OVX) is a canonical means to create PMOP model. Combined with GCs treatment, ovariecctomized animals not only rapidly develop OP, but also pertinently mimic glucocorticoid-induced postmenopausal osteoporosis (GI-PMOP) [4]. Therefore, we introduce this combined OP model by summarizing the advantages of its establishment, characteristics of animals selected, routine experimental protocols and evaluation of OP, and attempt to deduct the underlying pathogenesis of GI-PMOP on the basis of existing study.

2. Strengths and limitations of OVX and/or GC treatment

2.1. Ovariectomy

Varieties of OVX animals have been employed to investigate OP. In rats, bone loss occurs some days after OVX at different bone sites in both cancellous and cortical bone [5–7]. OVX causes enlarged bone marrow cavity, showing the similar pathologic regression of bone in postmenopausal women. Therefore OVX has been regarded as an canonical protocol to create the OP model in animals. However, in the other species, such as rabbits and sheep, etc. OVX alone is incapable of developing sufficient osteoporosis [8–10].

2.2. Glucocorticoid treatment

Clinical studies have shown that patients who take GCs for 6 months or longer develop OP [11]. OP also occurs in animals like rat, rabbit, and sheep after GCs treatment due to enhanced bone resorption and reduced bone formation. Effects of GCs on bone metabolism had been extensively studied. Most of researches have demonstrated that GCs administration lead to bone loss [12,13]. However, paradoxical result that dexamethasone increased the bone mass had been revealed when treating rats with GC at relative low dose [14]. Additionally, high dose treatment of GC may cause additional detrimental effects (like osteonecrosis) on bone [15], even lead to animal’s death [16] attributable to the increased risk of infection on account of immunosuppression of GC. Besides, although GCs causes a more progressive bone loss compared to OVX, the bone loss reverse after GC cessation [17]. These limitations increase the chance of failure establishing OP model.

2.3. Union of OVX and GC treatment

Due to the limitations of either OVX or GC treatment alone to induce osteoporosis, like insufficiency, inconsistency, and time consumption, combined methods including ovariectomy combined with deficient diet [18,19], OVX combined with hindlimb unloading [20], and with GCs treatment, have been applied to create OP models. While increasing researches preferred to combining OVX and GCs treatment due to the strengths. On the one hand, combination of OVX and GCs treatment rapidly and severely induced significant bone loss [21–23]. On the other hand, bone loss continues after GC treatment cessation [24,25], without rebound like that caused by GC intervention alone [26].

Altogether, OVX is a canonical means to produce PMOP model, whereas it cannot induce sufficient bone loss alone in common model animals such as rabbits and sheep. GC treatment can notably decreases cancellous bone in humans and large animals, but not consistently in rodents [27]. Moreover, GC-induced osteoporosis might show a reversal of bone loss after GC cessation. Compared with OVX or GC intervention alone, combination of them showed more potency in inducing OP and no reversal.

3. Creation of GI-PMOP model

3.1. Animals

Rodents, rabbits, sheep, goats, dogs, pigs and primates are familiar animals utilized to establish models for OP study. They have quite a few merits and respective characteristics in application. However, goats, dogs, pigs, and primates are not preferential in OP-related research due to their inherent shortcomings including high cost, inconvenience to feed and care, anatomical or physiological difference from human, etc. In contrast, rats, rabbits and sheep are most commonly used for OP models, particularly for GI-PMOP model. Therefore we focus on depicting characteristics of these three animals.

3.1.1. Rat

The satisfactory reproducibility is paramount in choosing the most proper animal model for the disease study. First, in adult humans, the prevailing activity of bone includes modeling and...
remodeling. In rat skeleton, remodeling follows modeling after certain time of “transition” in different sites including cancellous of lumbar vertebrae and proximal tibial metaphysis, and endocortical bone of lumbar vertebrae and of proximal tibial metaphysis [28,29]. This indicates discrepancies may exist among different ages and different bone sites in response to ovariectomy [30,31]. Therefore the appropriate bone site and age of this animal, with remodeling being the predominant activity, should be selected. Second, ovariectomized rats cherish pathological similarities with postmenopausal women in bone loss: bone resorption exceeding bone formation, enhanced bone resorption in endosteum and bone formation in periosteum [32] thus resulting in enlargement of marrow cavity, low calcium absorption in intestines etc. Moreover, rats resemble humans in responding to application of estrogen, parathormone and bisphosphonate [33,35]. These contribute to the satisfying reproducibility of rat models. Meanwhile, there are drawbacks such as the short period of remodeling and incapability of achieving truly skeletal maturity.

3.1.2. Rabbit

Adult rabbits have Haversian system, which is crucial for strength maintenance of cortical bone and enables the observation for bone mass change in cortical bone. Due to the high turnover rate with predominant remodeling over the modeling process, rabbit OP model is useful for anabolic agent investigation [36]. Moreover, rabbits are affordable and convenient to feed [37]. Rabbits aged six- to eight-month (sexually mature) were often chosen for GI-PMOP because calcification and closure of epiphysis plates occurs shortly after sexual maturity. Compared to rats, rabbits can provide more abundant serum samples and are more suitable for study on osteoporotic fracture treatment owing to the relatively big bone size. Despite these advantages, rabbits have relatively less cancellous bone resulting in inconvenience for bone densitometry.

3.1.3. Sheep

Sheep, as large animal with great docility, have long lifespan and can provide abundant samples such as blood and bone tissues. Due to their adequate bone size, sheep have become a favored experimental animal for study on biomaterials or medical devices [38], satisfying the increasing demand for an appropriate large animal model for research on treatment of osteoporotic fracture which is a clinical challenge because of the complicated fixation of the fracture and implants by the poor bone quality [39]. However sheep have the major drawback fluctuant bone mineral density (BMD), which varies in different seasons with the lowest value in winter. Limited by the long induction period up to 6 months and high cost, sheep has become less used as the GI-PMOP model.

Rats, rabbits, and sheep have their respective characteristics (as Table 1) and have been used for the study on GI-PMOP. OP is successfully induced in them by GCs treatment combined with OVX. Sheep are especially suitable for study on treatment of osteoporotic fracture which involves not only the medical treatment but surgical intervention. Other factors like the high cost and time-consumption (even though treated with GCs combined with OVX) make sheep used less. Therefore, more animals are necessary in accordance with the U.S. Food and Drug Administration Agency (FDA) guidelines that new anti-osteoporosis agents must be tested preclinically at least in two animal species [40]. Fortunately, several other animals had been proved promising in mimicking OP in human. For example, zebra fish, the animal possessing high genetic homology with human demonstrated well similarity to human GIOP in response to bisphosphonates treatment and proved significant for pathway dissection through genetic knockdown and overexpression studies [41]. Additionally, mice have been extensively utilized for GIOP study due to their advantages such as early sexual maturity, early senility and high sensitivity to GCs. However, no report seems to be available about zebra fish or mice model for GI-PMOP to the best of our knowledge.

3.2. Experimental protocol

In general, OVX was prior to GC treatment. After being anesthetized by intraperitoneal injection of 10% chloral hydrate or 3% pentobarbital sodium, or combination of ketamine and xylazine (also by intravenous), the animal underwent laparotomy and following bilateral ovariectomy. As usual, two or three weeks later, GC exposure begins. Dexamethasone and methylprednisolone are the most common GCs applied. The administration dosage and frequency vary between different types of GCs, and subcutaneous injection is the most common used route of administration.

In ovariectomized rats, dexamethasone at a dose of 0.3 mg/kg injected once every 2 weeks for at least 1 month caused significant decrease in BMD in vertebral, while for 3 months in pelvis BMD [42]. With the same dose and interval, dexamethasone induced significant BMD decline of femur after 1-month treatment and tibia after 3-month treatment [43]. In ovariectomized rabbits, daily intervention of methylprednisolone at dose of 1 mg/kg for 4 weeks was able to induced significant BMD decline of lumbar spine, whereas either OVX or methylprednisolone intervention alone but in the same way showed no notable BMD decline [16,22,44]. While in ovariectomized sheep, a much longer period up to 6 months was taken to induce marked bone loss or trabecula attenuation [45]. Based on these data, administration methods of GCs are recommend as listed below (Table 2).

3.3. Methods to evaluate bone mass

Parameters of bone mass, microarchitecture, biomechanics and metabolism are to be acquired, generally through methods similar with those used in humans for better understanding of the osteopenic status and bone metabolism.

3.3.1. Noninvasive methods

3.3.1.1. Densitometry. Dual-energy X-ray absorptiometry (DXA), considered as the gold standard for diagnosis of OP [46] is currently most commonly used to measure total and local BMD and bone mineral content (BMC) of humans and animals. However, the BMD measurement is affected by some factors such as bone

<table>
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<th>Table 1</th>
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<td>Characteristics of the three animal used currently for GI-PMOP model.</td>
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<tr>
<td>Advantages</td>
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<td>Rat</td>
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<td>Rabbit</td>
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<td>Sheep</td>
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degeneration, injury, ectopic calcification, inhomogeneous distribution of fat in extraosseous soft tissue and variable content of adipose in marrow [47], leading to measurement deviation of some degrees. Additionally, since BMD is responsible for 50% ~ 70% of total bone strength, and BMD alone does not completely account for bone quality [48], the value acquired from DXA may not exactly indicate the osteoporotic or osteopenic status in animals. Therefore, other parameters like microstructure need to be known, encouraging the introduction of other diagnostic techniques as supplement.

QCT is capable of measuring BMD of not only cancellous bone, but also cortical bone. QCT with the accuracy of 97% ~ 98% can precisely measure BMD of specific bone site and is the only tool to separately quantify the BMD of cancellous and cortical bone at present. However, like DXA, influenced by the varying adipose content with time in vertebrae, QCT tends to presents a lower BMD value than the real. Moreover, peripheral quantitative computed tomography (pQCT) which is applicable for the measurement of BMD both at spine and tibia [45] possesses an even higher accuracy and low radiation.

Overall, imaging techniques including DXA, QCT, and pQCT are successfully employed to determine bone quantity, with discrepant traits as presented in Table 3.

### 3.3.2. Invasive methods

#### 3.3.2.1. Histomorphometry

Bone histomorphometry provides images with the most high resolution compared to above-mentioned imaging techniques. Abundant parameters mainly including static parameters and dynamic parameters are tested by bone histomorphometry. Static parameters depicting the characteristics of bone architecture at certain time point includes trabecular number, trabecular thickness, trabecular separation, osteoblast or osteoclast number, osteoblast or osteoclast surface/bone surface, bone volume/tissue volume and so on. Dynamic parameters, like mineralization apposition rate and mineralization lag time, reveal the bone changes during a period and explain the change of static parameters through presenting the mineralization rate in bone surface. Combining two groups of parameters, a comprehensive knowledge on characteristic of bone is acquired.

<table>
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<th>Strengths</th>
<th>Limitations</th>
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<td>DXA</td>
<td>Vulnerability to be affected by some factors</td>
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<tr>
<td>QCT</td>
<td>High cost, high radiation, influenced by adipose</td>
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<td>pQCT</td>
<td>High cost</td>
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3.3.2.2. Biomechanics evaluation. Since bone quality is not solely associated with bone mass, biomechanics test is indispensable for direct evaluation of bone quality. Parameters including strength, stiffness, energy absorption capacity, compressive displacement, torque are gained to quantify bone mechanical property. In addition, QUS may indirectly reflect the bone strength according to the broadband ultrasound attenuation and speed of sound. Quantitative ultrasound (QUS) could be modified for biomechanics evaluation in animal.

3.3.2.3. Other measurement. PET/CT uniting metabolism imaging and anatomy imaging [61] displays high sensibility for osteopathy and great capacity to quantitatively analyze and compare those two images [62,63] with the developing agent 18 F-NaF. The scanner technically for rodent experiments with high resolution and sensibility [64,65] μPET/CT helps to acquire comprehensive information on characteristics of animal. In active bone metabolism region, plenty of 18 F-F-18 is taken in and interacts with hydroxyapatite producing fluorapatite as a result of exchanging with hydroxyl. Ingestion of 18 F-F-18 in skeleton is positively correlated with increase in bone blood flow and bone surfaces, as well as the activity of bone metabolism [66]. Therefore the imaging intensity of the osteopenic region by O VX in microPET/CT may be negatively correlated with BMD of that, and microPET/CT serves as an alternative tool for evaluation of osteopenia accompanied by high bone turnover like that induced by O VX or GC.

4. The underlying mechanism of OP induced by GCs in O VX animals

Although great efforts have been devoted to the investigation of OP induced by menopause, GC exposure or both, the precise underlying mechanism has not been delineated. And few studies have explored the underlying mechanism of GI-PMOP, which has been preliminary disclosed in our previous study, where sclerostin and CT S K, the key regulators of bone resorption was up-regulated in the O VX-DEXA group rats compared with the DEXA and O VX groups rats [23].

Based on those reports, we conclude that the vital signaling pathway including the canonical Wnt signaling pathway and RANKL/RANK pathway signaling play a critical role in the development of GI-PMOP. Osteogenic related, adipogenic related factors, and miRNA homeostasis which affects bone metabolism are involved. Besides, effect of GC on serum estrogen level may also contribute to GI-PMOP.

4.1. Regulation of vital signaling pathway of bone formation and resorption by estrogen and GC

4.1.1. Wnt/β-catenin signaling pathway

Wnt signaling is a ubiquitous system for intercellular communication [67]. However Wnt signaling plays its master role in bone development and homeostasis [68,69]. Wnt proteins bind to a frizzled receptor and a LRP co-receptor, activating the canonical Wnt signaling pathway. These receptors transduce a signal to scaffolding protein Dishevelled (Dsh), then activate the complex Axin-Adenomatous Polyposis Coli (APC)-glycogen synthase kinase-3β (GSK-3β), resulting in the excitation of intracellular signaling pathway.

Estrogen is a crucial regulator of bone metabolism that promoted osteoclastic differentiation in vitro [70,71], and its deficiency caused enhanced bone resorption leading to PMOP [72] through regulating factors in Wnt/β-catenin signaling pathway. Expression of the transcriptional regulator β-catenin was upregulated due to phosphorylations of glycogen synthase kinase 3β (GSK-3β, a cytosolic Wnt signaling inhibitor) at serine 9 induced by 17β-estradiol (E2), and was induced to accumulate in nucleus, leading to association with T-cell-specific transcription factor/lymphoid enhancer binding factor (TCF/LEF) transcription factors and the activation of a specific program of gene expression (like c-myc and cyclin D1) [73,74]. In vitro E2 stimulated the expression of Fhl1 that promoted MC3T3-E1 mineralization as a downstream molecule of the Wnt/β-catenin pathway [75]. Sclerostin is primarily detected in mature osteocytes embedded within the mineralized matrix in mouse and human bone [76,77]. As an inhibitor it blocked the Wnt/β-catenin signaling [78,79] by competitively binding to LRP5/6 [78].

However, sclerostin expression has been verified indirectly down-regulated by estrogen probably attributed to suppression of myocyte enhancer factor 2 (MEF2, a family of transcription factors expressed not solely in muscle lineages but also in adult rats [80]), which mediated inhibitory effect of PTH on SOST [80], or via interaction with BMP2 signaling since BMP2 potentially targets sclerostin gene [81].

In contrast, dexamethasone profoundly downregulated the mRNA expression levels of Wnt, β-catenin and LRP5, nevertheless upregulated the mRNA expression levels of sclerostin and Dkk-1 (Dkk1, a secreted Wnt inhibitor through interaction with LRP5) [82]. Besides, dexamethasone activates GSK-3β [83,24,97], thus inhibiting β-catenin nuclear translocation due to enhanced phosphorylation of β-catenin by GSK-3β.

Therefore, estrogen deprivation (E-D) and GC treatment may cooperate to affect phosphorylation of GSK-3β thereby down-regulating expression and compromising nuclear translocation of β-catenin, and upregulate sclerostin expression directly or indirectly (Fig. 1).

4.1.2. RANKL/RANK signaling pathway

RANKL/RANK signaling plays as a major regulatory system in the regulation of bone resorption [84]. Synthesized by the osteoblastic lineage cells, the immune cells, and some cancer cells, RANKL binds to the osteoclasts surface receptor, RANK, which transduces the signal to transcription factors c-Fos and tumor necrosis factor-receptor-associated factor6 (TRAF6) [85], triggering this critical signaling pathway and starting bone resorption.

Fig. 1. The Wnt/β-catenin pathway and its regulation by the synergic effect of GC and E-D.
through osteoclastogenesis and the activation of multinucleated mature osteoclasts [86].

In addition to involvement in the canonical Wnt signaling, sclerostin exerts influence on RANKL/RANK signaling as it stimulates the secretion of osteocyte-derived RANKL, thus activating osteoclast activity [87,88]. Since suppressed expression of sclerostin by estrogen reduces osteoclastogenesis, estrogen deficiency increases osteoclastogenesis and bone resorption. Moreover, osteoclast differentiation can be induced by the overexpressed c-Fos [89], which acts as an essential inducer of another transcription factor NFATc1, the major regulator of osteoclast differentiation [90]. However, this key regulatory gene in RANKL/RANK signaling pathway was down-regulated by E2 through modulation of beta3-integrin [91]. Although suppression of bone formation is a central feature in GIOP pathogenesis, the early and transient phase of enhanced bone resorption (the mechanism remains indefinite) had been also observed probably due to the increased expression of macrophage colony stimulating factor (M-CSF) and RANKL, but decreased expression of osteoprotegerin (OPG) which binds with RANKL preventing osteoclastogenesis, by GCs [92]. Consequently, RANKL secretion is raised resulting from the upregulated sclerostin expression by GCs in synergy with estrogen deficiency (Fig. 2). Sclerostin is a central factors affected by GC and estrogen, and serves as a crucial regulator in both RANKL/RANK signaling and Wnt/β-catenin signaling pathway, suggesting sclerostin inhibition may be a novel and promising therapeutic strategy for severe OP [93].

Other pathways such as PTH pathway, BMP/Smad pathway, and downstream pathways of RANKL/RANK like NF-κB pathway, MAPK pathway and P13-K/Akt pathway play important roles in the regulation of bone metabolism, providing more points of view for exploration of the underlying mechanism of GC-PMOP.

4.2. Effect of GC on osteogenic related and adipogenic related factors

Bone marrow stromal cell (BMSC) enjoys multiple differentiation potential and differentiates into certain lineages under rigorous control by several transcription factors. It is worth mentioning that there is a balance between adipogenesis and osteogenesis of BMSCs. Runt-related transcription factor 2 (RUNX2) and osteirix (Osx) are two major regulators of osteoblastogenesis [94,95]. As osteirix’s upstream controller [95], Runx2 also regulates some bone matrix protein genes, such as ColIα1, osteocalcin and osteoponin [96]. The zinc finger-containing transcription factor Osx is critical for the maturation, morphology as well as function of osteocytes [97]. However, both of these two transcription factors are downregulated by GC [82]. CCAAT enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) act as the triggers in regard to adipogenic differentiation [98] and prevent osteoblast differentiation [99]. In osteogenesis and adipogenesis, PPARγ binds the −1286 bp/−1065 bp region of the C/EBPα promoter to active its expression [100]. Overexpression of C/EBPα prevents osteoblastogenesis and DNA hypermethylation inhibits its expression. DEXA promoted C/EBPα expression by inhibiting DNA methylation of its promoter, thus shifting the differentiation potential of BMSCs to favor adipocytes over osteoblasts [101]. Intriguingly, PPARγ and CREB pathways were activated examined by mRNA microarray in skeletal tissues in ovariectomized mice [102]. Other factors like insulin-like growth factor-1 (IGF-1), a polypeptide that promotes bone formation is decreased by GCs [103].

4.3. Influence of GC on the homeostasis of microRNAs (miRNAs)

Non-coding RNAs (ncRNAs) that encompasses microRNAs (miRNAs) and long non-coding RNAs (LncRNAs) are emerging as key players in regulation of many cellular processes in both physiological and pathological condition [104]. MiRNAs regulate the cellular processes through the post-transcriptional regulation of gene expression homeostasis. Accumulating evidence suggests that microRNAs (miRNAs) possibly participates in regulating bone mass. As was delineated, miRNAs regulated bone formation or resorption mainly through targeting relevant factors of several signaling pathways related with bone metabolism like Wnt/β-catenin signaling pathway [105–107], OPG/RANKL/RANK signaling pathway [108,109], the noncanonical Wnt pathway [110], BMP/Smad signalling [111], and TGF-β/activin signaling [112]. MiR-199a-5p inhibits Wnt2 expression through binding a specific site within the 3′-UTR of Wnt2 and was remarkably increased in osteoblasts treated with DEXA, indicative of the important role in GC-inhibited osteoblast proliferation by regulating the Wnt signaling pathway [113]. In Shi’s study, in co-culture with osteoblast over-expressed microRNA-17/20a and osteoblast progenitors, DEXA-induced osteoblast differentiation and function through elevating RANKL level were markedly attenuated [114]. This implies DEXA may negatively regulate microRNA-17/20a. Moreover, the expression of miR-365 that regulates matrix metalloprotein 9 (MMP9) was downregulated by DEXA in vivo [115]. However, relationship between these miRNAs and estrogen level has not yet been invested or revealed to the best of our knowledge.

Another major member of nRNA long non-coding RNAs (LncRNAs) are acknowledged to participate in the osteoblast differentiation [116], and may act as competitive endogenous RNAs (ceRNAs) to decrease the amount of miRNAs available to target messenger RNAs (mRNAs) [117]. In recent studies several ceRNAs have been recognized to play roles in bone disorders [118,119]. Moreover, circular RNAs (circRNAs) that form through ‘back-splicing’ where a downstream splice donor links to an upstream splice acceptor may play roles in certain human diseases [120]. However, the study of complex functions of these RNAs in development of OP is still in its very early stages.

![Fig. 2. The RANKL/RANK signaling pathway and its regulation by the synergetic effect of GC and E-D.](Image)

Z. Zhang et al. / Biomedicine & Pharmacotherapy 84 (2016) 438–446

443
4.4. Effect of GC on estrogen level

Suppression of GC on serum estrogen has been observed. GC up-regulates expression of gonadotropin-inhibitory hormone (GnIH) gene by recruitment of glucocorticoid receptor (GR) to its promoter, thus lowering gonadotropin level due to the increased production of GnIH which inhibits gonadotropin [121]. Subsequent lowered sex hormone including estrogen exerting indirect inhibitory effect on osteoclast formation, and testosterone directly inhibiting osteoclast formation both through stimulating production of osteoprotegerin (OPG) respectively by estrogen receptor- and androgen receptor-mediated mechanisms, together exacerbate bone loss [122]. In addition, on the one hand, the secretion of follicle-stimulating hormone (FSH) which enhances osteoclastogenesis through its receptors at osteoclast and its precursors [123], increases in low estrogen status as a result of negative feedback. RUNX2, as a transcription factor plays a major role in osteoblast differentiation and maturation. Besides, RUNX2 can also stimulate the expression of aromatase, an estrogen-producing enzyme, causing the increased level of estrogen [124]. Therefore, under GCs intervention estrogen level futher descends due to down-regulated expression of RUNX2. This may account for the result of our previous study [23], where lower estradiol in OVX + DEXA group than that in DEXA group or in OVX group was demonstrated, though the exact molecular mechanism has not been unidentified.

Combining OVX with GCs treatment is a favored and popular means not only to cause severe OP rapidly, but also mimics GI-PMOP in humans with good reproducibility by establishing a suitable model for GI-PMOP research. Rat, rabbit and sheep have been used for simulating GI-PMOP. And sheep becomes less employed recently due to limitation of time consumption and high cost, thus other animals like mouse, zebra fish and so on are promising in GI-PMOP study. Invasive and noninvasive techniques are applied for direct or indirect evaluation of bone quantity. The pathogenesis of this severe OP is intricate and more efforts still need to be devoted to elucidating it for discovery of new therapeutic targets for precision treatment.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors contributions

Zhida Zhang and Hui Ren outlined the manuscript. Gengyang Shen, Ting Qiu, Jingjing Tang and Qishui Wei search for the related articles. Zhida Zhang scanned the literature, read all references and wrote the first draft. Xiaobing Jiang went through the whole text to correct logic errors between sentences or between paragraphs. De Liang, Zhidong Yang and Zhensong Yao discussed and revised the manuscript.

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