Adverse effects of the cancer therapy on osteoclast-mediated bone loss in patients with cancers: a challenge

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Abstract
It is well-known that cancer therapies, including chemotherapy drugs, aromatase inhibitors and gonadotropin-releasing hormone analogues, commonly contribute to remarkably reduce the bone mineral density, subsequently increase the rate of bone loss. For instance, in the patients with prostate cancer treated with androgen deprivation therapy (ADT), or chemotherapy drugs such as doxorubicin and cisplatin the calcium levels were significantly decreased in the body, thereby leading to bone loss. Besides, the aromatase inhibitors widely used to treat breast cancer, and antiresorptive agents targeting the receptor activator of nuclear factor κB ligand can also trigger bone loss. Osteoclasts (OCs), derived from monocyte/macrophage lineage, are deifferentiated into mature, multinucleated OCs (a process also known as osteoclastogenesis) via a series of osteoclastogenic signaling pathways, are responsible for resorbing bone. This review article characterizes and summarizes the adverse effects of chemotherapy drugs on accelerating OC-induced bone defects such as the increased bone resorption and the impaired bone mineral density (BMD) in the patients with cancers.

Key words chemotherapy drugs, osteoclastogenesis, bone loss, osteoporosis
Introduction

Bone loss in cancer patients are either resulted from a direct effects of cancer on their bones or from off-target effects of cancer therapies applied. Basically, bone loss is commonly found in the patients with breast or prostate cancers, who face a greater risk for osteoporosis, a skeletal-metabolic disease, characterized by low bone mineral density (BMD) and deterioration of the bone microarchitecture [1]. Etiologically, osteoporotic bone breaks are usually found in the hip, spine and/or wrist. To date, many cancer therapies and treatments lead to a severe loss of bone density including (1) aromatase inhibitors (anastrozole, letrozole and exemestane), (2) androgen deprivation therapy, (3) chemotherapies [doxorubicin (Adriamycin), methotrexate (Trexall), cyclophosphamide (Cytoxan) and 5-fluorouracil] and (4) corticosteroids [2].

Bone remodeling is stringently regulated by osteoblasts (OBs) and osteoclasts (OCs), which are responsible for bone formation and bone resorption, respectively. OC progenitors, derived from monocyte/macrophage lineage, are differentiated into mature, multinucleated OCs via a series of osteoclastogenic signaling pathways [3, 4]. In principle, OC differentiation and maturation, also known as osteoclastogenesis, are primarily induced by the binding of two critical cytokines, including (1) the receptor of nuclear factor kappa-B ligand (RANKL) to its specific RANK receptor and (2) the macrophage colony-stimulating factor (M-CSF) to c-fms receptor in cell surface of OC progenitors [5, 6]. RANKL/RANK signaling triggers the commitment of monocyte/macrophage precursors to the OC lineage and subsequently mature, multinucleated OCs via predominantly activating six downstream signaling cascades comprising (1) the nuclear factor of activated T cells cytoplasmic-1 (NFATc-1); (2) nuclear factor kappa B (NF-kB); (3) phosphatidylinositol 3-kinase (PI3K/Akt); (4) Jun N-terminal kinase (JNK); (5) extracellular signal-regulated kinase (Erk); and (6) p38 mitogen-activated protein kinase (MAPK) [7-9]. Nevertheless, the precise molecular mechanisms underlying the activation of these signaling pathways remains disputable. In addition to RANKL/RANK signaling axis itself, accumulating evidence illuminates that interferon (IFN) γ, IFN-β and immunoreceptor tyrosine-based activation motif (ITAM) play the specific roles in regulating osteoclastogenesis by cross-talking with one or more RANKL/RANK signaling pathways [10]. In bone, OBs and bone marrow stromal cells serve as primary sources of RANKL, M-CSF and osteoprotegerin (OPG). OPG functions as a decoy for RANKL, therefore inhibiting the binding of RANKL to RANK receptor, thereby alleviating osteoclastogenesis [11, 12]. Indeed, in vivo studies substantiated that mice deficient for the gene encoding either RANKL or RANK receptor developed osteopetrosis (a genetically etiological state of elevated bone mass) owing to the impaired OC formation [13]. Moreover, OPG-deficient mice developed early onset of osteoporosis [14] whereas transgenic mice overexpressing OPG exhibited osteopetrosis in mice [15]. In this review, I have struggled to summarize the published papers revealing the effects of cancer therapies on regulating bone cell-induced bone loss in the patients with cancers.

Aromatase inhibitors

Aromatase is a cytochrome P450 enzyme complex catalyzing the conversion of androgens to oestrogens is abundantly present in the ovaries of pre-menopausal women, placenta, fat and connective tissue both in men and in pre- and post-menopausal women [16]. Strikingly, high concentration of the enzyme complex was found in normal breast tissue as well as in breast cancer cells [16]. Increasing evidence suggests that oestrogen plays a pro-proliferative role in tumorigenesis, especially in estrogen receptor-positive (ER+) breast cancer after menopause [17]. Nonetheless, using aromatase inhibitors efficaciously abolished oestrogen production, but not modulated the turnover of its receptors in tumor cells. Notably, the serum level of oestrogen decreases by ~90% at the menopause, leading to a net bone loss and a greater risk of bone fractures. In post-menopausal women, aromatase inhibitors including anastrozole, letrozole and exemestane significantly reduced the serum levels of oestrogen by 81-94%, 88-98% and 52-72%, respectively [18].

Bone remodeling is tightly regulated by specialized cells including OBs, OCs and osteocytes. Oestrogen plays an important role in maintaining bone mass in adult women by suppressing bone remodeling and maintaining a balance between osteoblast and osteoclastic activities. Oestrogen alleviates the osteoblastic production of resorptive cytokines, including RANKL, M-CSF and tumor necrosis factor, and at the same time increases the production of anti-receptor cytokines (mainly osteoprotegerin) [11]. This leads to increased osteoclastic apoptosis and increased osteoblast activity. However, when the oestrogen levels are deficient, there is an increase in remodeling imbalance, resulting in osteoclastic activity, subsequently leading to deeper resorption spaces [19]. There is also some evidence that the ability of the OBs to refill these spaces may be impaired. Moreover, the deeper resorption spaces result in perforation of trabecular plates and loss of architectural elements, further weakening the skeleton in regions such as the vertebrae and distal forearm which contain large amounts of cancellous bone. These abnormalities in postmenopausal women appear to be more severe in women with postmenopausal osteoporosis [20]. Furthermore, the intestinal absorption and the renal re-absorption of calcium are also diminished. This triggers a rise in the serum-parathyroid hormone that activates OCs, thereby ruling in an increase in a net bone loss. Besides, a recent study has reported that aromatase inhibitor (exemestane) had a stimulatory effect on osteoblastic activity in human OB-like cells expressing aromatase genes [21]. On the contrary, whether aromatase inhibitors regulate osteoclastic activity in vitro is completely unknown.

In ovariectomized rats and mice, it was found that the aromatase inhibitors increased bone mass, and bone strength [22]. However, clinical data showed that aromatase inhibitors increased bone resorption and decreased BMD at the hip and lumbar spine, suggesting that it is likely these inhibitors potentially increase the risk of bone fractures. More crucially, from these data, it is suggested that there are remarkable differences in bone metabolism between rats and human. Tamoxifen, also named nolvadex or soltamox, a selective estrogen receptor modulator (SERM), used to trat all stages of hormone receptor-positive breast cancer in women and men, had a positive effect on BMD, but not linearly reduce the risk of bone fractures [23]. Nevertheless, aromatase inhibitors (letrozole, anastrozole and exemestane) had negative effects on BMD and enhanced the risk of bone fractures in the patients with breast cancers. Interestingly, previous studies have shown some drugs such as cathepsin K antagonists and RANKL antibodies (denusomab), might be of potential interest in aromatase inhibitor-induced osteoporosis in the patients with the post-menopausal osteoporosis.

Androgen deprivation therapy (ADT)

Recent studies have apparently demonstrated that OCs are the direct target for estrogen. Specifically, OC-specific deprivation of estrogen receptor (ER) alpha (ERα) triggered a decrease in trabecular bone mass [24]. This was, at least in part, owing to an increase in OC lifespan as a result of diminished OC apoptosis. In addition to estrogen-mediated induction of OC apoptosis, estrogen markedly blocks RANKL/M-CSF-induced activator protein-1-
dependent transcription, via a alleviation of c-jun transcriptional activity (reduces c-jun expression and decreases phosphorylation), and suppresses RANKL-induced OC differentiation [25]. Notably, estrogen declines ER alpha binding to a scaffolding protein, BCAR1; the ERα/BCAR1 complex then sequesters TNF receptor associated factor 6 (TRAF6), leading to inhibit the activation of NFκB and weaken RANKL-induced osteoclastogenesis [26]. Furthermore, estrogen also appears to indirectly regulate OC formation and activity. In combination of the in vitro and in vivo studies, it is suggested that estrogen suppresses RANKL production by osteoblastic, T- and B-cells and also promotes the production of osteoprotegerin (OPG), the decoy receptor for RANKL [27]. In mouse model, estrogen was shown to regulate the production of various bone-resorbing cytokines such as, for instance, interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), macrophage-colony stimulating factor (M-CSF), and prostaglandins. Combined together, it is demonstrated that estrogen play a multiple role in regulating OC differentiation, formation and activity either via the direct effects mediated by ERs or via the indirect effects initiated by OBs and T cells.

ADT is considered to be the primary or adjuvant treatment for non-metastatic prostate cancer [28]. Owing to decificiency of adrogens and estrogens that are crucial for bone remodeling and maintenance, the skeleton is virtually compromised in the male patients with prostate cancer during ADT. In fact, the ADT-treated patients sustained variable degrees of bone loss with an enhanced risk of fragility fractures [29]. Mechanistically, it was because of a remodeling imbalance between osteoclastic and osteoblastic activities wherein OCs resorbed the deeper resorption bone spaces. Besides, it was feasible that OBs were incapable of refilling these OC-resorbed bone spaces. It was reported that male patients undergoing ADT are four time more likely to develop considerable bone loss [30]. A previous study of prostate cancer patients who survived more than 5 years after diagnosis of prostate cancer, 19.4% of those receiving ADT had a higher tendency to develop the fragility fracture [30]. Consequently, bone health management in the patients with prostate cancer receiving ADT is urgently required to control bone loss-associated fractures. The antiresorptive drugs that weakened osteoclastic bone destruction were tested in controlled randomized patient groups receiving ADT. For example, the oral bisphosphonates, pyrophosphate analogs (alendronate and risedronate) and intravenous bisphosphonates (pamidronate and zoledronic acid) suppress bone turnover via interfering with the internal enzymatic system and disrupting the cytoskeleton of OCs, thereby triggering OC apoptosis, stabilizing bone mass and reducing the risk of fragility fracture in the osteoporosis patients, in general, and the patients with prostate cancer receiving ADT, in particular [31]. Accordingly, bisphosphonates have become the primary therapy for managing skeletal conditions characterized by increased OC-

![Figure 1. Cancer therapies causes the OC-induced bone loss in the patients with cancers. The chemotherapeutic agents such as DOX, MTX and 5-FU, and ADT commonly trigger the activation of TGF/Smad signaling pathway, which is crucial for stimulating osteoclastogenesis. Besides, the levels of RANKL and M-CSF are elevated, accelerating OC differentiation and maturation. Besides, these chemotherapeutic drugs also promoted the production of T cell-secreted cytokines such as IL1, IL6 and TNFα, which lead to osteoclastogenesis, thereby triggering bone loss in the patients with cancer.](image_url)
mediated bone resorption.

Chemotherapy drugs

Albeit chemotherapy drugs have been widely used to treat the patients with cancer in order to promote survivorship, it also trigger the severe bone-related adverse effects, consisting of arrested bone growth in patients with pediatric cancer and significant decrease in BMD in both pediatric and adult patients with cancer after chemotherapeutic treatments. In the animal model-based experiments of chemotherapy-induced bone defects, it was shown that single chemotherapeutic agents commonly reduced bone mass and disrupt trabecular bone architecture, leading to alleviated bone mechanical strength. To date, a variety of chemotherapy drugs have been discovered, and clinically used in the treatment for the patients with cancer; in this review, I briefly list and describe several widely used chemotherapeutic agents that have severe effects on OC-induced bone loss in the patients with cancers:

**Methodrexate (MTX)**

A commonly used anti-metabolite, causes a significant reduction of BMD [32]. In chemotherapeutic mechanism, MTX inhibits RNA/DNA synthesis via weakening the dihydrofolate reductase. After MTX treatment, bone formation is attenuated, and bone loss is elevated. These outcomes have been associated with several specific factors such as, for example, (1) abolishing circulating levels of vitamin D3, (2) depressed of OB precursor pool within bone marrow, and (3) varied response of bone cells towards mechanical loading [33]. Though these studies indicated BMD is impaired as a major result of a decrease in total bone synthesis, increasing evidence, however, it was suggested that impaired BMD was also cause by enhanced OC-mediated bone resorption. Indeed, it was reported that an increase in the density of OC precursors and an elevation of OC maturation in bone marrow in rats after short-term MTX treatment [34]. Furthermore, prolonged administration of MTX at a low dose resulted in osteopenia, linked to enhanced OC activity and recruitment [35]. Together, these studies indicate that the major reason leading to bone loss after MTX chemotherapy originates from increased OC differentiation, maturation and bone-resorbing activity.

**Doxorubicin (DOX)**

An anthracycline drug originally isolated from the fungus Streptomyces peucetius. This drug has been clinically used to treat a variety of cancers, comprising breast cancer, bladder cancer, lymphoma, and acute lymphocytic leukemia. DOX has been reported to inhibit topoisomerase II and induce cell death by generating reactive oxygen species (ROS) [36]. In spite of its role as a chemotherapeutic agent, emerging proof has demonstrated its side effects on bone physiology. For instance, DOX-treated breast cancer patients exhibited low bone mineral density, and especially DOX-treated children underwent a long-term bone damage [37]. At the cellular level, DOX was reported to diminish cell division and differentiation derived from MC3T3 mouse [38]. Also, DOX was found to reduce trabecular bone volume and cortical bone thickness in rabbits, mice and rats. DOX treatment promoted the activation of TGFβ/Smad signaling pathways, which played a critical role in accelerating OC maturation, as well as suppressing OB differentiation [37]. Additionally, TGFβ may also regulate the physiological characteristics of bone via modulating bone mass and bone milieu. Indeed, other studies have demonstrated that blocking excess TGFβ in bone milieu, either by the specific antibodies or by small molecule inhibitors, promoted bone formation. Ultimately, increase in TGFβ level was associated with the generation of reactive oxidative species (ROS), which is also consistent with the fact that DOX increases oxidative stress.

**1,3-Dibenzyl-5-fluorouracil (5-FU)**

It and its oral produgs including S1 and capecitabine are the important components of most chemotherapeutic regimens of which efficiencies was reported in the treatment of the patients with various neoplasms such as, for instance, head and neck squamous cell carcinoma (SCC), gastrointestinal SCC and adenocarcinoma (ADC), breast cancer, stomach cancer, colon cancer, etc. 5-FU inhibits thymidylate synthase, an enzyme required for the

### Table 1. The summary of the drug conjugates currently used in combination with cancer therapies to treat the patients with cancers.

<table>
<thead>
<tr>
<th>Cancer Therapies</th>
<th>Types of cancers</th>
<th>Drug conjugates</th>
</tr>
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<tbody>
<tr>
<td>Aromatase</td>
<td>Breast cancer</td>
<td>Aromatase inhibitors (letrozole, anastrozole and exemestane) [22]</td>
</tr>
<tr>
<td>Androgen Deprivation Therapy (ADT)</td>
<td>Prostate cancer</td>
<td>Oral bisphosphonates, pyrophosphate analogs (alendronate and risedronate), intravenous bisphosphonates (pamidronate and zoledronic acid) [31]</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>Leukemia, osteosarcomas, bladder cancer, breast cancer, etc.</td>
<td>Alendrolate (ALN), zoledronic acid (ZA) are third-generation bisphosphonates [41]</td>
</tr>
<tr>
<td>Doxorubicin (DOX)</td>
<td>Leukemia, bone sarcoma, breast cancer, gastric cancer, head and neck cancer, liver cancer, etc.</td>
<td>Anti-TGFβ antibody [37], resveratrol [42], MitoTEMPO [42]</td>
</tr>
<tr>
<td>1,3-Dibenzyl-5-fluorouracil (5-FU)</td>
<td>Breast cancer, head and neck cancer, colon cancer, skin cancer, etc.</td>
<td>Emu oil (EO) [40], Complement nutritions (calcium and vitamin D), [43], bisphosphonate zoledronic acid (ZA) [44, 45], aromatase inhibitors [46]</td>
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synthesis of thymine nucleotide, an important component of DNA and RNA [39]. Mechanistically, 5-FU and other chemotherapeutic agents including cisplatin, etoposide, cyclophosphamide have been shown to cause severe osteopenia by promoting OC differentiation as well as suppressing OB formation [40]. Nonetheless, it was the fact that the underlying molecular mechanisms of chemotherapy-induced bone loss still remain largely unknown. Moreover, no safe and coss-effective treatments against chemotherapy-induced bone loss are available. As abovementioned, only the antiresorptive therapies using bisphosphonates are well documented to abolishe bone resorption, and remarkably elevate bone mass, and therefore possess some potential efficacies in reducing osteoporosis.

Though the mechanisms underlying the augmentation of OC-induced bone loss in the patients with cancers during the period of chemotherapeutic treatments is little known (Figure 1), it has a severe impact on bone metabolism and architecture. Altogether, it seems to be unavoidable that cancer therapies lead to OC-induced bone defects in the patients with cancers. It is, however, urgently required to develop the special programs of drug development that is conjugated with chemotherapeutic agents to treat cancer patients to alleviate OC-induced bone resorption and/or enhance OB-induced bone formation (Table 1), thereby maintaining bone metabolism at the physiological level in these patients. The followings are several drug conjugates, even limited, which possess the potential impact on abolishing OC-induced bone loss in the cancer patients during cancer therapies.

Conclusion

In this review, I briefly list and describes the off target effects of several commonly used chemotherapeutic drugs that alter bone physiology, and damage the bone architecture in the patients with cancer. Although well-documented, it is largely unknown how these drugs have a negative impact on bone metabolism, and little is known about the drugs that could be used in conjugation with chemotherapeutic agents in order to inhibit OC-induced bone resorption and/or enhance OB-induced bone formation, thereby reducing the severity of bone fractures in the patients with cancer.

Ethical policy
Not applicable.

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References


