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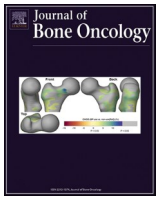

Role of the osteocyte in bone metastasis – The importance of networking

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HIGHLIGHTS

- Cancer effects on bone structure and the lacunar-canalicular network.
- Mechanical stimulation and osteocyte signalling networks in metastasis.
- Networking metastatic molecular crosstalk.
- Therapeutic strategies targeting osteocyte-cancer cell crosstalk.
- Major outstanding questions in the field of cancer-osteocyte interactions.

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ABSTRACT

Metastatic bone disease is a complex condition resulting from the migration and colonization of cancer cells from their primary site to the bone microenvironment, where they typically develop a metastatic niche. Osteocytes, the most abundant cells in bone tissue and the master regulators of bone remodelling, are increasingly thought to play a crucial role in this process through intricate interactions with cancer cells. This review covers the recent progress made in exploring the multifaceted interactions between osteocytes and cancer cells in the metastatic microenvironment, highlighting the importance of signalling networks in bone metastases. Though these interactions are particularly complex, the renewed focus of researchers on osteocytes within the last 5 years has uncovered multiple new potential molecular mechanisms underlying osteocyte-mediated regulation of cancer cell survival, proliferation, and invasion. A number of key papers will be discussed in detail, emphasizing the significance of signalling pathways and molecular crosstalk, and exploring potential therapeutic strategies targeting osteocyte-cancer cell interactions to improve patient treatment and outcomes.

1. Introduction

Bone metastases represent a significant clinical challenge in the management of various cancers, including breast, prostate, and lung. Multiple myeloma, a blood cancer that develops within cells of the haematopoietic lineage in the bone marrow, does not metastasise to bone, but induces similar bone degradation to other metastatic cancers. The establishment of tumour colonies in bone has a profound effect on the bone marrow microenvironment, disrupting normal balance of bone remodelling. The traditional paradigm to explain the success of disseminated tumour cells from such different primary tissues has been the generation of a "vicious cycle" between tumour cells and other cells within their local marrow microenvironment spurring tumour growth [1]. In bone-resorbing (lytic) lesions, typically found in breast cancer or multiple myeloma patients, osteoclast-promoting factors are secreted, e.g. macrophage colony stimulating factor (M−CSF), receptor activator of nuclear factor kappa-β ligand (RANKL), parathyroid hormone-related protein (PTHRP), interleukin 6 (IL-6), resulting in increased bone destruction, releasing tumour promoting growth factors sequestered in the bone matrix, further exacerbating the vicious cycle. In a similar manner, osteoblastic tumours typically presenting in metastatic prostate cancer or osteosarcoma patients can form large hard bony lesions, compromising bone integrity, with increased osteoblast formation releasing factors that promote cancer cell proliferation. However, this accepted understanding of tumour behaviour in bone does not generally consider the osteocyte.

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Osteocytes, the most abundant cells in bone making up more than >90 % of our bone cells, have recently emerged as key players in the metastatic cascade. Derived from bone-forming osteoblasts, osteocytes are embedded within the bone matrix and form an extensive network of linked dendritic processes, residing in lacunae and canaliculi, respectively. These cells are pivotal in regulating bone remodelling, maintaining bone strength, and sensing and responding to mechanical stimuli [2–4]. Long neglected in the field of bone oncology, recent endeavours by researchers have demonstrated multiple crosstalk mechanisms through which they may regulate the tumour microenvironment. A number of excellent reviews of have delved into the detail of the osteocyte's interactions in the microenvironment of various metastatic cancers affecting bone [5,6], as well as its potential role in the cellular origins of osteosarcoma [1]. These in-depth reviews are listed for further reading in Table 1, alongside a number of other articles that explain what is known about the vicious cycle [1,5–7], osteocyte biology and mechanobiology [2–4], the role of osteoblasts and osteoclasts in metastasis [8–11], and the bone remodelling cycle [12]. This perspective instead shines a spotlight on key recent findings in the last 5 years that underline emerging role of the osteocyte as the central cell in a complex network of signalling crosstalk in the metastatic microenvironment.

2. Cancer effects on bone structure and the lacunar-canalicular network

While bone tumours have been known to degrade nearby lacunar-canalicular architecture since the 1970s [13], a fascinating new study has characterised this in detail for the first time [14]. By inoculating mice with fluorescently tagged syngeneic breast and prostate cancer cell lines (Fig. 1A – D), the researchers observed disruption of the canalicular network as well as direct cancer cell-osteocyte contact clearly visible within the tissue [14]. This provides the strongest evidence yet that osteocytes, far from being distant from the action, can interact directly with cancer cells. Furthermore, they showed a significant increase in lacunar size within the bone tissue, regardless of whether the tumour was osteoblastic or osteolytic [14] (Fig. 1E and F). Coupled with the disruption seen in the canaliculi, this indicated that cancer cells can induce significant disruption of the bone structure and lacunar-canalicular network through signalling crosstalk (Fig. 2).

Recent *in vivo* work by the McNamara group has demonstrated that, even in early metastasis when tumour volume is not apparent in a mouse model and significant osteolysis cannot be detected, degeneration of bone structure and mechanical properties has already begun (Fig. 1G), with the team postulating that the osteocyte may play a role in this process [15].

Table 1
Further in-depth reviews of bone remodelling, the role of bone cells in metastatic bone disease, and osteocyte mechanobiology.

Review Paper Title	Author – Year
Skeletal remodeling in health and disease	Zaidi, 2007 [12]
The Amazing Osteocyte	Bonewald, 2011 [3]
Cancer to bone: A fatal attraction	Weilbaecher et al., 2011 [9]
Osteocytes: Master orchestrators of bone	Schaffler et al., 2014 [2]
Quantifying the osteocyte network in the human skeleton	Buenzli et al., 2015 [4]
Mechanisms of osteolytic and osteoblastic skeletal lesions	Roodman et al., 2015 [11]
The bone microenvironment: a fertile soil for tumor growth	Buenrostro et al., 2016 [8]
Osteocytes and Bone Metastasis	Riquelme et al., 2020 [5]
Osteocytes and Cancer	Pin et al., 2021 [1]
Mechanobiology of Bone Metastatic Cancer	Sarazin et al., 2021 [10]
Osteocytes: New Kids on the Block for Cancer in Bone Therapy	Anloague et al., 2023 [6]

Additional cancer-related disruption of the osteocyte network is known to occur in multiple myeloma *in vivo*, with more apoptotic osteocytes observed in multiple myeloma patients than in healthy patients [16]. Osteocyte apoptosis is strongly linked to downstream degradation of bone tissue, and this was confirmed in pre-clinical *in vivo* models of multiple myeloma, displaying increased osteocyte apoptosis and degeneration of tissue [17].

Perhaps the most startling example of the ability of cancer cells to cause bone degradation by disrupting osteocyte signalling was recently demonstrated by the Bonewald group when investigating non-bone metastases *in vivo*, tumours that had metastasised from a primary to a metastatic site, neither of which were bone. They found that these secondary tumours, despite no presence within bone tissue, could induce bone damage distant from the tumour sites [18]. In rodents bearing Colon-26 adenocarcinoma (C26), the ES-2 ovarian cancer (ES-2), and Lewis lung carcinoma (LLC), but which did not have any bone metastases, the group observed widespread osteocyte death and empty lacunae, as well as evidence of osteocytic osteolysis inducing damage from peri-lacunocanalicular remodelling (PLR) [18]. This may in part explain the development of osteoporosis in patients with non-bone tumours, though further research is required in order to unpick the underlying molecular mechanisms.

3. Mechanical stimulation and osteocyte signalling networks in metastasis

Given the key role played by the osteocyte in the exquisite ability of bone to adapt its structure in response to changing mechanical demands [2], it is unsurprising that researchers have probed the effect of mechano-signalling in cancer-osteocyte crosstalk. This is likely due to the complexity of the intracellular signalling networks, and thus recent *in vitro* studies have attempted to further unpick this relationship. There is much conflicting evidence in the literature on the effect of mechanical stimulation [1,10], with some studies finding that mechanical stimulation of osteocytes increases migration of breast cancer cells [19,20], and others showing decreased migration/invasion [21–23]. Recent advances in the use of *in vitro* studies to identify the likely mechanobiological molecular mechanisms involved are discussed in detail in this section.

Pioneering work by the You group showed using conditioned media experiments that mechanical stimulation of osteocytes can regulate the behaviour of breast cancer cells *in vitro* [24], both directly and via intermediate cells, such as endothelial cells [24]. Another recent *in vitro* study explored potential molecular mechanisms driving this behaviour, applying oscillatory fluid shear stress to osteocytes and finding that the resulting conditioned media enhance proliferation and migration of breast cancer cells with loading, identifying chemokine (C-X-C motif) ligand 1 and 2 (CXCL1 and CXCL2) as mediating the migration [19]. Comparatively less work has been carried out on prostate cancer bone metastases, relative to breast cancer, and therefore a welcome recent addition are *in vitro* experiments showing that shear stress stimulation of osteocytes decrease prostate cancer invasion without affecting proliferation [21].

A key challenge of investigating osteocyte mechanobiology in metastasis has been the need to selectively apply mechanical stimulation to osteocytes alone, which is not feasible in traditional co-culture experimental set-ups such as Boyden chambers. The development of microfluidic co-culture models, or organ-on-a-chip technology, provides an opportunity to achieve this, with the You group developing the first of these models [25], applying it to find that low-magnitude high-frequency vibrations can activate osteocyte signalling to reduce breast cancer extravasation [26]. Further work by the You group found that, while this vibration stimulation alone appears insufficient to reduce bone loss caused by breast cancer, activation of the mechano-sensitive Piezo1 channel, via treatment with Yoda1, significantly enhances the effect of the mechanical stimulation [27].

Additional *in vitro* organ-on-a-chip studies from our group applied

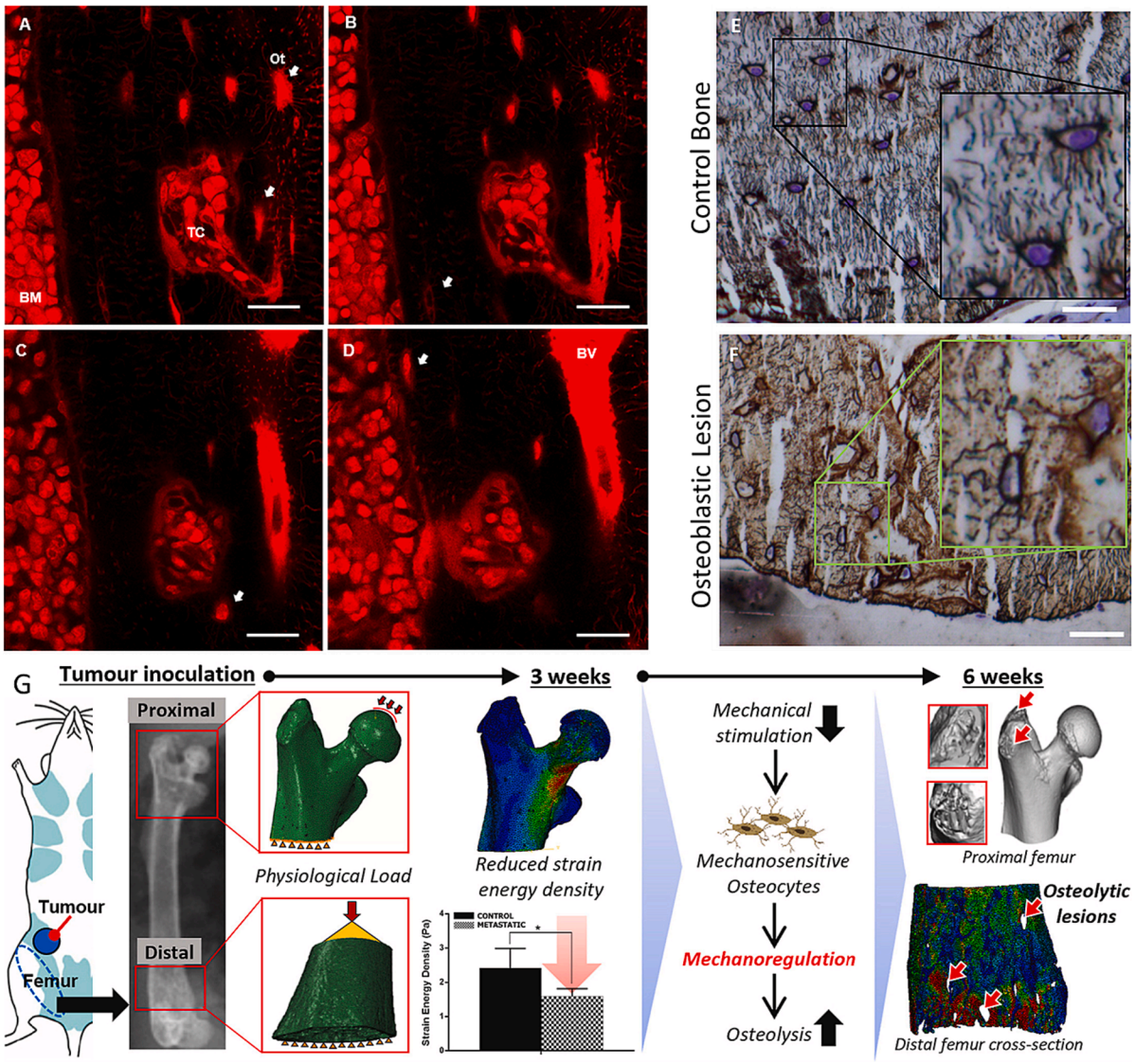

Fig. 1. Degeneration of the bone structure and lacunar-canalicular network architecture *in vivo* during metastasis. (A-D) Confocal microscopy scans of tumour cell-osteocyte interface, rhodamine 6G fluorescence showed the presence of infiltrating tumour cells into the cortical bone and highlighted direct contact between tumour cells and (B) empty lacuna, (C) late, more deeply embedded osteocytes as well as (D) early embedded osteocytes (white arrows). BM: bone marrow; TC: tumor cells; Ot: osteocyte; BV: blood vessel. RM1-Luc-injected tibia. Scale bar = 20 μm. [14] (E-F) Histological evidence for locally impaired osteocyte connectivity in osteoblastic regions. Ploton silver nitrate-stained images of canalicular connectivity in (C) control-site and (D) osteoblastic region from EO771-Luc-injected tibia. Inserts magnified 2.5×. Scale bar = 25 μm. [14] (G) Evidence that 3D computational biomechanics models of bone strength generated from micro-CT scans can detect reduced strain in early metastasis (at 3 weeks post-inoculation with tumour cells) before osteolysis can be detected (visible later at 6 weeks post-inoculation), with these early changed potentially perpetuating osteolysis via disruption of osteocyte signalling [15]. Figure reprinted with permission.

selective mechanical stimulation of just osteocyte cells in co-culture, without stimulating cancer cells, also investigated breast cancer cell-osteocyte crosstalk, finding that shear loading of osteocytes can actually increase breast and prostate cancer cell invasion [22]. In a further study, we found that conditioned media from multiple breast and prostate cancer cell lines can reduce osteocyte mechano-sensitivity to fluid shear stress, as measured by cyclooxygenase-2 (COX-2) expression, an early mechano-transduction messenger [28].

Separately, much work on the effect of breast cancer cells on osteocyte mechano-sensitivity has been carried out by the Lynch group [10], demonstrating that mechanical loading of breast cancer cells produces factors that reduce both osteocyte dendrite formation and bone resorption downstream of osteocytes [29]. Building on this work, they recently showed using an *in vitro* scaffold loading model that breast cancer cells can directly impair the mechano-sensitivity of osteocytes using soluble signals in conditioned media [30]. They further showed that a bone-homing subclone of this breast cancer cell line (i.e. selected sub-cultures that preferentially metastasise to bone in an *in vivo* animal model) disrupted osteocyte mechano-responsiveness to an even greater extent [30].

In an interesting additional study, another group investigated lipoprotein receptor-related protein 5 (Lrp5), which is expressed in osteocytes and is necessary to induce loading-driven bone formation, finding that loading-driven breast tumour suppression is partially regulated by an Lrp5-dependent mechanism [31]. Further research is required, but these osteocyte-specific pathways represent a promising avenue of enquiry for clinical combination mechano-therapy treatments.

It should be noted that there are significant differences in the type, frequency, magnitude and rest period for the mechanical stimulation in the above studies, which may explain some of the discrepancies in findings between the various experiments. A potential resolution to the contradictions in the broader literature may have recently been

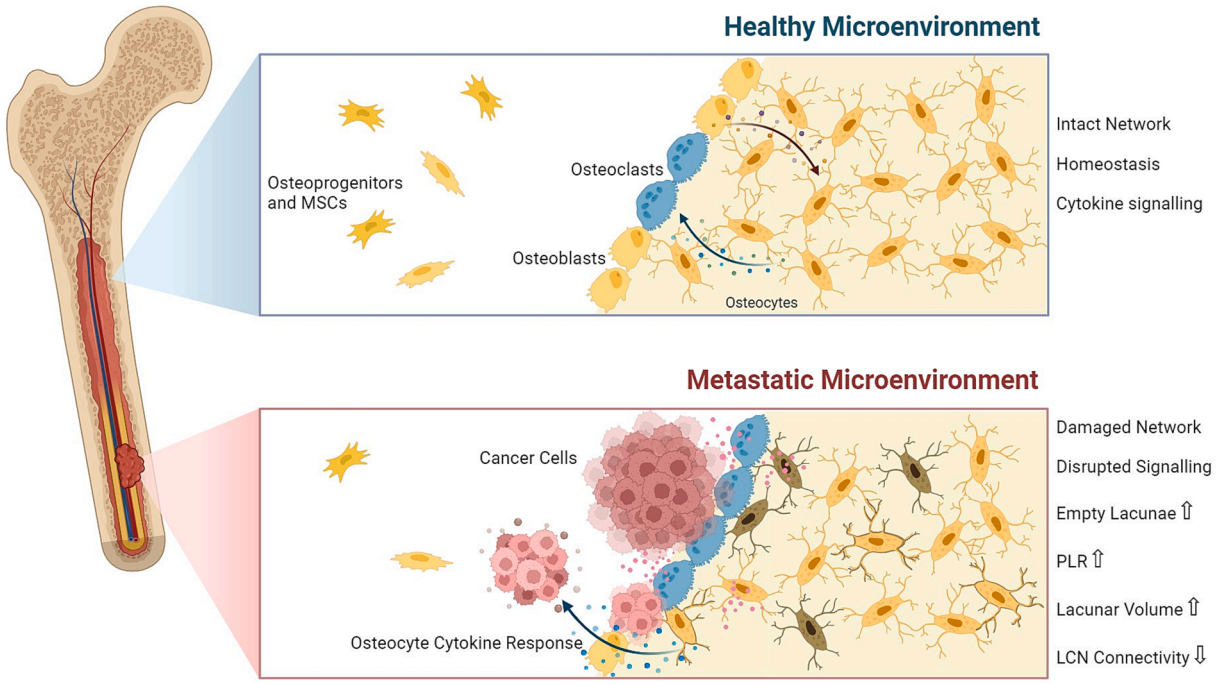

Fig. 2. Schematic of signalling interactions between the osteocyte network and surface bone cells during healthy bone remodelling, and disruption of both signalling and network architecture in the metastatic niche. This results in degeneration of the osteocyte network, lacunae empty of osteocytes, increased peri-lacunocanalicular remodelling (PLR), increased lacunar volume and decreased connectivity of the lacuna-canalicular network (LCN).

elucidated by two groups who found separately, both *in vitro* and *in vivo*, that a bi-modal response or a threshold may exist in metastatic mechano-responsiveness. One group found that 0.25 Pa of shear stress stimulation on osteocytes induced mesenchymal to epithelial transition, while 1 Pa shear stress induced an aggressive response in the reverse direction [23]. Similarly, moderate *in vivo* tibial loading (4.5 N) in metastatic mice reduced breast cancer metastatic burden, while overloading (8 N) accelerated cancer-induced osteolysis [32]. This information could be crucial to further studies, and any attempts to develop mechano-therapies for clinical interventions.

4. Networking metastatic molecular crosstalk

Given the importance of sclerostin as an osteocyte-secreted mechano-regulated signalling molecule that in part orchestrates the bone remodelling cycle, it presents a natural target for lytic breast cancer lesion studies. However, two recent works have developed conflicting findings, with one group observing that either genetic deletion of SOST in breast cancer cells or administration of a sclerostin inhibitor reduced bone metastases [33]. In contrast, the other group found that administering anti-sclerostin one week before tumour inoculation resulted in no major changes in tumour volume/area, while a sclerostin blockade actually promoted bone metastases [34]. It is possible that timing may play a role in the conflicting observations, and further study is necessary to elucidate the role of this key protein.

One of the important mechano-sensing mechanisms for osteocytes is connexin 43 (Cx43), a transmembrane protein that can form hemichannels sensitive to mechanical stimulation and that is highly enriched in osteocytes. Cx43 seems to play a protective role against breast cancer cell metastasis, and indeed has recently been show to do so via prevention of oxidative stress [35]. This study found, using an ovariectomised mouse model, that osteocytes can modulate the oxidative microenvironment via expression of Cx43, and thus can control the growth of tumours cells under oxidative stress in the local microenvironment [35].

A novel signalling pathway recently identified in the multiple myeloma lesion microenvironment is the major histocompatibility complex class II transactivator (CIITA) in osteocytes, which myeloma cells can activate by producing 2-deoxy-D-ribose (2DDR) [36]. CIITA activation promotes RANKL expression and osteoclastogenesis, presenting promising new target pathways, although blockade of CIITA signalling only partially prevented myeloma-induced RANKL upregulation in osteocytes [36]. Also in multiple myeloma, another group has found that signalling from the cancer cells upregulates osteocyte production of vascular endothelial growth factor (VEGF-A) and fibroblast growth factor (FGF23), both of which promote angiogenesis and cancer cell proliferation [37]. Indeed, this bears similarity to a recent study that found increased Fgfr3 expression and associated angiogenesis may play a role in the initiation of osteocarcomas [38].

In a parallel with the vicious cycle paradigms traditionally associated with osteoblast and osteoclast cells, recent work from our group has identified a potential feedback loop between osteocytes and cancer cells [28]. In a series of conditioned media, cancer spheroid and organ-on-a-chip experiments, we found that tumour necrosis factor alpha (TNF-α) is secreted by osteocytes, which in early metastasis vastly outnumber cancer cells, suppressing proliferation of breast and prostate cancer cells, while encouraging migration. This behaviour is dependent on the osteocyte's primary cilium (a key organelle involved in osteocyte mechano-transduction [39]) and associated intraflagellar transport protein 88 (IFT88), which are inhibited in established metastatic colonies by increased transforming growth factor beta (TGF-β) secreted by the higher number of cancer cells. This disruption of the cilia/IFT88 expression blocks TNF-α secretion from osteocytes, thereby switching off both the inhibition of cancer cell proliferation and the up-regulation of migration. Hence, increased numbers of cancer cells produce more TGF-β, further disabling osteocyte TNF-α secretion in a positive feedback loop reducing cancer cell migration and increasing proliferation, thereby accelerating metastatic tumour growth [28]. Most interestingly, this osteocyte suppression mechanism was found to be shared to varying degrees between breast and prostate cancer cells, indicating that further exploration could identify broad-based anti-metastatic treatments.

5. Therapeutic strategies targeting osteocyte-cancer cell crosstalk

Understanding the complexities of osteocyte-cancer cell interactions opens avenues for developing targeted therapies to disrupt this relationship and inhibit bone metastasis. Various additional therapeutic approaches to those mentioned here, including targeting specific signalling pathways, immune modulation, and bone-targeted therapies, have been explored elsewhere [1,5,6]. Additionally, a number of recent studies have suggested that many of the findings elucidated through mechanical loading studies discussed in Section 3, e.g. vibrational loading [26], could be applied to reduce metastatic progression. Indeed, broader clinical evidence has shown previously that targeted resistance exercise programs can improve outcomes, quality of life, fatigue, bone mineral density and strength in breast cancer patients [40], [41], and mechanical loading also decreases metastasis-induced osteolysis in preclinical xenograft cancer models [42]. Taken together, this opens up the exciting potential of mechano-therapy as a standard treatment for metastatic cancer patients.

A particularly interesting finding from a recent study revealed some beneficial effects of osteocyte-derived factors in the treatment of breast cancer metastases in the brain [43]. By taking the conditioned medium collected from MLO-A5 osteocyte–like cells, they found that direct injecion of the media could inhibit tumour growth in both mammary fat pad and metastatic tibia lesions in an *in vivo* model. Furthermore, the direct injection of osteocyte conditioned media into the frontal lobe of the brain resulted in a significant suppression of metastatic tumours in the brain. Through over-expression knock-ins in the osteocytes (Lrp5, β-catenin, and IL1r), the suppression was further enhanced. The authors further postulated that histone H4, highly secreted by osteocytes in the conditioned medium, may also play a role, finding upregulation of tumour suppressors TNF-related apoptosis-inducing ligand (TRAIL), p53, LIM domain and actin-binding protein 1 (LIMA1) and desmoplakin (DSP), as well as down regulation of oncogenic promoters (CXCL1 and CXCL5). Taken together with the previously discussed findings of a suppression role for osteocytes [28], this study points to conditioned media–based therapy as a viable option in the treatment of breast cancer metastases. Further exploration is required in other cancers, as work in our lab has indicated suppression signalling may be shared across cancer types [28].

6. Future perspectives – Major outstanding questions in the field

The recent and exciting exploration of osteocyte-cancer cell interactions in the context of bone metastases has revealed some of the intricate web of signalling pathways and molecular mechanisms that govern this process. Still, a number of major outstanding questions remain for the field as a whole:

- What is the extent of cancer-osteocyte crosstalk? While we have seen evidence of both distant endocrine crosstalk and direct cancer-osteocyte contact, we still know very little about the degree of communication occurring in the microenvironment, especially as most osteocytes are not located immediately adjacent to the growing tumour.
- How, precisely, do these tumours affect our bone macro- and micro-structure? Clinical evidence of deterioration in bone properties is highly variable, and we now know that there may be enormous changes happening to the micro-architecture of the lacunar-canalicular network. Quantifying and predicting this degeneration could provide enormous diagnostic and prognostic value for patients.
- Are there exploitable commonalities between osteocyte crosstalk with different cancers? While there is now some evidence of common signalling mechanisms across different cancer types, the complexity of the pathway networks is confounding. Nevertheless, research should continue in pursuit of broadly applicable treatments for this debilitating disease.
- How can we effectively model this microenvironment *in vitro* or *in vivo*? Many current models leave much to be desired, and likely contribute to the paucity of developed treatments specifically targeting the metastatic microenvironment. More complex predictive *in vitro* or *in silico* models may accelerate scientific enquiry and speed the discovery of now therapies.
- How does mechanical loading affect the metastatic cascade in bone tissue? Given the key role of the osteocyte as the primary mechanosensing cell in bone tissue, and a growing body of evidence that mechanical stimulation plays a role in cancer cell-osteocyte crosstalk, mechanobiology presents a further avenue of enquiry for both fundamental science and therapeutic discovery.

Future research efforts should focus on unravelling the specific molecular targets within osteocytes and deciphering the dynamic nature of their interactions with cancer cells. Additionally, the development of innovative therapeutic approaches tailored to disrupt these interactions holds promise for enhancing the efficacy of existing treatments for bone metastases. In conclusion, understanding the importance of networking between osteocytes and cancer cells provides valuable insights into the development of targeted therapies, ultimately improving the quality of life and survival rates for patients with metastatic bone disease.

Declaration of competing interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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