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Title page

The endocrine influence on the bone microenvironment in early breast cancer

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Abstract

Multiple factors influence the survival of disseminated breast tumour cells (DTCs) in bone. Whilst gene signature studies have identified genes that predict a propensity of tumours to metastasise to bone, the bone environment is key in determining the fate of these tumour cells. Breast cancer cells locate to specific niches within the bone that support their survival, regulated by host factors within the bone microenvironment including bone cells, cells of the bone micro vasculature, immune cells and the extracellular matrix. Reproductive endocrine hormones affect bone and clinical studies across the menopausal transition have provided comprehensive understanding of the changes in the bone microenvironment during this time. Menopause is characterised by a decrease in ovarian oestradiol and inhibins, with an increase in pituitary follicle stimulating hormone and this review will focus on the role of these 3 hormones in determining the fate of DTCs in bone. Both *in vivo* and clinical data suggest premenopausal bone is a conducive environment for growth of breast cancer cells in bone. Adjuvant cancer treatment aims to reduce the risk of tumour recurrence by targeting DTCs and drugs targeting the bone resorbing osteoclasts, such as bisphosphonates, have been evaluated in this setting. Both preclinical and adjuvant clinical studies and have shown that bisphosphonates ability to decrease tumour growth in bone is influenced by levels of endocrine hormones, with enhanced effects in a postmenopausal bone microenvironment. The challenge is to understand the molecular mechanisms behind this phenomenon and to evaluate if alternative adjuvant bone targeted therapies may be effective in premenopausal women.

Introduction

The process of metastatic spread from the primary breast tumour to bone is undoubtedly inefficient, with less than 0.01% of tumour cells released into the circulation able to form bone metastases (Cameron, et al. 2000). Multiple factors influence the success or failure of these tumour cells, including their intrinsic properties and the myriad of environmental factors encountered on the journey from breast to bone. Gene signature studies have identified genes that predict a propensity of tumours to metastasise to bone, including CAPG, GIPC1, TFF1 (Smid, et al. 2006; Westbrook, et al. 2016), but whilst these have a predictive value they cannot determine bone related disease outcomes for individual patients and are therefore not yet utilised in the clinical setting. The environment plays an important role in determining the fate of tumour cells; they have to overcome shear forces and oxidative stress in the circulation before extravasating (Massague and Obenauf 2016), once in the bone microenvironment they are vulnerable to immune surveillance. There is evidence that breast cancer cells locate to specific niches within the bone microenvironment that will support their survival, using chemokine receptors such as CXCR4 to select for areas of ligand (CXCL12, also known as SDF-1) rich bone marrow (Muller, et al. 2001). Tumour cells secrete various factors to modify this new environment and promote their survival, supporting dormancy and recurrence years after the primary tumour (Kang and Pantel 2013). Adjuvant therapy after removal of the primary breast tumour aims to reduce the risk of tumour recurrence by targeting single cell/small volume micrometastases that have not yet acquired the ability to sustain autonomous growth. However, it is now recognized that these small volume disseminated tumor cells (DTCs) may be in a non-proliferative state and not responsive to anti-proliferative agents (Massague and Obenauf 2016). There is therefore a need for alternative therapies that target DTCs to either initiate or maintain them in a dormant state, or that modify the microenvironment to make it

less hospitable and thereby promote tumour cell death. The bone-targeting bisphosphonates have been evaluated in large phase III adjuvant breast cancer trials and trials are ongoing with the newer agent denosumab. Both types of agents target the bone resorbing osteoclast (Oc). The bisphosphonate trials showed an interesting interplay between the prevailing endocrine environment and the efficacy of bisphosphonates; only women with postmenopausal levels of ovarian hormones (natural or chemically induced with the ovarian suppressor goserelin) showed improvements in disease outcomes with addition of bisphosphonates to standard adjuvant therapy (Coleman, et al. 2015). This suggests that the endocrine influence on bone creates two distinct bone microenvironments (pre- vs postmenopausal) that differentially affect DTCs within them and influence therapies that target the bone resorbing Ocs.

Tumour dissemination to bone and the bone microenvironment

Bone metastases from breast cancer are currently incurable with a median survival of 2.3 years following diagnosis (Harries, et al. 2014). The formation of clinically evident bone metastases represents the final part of a process of interactions between tumor cells and bone cells that may have lasted decades in patients who experience disease relapse many years after surgical excision of their primary tumour. Disseminated tumour cells (DTCs) are found in the bone marrow of a third of patients and 50% of these will develop metastatic disease during the first 10 years post diagnosis (Braun, et al. 2005). Thus the presence of DTCs confers a poorer prognosis, but there is also a significant proportion of patients in whom tumour cells reach the bone marrow but do not develop into metastases, hence DTCs either die or are maintained in a state of dormancy. When DTCs arrive in bone they interact with the host cells, including vessels, osteoblasts and osteoclasts, in a putative metastatic niche. The bone forming osteoblasts (Ob) are derived from mesenchymal progenitor cells and lay down new unmineralised matrix in the

resorption pits formed by the bone resorbing osteoclasts (Oc). The presence of tumour cells alters the bone microenvironment, increasing numbers/activity of Oc and decreasing numbers/activity of Ob, even prior to the development of overt bone lesions (Brown, et al. 2012). These early interactions may influence the outcome for tumour cells, determining whether they die, enter a dormant non-proliferative state or undergo early expansion to macrometastases. It is now well recognized that tumour cell fate can be influenced by multiple host factors within the bone microenvironment (see figure 1).

Osteoblasts and the haematopoetic stem cells (HSCs); In normal physiology HSCs function is to contribute to haematopoesis for months or even a lifetime. They respond to extrinsic (microenvironmental) signals to remain quiescent, to self-renew, or to undergo differentiation. The dormant state may in part be controlled by the Ob with evidence that increasing the number of Obs with parathyroid hormone (PTH) also increases the number of HSCs and the number of DTCs from subcutaneous prostate tumours (Shiozawa, et al. 2011). These DTCs are thought to be subject to the same Ob-derived signals that maintain HSC dormancy, thus maintaining tumour cell survival and contributing to relapse in bone many years after the initial diagnosis of the primary tumour. A recent study in mice has demonstrated that breast cancer cells form heterotypic adherence junctions with osteoblastic cells, resulting in activation of mTOR in the tumour cells and their proliferation to form micrometastases, implicating the osteoblasts in early progression (Wang et al, 2015).

Osteoclasts; These bone resorbing cells are activated by various factors produced by tumour cells in bone, including receptor activator of nuclear factor-κB (RANK) ligand, PTH-related protein (PTHrP), interleukins 1 and 6 and macrophage inflammatory protein-1-alpha (Roodman 2001). The activation of Oc induces bone resorption and release of tumour growth factors from

the bone matrix including TGFβ, BMPs, calcium, PDGF and IGF which promote tumour growth and expansion in bone. The activity of Oc is tightly controlled under normal physiological circumstances and is coupled with the activity of Ob through coupling mechanisms like the RANKL-RANK interaction; Ob's produce RANKL to activate Oc's in addition to the soluble decoy receptor for RANKL, osteoprotegerin (OPG), which inhibits Oc development (Cross, et al. 2006). OPG is also secreted by numerous breast cancer cells and the expression of OPG decreases as tumour grade increases (Holen, et al. 2005). Although the main driver of cancer-induced bone disease, the precise role of the Oc in the early stages of tumour cell dissemination to bone remains to be established. Interestingly, Ocs appear to be dispensable for hematopoietic stem cell maintenance and mobilization (Miyamoto et al., 2011), whereas Oc activity triggers growth of disseminated breast tumour cells to form overt metastases (Ottewell et al. 2014 and 2015). These data suggest that different cell populations residing in bone marrow niches have differential interactions, potentially regulated by distinct signaling pathways.

Bone microvasculature; Blood vessels within bone express adhesion proteins including P-selectin and E-selectin which are able to act as anchors for tumour cells (Nguyen, et al. 2009) and the highly fenestrated endothelial cell layer of blood vessels in bone promotes the extravasation of circulating tumour cells out of circulation and into bone (Mastro, et al. 2003). Once in bone, tumour cells are seen in close to blood vessels (see figure 2) and endothelial cells of the mature microvasculature have been shown to promote tumour cell dormancy through secretion of thrombospondin 1 (Ghajar, et al. 2013). Dormancy is also maintained by the secretion of anti stromal derived growth factor -1 (SDF-1) micro-RNAs (miRNAs) from either endothelial cells or other stromal cells within close proximity to blood vessels. These anti-SDF1 miRNAs (miR-127, -197, -222, and -223) are transferred to the tumour cells by use of gap junctions and induce

tumour cell growth arrest (Lim, et al. 2011). miRNAs have further roles in metastases and silencing of expression of miRNA-126 in breast cancer cells increased metastases to multiple sites including bone by promoting endothelial cell recruitment to breast cancer cells (Png, et al. 2012).

Inflammatory and immune cells; Macrophages within tumours have a dual role with some having a tumor growth suppressive action (M1) and some a growth promoting action on both tumours and blood vessels (M2). Tumour associated macrophages (TAMs) are also able to limit the effect of anti-cancer therapies by associating with tumour blood vessels and promoting revascularization after chemotherapy in both primary breast tumours and bone metastases (Hughes, et al. 2015). In this study TAMS were attracted to these sites by the interaction of tumour expressed CXCL12 and TAM expressed CXCR4. Within bone there is a plethora of inflammatory cells that can influence tumour cell homing and survival in bone including myeloid cells which express integrin $\alpha 4\beta 1$ and facilitate tumour cell homing to bone (Papayannopoulou, et al. 2001). Both tumour cells and associated myeloid cells produce inflammatory cytokines, including interleukins 1β and 6, TNFα (Kinder, et al. 2008), which induce Ob and bone stromal cells to secrete factors which attract more myeloid cells and perpetuate the pro-tumourigenic effects by stimulating RANKL expression on Ob (Lam, et al. 2000) which activates Oc and promotes the 'vicious cycle' of bone metastases. In addition, PTHrP secreted from breast caner cells induces IL-6 and VEGF-A expression in Ob which enhances angiogenesis and induces expression of matrix remodeling proteases which support tumour growth in bone (Park, et al. 2013).

Extracellular matrix and tumour hypoxia; Breast cancer cells that are cultured *in vitro* with mesenchymal stem cells (MSCs) demonstrate up regulation of lysyl oxidase (LOX), an enzyme that catalyses cross-linking between collagens and elastins in the extracellular matix. LOX changes the behavior of the cancer cells from an epithelial to a more invasive mesenchymal phenotype, which may promote the spread of these cells to the bone (El-Haibi, et al. 2012). In a cohort of patients with oestrogen receptor (ER) negative (ER-) breast cancers, primary tumours that showed up regulation of genes involved in tumour hypoxia, including LOX, was associated with metastases to bone rather than lung, liver and brain (Cox, et al. 2015), suggesting that LOX may be involved in preferential homing of tumour cells to bone. A murine model of spontaneous ER-ve breast cancers which express LOX showed that the appearance of focal osteolytic lesions preceded the arrival of tumour cells in bone, suggesting that hypoxia-induced tumour secreted factors, including LOX, 'primes' the bone to receive tumour cells through an increase in Oc mediated bone resorption (Cox et al. 2015). Numerous additional extracellular matrix molecules are involved in the formation of a pre-metastatic niche, including periostin (Wang 2016), tenascin (Chiovaro 2015) and thrombospondin (Gahjar 2013) but how their levels/activity are affected by endocrine hormones remain largely unexplored. Another component of the extracellular matrix are cancer associated fibroblasts (CAF), which promote tumour growth and angiogenesis in breast cancer cells through recruitment of bone marrow derived endothelial cells through production of SDF-1 (Orimo, et al. 2005).

Endocrine effects in bone

The effects of endocrine hormones on bone are numerous and clinical studies across the menopausal transition have provided comprehensive understanding of the changes in the bone microenvironment during this time (Perrien, et al. 2006). As menopause is characterised by a

decrease in ovarian oestradiol and inhibins, with an increase in pituitary follicle stimulating hormone (FSH), this review will focus on the role of these 3 key hormones. The role of other hormones may also be important, with a recent study reporting a role for prolactin in the development of breast cancer bone metastasis and the associated bone disease (Sutherland et al 2016). Prolactin has been shown to enhance bone turnover (2008), however levels decrease during menopause (Tanner et al 2011) and whether bone-targeted agents directly influence prolactin levels remain to be determined.

Oestrogen has a well documented effect on osteoblasts and osteoclasts as both cell types express oestrogen receptors (ER) α and β. Oestrogen exerts its effect on bone cells by direct inhibition of osteoclastogenesis, promotes Ob-mediated bone formation, in addition to inhibiting Ob production of osteoclastic cytokines such as TGFβ (Krassas and Papadopoulou 2001). Oestrogen also maintains the number and activity of HSCs in bone (Qiu, et al. 2012), and increases the ratio of OPG/RANKL (Yan and Ye 2015). The ability of oestrogen to affect the bone vasculature is not well described, but there is close association between osteogenesis and vasculogenesis suggesting that modification of bone will influence vessels and vice versa. 17β-oestradiol can influence sub-cutaneous tumour vasculature, increasing vessel density and maintaining a more structured vasculature (Pequeux, et al. 2012). The immune system can be modified by oestrogen with evidence that oestrogen inhibits the secretion of pro-inflammatory cytokines including IL-6, TNFα and macrophage inhibitory factor from monocytes mediated through the ERα36 receptor on the surface of human peripheral monocyte (Pelekanou, et al. 2016). Oestrogen also affects miRNA expression and CAFs with evidence that 17β-estradiol induces miR144 expression resulting in down-regulation of the onco-suppressor Runx1 (Vivacqua, et al. 2015).

Inhibins are not abundantly expressed in bone, but radiolabelled inhibin A administered intravenously *in vivo* accumulates rapidly in the bone marrow (reviewed in Wilson *et al*)(Wilson, et al. 2012). Its effects on bone turnover was highlighted in a cross sectional study of women aged 21-85 (n=188) where endocrine hormones were correlated to changes in serum markers of bone formation; bone specific alkaline phosphatase (BSAP), and bone resorption; carboxyterminal telopeptide of type I collagen (CTX). Inhibin A was the most accurate predictor of changes in bone formation and resorption being negatively correlated with levels of BSAP and CTX (Perrien et al. 2006), thus declining inhibins in early menopause will lead to increased bone turnover. Inhibins also influence both the adaptive and innate immune systems, affecting the development and function of immune cells (Aleman-Muench and Soldevila 2012). The specific effects of inhibin on the immune system within bone has not been defined, however, inhibins do not have an identified downstream signaling pathway but instead bring about their effector functions by binding to the activin receptor (ACTRIIA) and inhibiting the biological actions of activin (Jeruss, et al. 2003). Activin, secreted from monocytes and bone fibroblasts, has been shown to suppress immune processes in bone (Abe, et al. 2002), suggesting the inhibin/activin pathway may be important for bone specific immune /inflammatory processes.

Inhibins inhibit the secretion of *follicle stimulating hormone* (FSH) from the anterior pituitary. In the previously discussed cross sectional study, FSH correlated with bone resorption markers (CTX) but not bone formation markers (BSAP) in perimenopausal women only (Perrien et al. 2006). *In vivo* treatment of ovariectomised 14-week old mice with an antibody to β-subumit of FSH prevented OVX-induced bone loss after 4 weeks of treatment associated with increases in bone formation and inhibition of bone resorption (Zhu, et al. 2012). There is however reports suggesting that lowering FSH increases bone resorption; a prospective study of changes in bone

turnover in postmenopausal women (n=46) with inhibition of FSH, using GnRH agonists, showed a significant increase in CTX and TRAP5b (serum markers of bone resorption) with suppression of FSH, in addition to a significant increase in P1NP (a marker of bone formation)(Drake, et al. 2010), thus the specific effects of FSH on bone turnover still need to be defined. FSH has effects on HSC in bone, with evidence from *in vivo* models that bone derived HSC express FSH receptor and that treatment with FSH enhances haematopoetic recovery after chemotherapy (Shaikh, et al. 2016). FSH also influences the vasculature; the FSH receptor (not normally expressed on vascular endothelium) is upregulated in the vasculature of bone metastases (Siraj, et al. 2013). The influence of FSH on bone mass has also been linked to its effects on the immune system; production of bone resorbing cytokines and BMD was evaluated in 36 healthy women (aged 20-50) and showed BMD was inversely proportional to FSH levels and endogenous FSH levels correlated with circulating levels of IL-1beta, moreover, exogenous FSH induced isolated monocytes to secrete IL-1beta, TNF-alpha, and IL-6 (Cannon, et al. 2010).

These data show that the endocrine hormones oestrogen, inhibin and FSH can modify multiple components resulting in very different bone microenvironments in pre- and post menopausal women. At the time of the final menstrual period (FMP), the majority of women will have undetectable levels of inhibins (Burger, et al. 2002) however oestradiol remains detectable in serum for up to 5 years (Burger, et al. 1999). The rise in FSH can occur up to 3-10 years before menopausal transition (Burger, et al. 2007), attributed to the decline in inhibins (Klein, et al. 2004). The differing levels of endocrine hormones in pre- and postmenopausal women will therefore exert differential effects on disseminated tumour cells (DTCs) in bone, with the potential to modify both tumour growth and response to bone targeted therapy.

Endocrine effects on tumour cells in bone

In vivo the influence of endocrine hormones within the bone microenvironment in regulating the fate of DTCs has been described. 12 week old BALB/c nude mice underwent ovariectomy (OVX) and the bone microenvironment evaluated. Within 2 weeks post procedure the bone microenvironment of OVX animals was significantly altered compared to sham animals with an increase in Oc activity and decrease in Ob activity (Ottewell, et al. 2014). This alteration in the bone microenvironment affected breast tumour cell homing to bone following intracardiac (IC) injection of MDA-MB-231 cells 7 days post OVX, with significantly higher numbers of tumor cells in bone of control compared to OVX animals. However, at a later time point tumour growth in bone was detected in 18% of sham animals and 89% of OVX animals indicating that whilst OVX bone may be less attractive for tumour cell homing it is more conducive to survival and growth of cells that do colonise bone (Ottewell et al. 2014). This data is supported by the results from clinical studies evaluating disease recurrence in patients following a diagnosis of breast cancer. Combined data on the incidence of bone marrow micrometastases in over 4,000 women showed that premenopausal women had significantly higher incidence of bone marrow micrometastases than postmenopausal women (32.7% vs 29.5% p=<0.001) (Braun et al. 2005), indicating that premenopausal bone offers more favourable conditions for DTCs. A further study evaluating the incidence of clinically overt bone metastases in over 7,064 women showed the incidence to be significantly higher in younger women (Harries et al. 2014) suggesting that premenopausal bone can support the growth of DTCs into bone metastases. Further data on recurrence patterns of 6,792 breast cancer patients entered into trials conducted by the International Breast Cancer Study Group showed that younger patients (<35 years) had significantly higher incidences of bone metastases occurring during the course of their disease (Colleoni, et al. 2000). These data suggest that younger women may be at increased risk for

bone metastases but how and which endocrine hormone is affecting the bone microenvironment and DTCs is yet to be defined.

Endocrine influence on bone targeted therapies

Both preclinical and clinical studies have found that the anti-tumour efficacy of osteoclast-targeted agents is influenced by levels of endocrine hormones, with differential effects according to menopausal status. These bone-targeted therapies have included bisphosphonates, the RANKL inhibitor denosumab and the soluble decoy receptor OPG-Fc.

In vivo studies have shown that tumour growth in bone of 12-week old BALBc/nude mice injected IC with the breast cancer cell line MDA-MB-231 was significantly decreased with zoledronic acid (Zol) in animals who had undergone OVX (modeling postmenopausal) but not sham-OVX (modeling premenopausal) (33% vs 86%). This effect on tumour cells was independent of the effect of Zol on bone volume with drug-induced increases in both groups and no significant difference in bone volume between Zol treated groups (Ottewell et al. 2014). This indicates that the effect of Zol on formation of bone metastases is independent of bone volume but dependent upon other factors in the bone microenvironment that are differentially affected by Zol according to the prevailing level of endocrine hormones. Further *in vivo* data has supported an Oc mediated differential effect on breast cancer bone metastases according to menopausal status using the potent inhibitor of osteoclastogenesis, OPG-Fc, which prevents Oc activation by preventing RANKL-RANK binding similar in mechanism to denosumab (Ottewell, et al. 2015). OPG-Fc increased bone volume in 12-week old BALBc/nude mice post OVX and reduced number and activity of both Oc and Ob. Tumor growth in bones after IC injection of MDA-MB-231 cells was decreased by OPG-Fc in OVX animals (7% vs 78.5%) but no effect was seen in sham-OVX animals. These data suggest that pharmacological inhibition of Oc's

283 decreases breast tumour growth in bones but only in a bone microenvironment that mimics
284 postmenopausal (OVX) with low levels of oestradiol and inhibin and high levels of FSH.
285
286 Clinical studies of adjuvant bisphosphonates for early breast cancer have included thousands of
287 patients treated with both oral and intravenous bisphosphonates. The largest of the zoledronic
288 acid studies included AZURE (n=3340)(Coleman, et al. 2011), ABCSG-12 (n=1803)(Gnant, et al.
289 2011) and ZO-FAST (n=1065)(Coleman, et al. 2013). AZURE recruited patients with a mixed
290 menopausal status; premenopausal (45%), unknown menopausal status (9.7%), <5 years since
291 menopause (14.7) and >5 years since menopause (31%). Patients were randomised to receive
292 standard adjuvant therapy +/- zoledronic acid for 5 years and the primary endpoint was disease
293 free survival (DFS). Patients who were >5 years postmenopausal showed a significantly
294 improved DFS with the addition of zoledronic acid (ZOL) to standard therapy (ST) (DFS; ST 71%,
295 ST+ZOL78.2%, HR 0.75, 95%CI 0.59-0.96 p=0.02), but this effect was not seen in any other
296 patient groups. ABCSG-12 recruited premenopausal women that all received the goserelin,
297 which induced a chemical menopause, prior to being randomised to receive
298 tamoxifen/anastrazole +/- zoledronic acid every 6 months for 3 years. The primary endpoints
299 were DFS and zoledronic acid significantly improved DFS compared to endocrine therapy alone
300 (92% vs 88% p=0.008). A pre-planned subgroup analysis according to age demonstrated a
301 significant beneficial effect of zoledronic acid on DFS in women >40 years (HR 0.58, 95% CI 0.4-
302 0.83), and these effects were not seen in women <40 years (HR 0.94, 95%CI 0.57-1.56) which
303 may be due to incomplete ovarian suppression by goserelin in very young women. ZO-FAST
304 evaluated addition of zoledronic acid to the aromatase inhibitor letrozole for 5 years in
305 postmenopausal women with zoledronic acid initiated either at the start of the letrozole (early)
306 or delayed until evidence of bone mineral density (BMD) loss or fracture. The primary endpoint

of this study was change in BMD at 12 months but pre-planned secondary analyses included DFS and OS. Patients who started zoledronic acid with letrozole had a 34% decrease in DFS events (HR 0.66 95%CI 0.44-0.97 p=0.0375) and exploratory analyses according to menopausal status at randomisation showed that women >5 years postmenopausal or >60 years has a substantially improved OS with early vs delayed zoledronic acid (HR 0.5; p=0.0224). This effect was not seen in women who were recently postmenopausal due to chemotherapy induced ovarian toxicity, oophorectomy or ovarian suppression. These trials indicated that adjuvant zoledronic acid was able to improve disease outcomes only when started in women who had a very suppressed hypothalamic-pituitary-gonadal (HPG) axis either naturally or chemically. Similar results were reported with the adjuvant clodronate trials (Paterson, et al. 2012; Powles, et al. 2006) and a large meta-analysis of all adjuvant bisphosphonate trials involving >18,000 breast cancer patients has recently reported and showed that women who were postmenopausal at initiation of bisphosphonates had fewer recurrences in bone (RR 0.72, 0.60–0.86; 2p=0.0002), at other distant sites (RR 0.82, 0.74–0.92; 2p=0.0003) and an improved breast cancer mortality (RR 0.82, 0.73–0.93; 2p=0.002) (Coleman et al. 2015). Inhibiting Oc's by inhibiting the RANKL-RANK interaction is currently being evaluated in phase III trials of the RANK ligand inhibitor denosumab and recent data from the ABCSG-18 trial has shown that adjuvant denosumab reduces the risk of disease recurrence in postmenopausal patients with early stage hormone receptor positive breast cancer (Gnant M 2015), suggesting that osteoclast inhibition with an alternative pharmacological agent to bisphosphonates also improves outcomes in patients with a quiescent HPG axis. Further data is awaited from the D care study, which is a phase III trial evaluating addition of denosumab to standard adjuvant therapy for 5 years in pre- and postmenopausal women to define the population of patients who will derive most benefit.

Summary

It is evident that endocrine hormones play a key role in modifying multiple cells within the bone microenvironment, including bone cells, immune cells, stromal cells and the vasculature, as well as systemic factors and extracellular matrix components (Holen I 2016a). This plethora of cellular effects will undoubtedly have an influence on the homing to and survival of DTCs within the bone microenvironment, with evidence to suggest this process is enhanced in a premenopausal bone microenvironment with high oestradiol and inhibin and low FSH. The clinical utility of Oc inhibitors, used in early breast cancer, with the aim to prevent bone metastases and improve disease outcomes (DFS and OS) has been confirmed in a meta-analysis of large phase III clinical trials involving thousands of women (Coleman et al. 2015). These trials have shown that inhibition of the Oc is only effective in preventing metastases when there is a suppressed HPG axis, either due to natural menopause or chemically induced with GnRH analogues. The challenge now is to understand the molecular mechanisms behind this phenomenon and to evaluate if alternative bone targeted therapies, which act on other cellular components of the bone microenvironment, may be effective in premenopausal women where there is a clear need for bone targeted adjuvant therapy.

Declarations of Interest

The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the research discussed in this review

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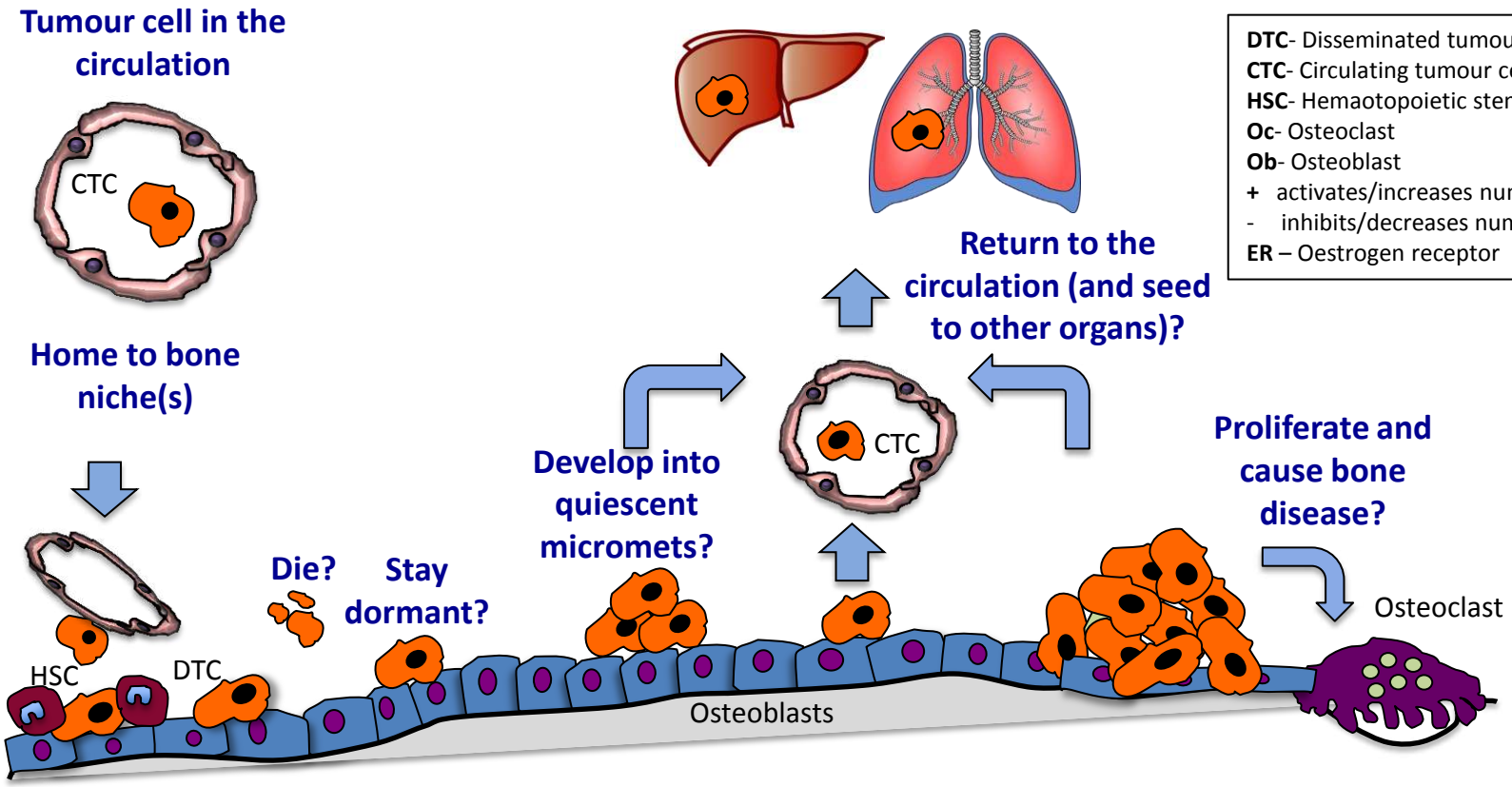
Figure legends.

Figure 1. Tumour cell interactions with the bone microenvironment and the effect of endocrine hormones.

Tumour cells home to vascular areas within bone where they interact with bone cells and can enter a state of dormancy/quiescence for many years prior to growth. They are in close contact with bone and are thought to occupy the HSC niches. Following unknown triggers, the tumour cells re-gain the ability to proliferate and ultimately form bone metastases or spread to other metastatic sites. The table provides a brief overview of the influence of endocrine hormones on bone cells involved in the metastatic niche.

Figure 2. Breast tumor cells visualized in close proximity to osteoblasts and blood vessels in mouse bone

Genetically engineered mice with GFP-expressing osteoblasts injected via the intracardiac route with CMDii-labelled human MDA-MB-231 cells before sample collection and processing for paraffin sections. Histological sections of tibias were stained with markers for endothelial cells using antibodies and DNA was visualized using DAPI. Tumor cells, osteoblasts and blood vessels were seen in close proximity to each other within the bone marrow compartment.



Hormone	Change in serum levels with menopause	Effect on bone cells	Effect on tumour cells in bone
Oestradiol	Decrease	- Oc + Ob	Promotes spread and growth of ER-positive breast cancer cells to bone but not ER-negative (Holen 2016b)
Inhibins	Decrease	- Oc + Ob	Inhibin A does not affect spread of ER-negative cells to bone (Wilson 2016). Effects on growth in bone not defined.
Follicle stimulating hormone	Increase	- Oc (+ Oc in vivo) - Ob	Effects on spread and growth of cancer cells in bone not defined.
Prolactin	Decrease	- Oc + Ob	Prolactin induces breast cancer cell secretion of osteolytic factors (Sutherland 2015). Effects on spread and growth of cancer cells in bone not defined.

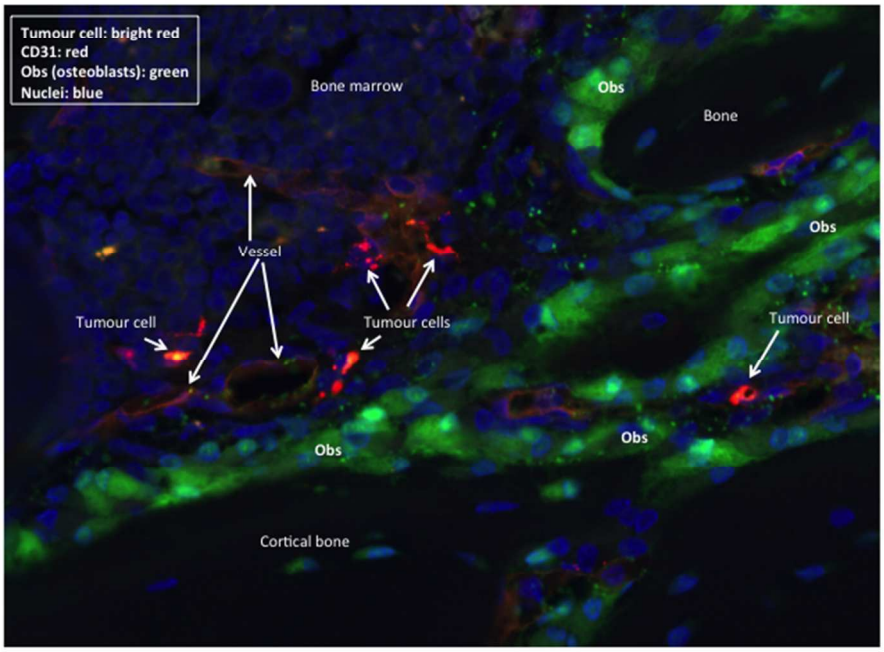


Figure 2

254x190mm (72 x 72 DPI)