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REVIEW

Chronic stress, sympathetic activation and skeletal metastasis of breast cancer cells

Florent Elefteriou

Vanderbilt University School of Medicine, Pharmacology and Cancer Biology, Nashville, TN, USA.

Improved detection programs and new therapies significantly improved the 5-year survival rate of women with breast cancer. However, some women still relapse and succumb to cancer because of metastatic disease. In particular, chronically depressed patients do not seem to benefit from newly developed treatments and present with shorter survival. The reason for this association is unclear, but recent cues from preclinical studies point to the possible contribution of neuroendocrine factors generated in response to chronic stress and depression. Retrospective clinical studies also suggest a beneficial effect of sympathetic blockade in terms of less advanced disease at diagnosis, lower cancer-specific mortality, longer disease-free survival and reduced metastasis development and tumor recurrence, especially in patients who have taken propranolol before diagnosis. Therefore, β-blockers or therapies normalizing sympathetic tone might be beneficial as early adjuvant therapies to limit skeletal metastases and growth and eventually to improve prognosis in patients with breast cancers.

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Introduction

Although as many as 22% of women with stage IV breast cancer survive at least 5 years with optimal therapies, metastasis to distant organs remains a common and a still deadly complication associated with advanced breast cancers. As many as 70–80% of patients with breast cancer present with skeletal metastases. Metastatic breast cancer commonly arises months or years after treatment completion for early and localized breast cancer, and the risk of recurrence varies between patients, depending on the type of primary tumor, its stage at the time of initial diagnosis and likely on a number of intrinsic and extrinsic factors that affect host response. This condition cannot be cured and is currently managed by palliative interventions, focused on length and quality of life.

Cancer metastasis is overall an inefficient process through which cells acquire genetic and/or epigenetic characteristics that allow them to gain a proliferative advantage and higher migratory and survival properties. Although the cellular genetic and phenotypic make-up of a tumor is a major determinant of metastatic efficiency, oncogenic transformation is not sufficient for metastatic competence. A receptive microenvironment is indeed a prerequisite for establishing secondary tumor growth.[1]

The skeleton is a preferential organ for the homing of disseminated breast tumor cells. In the case of breast cancer, the interaction of cancer cells with the bone microenvironment is crucial for their ability to colonize this tissue and their subsequent survival, growth and promotion of the feed-forward cycle of bone destruction, first described by Mundy and collaborators.[2–4] Thus, pharmacological interference with the microenvironmental support is an attractive strategy for repressing early bone metastasis. Toward that end, there is a need to identify the conditions and factors that make the bone microenvironment a hospitable tissue for breast cancer cell colonization and growth.

Understanding the early events and mediators promoting establishment of metastatic cells in distant organs, especially bone, is challenging in the setting of human clinical studies. This is because the steps involved occur at a time the primary tumor may not yet be detected, and also because one cannot easily detect low numbers of metastatic cells within the skeleton at the time they colonize this dense tissue. For lack of techniques to detect early metastatic cells within the skeleton, retrospective clinical studies can be useful in pointing to conditions or factors associated with reduced survival or increased recurrence, as a way to identify possible mechanisms priming the process of skeletal metastasis.

Long before epidemiological studies were performed, ancient medical writers such as Galen (AD 132) noticed that cancer was dependent on 'black bile', which in Greek is synonymous of melancholia.[5] This observation led to the idea that cancer incidence was associated with emotional factors, which was eventually scientifically refuted. However, a number

Correspondence: Dr F Elefteriou, Vanderbilt University School of Medicine, Pharmacology and Cancer Biology, 2215 Garland Avenue, Light Hall, Room 1255D, Nashville, TN 37232, USA.
E-mail: florent.elefteriou@vanderbilt.edu

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of studies provided preclinical and clinical evidence that psychosocial factors may contribute to the progression of the disease and that the activation of neuroendocrine pathways may be involved. I will briefly review here the evidence supporting the contribution of sympathetic cues to the process of skeletal metastasis and discuss the potential of β-blockers as adjuvant drug during treatment of the primary cancer to prevent bone metastasis and possibly increase the prognosis of women with breast cancer.

Neuroendocrine Factors and Bone Metastasis

Chronic psychosocial stress and severe depression are two conditions that have been linked to increased breast cancer recurrence, reduced survival and poor prognosis.[6–12] These conditions are particularly relevant to both breast and ovarian cancer, as women are more predisposed to depression than males.[11,13,14] In addition, major depression is a frequent but under-recognized and under-treated condition among breast cancer patients.[15,16] The use of β-blockers, on the other hand, has been associated with prolonged survival in women treated for breast cancer,[17–20] as well as in patients affected with prostate,[21,22] lung,[23] ovarian[24] and cutaneous melanoma.[25,26] A commonality among these studies is that the conditions and drugs involved affect the sympathetic nervous system (SNS), whose activity is stimulated by chronic stress and depression and inhibited by β-blockers.

The SNS is part of the autonomic nervous system that controls involuntary body functions. Its main mediator is norepinephrine (NE), a neurotransmitter that acts via β-adrenergic receptors (βARs). These receptors are expressed broadly, including in bone cells and in cancer cells of multiple origins. The skeleton is richly innervated by sympathetic nerves and, as indicated above, a preferential organ for the metastasis of breast and prostate cancer cells. Studies in mice have shown that β1/2AR non-selective agonists, such as isoproterenol (ISO), or chronic unpredictable stress, known to stimulate sympathetic outflow and the Hypothalamic-Pituitary-Adrenal axis (HPA), alter bone remodeling and lead to bone loss. These effects are mediated by the stimulation of bone resorption and the inhibition of bone formation.[27,28] On the other hand, mice receiving the β-blocker propranolol, a β1/2AR non-selective antagonist, gain bone, especially in conditions associated with high bone turnover.[27] Accordingly, mutant mice lacking the β2AR display a late-onset increase in bone mass caused by high bone formation and low bone resorption.[28,29] Adrenergic agonists promote osteoclastogenesis via action on osteoblasts and stimulation of *Rankl* (*Receptor Activator of Nuclear Factor Kappa-B Ligand*) expression.[28] It also regulates hematopoietic stem cell bone marrow trafficking via CXCL12/SDF1 (stromal cell-derived factor-1),[30] as well as insulin secretion via its effect on osteoblasts.[30–32] Therefore, the bone marrow environment is innervated and responsive to sympathetic outflow or β1/2AR pharmacological stimulation, and the osteoblast lineage is critical to this response.

Remarkably, the cytokines involved in these actions of sympathetic nerves on bone cells are known to contribute to skeletal cancer cell metastasis.[33–35] SDF1 for instance is expressed by osteoblasts and is considered a major cytokine for successful homing and survival of prostate and breast cancer cells in bone.[36,37] High CXCR4 expression has also been correlated to poor clinical outcome in patients with breast cancers.[38,39] Similarly, RANKL is expressed by osteoblasts and osteocytes (as well as T cells and chondrocytes) in bone. Its role in osteoclastogenesis is well established in the setting of bone remodeling as well as osteolytic bone destruction associated with breast metastatic tumors. It also stimulates melanoma, breast, prostate and lung cancer cell migration *in vitro*[40,41] and melanoma cancer cell bone metastasis *in vivo*.[35] Finally, RANK immunohistochemical positivity in combination with CXCR4 positivity identified breast cancer patients whose disease has a high probability to metastasize to bone.[38] Collectively, these observations suggested that sympathetic activation, in response to chronic stress or depression, may transform the bone marrow environment into a favorable tissue for metastatic establishment.

Chronic Stress Favors Ovarian, Prostate and Breast Cancer Bone Metastasis

The aforementioned hypothesis is difficult to address through clinical studies; thus, several groups relied on preclinical models, which allow investigators to use a number of consistent and controlled experimental paradigms of stress or depression and to inoculate and track metastatic cancer cells at different stages of the metastatic process. Although these models are imperfect and plagued with several limitations, they can provide useful mechanistic information if results are interpreted with these limitations in mind and inform the design of future clinical intervention studies.

Studies focused on ovarian cancers suggested that ovarian cancer cells are directly influenced by sympathetic cues. In xenograph models based on the use of HeyA8 and SKOV3ip1 cells, sympathetic activation by restrain stress increased weight and vascular endothelial growth factor (VEGF)-mediated vascularization of intraperitoneal tumors.[42] The observation that *Adβr1* and *Adrβ2*-deficient ovarian cancer cell lines did not respond to mouse chronic immobilization stress with an increase in tumor weight indicated that this effect of sympathetic activation on primary tumor growth was mediated via the βARs expressed in cancer cells. Sood *et al.* also reported that chronic stress induced by daily restrain or ISO injections protected tumor cells from apoptosis upon loss of anchorage and detachment of the extracellular matrix in an orthotopic model of human ovarian cancer.[43] Other studies indicated that βAR stimulation increases the expression of VEGF and angiogenesis in human breast ovarian and melanoma cancer cells[42,44,45] and pro-metastatic matrix metalloproteinases and other inflammatory mediators in gastric tumors.[46] αAR blockade was also shown to block the proliferative effect of catecholamines on breast cancer cells *in vitro*,[47] although the stimulatory effect of catecholamine on breast cancer cells is not seen in all studies.[48] These effects of sympathetic nerves on primary tumors are consistent with the known innervation of the prostate,[49,50] ovaries[51] and the skin.[52] It is still unclear whether these direct effects of catecholamines on primary tumor cells have any repercussion on their ability to disseminate to secondary sites, although studies on prostate and ovarian cancer support a role for the β2AR and β3AR in tumor development and dissemination to distant organs.[42,53] It is possible that the activation of sympathetic nerves in the primary tumor leads not only to increased tumor growth but also to

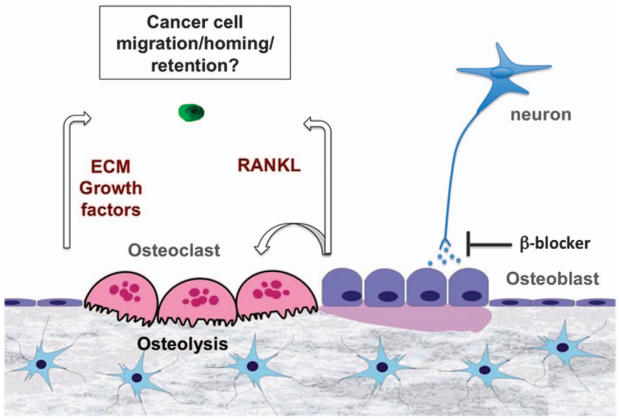

Figure 1 Sympathetic neurons act on osteoblasts to stimulate breast cancer cell establishment in bone, via the pro-migratory and pro-resorptive effect of RANKL.

remodeling of the host stroma to lead to a tissue, cell and cytokine profile that is favorable for cancer cell egress and dissemination to distant organs.

Studies related to breast cancer provided strong evidence for indirect effects of sympathetic cues on key host metastatic sites, including bones, lymph nodes and lungs, which are known to receive sympathetic innervation. A study by Sloan *et al.*,[63] based on the use of 66cl4 breast cancer cells injected in the mammary fat pad of mice subjected to daily restrain or ISO injections, showed that sympathetic activation in mice stimulated the infiltration of activated macrophages (as well as myeloid-derived suppressor cells) into the parenchyma of the breast primary tumor and thereby induced a pro-metastatic gene expression signature that favors dissemination to distant organs. *In vivo* macrophage suppression, but not T-cell absence, inhibited metastasis of these cells to the lung under stress conditions, demonstrating the functional contribution of macrophages to this increase in lung metastasis. Whether a similar mechanism involving macrophages could be involved in metastasis to the skeleton remains to be determined. These studies showed that psychological factors, via activation of the SNS, can activate a metastatic switch within a growing primary tumor and that macrophages/monocytes could functionally extend the influence of sympathetic signaling beyond the distance of neurotransmitter diffusion from neuronal fibers, especially in tissues with nerve terminal densities lower than the adrenal or the central nervous system (like breast or bone), to help the dissemination of cancer cells to distant organs. Another study by Szpunar *et al.* showed that, although mouse 4T1 breast cancer cells do not express αARs and βARs, modulation of NE levels by NE reuptake inhibition with desipramine promoted tumor growth (but not metastasis to the lung), suggesting that sympathetic outflow can affect tumor growth without a direct input on cancer cells.[54]

To investigate the mechanism by which sympathetic activation may alter the establishment of metastatic breast cancer cells in the skeleton, we have used intracardiac injections of the human osteotropic breast cancer cell line MDA-MB-231-VU in mice subjected to ISO treatment or daily immobilization stress (CIS). In this experimental setting, chosen for its focus on skeletal metastasis, we found that ISO and SNS activation by CIS stimulated the establishment of MDA-MB-231-VU cells within the skeleton.[48] By subjecting mice to CIS or ISO treatment prior to MDA-MB-231-VU cell intracardiac inoculation, we were able to show that sympathetic nerves or βAR agonists promoted bone colonization via an indirect effect on the host stroma rather than via a direct effect on cancer cells. In this experimental paradigm, the number of lesions and metastatic foci was increased compared with control, whereas it was not if stimulation occurred after cancer cell inoculation. Tumor burden, however, was increased in both conditions compared with controls, most likely because CIS and ISO treatment promoted osteoclastogenesis, increased bone turnover and bone matrix growth factor release, thereby feeding the 'vicious' feed-forward cycle of bone destruction. These effects were also observed with an osteotropic murine 4T1 clone in Balb-c mice, broadening these results to a distinct cancer cell line and to an immunocompetent host (unpublished data). Collectively, these data suggest that the affinity of breast cancer cells for the skeleton can be significantly increased by the action of sympathetic cues on this tissue.

The strong stimulatory effect of sympathetic activation and ISO treatment on the expression of *Rankl* in osteoblast cultures *in vitro*, as well as in bone *in vivo*, as well as the specificity of this increase in tissues where breast cancer cells metastasize, i.e. bone, liver and lung,[48] led us to address whether this cytokine could mediate the stimulatory effect of sympathetic activation on breast cancer cell metastasis to the skeleton. Several studies demonstrated the direct contribution of RANKL to mammary tumorigenesis[55–57] and melanoma bone metastasis.[35] In the context of bone and breast cancer cells, we found that βAR agonists did not promote breast cancer cell proliferation *in vitro* and in a subcutaneous tumor growth model *in vivo*, thus supporting a main action on the host to promote skeletal establishment, rather than a direct effect on cancer cells. However, ISO stimulated the migration of MDA-231-VU cells *in vitro*, and this effect was inhibited by osteoprotegerin (OPG), the decoy receptor for RANKL, but not by AMD3100, a blocker of the receptor for SDF1/CXCL12.

The *in vivo* contribution of host-derived RANKL to breast cancer bone metastasis could not be investigated by using a classical systemic loss of function experiment using OPG for instance, as this approach would have affected osteoclastogenesis/bone turnover and the release of skeletal growth factors that could impact breast cancer cell establishment and proliferation in bone, in addition to the putative pro-migratory effect of RANKL on breast cancer cell homing to bone. Therefore, we chose to silence expression of the receptor for RANKL (RANK) in osteotropic MDA-MB-231-VU cells with the hypothesis that it should reduce their dissemination into the skeleton. This knockdown diminished MDA-MB-231-VU cell migration *in vitro*, did not affect cell proliferation and most importantly significantly decreased the establishment of these cells in bone *in vivo* following intracardiac injection and ISO pretreatment.[48] These results suggest that sympathetic signals promote the skeletal establishment of breast cancer cells via a host-derived, RANKL-mediated mechanism (**Figure 1**). Of note is that nearly 40% of breast cancer metastatic tumors do not express RANK;[58] hence, additional mechanisms likely contribute to breast cancer cell metastasis to bone. High levels of catecholamines and corticosteroids generated upon chronic stress for instance have immunosuppressive effects on natural killer (NK) cells, lymphocytes and macrophages, by affecting proliferation,

differentiation or function of these cells.[59–62] βAR loss of function experiments, targeting the β2AR in either osteoblasts, specific immune cell lineages or in cancer cells, will be one experimental strategy to determine the relative contribution of the major cell type(s) responding to the effect of sympathetic activation in the setting of bone metastasis. It will also be informative to determine whether tissue distribution of a non-osteotropic disseminating cancer clone could be altered by sympathetic activation in favor to skeletal sites versus soft tissues, such as liver or brain.

In conclusion, breast cancer metastatic cells may be affected by sympathetic cues in various manners, directly or indirectly, depending on their origin (breast, prostate, ovary, skin and so on) and stages of tumor development (primary growth, egress into the blood stream, survival, establishment at distant sites, dormancy and growth at these sites). Regardless of the mechanism, it appears from these preclinical studies that interventions aimed at reducing sympathetic nerve activity or downstream signals (including RANKL) may hold promise for limiting tumor cell dissemination to distant organs and thus for improving the prognosis of patients with cancers at risk for metastasis.

Stress, β-Blockers and Survival in Patients with Breast Cancer

An interesting observation in the course of these studies was that the β-blocker propranolol could prevent CIS-induced lung and bone metastasis of 66cl4 and MDA-MB-231-VU cells, respectively.[48,63] These results support the specific role of catecholamines and βARs in these effects of chronic stress on lung and skeletal metastasis and also suggest that activation of the HPA axis is not preponderant in this stimulatory effect, although it certainly does not exclude its contribution. In that regards, the stimulatory effect of high-dose glucocorticoids on β2AR expression in osteoblasts for instance suggests that SNS and HPA activation may synergize to favor skeletal metastasis of breast cancer cells.[64]

The observation that propranolol reduced skeletal and lung establishment of breast cancer cells also suggests that β-blockers could have a beneficial effect clinically in women with breast cancer. This was indeed observed in a small number of retrospective studies.[65] β-blockers are safe and well-characterized drugs, used clinically for the treatment of congestive heart failure and high blood pressure. Although these drugs do not seem to affect breast cancer incidence and tumorigenesis, a beneficial effect in terms of less advanced disease at diagnosis and lower cancer-specific mortality was observed in patients who have taken propranolol 1 year before diagnosis.[19] Longer disease-free survival[18] and reduced metastasis development and tumor recurrence were also observed in several studies,[17,20] whereas some studies did not measure a significant effect of β-blockers on breast cancer recurrence or survival.[66,67] In these latter studies, the majority of patients were taking β1AR-selective antagonists, which is different from all preclinical studies mostly based on effects via the β2AR and the use of β1/β2 non-selective antagonists. Finally, the observation of no association between post-diagnostic use of β-blockers and breast cancer-specific mortality further reinforces the notion that the early stages of metastatic dissemination represent the best window of opportunity for treatment to increase prognosis.[68]

Conclusion and Future Directions

The contribution of psychosocial factors to the clinical progression of breast cancer is supported by an increasing amount of preclinical data in mouse models and by retrospective clinical studies. Collectively, these studies support the pathophysiological relevance of the cross-talk between the neuronal and skeletal systems, provide new therapeutic opportunities to reduce or prevent metastatic spread in patients with low grade primary tumors and raise a number of interesting questions. For instance, experimental data support an effect of sympathetic signals on breast cancer bone metastasis via main effects on the host stroma; however, a direct effect of catecholamines on β2AR-positive breast cancer cells remains possible. This could affect early metastatic events such as tumor cell invasion, angiogenesis of the primary tumor, cancer cell survival or resistance to chemotherapy, as shown in studies related to other types of solid cancers.[42,43,69–75] A stromal-mediated mechanism also implies that other types of metastatic cancers may be influenced by depression, chronic stress and subsequent sympathetic activation.[42,70–72,75] The relative contribution of activation of the HPA axis and adrenal-derived epinephrine (versus nerve-derived NE) to bone remodeling and metastasis remains to be better characterized. As not every breast cancer cell line or tumor biopsy expresses the RANKL receptor (RANK), it will be important to determine whether and how RANK expression/signaling is regulated in cancer cells at the various stages of the metastatic process and to investigate the possible existence of additional mechanisms by which sympathetic activation may stimulate tumor metastasis. The molecular mechanism whereby RANKL promotes cancer cell migration also remains unclear. In addition, it is unknown whether the inhibitory effect of β2AR blockade on bone and lung metastasis observed in preclinical models contributes to the increase in relapse-free survival observed in patients with breast cancer who were taking β-blockers before breast cancer diagnosis. Lastly, the stimulatory effect of sympathetic activation on the production of inflammatory cytokines (tumor necrosis factor-α, interleukin-1, interleukin-6 and interferon-γ) that signal in neurons of the central nervous system controlling energy metabolism is also to consider in the context of cachexia seen in patients with metastatic cancers. Collectively, these studies warrant further investigations to address the potential of β-blockade or behavioral therapies to limit breast cancer spread to distant organs and to increase the prognosis of women diagnosed with breast cancer.

Conflict of Interest

The author declares no conflict of interest.

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