

A Review of the Literature on Metastatic Spine Disease

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Abstract

Metastatic spine disease is increasing in incidence and is a major cause for patient morbidity and mortality. The clinical presentation is often characterised by pain and spinal deformity and often progresses to neurological deficits without treatment. The cornerstones of treatment have been chemotherapy, radiation therapy and surgery to debulk and stabilize the spine. However, these are considered palliative procedures being limited by damage to normal healthy tissues. Recent studies have focused on the key pathways that mediate tumour progression and spread to bone and more targeted therapies that may reduce the injury to normal cells. This article reviews the key features, clinical presentation, workup and treatment options of spinal metastases.

Keywords: Spine; Metastasis; Chemokines treatment

Introduction

The word “metastasis” (from the Greek for “displacement”) refers to the migration of malignant cells to areas distant from the primary tumour. Due to a worldwide increase of cancer incidence and a longer life expectancy of patients with cancer, a rise in the incidence of bone metastases has been observed [1-4]. Bone is the third most common site of metastasis, behind lung and liver [5]. Prostate and breast cancer are responsible for the majority of these bone metastases (up to 70%), largely attributed to both the high incidence and the insidious clinical course of these tumours [5]. Bone metastases, especially involving the spine, are a major cause for mortality and morbidity; characterized by poor patient quality of life due to severe and constant pain, poor mobility, pathological fractures, spinal cord compression, bone marrow aplasia, and hypercalcemia [6].

Spinal metastases can affect all age groups; however, the highest incidence occurs between the ages 40 to 65 years [7]. They can be classified according to their anatomic location as intradural (intradural or extra medullary) or extradural [7]. Extradural lesions account for up to 95% of spine lesions and can be further divided into pure extradural lesions or those originating from the vertebra and subsequently impinging on the thecal sac. Pure epidural metastases are rare [8]. The thoracic spine is the location of predilection (60% to 80%), followed by the lumbar spine (15% to 30%), and finally the cervical spine (less than 10%) [9].

A post-mortem examination study showed 30% to 70% of patients who die of cancer have spinal metastases, however, only 14% will have symptomatic lesions during their illness [10]. The majority of patients with symptomatic spinal metastases receive palliative radiotherapy, while less than 10% of patients undergo surgical treatment [11]. Depending on their location, these symptomatic spinal lesions can have debilitating and potentially life-threatening consequences. One of these consequences is metastatic spinal cord compression (MSCC), defined as the compression of the dural sac (spinal cord and/or cauda equina) and its contents by an extradural tumour mass [12]. MSCC is a catastrophic complication of spinal metastases and is often considered a surgical emergency. If not treated in a timely fashion, it can lead to instability, relentless and progressive pain, severe neurological dysfunction, and impaired quality of life [13,14].

For long period of time the cornerstones of cancer treatment were surgery, radiation therapy and chemotherapy [15]. However, surgery is a palliative procedure in most cases of metastatic disease, and effective radiation and chemotherapy are limited by significant adverse effects

associated with damage to the healthy tissues. Current research and treatment is focused on the key pathways that mediate tumour migration and establishment at bone sites. This more targeted therapy may reduce the injury to normal cells and prevent or delay the irreversible effects of bone fractures and onset of pain that eventuate, greatly enhancing the prognosis and quality of life for patients with spinal metastases.

Pathophysiology

Tumour migration (metastasis)

Bone metastasis is not a random event, but an organized multistep process that involves

- Tumour intravasation
- Survival of tumour cells in the blood circulatory system
- Successful extravasation into the surrounding tissue
- Initiation and maintenance of growth
- Angiogenesis [16].

To execute this complex operation, an organised cooperation and interplay of gene mutations, protein expression, and signalling of abnormal pathways must occur. In a landmark paper, Kang and co-authors [17], in a multigenic examination of breast cancer bone metastases identified some key gene expression involved in this process. These included C-X-C chemokine receptor type 4 (CXCR4), fibroblast growth factor 5, connective tissue-derived growth factor, interleukin-11 (IL-11), matrix metalloproteinase (MMP)-1, folistatin, ADAMTS1, and proteoglycan-1, all of which were overexpressed by at least four times when compared with the same cell line that had not metastasized to bone [17].

MMP and chemokines are the two main proteins crucial for metastatic breast cancer in transit to bone. MMP is a superfamily of at least 28

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proteinases that breakdown the extracellular matrix [18]. MMP-2 is the most studied in breast cancer and along with MMP-9 is associated with poor prognosis when found in high levels [19,20]. It works closely with chemokines and adhesion molecules, such as E-cadherin, resulting in tumour cell attachment and invasion of the basement membrane [18].

Chemokines, on the other hand, are small molecular cytokines essential for the generation of tumour angiogenesis, and homing of tumour cells to target end-organs [21]. CXCR4 and C-C chemokine receptor type 7 (CCR7) are the key receptors which are important for breast cancer migrating to bone. Immunohistochemistry shows that CXCR4 receptor is expressed in 67% of breast cancer bone metastases compared with 26% without bone metastases, while CCR7 is expressed exclusively in breast cancer bone metastases (27% versus 0%) [22]. The connection between CXCR4 and the development of bone metastasis has made CXCR4 an attractive therapeutic target. Multiple preclinical studies have now demonstrated the efficacy of CXCR4 antagonists in inhibiting bone metastases of breast cancer [23-25].

Cancer metastasis to bone is a very organised and well-coordinated process requiring high-level communication between cancer cells and the host to establish bone metastasis. Therefore, it is likely that multiple pathways need to be targeted to reduce the occurrence of bone metastasis.

Model of tumour establishment

The dissemination and extravasation of cancer cells to secondary sites are very efficient processes provided the cells escape the immune system. On the other hand the establishment and persistence of growth is relatively inefficient [16]. Hence, optimal conditions for tumour cells to resettle are paramount after they have lodged in the bone microenvironment. Although bone is macroscopically a hard organ compared to other soft and spongy structures like the liver, lung and brain, it has some unique qualities that favour tumour engraftment. Firstly, bone is a highly vascular organ. The axial skeleton contains large amounts of red marrow, which is demonstrated to have high blood flow [26]. Secondly, bone is susceptible to metastases because of its acidity, intramedullary oxygen, and extracellular calcium levels which make it a favourable host to certain tumours. Lastly, bone harbors an abundance of growth factors, including transforming growth factor beta (TGF- β), insulin-like growth factors (IGF), hypoxic-inducing factor, interleukins, and chemokines, all of which are vital to cancer cell survival and proliferation.

In essence, both the tumour and the host microenvironment contribute to the successful tumour engraftment from primary site to bone. Over 100 years ago, Paget described the “seed and soil” model, where the seeds (tumour cells) can only live and grow if they fall on congenial soil (an optimal microenvironment) [27]. Recently, Psaila and Lyden expanded on Paget’s original concept, postulating the “metastatic niche” model [28]. Firstly, the primary tumour prepares a “premetastatic niche” (the eventual site of bone metastases) by secreting a plethora of growth factors, such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF), TGF- β , S100 chemokine, and serum amyloid A3, even before tumour migration [28]. Then once the tumour has engrafted on the “metastatic niche”, a continuing supply of growth factors from the microenvironment, loss of death signals, and recruitment of endothelial progenitor cells, enable the evolution of a tumour population from micrometastases to macrometastases [28]. Thus, the symbiotic relationship between tumour and bone is pivotal to the settlement of metastases in new distant sites.

Clinical presentation

Pain is the most common complaint from spinal metastases and can occur due to tumour activity (non-functional) or due to instability and fracture (functional). Non-functional pain is usually localized dull pain that first arises only at night and gradually increases in intensity to become constant. It is often due to elevated intraosseous pressure from the rapid increase in size of the space occupying lesion. It’s been shown that the size of the tumour and a rapid increase in size is correlated with pain intensity [29]. This type of pain often results in hospital admission and is associated with progressive impairment in quality of life, increased psychological distress and decreased physical and social functioning [29]. Non-functional pain is usually treated with medical treatment and/or radiotherapy, while surgery to debulk the tumour is occasionally used.

Functional pain is due to loss of structural stability of the vertebral column and may result from bone destruction, pathological fractures and/or ligamentous insufficiency [30]. This type of pain increases with movement and improves with bedrest or an orthosis. Surgery is an excellent treatment option for functional pain because it immobilizes the spine and restores stability [30]. Radicular pain may develop as a result of compression of nerve roots by the tumour or due to the deformity resulting from a pathological fracture. This is a shooting type pain that radiates along the distribution of the affected nerve root [31]. It can be burning in nature and increases by passive or active stretching of the nerve root. It may be associated with sensory or motor deficit depending on the nerve root affected.

Tumour induced compression of the spinal cord can cause long tract deficits or conus medullaris syndrome and compression of the cauda equina cause can result cauda equina syndrome. The mass effect produced by a tumour comes from the vertebral body in approximately 90% of cases; thus, the corticospinal tracts are often the first long tracts of the spinal cord to be affected, as they are ventrally located. This explains why dramatic spastic paraparesis often arises before any sensory abnormalities are present, however when this occurs it carries a poor prognosis [32]. Bladder and/or bowel dysfunction resulting from compression of the conus medullaris, cauda equina, or both is often misinterpreted as a sequela of prostatic hypertrophy or weakness of the pelvic floor, particularly in elderly patients. Autonomic dysfunction may also result from spinal cord compression or cauda equina compression, while painless urinary retention suggests a neurologic cause [33]. Compression of the spinal cord in the cervical or thoracic region often results in myelopathy, beginning as hyperreflexia and upper motor neuron signs and progressing to weakness, proprioceptive sensory loss, and loss of pain and temperature below the level of the spinal cord compression [34].

Patients with thoracic or thoracolumbar compression fractures often present with kyphosis and progressive deformity of the spine. They also often have severe pain in recumbence and often give a history of sleeping upright in a chair for several weeks. The presumed mechanism is extension of the unstable kyphosis. This pain does not usually respond to steroids but may be relieved with narcotics or an external orthosis, pending definitive therapy.

In general, spine metastatic disease is the terminal clinical stage of the cancer course. Patients often shows general health decline including loss of appetite, lethargy, and unintentional weight loss. The defining criteria for unintentional weight loss is >5% reduction in body weight over a period of 6-12 months, or at least two of the following: evidence of change in clothing size, reported weight loss by a relative or friend, or a numerical approximate of the amount of weight loss [35]. Malignancy

has been shown to be the cause of unintentional weight loss in around 33% of cases [36].

Clinical evaluation

The evaluation of patients with spinal metastasis should include a quantitative pain assessment, quantitative neurologic score, and a general performance score. Pain assessment can be most readily performed with a visual Analog scale which is familiar and easy to many patients. The score can be converted to reflect mild (0 to 4), moderate (5 to 6) and severe (7 to 10) pain [37]. The motor grading system by MRC grading provides the baseline for assessment of motor function (Table 1) [38]. The two most widely used neurologic scales assessment includes the Frankel grading system [39] and the American Spinal Injury Association (ASIA) score (Table 2) [40]. The ASIA score incorporated the MRC grading system into its scoring. Both assess motor function with a score of (E) being normal and (A) being complete paralysis. Performance status incorporates ambulation, medical co-morbidities and extent of disease. A patient may have normal motor strength, but be unable to ambulate from loss of proprioception, fracture in the lower extremity, poor nutritional status, poor pulmonary function and a variety of other symptoms. The Eastern Cooperative Oncology Group (ECOG) performance status [41] and Karnofsky performance status [42] (Table 3) are the most commonly used in the functional assessment of cancer patients. It is important to include both neurologic and performance status when reviewing outcomes in cancer patients because together they provide a comprehensive patient assessment.

Imaging

Plain radiography

X-rays are the initial scanning modality, but are insensitive to small lytic lesions and struggle to assess canal compromise. They necessitate a reasonably large (1 cm) diameter lesion with 50% bone mineral loss at minimum for detection [43,44]. Furthermore, up to 40% of lesions can be missed by X-rays, presenting false-negative results [45]. Radiography may be a provide rough assessment of the risk of pathologic fracture, with the risk being high if 50% of the cortex is destroyed by tumour [46]. Extension of the lesion to the epidural space may demonstrate osseous erosion along the posterior vertebral body margin or pedicles. Rarely, metastases may cause scalloping of the adjacent bone [47].

Metastatic lesions can be osteolytic, sclerotic or mixed. The most frequent type observed in metastases are pure osteolytic lesions. Lodwick et al. reported three different types:

- Geographical osteolytic lesions refer to focal destruction of bony tissue by tumor.
- A moth-eaten osteolytic lesion refers to the presences of multiple small irregular holes.
- Pervious osteolytic lesions are characterized by the presence of smaller, millimetre-sized holes that reduces the density of bone on x-ray films [48].

Other suggestive features to look for in metastatic lesion include blurred outlines on a vertebral body which indicates cortical involvement, loss of cortical bone in the posterior wall of the vertebral body, along with loss of its posterior convexity [49]. As metastases have a predilection for involving the posterior vertebral body and pedicle, a missing pedicle is a useful and subtle sign to seek on AP films (Figure 1). Vertebral collapse is also frequently observed with metastatic lesions of the breast, lung, and prostate [50]. Several patterns are indicative of a malignant lesion: one-sided damage; angular or irregular distortion of the vertebral

endplates; involvement of the upper thoracic spine; and associated soft-tissue mass or pedicle destruction. A noteworthy finding, useful for differentiation of a malignant tumour from spondylitis, is preservation of vertebral disc height.

Computed Tomography (CT) Scans

CT offers images with a density resolution ten times higher than plain films, allowing for a precise study of trabecular bone, and without superposition. Laredo et al. published the sensitivity and specificity of CT features in a metastatic process affecting the spine and vertebral body (VB) [51] (Table 4). Commonly a tumour mass or tissue replace normal soft cancellous bone tissue such as trabeculae; the resultant lesion can be seen with better delineation on CT than on plain films especially in very small tumour size (Figure 2). Depending on the type of the tumour, lesion evolution and host respond, some trabeculae may remain visible. Sometimes, necrosis and more rarely, calcifications can be seen as well as cortical or pedicular destruction, epidural involvement or a paravertebral mass [49]. One of the main criteria to distinguish a malignant metastasis from benign osteoporotic lesions is cortical involvement [51,52]. With contrast intravenous injection, intra or extracanal spread of the tumour can be easily studied [49]. Following intravenous contrast-media injection, CT scan can demonstrate soft-tissue masses in about two-thirds of cases [49]. A “double-bag” configuration may also be noted in cases of epidural encroachment [49].

Magnetic Resonance Imaging (MRI)

MRI is excellent radiological method to demonstrate the soft tissue



Figure 1: AP X-ray showing missing of the pedicle.



Figure 2: Axial CT scan showing moth eaten osteolysis.

details including the spinal cord and nerve roots, the intervertebral disc and extra-osseous extent of the metastasis. On T1-weighted imaging, normal bone marrow appears hypointense in children as it's very active with high vascularity and hence high-water content. This becomes progressively more hyperintense in advancing age population [49]. Signals for intravertebral metastatic lesions are of low intensity. The visibility of the extent of spine metastasis can be significantly improved by intravenous gadolinium administration, especially when they are extra-vertebral. However, the degree of tumour enhancement is variable and sometime absent in sclerotic metastasis. Furthermore, enhancement may be random and often not follow a specific pattern, but mostly start peripheral with subsequent central spread, or homogeneous [49]. On T2-weighted imaging, the signal characteristics of intravertebral lesions are variable; however increased signal intensity is most likely appearance [49]. STIR (T2) is more sensitive than T1- and T2-weighted images for detecting intra spinal metastases as it improves lesion visibility [52]. This is because fast spin-echo sequencing results in high signal intensity of fat, causing metastases to become isointense in adult bone marrow (Figure 3).

Nuclear Medicine

The sensitivity of bone scan is superior to plain radiographs for detecting spinal metastases. They rely on increase uptake of (99mTc-MDP) by new bone formation or bone deposition to detect spinal metastases [53]. The advantage of bone scan is the ability to identify intra-spinal and extra-spinal skeletal system in one image, making it a useful screening tool. It can also help in working up the patient prior to treatment and monitoring a treatment response. However, bone scan is not specific and can fail to detect lesions, especially in patients with predominantly bony destructive tumours with minimal or no osteoblastic reaction [54]. It has been reported that 52% of the patients with spine metastasis had negative bone and CT scans, however MRI detected the presence of tumour [55].

[¹⁸F] fluoro-2-deoxy-d-glucose positron emission tomography (FDG-PET) is more sensitive than bone scan and replaced bone scan as screening and staging tool in many centres. It relies on the principle of increase glucose uptake by tumour cells due to a high metabolic rate. It can detect increased glucose metabolism of metastasis and primary tumour cells making it a sensitive method for assessment of bone and bone marrow metastases. Furthermore, [¹⁸F]-FDG PET can be combined with CT scan to improve the sensitivity [56].

In conclusion, workup of a patient with suspected spine or bone metastasis requires both local and systemic staging before considering

tissue biopsy. Local staging requires CT scan to determine the bony extent of the lesion and MRI with variable sequences to assess the soft tissue and extra-osseous extension of the tumour. Systemic staging ideally requires whole body [¹⁸F]-FDG PET scan or Tc-99m HDP bone scan which has lower sensitivity.

Laboratory Studies

Laboratory tests form a part of the work up of patients with suspected or confirmed spine metastasis, but are rarely diagnostic. Routine laboratory tests required for spinal metastasis patients are complete blood count with differential count, erythrocyte sedimentation rate, urinalysis, electrolyte, specific tumour marker, and electrophoresis of serum and urine [57]. Electrophoresis of serum and urine protein, along with the presence of Bence Jones protein in urine, is useful for diagnosis of myeloma. Bone metastasis can result in anaemia and thrombocytopenia because of the deteriorating general health of the patient. Furthermore, extensive metastasis may replace the normal bone marrow and hematopoietic elements resulting in neutropenia or pancytopenia [57]. Leukocytosis may be reflecting the development of leukemia or disseminated cancer. Erythrocyte sedimentation is usually non-specific, but very high rates without a clear explanation or identifiable focus of infection may be due to metastatic spinal tumour or myeloma [57]. Hypercalcemia is a common complication of bone metastasis, present in about 17% of patients with bone metastases from breast cancer [58]. Undetected hypercalcaemia can have catastrophic consequences including sudden cardiac arrhythmias and death.

In metastatic lesions of the spine, tumour marker can be useful for confirming the primary lesion. Increase in prostate specific antigen (PSA) is associated with prostate cancer. Carcinoembryonic antigen (CEA) is typically associated with colon cancer, however, increased levels can also be found in breast cancer. Alpha-fetoprotein (α -FP) may increase in cases of hepatocellular carcinoma, and beta-human chorionic gonadotropin (β -HCG) increases in urogenital malignancies [57]. Bone metastasis is associated with increase bone destruction and new bone formation and hence with increase N-telopeptide in urine (bone destruction marker) and serum alkaline phosphatase level (bone formation marker) [59].

Prevention of Bone Metastases

It's clear that bisphosphonates inhibit malignant osteolysis, prevent bone resorption and render the bone more resistance to invasion by tumour metastasis. Bone marrow is a highly vascular structure and the site for normal hematopoietic stem cells. However, it can also provide a safe soil for tumour cells from immune-surveillance and cytotoxicity from chemotherapeutic agents [60]. Furthermore, metastatic cells which have settled in bone marrow can stay dormant for long periods of time before reactivating and metastasizing to other sites [60]. Changing this safe soil microenvironment for metastatic cancer cells in the bone marrow with a substance like bisphosphonates is emerging as an important anticancer strategy [61]. Studies have shown that the risk of distant disease recurrence and poorer prognosis is associated with circulating tumour cells (CTCs) >5 mL in 7.5 mL of peripheral blood, along with negative bone marrow biopsy testing for the presence of disseminated tumour cells (DTCs) [62-65]. Other clinical studies have shown that the potent nitrogen-containing bisphosphonates zoledronic acid can reduce DTC levels in breast cancer adjuvant therapy [66], highlighting its role in reducing risk of distant metastases. Bisphosphonates have also been shown to break the previously described pre-metastatic niche by inhibiting growth-factor release from the bone matrix and further preventing the mobilization of the cells [61]. There are further suggestions that bisphosphonates may directly inhibit cancer cell proliferation and induce apoptosis [67-69].



Figure 3: MRI thorax before and after administration of fat suppression (STIR) demonstrating improved visibility of metastasis.

Medical Research Council (MRC) Grading of muscle strength	
Grade	Description
0	No Contraction
1	Flicker or trace of contraction
2	Active movement, with gravity eliminated
3	Active movement against gravity
4	Active movement against gravity and resistance
5	Normal power

Table 1: Medical Research Council grading of muscle strength. (Used with the permission of the Medical Research Council [38]).

Frankel Grading System		ASIA Score	
Grade	Description	Grade	Description
A	Complete motor and sensory loss below the level of the lesion	A	Complete motor and sensory loss below the level of the lesion
B	Complete motor and incomplete sensory loss below the level of the lesion	B	Complete motor and incomplete sensory loss below the level of the lesion
C	Incomplete motor loss below the level of the lesion with no practical use.	C	Incomplete motor loss below the level of the lesion with more than half of the key muscles below the injury level have less than grade 3 function (not useful for ambulation)
D	Incomplete motor loss below the level of the lesion with practical use	D	Incomplete motor loss below the level of the lesion with more than half of the key muscles below the injury level have grade 3 or more function (useful for ambulation)
E	Normal motor and sensory function	E	Normal motor and sensory function

Table 2: Frankel Grading System and ASIA score [39,40].

ECOG Performance Status	KARNOFSKY Performance Status
1- Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	80- Normal activity with effort, some signs or symptoms of disease 70-Cares for self but unable to carry on normal activity or to do active work
2- Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of working hours	60- Requires occasional assistance but is able to care for most of personal needs 50- Requires considerable assistance and frequent medical care
3- Capable of only limited self-care; confined to bed or chair more than 50% of waking hours	40- Disabled; requires special care and assistance 30- Severely disabled; hospitalization is indicated although death is not imminent
4- Completely disabled; cannot carry on any self-care; totally confined to bed or chair	20- very ill; hospitalization and active support care necessary
5- Dead	0- Dead

Table 3: Comparing ECOG and KARNOFSKY performance status [41,42,87].

CT findings	Sensitivity (%)	Specificity (%)
Cortical fracture on VB side	94	91
Cortical fracture on posterior aspect	56	91
At least once cortical fracture	94	97
Cortical fragment inside medullary canal	35	97
Fracture inside VB	85	72
Circular fracture	26	97
Vacuum sign	15	100
Circular thickness of soft tissue<8mm	41	87

Table 4: The sensitivity and specificity of CT scan in a benign osteoporotic process affect the spine and Vertebrate body (VB) [51].

Treatment

For long period of time the cornerstones of cancer treatment were surgery, radiation therapy and chemotherapy [15]. Surgery has been shown to improve pain, function and the quality of life in selected patients with symptomatic spinal metastases [70-72]. However, current surgical treatments are limited since they involve only partial removal of the tumour i.e., surgical debulking, in combination with decompression and spinal stabilisation and are therefore purely palliative [73-76]. Furthermore, surgery is associated with other challenges and an overall complication rate of 25%, which may also lead to prolonged hospitalization. Therefore, surgery is preferentially reserved for patients anticipated to have greater than 3 to 6 months survival [75].

Radiotherapy and chemotherapy are limited by significant adverse effect associated with damage to the healthy tissues.

Over the past decade our increased understanding of the aetiology of cancer at the molecular level has shifted the focus away from non-specific chemotherapeutics which target all rapidly dividing cells including cancer and towards new drugs that target cancer-specific pathways. These new drugs are designed to spare normal cells and thereby offer improved safety benefits over standard chemotherapeutics, while also providing a higher therapeutic index. A classic example of this is the multi-kinase inhibitor, Sorafenib, which works by inhibiting a number of tyrosine kinase receptors including VEGFR1-3, PDGFR, KIT, and RET. It also works by down streaming the Raf signalling molecules

(Raf-1 and B-Raf). These are the first kinases in the MAPK (mitogen-activated protein kinase) cascade which regulates normal cellular function including proliferation, survival, differentiation, adhesion and motility [76]. Dysregulated activation of these direct Raf pathways has been shown to play an important role in tumorigenesis and the progression of several solid tumour types [77,78]. To address these complications, more targeted therapy focusing on the multistep process of spinal metastasis has been studied.

In-vivo Animal Studies

In vivo animal studies are very important to understand the biology, behaviour and sequence of events those results in successful establishment of spine metastasis. They provide the link between *in-vitro* and human studies and help monitor metastasis progression and response to treatment. Most animal studies utilize either rodent or rabbit host species, with rodents having the advantage of being available, cheap, easy to handle, allowing easy detection of neurological deficits, and having similar anatomical organs to humans and a high degree of gene sequence homology with humans. In addition, rodents can be manipulated for investigation of specific cancer pathways by producing knockout, transgenic, or over-expressing strains [79-81]. Immune competent animal host can reject human cancer cells and tissue, which will prevent the development of spine metastasis. Therefore, the animal used in most xenograft cancer studies are immune-deficient animals such as Balb/Cathymic nude mice and severe combined immunodeficiency (SCID) mice [79-81].

Spine metastases in animal models can be induced by inoculating cancer cells either systemically or locally [79]. Systemic administration of cancer cells can be done directly into the circulation intravenously via the rodents lateral tail vein and intra-cardiac via the left ventricle of the heart. With systemic inoculation, the location and number of metastases is unpredictable, and thus the disease course is not reproducible, making it difficult to assess therapeutic interventions. Local inoculation of cancer cells involves direct injection into the desired site for the metastasis. This method is more reliable and reproducible compared to systemic inoculation with regard to the location and timing of metastasis development in the spine [79]. However, it is more invasive and can be technically challenging given the size of the animal and the unfamiliar anatomy, which can lead to complications.

Chemokines

Chemokines are involved in tumor growth, senescence, angiogenesis, metastasis and immune evasion. The expression of chemokines and their receptors is altered in many malignancies and subsequently leads to aberrant chemokine receptor signaling.

In normal physiological functions, homeostatic chemokines regulate the migration of leukocytes by recruiting specific populations of lymphoid cells to certain tissues in either innate or acquired immune responses. Recent studies suggest that metastatic cancer cells simply co-opt these chemokine pathways to migrate to distant sites. The bone marrow is a common destination for many malignant cancers, including breast carcinoma, prostate carcinoma, multiple myeloma, lung carcinoma, uterine cancer, thyroid cancer, bladder cancer, and neuroblastoma.

The CXCR4/CXCL12 axis is one of the most studied chemokine receptor axis and has been shown to play a vital role in metastasis. Studies show that metastatic breast cancers selectively express CXCR4 and migrate to organs that express high levels of its respective ligand CXCL12, also known as SDF-1. Ligand CXCL12 is preferentially expressed in the most common sites of breast cancer metastasis, lung, brain, lymph

nodes, liver, and bone marrow. Muller et al. in a landmark study injected the tail vein of severe combined immuno-deficient (SCID) mice with the human breast carcinoma cell line MDA-MB-231 [82]. They then gave twice weekly treatment with either neutralizing anti-human CXCR4 monoclonal antibody or an isotype control. They found that *in vivo* inhibition of CXCR4-CXCL12 interactions significantly reduces metastasis of breast tumor cells to the lymph node and lungs. Furthermore, inhibition of CXCL12- CXCR4 interactions using anti-CXCR4 or CXCL12 antibodies significantly impairs these migratory responses by 63-76% and 60-62%, respectively [82].

CCL2 (also called monocyte chemoattractant protein/MCP-1) is the primary ligand for the CCR2 receptor which is normally expressed on monocyte/macrophages. The importance of the CCL2-CCR2 axis in breast and prostate cancer has been well documented and there is solid evidence for this pathway in mediating tumour growth in the bone microenvironment [83]. Preclinical studies have shown the effectiveness of CCL2 neutralizing antibodies in blocking prostate cancer tumour growth in bone both as a single agent and in combination therapy [84]. Recently, carlumab (CNTO-888), a CCL2 neutralizing antibody, was tested in Phase 2 clinical trials in patients with metastatic castration-resistant prostate cancer (NTC00992186) [85]. Unfortunately, CCL2 levels were only transiently blocked and no stable inhibition of CCL2/CCR2 signalling was observed in these patients.

Interleukin-8 (IL-8/CXCL8) is a member of the CXCL class of chemokines which bind to the receptors CXCR1 and 2. IL-8 also has potent pro-osteoclastogenic activity and has been identified as an osteolytic factor. Overexpression of IL-8 has been observed in breast cancer tumour samples and an elevated serum IL-8 level is associated with osteolysis and bone metastasis in breast cancer patients [86]. A recent study showed that disruption of IL-8-mediated signalling through use of neutralizing antibodies slowed the growth of bone tumours in mice injected with MDA-MET BC cells [86,87].

Conclusion

The spine is the most common site of skeletal metastases, with spinal metastases present in up to 36% of patients with terminal cancer. Furthermore, with an ageing population, this number is set to increase and currently the treatment of metastatic spinal disease remains largely palliative. Additionally, it is associated with morbidities such as pathological fractures, paralysis, incontinence and severe pain. Recent studies have focused on the key pathways that mediate tumour progression and spread to bone and more targeted therapies that may reduce the injury to normal cells. Further research is required to decrease the burden of this disease process.

Future Directions

Therapies that target key pathways mediating tumour migration and establishment at bone sites may prevent or delay the irreversible effects of bone fractures and onset of pain that eventuate, as well as prolong life. Chemokines are an attractive target for metastatic bone cancer. Not only are chemokines involved in most steps of the metastatic cascade, including survival, angiogenesis, invasion, and trafficking to bone but are also strongly associated with bone disease and tumour growth. Chemokine receptors are also amenable to inhibition by small pharmacological compounds. The uses of neutralizing antibodies against soluble ligands and small molecule pharmacological inhibitors that target the relevant chemokine receptors have yielded encouraging results in a number of pre-clinical trials. However, in most of these targeting a single receptor has failed to completely inhibit bone metastasis and the effect on prolonging animal survival is modest.

Advanced cancers are notorious for adapting and developing chemoresistance, possibly by switching from one chemokine system to another. Given that multiple chemokine ligand/receptor pairs may be driving bone metastasis, a combinatorial approach targeting multiple chemokine pathways simultaneously, may be required for effectively preventing bone metastasis.

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