Review Article

Lactate and cancer: spinal metastases and potential therapeutic targets (part 2)

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Abstract: Metastatic spine disease is a heterogeneous clinical condition commonly requiring surgical intervention. Despite this heterogeneity, all cases share the common theme of altered tumor metabolism, characterized by aerobic glycolysis and high lactate production. Here we review the existing literature on lactate metabolism as it pertains to tumor progression, metastasis, and the formation of painful bone lesions. We included articles from the English literature addressing the role of lactate metabolism in the following: (I) primary tumor aggressiveness, (II) local tissue invasion, (III) metastasis formation, and (IV) generation of oncologic pain. We also report current investigations into restoring normal lactate metabolism as a means of impeding tumor growth and the formation of bony metastases. Both *in vivo* and *in vitro* experiments suggest that high lactate levels may be necessary for tumor cell growth, as small molecules inhibitors of lactate dehydrogenase (LDH5/LDHA) decrease both the rate of tumor growth and formation of metastases. Additionally, *in vitro* evidence strongly implicates lactate in tumor cell migration by driving the amoeboid movements of these cells. Acidification of the local bony tissue by excess lactate production activates $CGRP^+$ neurons in the bone marrow and periosteum to generate oncologic bone pain. High lactate may also increase expression of acid sensing receptors in these neurons to generate the neuropathic pain seen in some patients with metastatic disease. Lastly, investigation into lactate-directed therapeutics is still early in development. Initial preclinical trials looking at LDH5/LDHA inhibitors as well as inhibitors of lactate transporters (MCT1) have demonstrated promise, but clinical work has been restricted to a single phase I trial. Lactate appears to play a crucial role in the pathogenesis of metastatic spine disease. Efforts are ongoing to identify small molecules inhibitors of targets in the lactogenic pathway capable of preventing the formation of osseous metastatic disease.

Keywords: Lactic acid; spinal metastases; Warburg effect; neoplasms; tumor metabolism

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Introduction

Each year more than 1.7 million Americans receive a new cancer diagnosis, most commonly of the prostate, lung, or breast (1). Though prognosis varies wildly, for the vast majority of patients, disease is progressive and forms metastases, most commonly in the lungs, liver, and bones (2). More importantly, between 40% and 70% of patients will have metastatic cancer to the spine at some point during the course of their disease (3,4). Though the symptoms of

metastatic spine disease can have significant overlap with the symptoms produced by primary vertebral body pathologies e.g., osteosarcoma, this clinical entity represents a highly heterogeneous group with a varied array of dysregulated cell signaling pathways, genetic mutations, and clinical interventions. Despite this, some commonalities have been observed, notably shared metabolic disruptions (5). Here we review the role of these metabolic perturbations, specifically the proposed mechanisms involved in lactate metabolism, as they pertain to primary tumor growth, the formation of osseous metastasis, and the generation of oncologic bone pain.

Proposed mechanisms

From the Greek *karkinos*, or crab, cancer has been known to humans since pre-literary times. Despite this relatively benign nomenclature, advances in sanitation, childhood mortality, and preventative medicine, have led cancer to become a common clinical entity that is now the second leading cause of death in the US behind only heart disease (6). Much of this increase occurred during the 20th century, the same period during which cancer was demonstrated to be a disease of genetic mutation (7). Occurring concurrently with this research on cancer genetics were investigations into neoplastic metabolism with the goal of identifying how tumor cells fed and could thus be killed (8). This work culminated in the description of the Warburg effect, which posits that tumor cells are glucose-consuming and lactate-producing, despite normoxic conditions (9,10).

During this same time, research in muscle physiology and lactate metabolism was transitioning from an era where lactate was thought to be a "dead-end waste product" formed under hypoxic conditions, to our current understanding of it being a dynamic metabolite seen under normoxic conditions (5). Lactate is readily mobilized during times of stress and is the mechanism by which whole body metabolism is coordinated. In fact, lactate flux often exceeds glucose flux, and lactate accumulation is rarely if ever due to hypoxia *in vivo* (5). For a more detailed look at normal lactate metabolism, the reader is encouraged to see part 1 of this review.

The first work demonstrating a role for lactate in tumor metabolism was generated by the Cori's and Warburg in the 1920s. The latter demonstrated increased venous lactate levels in tumor-bearing limbs of rats (11) as compared to control limb, as well as increased lactate production by tumor cells cultured in the presence of glucose (12). Incorporating contemporaneous research, lactate was suggested to be a metabolic waste product attributable to anaerobic cellular respiration within tumor cells, demonstrating a selective preference for anaerobic metabolism (5). Subsequent studies also reported increased glycolysis within cancer cells, which led to the formalized description of the Warburg effect as a key part of tumor metabolism in the 1970s by Racker (9,13).

Research by Sonveaux *et al.* intimated that the increased lactate production might occur in the hypoxic tumor regions and then get shuttled to normoxic regions for utilization as a metabolic fuel (14). This shuttling of lactate between tumor cells was in line with work in muscle physiology, where numerous cell-to-cell lactate shuttles had been described by Brooks and others (15). Work to determine which tumors cells produce lactate, and which tumor cells use lactate is ongoing and dependent on numerous dynamic factors (see part 1).

More recently, tumor cells have been demonstrated to preferentially express lactate dehydrogenase type A (LDHA or LDH-5) (13), an isoform thought to more efficiently convert pyruvate to lactate in the presence of high pyruvate levels as may be seen in Warburg-type cancer cells (5). Consistent with this, both LDHA knockdown (16,17) and administration of LDH inhibitors seem to hinder the growth of tumor cells *in vitro* (18-20) suggesting lactate metabolism to be an essential component of carcinogenesis (13). Specifically, lactate has been implicated in the role LDH plays in promoting tumor aggressiveness and metastatic disease. Tumor aggressiveness has been described as the ability to grow or spread quickly, forming metastatic foci throughout the body. Three main steps characterize this process of progressive tumor invasion: (I) angiogenesis, (II) immune system evasion, and (III) extracellular matrix (ECM) degradation and tumor cell migration. The role of lactate in each of these will be discussed in turn (*Figure 1*).

Angiogenesis

Angiogenesis is an essential component of bone tumor growth and the formation of metastases (21), especially metastases to the vertebral column, which are commonly attributed to retrograde migration through Batson's plexus (22,23). In support of this, it has been demonstrated that increases in the density of tumor microvessel formation are directly correlated to the risk of metastasis (24).

The mechanisms by which lactate mitigates angiogenesis are largely derived from investigations into the mechanisms

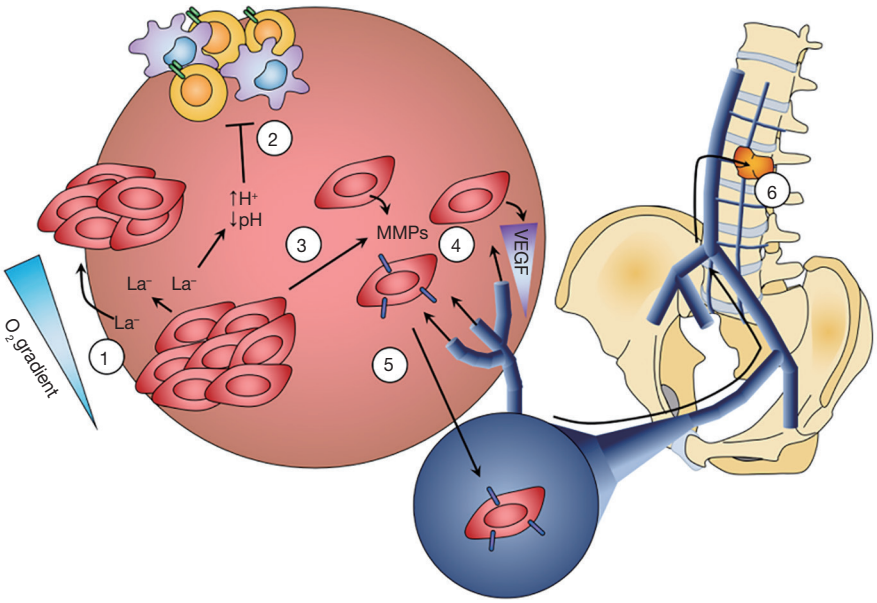

Figure 1 Lactate contributes to local tumor invasiveness and the formation of metastatic disease. 1: Warburg-type cancer cells, commonly thought to be enriched in the hypoxic regions, upregulate proteins essential for the production (LDHA/LDH5) and export (MCT4) of lactate. By contrast, reverse Warburg cells—thought to be more enriched in normoxic tumor regions—upregulate enzymes and transporters (e.g., MCT1) important for using lactate as a metabolic fuel, setting up a lactate shuttle within the tumor based upon the tumor's oxygen gradient. 2: Increased lactate production and export creates a strong ion gradient and concurrent drop in extracellular pH. This acidification leads to inhibition of immunosurveillance by impairing recognition of phenotypically abnormal cells by T-cells and NK cells, by impairing NK cell generation, and by decreasing monocyte motility. 3: Additionally, extracellular acidification disrupts the binding of cell surface integrins to the surrounding ECM, which in conjunction with downregulation of surface cadherins allows tumor cells to fragment from the central mass, leading to local invasion. This is further compounded by increases in the activity of tumor "invadopodia", increasing tumor cell motility. 4: Further facilitating tumor cell invasiveness is upregulation of matrix metalloproteinases, which digest the local ECM, and CD44, enabling tumor cells to bind and migrate along hyaluronan-based proteoglycans. By inhibiting the citric acid cycle, lactate indirectly results in upregulation of vascular endothelial growth factor (VEGF), which drives angiogenesis and vascularization of the tumor mass. 5: A secondary function of VEGF is to promote the detachment of pericytes from local blood vessels, creating fenestrations in the vessel wall that facilitate tumor cell diapedesis and subsequent dissemination to distant sites, including the bones of the vertebral column through the veins of Batson's plexus (6) (credit Z Pennington).

of wound healing (25). Local increases in extracellular lactate diffuse down their concentration gradient into the cell via monocarboxylate transporters (MCTs). Once intracellular, the law of mass action drives lactate to form pyruvate, catalyzed by LDH (26). Pyruvate accumulation inhibits the formation of α-ketoglutarate (2-oxoglutarate). In the canonical view of angiogenesis, oxygen-rich environments prevent accumulation of pyruvate and consequently α-ketoglutarate levels are high. Alpha-ketoglutarate then binds to prolyl hydroxylases, resulting in hydroxylation and subsequent degradation of hypoxia-inducible factor 1α (HIF1α) (27,28). As α-ketoglutarate is depleted though, prolyl hydroxylase activity drops, decreasing HIF1α degradation (5). HIF1α then migrates to the nucleus and upregulates pro-angiogenic genes, including vascular endothelial growth factor (VEGF). HIF1α upregulation can additionally feedback to shift tumor cell metabolism towards glycolysis by inactivating pyruvate dehydrogenase, resulting in additional lactate production (13). This is further compounded by HIF-1α-mediated upregulation of LDH type A (LDHA) (29) and MCT4 (30), leading to increased lactate efflux from tumor cells (31).

HIF1α upregulation increases VEGF expression by tumor cells. High VEGF levels promote vascular pericyte detachment, degradation of the surrounding basement membrane, and blood vessel dilatation (32). This fenestrates the vessel, allowing plasma proteins to extravasate and lay

down ECM into which endothelial cells migrate, creating new vessels that feed the tumor. Inhibition of VEGF signaling has proven to offer selective survival benefits in patients with advanced disease (32), though it remains to be seen whether such benefits are associated with changes in lactate metabolism.

Immune system evasion

At baseline, the intrinsic immune system, chiefly natural killer cells, regularly monitors the body's tissues for alterations in cell phenotype, such as those that characterize neoplastic cells (33). When said cells are encountered, they are targeted for death through either the expression of Fas/Fas ligand or release of granzymes/perforin, both of which activate the apoptotic machinery (34). Successful downregulation of these pathways through loss of heterozygosity or hypermethylation, along with upregulation of anti-apoptotic proteins, allows tumor cells to escape immune system-mediated death (35). Similarly, downregulation of the highly immunogenic antigens, the instigating entity in the extrinsic cell death pathway, allows for immune evasion.

Alternatively, tumor cells may escape immune surveillance by creating a microenvironment that silences cells of the immune system (anergy) (36). This can occur through upregulation of immune cell inhibitory molecules—PD1 (37) or CTLA-4 (38)—or alterations in the extracellular milieu (e.g., through acidification of the extracellular space) (9). Changes in extracellular lactate, a key player in this acidification, have been demonstrated to affect cells of both the lymphoid and myeloid lineages. Within myeloid-derived cells, monocytes demonstrate decreased motility in the presence of high extracellular lactate concentrations (contrasted with the increased motility seen in many cancer cells) (39). Lactate also biases tumor-associated macrophages towards a "tumor-friendly" M2 phenotype (40) and downregulates expression of both TNFα and IL-6, both of which help to mediate tumor cell immune escape (39). Similarly, high lactate levels impair IFNγ-producing T cells and NK cells of the lymphoid lineage by decreasing recognition of phenotypically abnormal cells and thus immunosurveillance within tumors (41). The acidic microenvironment created by lactate build-up compounds this issue by impairing the generation of natural killer cells responsible for tumor immunosurveillance (42).

Lactate specifically also impairs proper presentation of tumor cell antigens to members of the adaptive immune system by preventing normal differentiation of monocytes into dendritic cells (43). Similarly, high lactate levels inhibit proper function of $CD8^+$ T cells and promote T cell anergy (44) by impairing lactate exportation (45). Generation of lactate by tumor cells can also deplete extracellular glucose stores to the point of becoming insufficient to sustain the effector functions of local immune cells, such as tumor cell killing (9).

ECM degradation, local invasion, and metastatic potential

As tumor cells proliferate, they must generate new regions for expansion, which involves degradation of the ECM and invasion of local tissues. Decreased extracellular pH promotes *de novo* actin filament production, essential for cellular migration (46). It may also alter the binding properties of tumor cell surface integrins, improving the ability of tumor cells to bind ECM components and migrate along them (46). Furthermore, acidification of the extracellular space increases the number and size of tumor cell "invadopodia", themselves responsible for the amoeboid movements that underlie tumor cell migration (47). This relationship of pH to tumor growth has been demonstrated both directly *in vitro* and indirectly *in vivo* (48). Additionally, alkalization of the tumor environment *in vitro* directly inhibits tumor invasion, suggesting that acidification of the local tumor microenvironment is necessary for local invasion.

Decreased extracellular pH also activates proteinases released by tumor cells, such as matrix metalloproteinases-9 (49), cathepsin B, and hyaluronidase-2 (50). These enzymes degrade surrounding matrix, promoting tumor cell invasion, and their inhibition significantly impairs tumor cell invasion in preclinical studies, though clinical trials have been unsuccessful (51). ECM lysis by secreted proteinases may also free growth factors embedded in the matrix, including VEGF, transforming growth factor-β (TGFβ), and fibroblast growth factor-2 (FGF2), which can further promote tumor growth and angiogenesis (52). Acidification of the extracellular milieu *in vitro* leads to increased expression of hyaluronan and increased expression of tumor-specific varieties of CD44, which is responsible for binding hyaluronan and allowing tumor cell invasion (53,54).

To date, multiple *in vitro* experiments have implicated lactate or lactate metabolites either directly or indirectly in almost all of the above processes. Additionally, evidence suggests that lactate may help mitigate several of the key steps in the formation of metastatic disease, such as invasion of neighboring vessels (55,56). It is known that lactate

formation contributes to the strong ion difference (SID) *in vivo*, and consequently to acidification of the local tumor environment (5). This in turn both decreases the binding avidity of cell integrins to elements of the surrounding ECM (46) and downregulates the expression of E-cadherins on tumor cells, helping to free them from neighboring cells (57,58). Acidification of the tumor stroma also results in activation of the extracellular proteinases that degrade ECM, such as the matrix metalloproteinases (50), and increases the density and length of "invadopodia"—the foot processes used by migrating cells (47). Lactate specifically appears to upregulate cathepsin B (59) and matrix metalloproteinase-9 (60), the latter of which has been correlated with both survival and the formation of distant metastases in multiple pathologies, including breast (61), ovarian (62), and prostate cancer (63). To that end, direct inhibition of matrix metalloproteinases has prevented metastasis formation *in vivo* (64). Unfortunately, none of the early-stage clinical trials examining MMP inhibitor use have reported significant survival benefits (65).

Additionally, lactate has been demonstrated to increase the expression of CD44 (53), perhaps due to the colocalization of the lactate transporters MCT1 and MCT4 with CD44 in cultured cells (66). As stated above, CD44 is commonly upregulated in tumor cells (67) and has been associated with both tumor burden and metastasis (68). More importantly, CD44 upregulation has been demonstrated *in vitro* to potentiate the adherence of metastatic breast and prostate cells to bone marrow endothelium, a key step in the formation of bony metastases (69). CD44 may also underlie the increase in tumor migratory capacity that has been correlated with lactate and lactate metabolites (39,70,71).

Evidence from *in vivo* experiments has suggested that tumor microenvironment acidification and increased lactate concentrations are necessary for several of the key steps in the formation of tumor metastases. Rizwan *et al.* demonstrated in a murine model that both LDHA expression levels and overall lactate production correlated with disease severity. Knockdown of LDHA resulted in increased time to first metastasis and longer overall survival (72). Concomitantly, Robey *et al.* reported that use of alkalinized drinking water decreased matrix proteinase activity in a murine model of breast adenocarcinoma (73). Alkalinization of the tumor microenvironment through this mechanism also decreased the rate of spontaneous metastasis (74) and increased overall survival (73). However, the authors did not report whether the therapeutic benefits were also associated with decreases in lactate concentration. Most recently, Zhao *et al.* provided evidence suggesting that LDHA/LDH5 overexpression may mediate the epithelial-mesenchymal transition that characterizes metastatic disease (75).

Several clinical studies have also been published over the past two decades correlating tumor sample lactate concentration with the incidence of metastatic disease (*Table 1*). An early study by Schwickert *et al.* examined a cohort of patients with cervical cancer and reported significantly higher lactate levels in primary tumor samples harvested from patients with metastatic disease as compared to non-metastatic disease (76). A follow-up study by the same group also reported higher lactate concentrations to be correlated with poorer overall survival and poorer disease-free survival (78). Similar findings have been reported in patients with gastric cancer (81), head and neck cancer (77,79), and colorectal adenocarcinoma (80). These results are similar to *in vitro* findings which have demonstrated cells with higher lactate production to have greater propensity to metastasize (82).

Bone pain and osteolytic bone metastases

One of the chief concerns of metastatic spine disease to the spinal oncology surgeon is mechanical instability of the vertebral column. Progressive destruction of the anterior and middle columns steadily reduces the strength of the vertebral body, and can ultimately result in an incompetent body that is incapable of fully supporting the torso's mass (83-87). At said point the body begins to fracture and undergo height loss. Regardless of the extent of disease or collapse, 70% of patients will suffer from cancer-associated bone pain (88). Those with the most severe and concerning disease will also experience mechanical, or movement-related pain, which is commonly used as a clinical indicator of potential mechanical instability (89).

The role of lactate in osteolytic bone tumors can then be considered from two perspectives—in terms of its contribution to progressive osteolysis and mechanical instability, and in terms of its contribution to cancer-related bone pain (*Figure 2*). The latter is far more common and is thought to stem from a combination of acidification of the tumor microenvironment and stretching of the periosteum, which can occur with large or eccentrically-located tumors (88). Lactate is thought to be a major contributor to acidosis of the tumor extracellular microenvironment as lactate export can decrease extracellular pH to the 5.5–7.0 range (13). This low pH is sufficient to activate TRPV1 and ASIC3 ionotropic receptors found on the $CGRP^+$, SP^+ small,

Table 1 Studies examining serum lactate levels and metastatic disease

Study	Methodology	Findings
Schwickert *et al.*, 1995 (76)	❖ Cryostat sections of 11 tumor samples from 10 patients with cervical carcinoma of various stages	❖ Lactate levels significantly higher (P<0.05) in tumor samples from patients with documented metastases
	❖ Lactate levels in samples measured and compared between patients with and without clinically-documented metastasis	❖ Lactate levels significantly higher in viable tumor samples compared to necrotic samples
Walenta *et al.*, 1997 (77)	❖ Specimens from 15 patients with head and neck cancer	❖ Sample lactate levels significantly higher in patients with metastatic disease (12.3 *vs.* 4.7 µmol/g; P<0.005)
	❖ Quantitative bioluminescence imaging of lactate levels compared between patients with and without metastatic disease	❖ Lactate levels spread over a greater range in patients with metastases
Walenta *et al.*, 2000 (78)	❖ Cryostat sections of 35 tumor samples from 34 patients with cervical carcinoma (most stage II or III)	❖ Lactate levels were significantly higher (P=0.001) in tumor samples from patients with metastatic disease
	❖ Lactate levels in samples measured and compared between patients with and without detectable metastases	❖ High tumor lactate levels were associated with significantly worse disease-free (60.5 *vs.* 22.1 months; P=0.014) and overall survival (70.9 *vs.* 31.0 months; P=0.015)
	❖ Lactate levels correlated with overall and disease-free survival	
Brizel *et al.*, 2001 (79)	❖ Biopsies from 34 patients with head and neck cancer	❖ High tumor lactate concentrations correlated with poorer metastasis-free survival at 2 years (90% *vs.* 25%; P<0.0001)
	❖ Tumor samples analyzed for lactate concentration	❖ High tumor lactate concentrations correlated with poorer 2-year overall survival (90% *vs.* 35%; P<0.0001)
	❖ Sample lactate concentrations correlated with overall and metastasis-free survival at 2 years	❖ Median lactate concentration in tumors that metastasized significantly lower than those remaining local (12.9 *vs.* 4.8 µmol/g; P<0.005)
Walenta *et al.*, 2003 (80)	❖ 33 cryobiopsy samples from 24 patients with rectal adenocarcinoma	❖ Lactate levels significantly higher in patients with metastatic disease (13.4 *vs.* 6.9 µmol/g; P<0.005)
	❖ Lactate levels measured and compared between metastatic and nonmetastatic groups	❖ No significant difference in lactate levels between normal tissue or adenoma and non-metastatic disease
		❖ Nodal involvement did not correlate with lactate levels
		❖ All patients with metastatic disease had lactate levels greater than 8.0 µmol/g
Hur *et al.*, 2013 (81)	❖ Samples from 152 patients having undergone surgery for gastric adenocarcinoma	❖ PDK-1 levels correlated with overall and progression-free survival
	❖ Samples stained for PDK-1 and subjected to glucose uptake and lactate production assays	❖ *In vitro* levels of PDK-1 expression correlated with higher lactate production
		❖ 5-fluorouracil-mediated cell killing decreased PDK-1 expression which was correlated with decreased lactate production

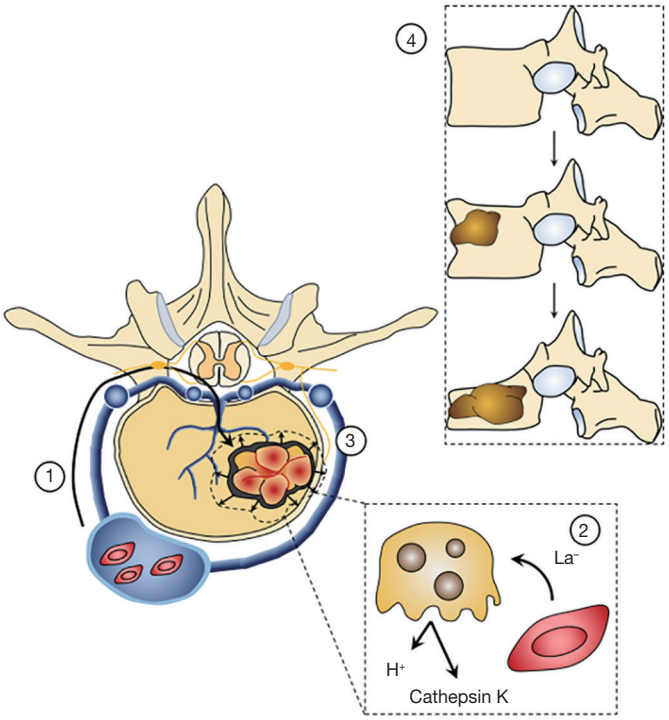

Figure 2 Increased lactate production contributes to progressive osteolysis, bone pain, and mechanical instability in the spine. 1: Circulating tumor cells can move retrogradely through lumbar intersegmental veins and into the vertebral venous plexus (of Batson). From there they progress through basilar veins to seed the vertebral body. Once the tumor cells have instantiated themselves in the local bone, they begin to proliferate. 2: The actively proliferating cells release lactate into the local microenvironment, which can be taken up and used as fuel by osteoclasts. Increased osteoclastic activity results in greater destruction of local bone through acidification and the release of proteases such as cathepsin K. The increased acidity of the bone milieu is thought to activate acid-sensitive receptors, such as ASIC1a and ASIC 1b, on CGRP$^+$ nociceptive afferents that innervate the bone, creating the oncologic pain seen in many patients with bony metastases. 3: Additionally, as the tumor continue to grow, it may compromise the cortical bone and displace the overlying periosteum. The latter is richly innervated by nociceptive afferents, giving rise to mechanical pain. 4: As the lesions continue to increase in size (top to bottom), the structural integrity of the vertebral body is compromised, leading to wedging and eventual vertebral body collapse. This mechanical instability may require surgical intervention and reconstruction (credit Z Pennington).

unmyelinated nociceptive afferents that innervate the bone marrow and periosteum (90-93). Proton release by activated osteoclasts may additionally contribute to activation of these fibers (88). Evidence for this latter mechanism of bone pain is equivocal at best though, as randomized controlled trials of bisphosphonates—inorganic molecules that inhibit osteoclast activity—have provided only weak evidence suggesting that they are effective at relieving oncologic bone pain (94).

CGRP$^+$ sensory neurons innervating the bone also appear to play a role in the neuropathic pain experienced by many patients with metastatic disease, possibly through the formation of neuromas at the sensory tips of neurons. These neuromas are thought to underlie the breakthrough pain experienced by many patients with metastatic disease (88), which is notoriously difficult to treat (95). Tumors may also directly injure nociceptive fibers, in part explaining the relative intractability of bone pain to conventional analgesics (95). Along these lines, experiments in a murine model of breast cancer have demonstrated upregulation of acid-sensing receptors—ASIC1a and ASIC1b—in sensory neurons innervating compromised bone, as compared to those innervating unaffected bone (96). These changes have previously been linked to hyperalgesia and allodynia (97). Phenotypic changes are also noted in the machinery of the dorsal horn with increased expression of immediate early genes (96) previously tied to the development of neuropathic pain (98).

Bone remodeling is normally characterized by the balanced activities of osteoblast and osteoclasts, which lay down and resorb bone matrix, respectively (99). In cases of metastatic disease to the spine, these activities can become unbalanced resulting in either net bony deposition, or more commonly, bony destruction, visualized radiographically as osteoblastic and osteolytic lesions, respectively. As the tumor progresses in size and net bone mineral density of the vertebral body decreases, loading of the spine can result in a series of microfractures (88). These destabilizing fractures have two effects: (I) progressive vertebral height loss and potential deformity, and (II) distortion of the overlying periosteum with application of load to the vertebral column (100). Periosteal distortion stretches and activates the nociceptive neurons, creating oncologic pain, which is often excruciating owing to the high density of neuronal input to the periosteum (88).

In vitro experimentation has suggested that lactate may also play a key role in generating this mechanical instability-related pain. Using a human-derived breast adenocarcinoma line, Lemma and colleagues demonstrated that lactate produced by the tumor cells feeds and consequently activates local osteoclasts (82). High lactate production was associated with upregulation of MCT4s and downregulation of MCT1s in tumor cells, consistent with the conclusion that the high extracellular lactate levels results from net lactate export (82). By contrast, osteoclasts demonstrated phenotypic changes more consistent with increased uptake of lactate. Inhibition of MCT1 led to significant decreases in osteoclast-dependent collagen 1 degradation where lactate-feeding increased degradation, suggesting that neoplasm-derived lactate feeds osteoclasts to promote osteolysis (101).

Proposed therapeutic targets

Drugs with the potential to block or alter lactate metabolism in such a way as to halt tumor progression, radiosensitize the tumor, or decrease vertebral column osteolysis may have clinical utility. In their recent review, Muir and Vander Heiden note that much of our understanding of the tumor microenvironment is limited by experimental models though (102). For example, cell cultures replicate neither the exact milieu that makes up the tumor microenvironment, nor do they recreate the three-dimensional relationship a tumor takes on in the *in vivo* environment. Nonetheless, therapies targeting various "metabolic checkpoints" are currently being explored.

Dichloroacetate, monocarboxylate inhibitors, and LDH inhibitors

To date several interventions directed at addressing lactate metabolism have been implemented with varying degrees of success. Pyruvate dehydrogenase kinase (PDK) inhibitors, originally employed for patients with lactic acidosis, have been tested for their ability to remove inhibition of the pyruvate dehydrogenase complex and allow pyruvate to be shuttled away from LDH and into the citric acid cycle (17). These drugs, notably dichloroacetate (DCA), have been demonstrated to inhibit tumor growth both *in vitro* and *in vivo* (103) as well as decrease metastatic progression *in vivo* (104). A recent phase II clinical trial investigating the use of DCA in refractory breast and non-small cell cancer found adverse effects to be unacceptably high though, leading to early termination (NCT01029925). By contrast, a subsequent phase 1 study in glioblastoma found it to be reasonably well tolerated (105). Other phase 1 studies in head and neck cancer (NCT01163487), squamous cell cancer (NCT01386632) and metastatic solid tumors (NCT00566410) have been conducted but have failed to report therapeutic benefit.

LDHA/LDH5 and lactate transporters (e.g., the MCT family) have also been targeted. *In vitro* experiments have demonstrated that LDHA knockdown impairs tumor growth (16,106) and *in vivo* experiments have confirmed that animals injected with LDHA-deficient tumors have significantly improved survival relative to control animals (16). Small molecule inhibition of LDHA was similarly effective at inhibiting tumor progression *in vitro* (18,107-112) and *in vivo* (20). As of yet, none of the small molecule inhibitors has progressed to the point of being a clinically viable treatment and no clinical trials have been registered (113), possibly due to the bidirectional, near equilibrium nature of LDH, regardless of isoform.

MCT inhibitors have demonstrated somewhat greater success. MCT1 inhibitors (e.g., AZD3965) have shown anti-tumor activity *in vitro*, significantly impairing lactate production and leading to massive tumor cell die off (14). Recently, *in vitro* and *in vivo* results of the inhibitor AR-C155858 have demonstrated mixed results with regard to its effects on tumor growth (114,115). Curiously, AR-C155858 demonstrates higher MCT1 affinity than AZD3965 (116,117), though it is the latter which has demonstrated more success *in vivo* (118,119) and is currently being tested in a phase 1 trial (NCT01791595). This contradictory result may be secondary to tumor adaptation as has been

demonstrated with other MCT1 inhibitors. In these studies MCT1 inhibition led to MCT4 upregulation and "tumor escape," which may prove problematic as MCT inhibitors continue to be moved along the pathway to clinical utilization (120). Our personal experience with several MCT inhibitors in mice demonstrated them to be ineffective at tumor control, and in some cases toxic, as some older inhibitors [e.g., α-cyano-4-hydroxycinnamate (CHC)] had low target specificity and multiple off-target effects, including inhibition of pyruvate transporters (personal communication with Andrew Halestrap, University of Bristol, 2013).

Although drugs that seek to lower lactate are promising, care must be taken to consider the model used and the various roles lactate has in normal metabolism. For example, pertinent to the spine, oligodendrocytes rely heavily on lactate as fuel (121) and as a precursor for making lipids. Murine and human oligodendrocytes highly express MCT1, the inhibition of which results in significant axonal damage *in vitro* and *in vivo* (122). Additionally, reduction of MCT1 expression is seen in both patients with amyotrophic lateral sclerosis (ALS) and animal models of ALS, suggesting a critical role for lactate in neuronal function. Therapies that non-specifically block lactate flux may therefore have profound deleterious effects on central nervous system white matter (123).

Finally, it should be emphasized that both LDH and MCTs readily operate in both directions. MCTs function via diffusion, while lactate-pyruvate interconversion is a near-equilibrium reaction regardless of LDH subtype (124). Although traditional thinking teaches that MCT1s favor import and MCT4s favor export, in actuality, both isotypes perform both activities (5). To this end, MCT1s may appear to favor import as they are commonly upregulated in tissues that consume lactate, as compared to MCT4s, which are preferentially upregulated in lactate-exporting tissues (5). Newsholme has similarly pointed out that LDH subtype is likely of minimal importance given that it does not alter the net free energy change of lactate-pyruvate interconversion (125). In summary, while LDH and MCT inhibitors offer tremendous potential, care should be used in experimental trials in light of their bidirectional nature.

Radioresistance

Several groups, including those of Quennet *et al.* (126) and Sattler *et al.* (127) have demonstrated that *in vivo* lactate concentrations directly correlate with tumor response to fractionated irradiation. Lactate is proposed to confer this radioresistance indirectly by means of pyruvate generation (127). Pyruvate is a potent free radical scavenger and so may prevent accumulation of these species, which mediates DNA damage and cell death (128). Along these lines, use of inhibitors that decrease pyruvate levels should increase lesion radiosensitivity. This has been demonstrated *in vivo* using MCT1 inhibitors in murine models of small cell (129) and non-small cell lung cancer (14). Most recently, Corbet *et al.* published the results of an experiment examining the effects of a mitochondrial pyruvate transporter on radiosensitivity in a murine model of cervical carcinoma (130). Blockage of this transporter decreased tumor cell lactate uptake and resulted in cell killing as opposed to cell senescence, which was seen with application of an MCT1 inhibitor (AR-C155858). Further evidence is required to evaluate the utility of this new clinical target.

Conclusions

Though held as a metabolic waste product for the better part of a century, lactate has steadily come to be appreciated as an essential component of tumor carcinogenesis. Both *in vitro* and *in vivo* work has demonstrated it to play key roles in tumor growth, angiogenesis, the epithelial-to-mesenchymal transition, and the formation of painful osteolytic metastases. Due to its relatively late appearance in the cancer literature, clinical interventions aimed at addressing dysregulated lactate metabolism are currently undergoing preclinical and early clinical investigation. Early preclinical results have been promising and suggest that restoration of normal lactate homeostasis may inhibit the formation of bony metastasis, a potential boon for the spinal oncologist. Additionally, blockage of lactate exportation may increase tumor radiosensitivity and thereby provide potential interventions for patients with mechanically stable metastatic spine disease who are too frail to undergo surgical intervention. Much additional research is necessary before any changes in clinical standard of care can occur. But success in these endeavors may present spinal surgeons with an option for prophylaxis against progressive spinal instability—an intervention which is currently unavailable.

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Footnote

Conflicts of Interest: ML Goodwin: Consultant for ROM3, Augmedics; DM Sciubba: Consultant for Orthofix, Globus, K2M, Medtronic, Stryker, Baxter. The other authors have no conflicts of interest to declare.

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