Biochemical and Biophysical Research Communications 391 (2010) 91–95

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cancer cells release glutamate via the cystine/glutamate antiporter

Mohit K. Sharma [1], Eric P. Seidlitz [1], Gurmit Singh *

Department of Pathology & Molecular Medicine, McMaster University, Ont., Canada
Research Department, Juravinski Cancer Centre, Hamilton, Ont., Canada

ARTICLE INFO

Article history:
Received 26 October 2009
Available online 5 November 2009

Keywords:
Bone metastasis
Breast cancer
Cell lines
Cystine
Glutamate
Glutamate transporters
Intercellular communication
Metabolism
Prostate cancer

ABSTRACT

Although the amino acid glutamate is used as an intercellular signaling molecule for normal bone homeostasis, little is known regarding its possible role in the metabolic disruption characteristic of bone metastasis. We have previously shown *in vitro* that cancer cell lines relevant to bone metastasis release glutamate into the extracellular environment. This study demonstrates the expression of multiple glutamate transporters in cancer cell lines of non-central nervous system origin. Furthermore, we identify the molecular mechanism responsible for glutamate export and show that this system can be inhibited pharmacologically. By highlighting that glutamate secretion is a common biological feature of cancer cells, this study suggests that tumor-derived glutamate could interfere with glutamate-dependent intercellular signaling in normal bone. Pharmacological interference with cancer cell glutamate release may be a viable option for limiting host bone response to invading tumor cells in bone metastasis.

Introduction

Breast and prostate cancer cells that metastasize to bone frequently provoke significant alterations in bone metabolic processes that result in pain, hypercalcaemia, and weakening of bone structure [1]. The mechanisms that generate these effects are unknown, although factors released by invading cancer cells are likely involved in signaling these responses [2,3]. The amino acid glutamate has been shown to be critical for normal bone metabolic processes [4–6], and we have shown that a variety of cancer cells secrete glutamate into their extracellular environment [7]. Tumor cell derived glutamate may have important functional implications for the bone microenvironment.

Although the expression of both vesicular and non-vesicular glutamate transporters have been demonstrated in tumors derived from central nervous system (CNS) and peripheral sites, it is not clear whether a common secretion mechanism is used between different tumor types. Although glial-derived cancers release glutamate via the glutamate/cystine antiporter mechanism known as system x_c^- [8–12], it is not known whether non-CNS derived cancers use the same mechanism. Other candidate glutamate transporters include the glutamate–aspartate transporter (GLAST-1 or the human homolog EAAT1) [13], glutamate transporter 1 (GLT-1 or the human homolog EAAT2) [14], and the vesicular glutamate transporters (VGLUT) [15–17].

This study was designed to evaluate the mRNA expression of GLAST, GLT-1, VGLUT1, and system x_c^- in a series of cancer cell lines. Secondly, protein expression of the transporters primarily involved in glutamate secretion was assessed *in vitro* and in a mouse model of breast cancer bone metastasis. As a functional measure, we quantified the amount of glutamate released from cancer cells *in vitro* using glutamate-free culture media and with specific pharmacological inhibition of vesicular and non-vesicular glutamate transporters.

Materials and methods

All protocols for animal studies were reviewed and approved by the Animal Research Ethics Board of McMaster University, Hamilton, Ontario, Canada, and adhered to the guidelines of the Canadian Council on Animal Care.

Cell lines. Mycoplasma-free cancer cells (obtained from the American Type Culture Collection, Manassas, VA, unless otherwise stated) were maintained in a humidified incubator with 5% CO_2 in room air using DMEM, DMEM/F12 (Ham), MEM, or RPMI-1640 (Invitrogen Canada, Inc., Burlington, Ontario, Canada) supplemented with 10% fetal bovine serum and antibiotics (100 U/mL

Abbreviations: CNS, central nervous system; EAAT1, excitatory amino acid transporter 1; EAAT2, excitatory amino acid transporter 2; GLAST-1, glutamate/aspartate transporter 1; GLT-1, glutamate transporter 1; VGLUT1, vesicular glutamate transporter 1; xCT, light-chain subunit of system x_c^- glutamate/cystine antiporter.

* Corresponding author. Address: Research Department, Juravinski Cancer Centre, 699 Concession Street, Hamilton, Ont., Canada L8V 5C2. Fax: +1 905 575 6368.
E-mail address: gurmit.singh@jcc.hhsc.ca (G. Singh).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.10.168

penicillin sodium and 100 μg/mL streptomycin sulfate, Invitrogen). Experiments were performed after switching cells to glutamate-free media after seeding, and using DMEM (Invitrogen) with no added serum and antibiotics. The cell lines used were MDA-MB-231 (human breast cancer), MCF-7 (human breast cancer), C3L5 (mouse breast cancer—a kind gift from Dr. P. Lala, University of Western Ontario), B16F1 (mouse melanoma), B16F10 (mouse melanoma), MAT-LyLu (rat prostate cancer), and CNS-1 (rat astrocytoma).

Transporter expression. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to evaluate the expression of transporters involved in secreting glutamate, VGLUT1 (vesicular glutamate transporter) and system x_c^- (the glutamate–cystine antiporter), and those involved in glutamate uptake, GLAST (glutamate–aspartate transporter) and GLT-1 (glutamate transporter). Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Mississauga, Ontario, Canada) according to manufacturer's instructions and quantified spectrophotometrically. cDNAs from 1 μg of RNA were generated using a Superscript III RT-PCR kit (Invitrogen). Gene sequences for glutamate transporters were obtained from the NCBI nucleotide database (GenBank®). Since the genes for glutamate transporters GLAST, GLT-1, VGLUT1, and the xCT subunit of system x_c^- are highly conserved, single primer sets were designed for each transporter and used for all species: GLAST (Accession IDs: NM_004172, NM_148938, and NM_019225, respectively): 5′-gttgtcttctccatgtgcttcg-3′ (forward) and 5′-gcttgcagcaaccctcca-3′ (reverse) (326 bp); GLT-1 (Accession IDs: NM_004171, NM_001077514, and NM_017215, respectively): 5′-ggtgtcatcctgggagcagt-3′ (forward) and 5′-caatgatggtcgtggacatgtaa-3′ (reverse) (235 bp); VGLUT1 (Accession IDs: NM_020309, NM_182993, and NM_053859, respectively): 5′-cagagactgtcggcctcataca-3′ (forward) and 5′-aggatcctcacgaagatgacac-3′ (reverse) (196 bp); xCT (Accession IDs: NM_003982, NM_011405, and NM_031341, respectively): 5′-taacctttgcaagctcacagca-3′ (forward) and 5′-aacacacc accgttcatggag-3′ (reverse) (427 bp). Amplification of cDNA was conducted by denaturing cDNA at 94 °C for 5 min, followed by a 35-cycle PCR under the following conditions: denaturing at 94 °C for 30 s, annealing at 60 °C (for GLAST, GLT-1, and VGLUT1; 62 °C for xCT) for 30 s, followed by extension at 72 °C for 30 s.

Western blot analysis for protein expression was performed with equal protein loading of whole cell lysates using 10% SDS–PAGE gels and subsequent electrophoretic transfer to nitrocellulose membranes (BioTrace NT, Pall Corporation, Pensacola, FL). Protein concentrations were measured using the Bio-Rad assay (Bio-Rad Laboratories, Hercules, CA). Expression of system x_c^- was evaluated using a polyclonal antibody for the light-chain subunit called xCT (Novus Biologicals, Littleton, CO) applied overnight at 4 °C with blocking in 5% non-fat milk. After washing, horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (Santa Cruz, CA) were applied and visualized by enhanced chemiluminescence using an ECL-plus kit (GE Healthcare, Buckinghamshire, UK) on Kodak Biomax XAR film (Kodak Canada). β-Actin was used as a loading control.

Cell growth and glutamate measurements. Growth was measured using a crystal violet assay in 96-well plates with all cell lines seeded at 10,000 cells per well. Glutamate levels were evaluated in cell culture media with the AMPLEX red® glutamic acid kit (Invitrogen/Molecular Probes, Eugene, OR) and analyzed on a fluorescence plate reader (Cytofluor Series 4000). The AMPLEX red® glutamic acid kit assay was optimized for measurement of glutamate concentrations above 0.5 μM by omitting L-alanine and L-glutamate pyruvate transaminase from the reaction, thus eliminating signal amplification via repeated cycling of glutamate through α-ketoglutarate. All treatment drugs were prepared in aqueous solutions as recommended by the manufacturer (Sigma–Aldrich, St. Louis, MO). Sulfasalazine (Sigma, S0883) was dissolved in 1 M NH_4OH. The dose of sulfasalazine was selected as the 50% inhibitory concentration (IC_{50}) in a previous dose–response study using MDA-MB-231 cells (data not shown). Preliminary studies with higher doses of Evans Blue (10 μM and 100 μM) showed significant cell death (data not shown). The highest dose of Evans Blue that did not have a significant effect on cell survival was 2.5 μM, and was thus used in transport inhibition studies. A concentration of 1 μM has been shown in other cell lines to effectively inhibit VGLUT1 [17].

Tissues. A model of experimental bone metastasis in mice was prepared as previously described [18,19]. Briefly, 10^5 MDA-MB-231 cells were percutaneously injected into the left cardiac ventricle of 5-week-old Balb/c nu/nu mice (Charles River, St. Constant, Quebec, Canada) using a 26-gauge needle. Animals were sacrificed after 28 days and the long bones were harvested and fixed in formalin. Following decalcification with formalin–EDTA, the tissues were embedded in paraffin and sectioned at a thickness of 4 μm for immunohistochemistry analysis. Polyclonal antibodies against xCT (Novus Biologicals, Littleton, CO) were applied and biotinylated goat anti-rabbit secondary antibodies were visualized using the Vectastain ABC and NovaRED kits (Vector Laboratories, Burlingame, CA). Samples were mounted for routine light microscopy using Permount (Vector).

Statistical analysis. Glutamate release data were tested for differences between means using one-way ANOVA and post hoc two-tailed Student's *t*-tests. Differences were considered significant at $p < 0.05$.

Results

Cancer cells express many glutamate transporters

Using semi-quantitative RT-PCR, mRNA expression for GLAST was identified in five of the tested cancer cell lines, while GLT-1 was expressed in the remaining three (Fig. 1A). MDA-MB-231 cells, however, expressed both the GLAST and GLT-1 transporters.

The mRNA for the vesicular glutamate transporter VGLUT1 was expressed in most of the cancer cell lines tested. As a positive control expected to show VGLUT1, the rat astrocytoma cells (CNS-1) demonstrated strong mRNA expression. The light-chain subunit of system x_c^- called xCT was consistently expressed in all the cancer cell lines (Fig. 1B). The relative expression levels of VGLUT1 and xCT were evaluated by densitometry in three cancer cell lines from different primary sites that are used in bone metastasis models: MDA-MB-231 (breast), MAT-LyLu (prostate), and B16F1 (melanoma). Expression was normalized to GAPDH as a house-keeping gene (Fig. 2).

MDA-MB-231 and B16F1 cells show similar levels of VGLUT1 and xCT transcripts, and MAT-LyLu cells show higher levels of xCT than VGLUT1. Overall, the only cell line to express all of the glutamate transporter mechanisms was the MDA-MB-231 human breast cancer line, cells that we have applied previously in an *in vivo* model of osteolytic bone metastasis [18].

Transporters involved in glutamate secretion are expressed at the protein level

Using Western blot analysis, the presence of xCT subunit was evaluated in all the tested cell lines and its expression was confirmed in all lines (Fig. 3A). Immunohistochemistry analysis of tissues from nude mice demonstrated clear xCT immunostaining in localized regions of a brain cross section (positive control, Fig. 3B). Also, from untreated mice, little or no xCT expression was observed in normal bone (b) or marrow (m) cells (Fig. 3C). However, when the bone was colonized with the strongly osteolytic MDA-MB-231 cells, there was marked xCT expression in both the tumor cells (t) and in the host

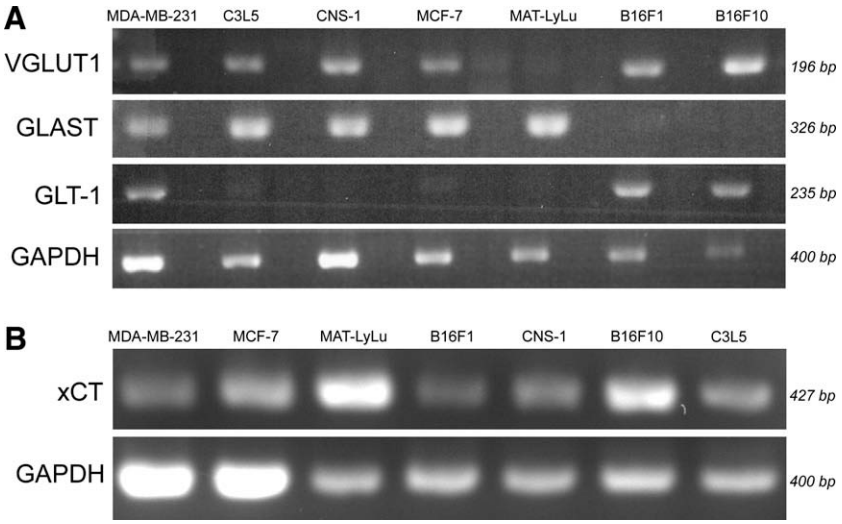

Fig. 1. Glutamate transporter mRNA expression in cancer cell lines. mRNA expression of glutamate transport mechanisms was analyzed by RT-PCR. (A) Gene expression of the vesicular glutamate transporter (VGLUT1), and the non-vesicular plasma membrane transporters called glutamate–aspartate transporter (GLAST) and glial glutamate transporter 1 (GLT-1). (B) Gene expression of xCT, the catalytic subunit of system x_c^- in all the tested cell lines. GAPDH was used as the house-keeping gene.

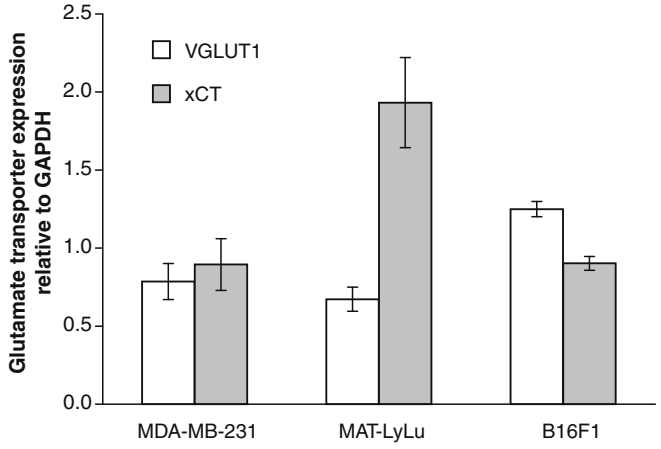

Fig. 2. Relative expression of VGLUT1 and xCT subunit normalized to GAPDH expression. The cancer cell lines analyzed were MDA-MB-231 (breast), MAT-LyLu (prostate), and B16F1 (melanoma). Data are expressed as means ± SEM and are generated from three independent experiments.

bone (b) (Fig. 3D). In comparison to the xCT protein, VGLUT1 expression was not as robust (data not shown).

System x_c^- is responsible for glutamate release

Glutamate release was measured from three cancer cell lines in the presence of the VGLUT1 inhibitor Evans Blue and the system x_c^- inhibitor sulfasalazine in DMEM. Glutamate release rate from each cell line (normalized to cell number) was significantly inhibited by sulfasalazine ($p = 0.028$, $p = 0.005$, and $p = 0.004$ for the MAT-LyLu, B16F1, and MDA-MB-231 cells, respectively) (Fig. 4). Evans Blue did not significantly change the glutamate release in any cell line. When both inhibitors were added together, the combination was not statistically different from the sulfasalazine-alone group (data not shown). No significant changes in cell growth were observed in any treatment group.

Discussion

The mechanisms through which metastasizing cancer cells cause functional disruption of bone are not fully characterized. Like the brain, bone uses the amino acid glutamate as a specific intercellular signaling molecule [5,20], and the tightly regulated glutamatergic processes in bone could become disordered if excess glutamate is present in the environment. Although the detrimental effects of tumor-derived glutamate on host organ functions are well established in malignant glioma [9,21], it is unclear whether an analogous process may occur in bone metastasis. We have previously shown that a variety of cancer cells relevant to bone metastasis secrete glutamate *in vitro* [7], but the transport mechanism responsible for this release was not known. This study establishes that several cancer cell lines relevant to bone metastasis actively secrete glutamate using a transport mechanism that is expressed at the mRNA and protein level, and that this glutamate release can be pharmacologically inhibited.

Several glutamate transport systems are known to be expressed outside of the CNS and may be capable of driving glutamate secretion. These included the plasma membrane transporters GLAST and GLT-1, the vesicular glutamate transporter VGLUT1, and the system x_c^- glutamate/cystine antiporter. GLAST and GLT-1, also called the excitatory amino acid transporters, are frequently expressed in a complementary fashion in the CNS [22]. This mutually exclusive expression pattern is evident in seven of the cell lines tested in this study, although MDA-MB-231 cells appear to express mRNA for both transport mechanisms (Fig. 1A). Aside from one report of GLAST expression in MG-63 osteosarcoma cells [23], this study clearly demonstrates that these transporters are expressed in non-CNS tumor cell types. Although primarily involved in transporting glutamate into cells [14], there is evidence that GLAST and GLT-1 may function in the CNS to release glutamate under certain ion gradient perturbations [24]. Since it is uncertain whether a peripheral tumor environment would support such conditions, the role of these transporters in tumor cell glutamate release *in vivo* is undetermined. The *in vitro* culture conditions required for this study, however, preclude a proper evaluation of this concept.

Vesicular glutamate transporters, involved in glutamate release from presynaptic neurons, have also been identified in a variety of peripheral sites, including pancreas, upper GI, adipose tissue, and testis [15,25,26]. This study is the first to clearly demonstrate VGLUT1 expression in a range of cancer cell types of peripheral origin (Fig. 1A). However, VGLUT1 does not appear to play a role in glutamate release from the tested cancer cell lines, as an inhibition

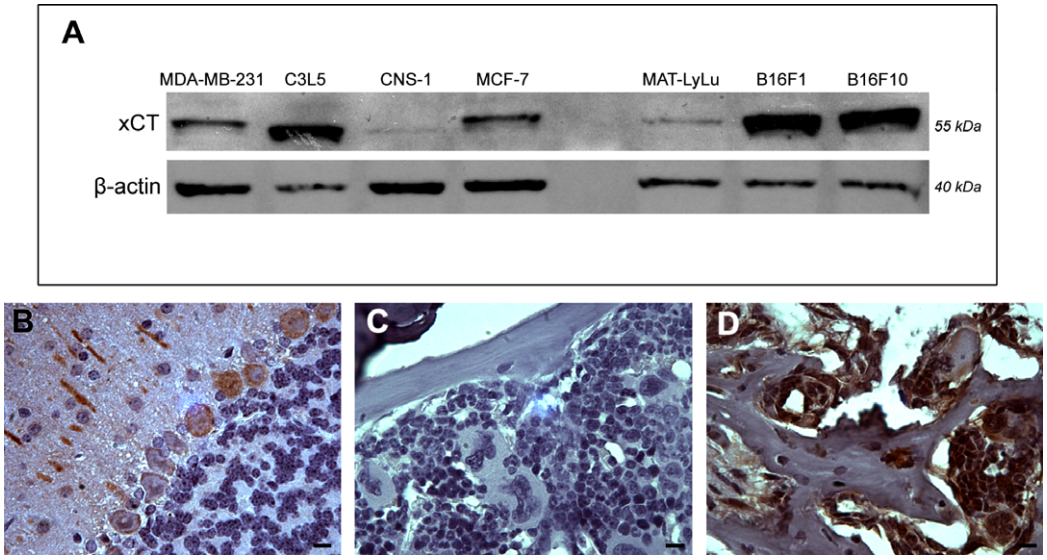

Fig. 3. Protein expression of the light-chain subunit of system x_c^- in cancer cell lines and in nude mice with MDA-MB-231 human breast cancer bone tumors. (A) Protein expression of xCT by Western blot analysis of the light-chain subunit of system x_c^- in a panel of human and murine cancer cell lines. β-Actin was used as a loading control. (B–D) Immunohistochemistry for xCT in tissues from nude mice, with brain used as a positive control (B). Normal bone (b) and marrow (m) from untreated mice is shown in (C), and bone with MDA-MB-231 human breast tumors (t) is shown in (D).

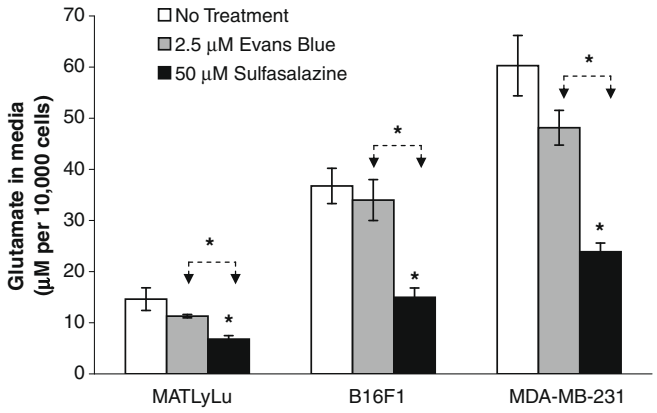

Fig. 4. Effect of glutamate transport inhibition on 48-h relative glutamate release and cell growth from three cancer cell lines grown in DMEM. Cancer cells were grown in 96-well plates for 48 h with glutamate transport inhibitors. Glutamate release was measured in the conditioned media and the relative glutamate release was calculated as the absolute glutamate concentration normalized by the number of cells remaining in each well at 48 h (μM glutamate per 10,000 cells). Data are expressed as mean ± SEM from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ but not $p < 0.001$. Cell growth was measured but there were no significant changes when compared to untreated cells. With Evans Blue treatment, cell growth was 106.8%, 114.6%, and 154.0% compared to control MAT-LyLu, B16F1, and MDA-MB-231 cells, respectively. For sulfasalazine, cell growth was 88.7%, 127.3%, and 99.4% compared to untreated MAT-LyLu, B16F1, and MDA-MB-231 cells, respectively.

of this transport mechanism with 2.5 μM Evans Blue had no significant effect on relative glutamate export (Fig. 4).

The system x_c^- glutamate/cystine antiporter, a mechanism that exchanges one molecule of intracellular glutamate for one molecule of extracellular cystine [27], functions primarily as a means of accumulating intracellular cystine for the subsequent synthesis of glutathione, the primary antioxidant in many cells [28]. Cancer cells require high levels of glutathione to counteract the oxidative stress arising from their increased metabolic rate, and the gene for the catalytic subunit of system x_c^- (xCT or *SLC7A11*) is expressed [29,30], and potentially upregulated [31] in many cancer cells. Although several reports show that xCT is expressed in breast cancer cell lines [29,30], we have confirmed that xCT is expressed in *all* cancer cell lines tested, regardless of their tissue of origin (Fig. 1B). Furthermore, our protein analysis verifies xCT expression *in vitro*, although the xCT antibody may not effectively cross-react with rat xCT (CNS-1 and MAT-LyLu cells are rat cell lines) (Fig. 3A).

Immunohistochemistry of mouse bone with human breast cancer tumors demonstrates *in vivo* that xCT is strongly expressed in the tumor cells as well as in host bone (Fig. 3D). An apparent increase in xCT expression in bone stroma suggests that system x_c^- may be upregulated in response to the presence of tumors in the bone environment. The predominant role of the glutamate/cystine antiporter in glutamate release is supported by our observation of a direct correlation between cystine availability in the culture media and the relative glutamate release rate in all three cell lines tested—when untreated cells were grown using MEM instead of DMEM (MEM contains 99 μM cystine; DMEM contains 201 μM cystine) the relative glutamate release over 48 h was virtually half the amount of glutamate released by cancer cells grown in DMEM (6.47 μM per 10,000 cells (±0.76) for MAT-LyLu, 20.13 (±0.41) for B16F1, and 31.11 (±4.05) for MDA-MB-231 cells). Furthermore, we have shown functionally that inhibition of system x_c^- by sulfasalazine significantly decreases glutamate release in all the tested cell lines (Fig. 4), although this low-dose effect was without the cell growth inhibition observed in breast cancer cells with higher drug doses [29]. High-dose sulfasalazine significantly reduces cell proliferation [32], decreases glutathione production, and may make cancer cells more sensitive to chemotherapy [33]. Although system x_c^- has been suggested as a viable therapeutic target for impeding the growth of primary cancers [34,35], preventing tumor cell glutamate release is an attractive concept for limiting glutamatergic disruption in bone metastasis.

Glutamate export from cancer cells is emerging as a general phenomenon rather than being specific to CNS tumor cell types. In many cancer cells, it appears that glutamate is secreted predominantly through the system x_c^- glutamate/cystine antiporter, and this transport mechanism is amenable to pharmacological inhibition. In the context of bone metastasis, large amounts of extracellular glutamate released from tumor cells could significantly disrupt glutamate-based homeostatic mechanisms operating in the normal bone environment. To further expand on this concept, we feel that an assessment of glutamate effects on bone cell func-

tions *in vitro* is essential before this compelling therapeutic approach can be tested using *in vivo* models of bone metastasis.

Acknowledgments

Experimental results have been used in portions of the graduate thesis of M.K.S. and doctoral dissertation of E.P.S.

This study was supported by operating grants from the Canadian Institutes of Health Research.

References

[1] R.D. Rubens, Bone metastases—the clinical problem, Eur. J. Cancer 34 (1998) 210–213.
[2] A.A. Rose, P.M. Siegel, Breast cancer-derived factors facilitate osteolytic bone metastasis, Bull. Cancer 93 (2006) 931–943.
[3] R.D. Bonfil, S. Chinni, R. Fridman, H.R. Kim, M.L. Cher, Proteases, growth factors, chemokines, and the microenvironment in prostate cancer bone metastasis, Urol. Oncol. 25 (2007) 407–411.
[4] C. Chenu, Glutamatergic regulation of bone remodeling, J. Musculoskelet. Neuronal Interact. 2 (2002) 282–284.
[5] E. Hinoi, T. Takarada, Y. Yoneda, Glutamate signaling system in bone, J. Pharmacol. Sci. 94 (2004) 215–220.
[6] T.M. Skerry, The role of glutamate in the regulation of bone mass and architecture, J. Musculoskelet. Neuronal Interact. 8 (2008) 166–173.
[7] E.P. Seidlitz, M.K. Sharma, Z. Saikali, M. Ghert, G. Singh, Cancer cell lines release glutamate into the extracellular environment, Clin. Exp. Metastasis (2009), doi:10.1007/s10585-009-9277-4.
[8] S. Kato, K. Negishi, K. Mawatari, C.H. Kuo, A mechanism for glutamate toxicity in the C6 glioma cells involving inhibition of cystine uptake leading to glutathione depletion, Neuroscience 48 (1992) 903–914.
[9] Z.C. Ye, H. Sontheimer, Glioma cells release excitotoxic concentrations of glutamate, Cancer Res. 59 (1999) 4383–4391.
[10] J.Y. Kim, Y. Kanai, A. Chairoungdua, S.H. Cha, H. Matsuo, D.K. Kim, J. Inatomi, H. Sawa, Y. Ida, H. Endou, Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells, Biochim. Biophys. Acta 1512 (2001) 335–344.
[11] H. Sontheimer, Malignant gliomas: perverting glutamate and ion homeostasis for selective advantage, Trends Neurosci. 26 (2003) 543–549.
[12] W.J. Chung, S.A. Lyons, G.M. Nelson, H. Hamza, C.L. Gladson, G.Y. Gillespie, H. Sontheimer, Inhibition of cystine uptake disrupts the growth of primary brain tumors, J. Neurosci. 25 (2005) 7101–7110.
[13] J.F. Huggett, A. Mustafa, L. O'neal, D.J. Mason, The glutamate transporter GLAST-1 (EAAT-1) is expressed in the plasma membrane of osteocytes and is responsive to extracellular glutamate concentration, Biochem. Soc. Trans. 30 (2002) 890–893.
[14] D.J. Mason, L.J. Suva, P.G. Genever, A.J. Patton, S. Steuckle, R.A. Hillam, T.M. Skerry, Mechanically regulated expression of a neural glutamate transporter in bone: a role for excitatory amino acids as osteotropic agents?, Bone 20 (1997) 199–205
[15] M. Hayashi, R. Morimoto, A. Yamamoto, Y. Moriyama, Expression and localization of vesicular glutamate transporters in pancreatic islets, upper gastrointestinal tract, and testis, J. Histochem. Cytochem. 51 (2003) 1375–1390.
[16] L. Tremolizzo, J.C. DiFrancesco, V. Rodriguez-Menendez, E. Sirtori, M. Longoni, A. Cassetti, M. Bossi, S. El Mestikawy, G. Cavaletti, C. Ferrarese, Human platelets express the synaptic markers VGLUT1 and 2 and release glutamate following aggregation, Neurosci. Lett. 404 (2006) 262–265.
[17] R. Morimoto, S. Uehara, S. Yatsushiro, N. Juge, Z. Hua, S. Senoh, N. Echigo, M. Hayashi, T. Mizoguchi, T. Ninomiya, N. Udagawa, H. Omote, A. Yamamoto, R.H. Edwards, Y. Moriyama, Secretion of L-glutamate from osteoclasts through transcytosis, EMBO J. 25 (2006) 4175–4186.
[18] W.C. Duivenvoorden, S.V. Popovic, S. Lhotak, E. Seidlitz, H.W. Hirte, R.G. Tozer, G. Singh, Doxycycline decreases tumor burden in a bone metastasis model of human breast cancer, Cancer Res. 62 (2002) 1588–1591.
[19] W.C. Duivenvoorden, S. Vukmirovic-Popovic, M. Kalina, E. Seidlitz, G. Singh, Effect of zoledronic acid on the doxycycline-induced decrease in tumour burden in a bone metastasis model of human breast cancer, Br. J. Cancer 96 (2007) 1526–1531.
[20] C. Chenu, C.M. Serre, C. Raynal, B. Burt-Pichat, P.D. Delmas, Glutamate receptors are expressed by bone cells and are involved in bone resorption, Bone 22 (1998) 295–299.
[21] T. Takano, J.H. Lin, G. Arcuino, Q. Gao, J. Yang, M. Nedergaard, Glutamate release promotes growth of malignant gliomas, Nat. Med. 7 (2001) 1010–1015.
[22] S.M. Williams, R.K. Sullivan, H.L. Scott, D.I. Finkelstein, P.B. Colditz, B.E. Lingwood, P.R. Dodd, D.V. Pow, Glial glutamate transporter expression patterns in brains from multiple mammalian species, Glia 49 (2005) 520–541.
[23] N. Kalariti, P. Lembessis, E. Papageorgiou, N. Pissimissis, M. Koutsilieris, Regulation of the mGluR5, EAAT1 and GS expression by glucocorticoids in MG-63 osteoblast-like osteosarcoma cells, J. Musculoskelet. Neuronal Interact. 7 (2007) 113–118.
[24] P. Marcaggi, N. Hirji, D. Attwell, Release of L-aspartate by reversal of glutamate transporters, Neuropharmacology 49 (2005) 843–849.
[25] T. Li, F.K. Ghishan, L. Bai, Molecular physiology of vesicular glutamate transporters in the digestive system, World J. Gastroenterol. 11 (2005) 1731–1736.
[26] A. Nicolaysen, R. Gammelsaeter, J. Storm-Mathisen, V. Gundersen, P.O. Iversen, The components required for amino acid neurotransmitter signaling are present in adipose tissues, J. Lipid Res. 48 (2007) 2123–2132.
[27] S. Bannai, Exchange of cystine and glutamate across plasma membrane of human fibroblasts, J. Biol. Chem. 261 (1986) 2256–2263.
[28] S. Okuno, H. Sato, K. Kuriyama-Matsumura, M. Tamba, H. Wang, S. Sohda, H. Hamada, H. Yoshikawa, T. Kondo, S. Bannai, Role of cystine transport in intracellular glutathione level and cisplatin resistance in human ovarian cancer cell lines, Br. J. Cancer 88 (2003) 951–956.
[29] V.S. Narang, G.M. Pauletti, P.W. Gout, D.J. Buckley, A.R. Buckley, Suppression of cystine uptake by sulfasalazine inhibits proliferation of human mammary carcinoma cells, Anticancer Res. 23 (2003) 4571–4579.
[30] Y. Huang, Z. Dai, C. Barbacioru, W. Sadee, Cystine–glutamate transporter SLC7A11 in cancer chemosensitivity and chemoresistance, Cancer Res. 65 (2005) 7446–7454.
[31] M. Lo, V. Ling, Y.Z. Wang, P.W. Gout, The xc− cystine/glutamate antiporter: a mediator of pancreatic cancer growth with a role in drug resistance, Br. J. Cancer 99 (2008) 464–472.
[32] R.S. Chen, Y.M. Song, Z.Y. Zhou, T. Tong, Y. Li, M. Fu, X.L. Guo, L.J. Dong, X. He, H.X. Qiao, Q.M. Zhan, W. Li, Disruption of xCT inhibits cancer cell metastasis via the caveolin-1/beta-catenin pathway, Oncogene 28 (2009) 599–609.
[33] V.S. Narang, G.M. Pauletti, P.W. Gout, D.J. Buckley, A.R. Buckley, Sulfasalazine-induced reduction of glutathione levels in breast cancer cells: enhancement of growth-inhibitory activity of doxorubicin, Chemotherapy 53 (2007) 210–217.
[34] D.W. Doxsee, P.W. Gout, T. Kurita, M. Lo, A.R. Buckley, Y. Wang, H. Xue, C.M. Karp, J.C. Cutz, G.R. Cunha, Y.Z. Wang, Sulfasalazine-induced cystine starvation: potential use for prostate cancer therapy, Prostate 67 (2007) 162–171.
[35] M. Lo, Y.Z. Wang, P.W. Gout, The x(c) (−) cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases, J. Cell. Physiol. 215 (2008) 593–602.