Galectin-1-Mediated Tumor Invasion and Metastasis, Up-Regulated Matrix Metalloproteinase Expression, and Reorganized Actin Cytoskeletons

Ming-Heng Wu,1 Tse-Ming Hong,2 Hui-Wen Cheng,3 Szu-Hua Pan,6 Yu-Ray Liang,3 Hsiao-Chin Hong,3 Wei-Fan Chiang,6 Tung-Yiu Wong,3 Dar-Bin Shieh,3 Ai-Li Shiau,4 Ying-Tai Jin,3 and Yuh-Ling Chen1,3

Institutes of 1Basic Medical Sciences, 2Clinical Medicine, and 3Oral Medicine and 4Department of Microbiology and Immunology, National Cheng Kung University College of Medicine; 5Oral and Maxillofacial Section, Chi-Mei Medical Center, Tainan, Taiwan and 6Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan

Abstract
Galectin-1 (Gal-1) is a β-galactose-binding lectin; its expression level has been reported to correlate with tumor progression. Gal-1 is highly expressed in the invasive front of primary tumors and in the cancer cells of metastatic lesions in the lymph nodes of patients with oral squamous cell carcinoma. However, the molecular mechanism of Gal-1 in tumor metastasis is not completely clear. We found that increased Gal-1 expression is closely associated with its high levels of invasion in lung adenocarcinoma and oral squamous cell carcinoma cell lines. Knocking down Gal-1 with small interfering RNA in highly invasive cancer cells reduced their invasion levels. Moreover, the invasion ability of poorly invasive cancer cells was significantly increased after Gal-1 overexpression of Gal-1. Mechanism studies revealed that Gal-1 promoted tumor invasion mainly by up-regulating matrix metalloproteinase (MMP)-9 and MMP-2 and by reorganizing actin cytoskeleton. Gal-1 enhanced the activation of Cdc42, a small GTPase and member of the Rho family, thus increasing the number and length of filopodia on tumor cells. Furthermore, Gal-1-overexpressing cells had higher metastatic abilities in tail vein metastasis assays in vivo. We conclude that Gal-1 is involved in tumor invasion and metastasis by increasing MMP expression and reorganizing cytoskeletons in oral cancers and lung adenocarcinoma. (Mol Cancer Res 2009;7(3):311–8)

Introduction
Metastasis is a multistep process and the leading cause of death from cancer. During metastatic conversion, cancer cells escape from their original tissue, invade the surrounding tissues of the primary tumor, and penetrate a lymphatic or blood vessel (1). Some survive in the circulation and reach distal sites. Finally, the survivors extravasate and colonize in the second organ (2). Increased invasiveness of cancer cells is a critical step in tumor metastasis and many changes are required; for example, cancer cells loosen their adhesion to neighboring cells and the extracellular matrix, degrade adjacent tissues, and elevate their motility (3). In tumor cells and their micro-environmental cells, genetic or epigenetic changes can alter the expression of many genes, such as matrix metalloproteinases (MMP; refs. 4, 5), integrins (6), and cadherins (7, 8), to promote cancer progression and metastasis. Therefore, understanding the molecular mechanisms of how changes in gene expression affect tumor invasion and metastasis is important for designing therapeutic approaches.

Galectin-1 (Gal-1) is a member of the β-galactoside-binding lectin family and exists as a noncovalent homodimer composed of two carbohydrate recognition domains to recognize a wide range of glycoprotein or glycolipid (9). Gal-1 acts extracellularly by binding to cell surface receptors and extracellular matrix, such as neuropilin-1 (10), integrins (11), fibronectin, laminin (12), and carcinoembryonic antigen (13). In addition, it exists intracellularly and interacts with cytoplasmic and nuclear proteins to regulate cell transformation (14) or pre-mRNA splicing (15). Immunohistochemical staining of Gal-1 in clinical cancer samples reveals that its expression is positively associated with the malignant progression of many tumor types, including glioma (16), prostate (17), colon (18), breast (19), cervical (20), and oral squamous (21) cells. The expression of Gal-1 in carcinoma-associated stromal cells was significantly correlated with breast tumor invasiveness and lymph node metastasis (19). The possible mechanisms of how Gal-1 contributes to cancer progression and metastasis have been

Received 6/24/08; revised 10/18/08; accepted 11/15/08; published OnlineFirst 3/10/09.

Grant support: National Science Council grants NSC-96-2311-B-006-005-MY3 (Y-L. Chen) and NSC-95-2314-B-002-119-MY3 (T-M. Hong), Department of Health grant DOH-TD-B-111-004 (Y-L. Chen), and National Cheng Kung University Landmark Project (Y-L. Chen).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Molecular Cancer Research Online (http://mcr.aacrjournals.org/).

Y-T. Jin and Y-L. Chen contributed equally to this work and are joint corresponding authors.

Requests for reprints: Yuh-Ling Chen, Institute of Oral Medicine, National Cheng Kung University College of Medicine, 1 University Road, Tainan 701, Taiwan. Phone: 886-6-235-3355, ext. 5364; Fax: 886-6-235-9885. E-mail: yuhling@mail.ncku.edu.tw

Copyright © 2009 American Association for Cancer Research.

doi:10.1158/1541-7786.MCR-08-0297

Published OnlineFirst March 10, 2009; DOI: 10.1158/1541-7786.MCR-08-0297
proposed: it regulates tumor cell growth (22), triggers the death of infiltrating T cells (23), suppresses T-cell-derived proinflammatory cytokine secretion (24), mediates cell-cell or cell-extracellular matrix adhesion (12, 25), is involved in tumor angiogenesis (26), and promotes cancer cell migration (16, 27). However, it is still unclear whether Gal-1 affects the invasive ability of oral squamous cell carcinoma (OSCC) and lung adenocarcinoma and their underlying molecular mechanisms.

We recently showed (21) that strong Gal-1 immunoreactivity was present in the cancer cells of metastatic lesions of lymph nodes and in the invasive fronts of primary tumors and was significantly associated with poor prognosis in early-stage OSCC. In the current study, we provide direct functional evidence that Gal-1 affects the ability of tumor invasion and metastasis in lung adenocarcinoma and oral cancer cell lines. Furthermore, the molecular mechanisms underlying Gal-1-enhanced cancer cell invasion were elucidated for understanding the mode of action.

Results
Increased Expression of Gal-1 Is Correlated with Elevated Invasiveness

We used an invasion chamber assay to compare the invasiveness of three OSCC cell lines, HSC-3, OC-2, and OEC-M1. HSC-3 is a highly malignant and invasive OSCC cell line, isolated from metastatic tumors in a lymph node (28), whereas OC-2 and OEC-M1 were established from primary tumors of OSCC patients. We found that HSC-3 cells were 2.6 and 8 times more invasive than OC-2 and OEC-M1 cells, respectively (Fig. 1A). Western blot analysis of the three cell lines showed that Gal-1 expression was higher in HSC-3 cells than in OEC-M1 and OC-2 cells and that there was no obvious difference in Gal-1 expression between OC-2 and OEC-M1 cells (Fig. 1A, bottom). To rule out the possibility that the result was due to different genetic backgrounds, Gal-1 expression was also analyzed in several lung adenocarcinoma cell sublines, CL1-1, CL1-5F4, and CL1-5, with different levels of invasiveness (29). Although Gal-1 was detectable in the two highly invasive sublines (CL1-5F4 and CL1-5), it was almost undetectable in the least invasive cell line (CL1-1; Fig. 1B). These results showed that Gal-1 expression was correlated with the invasiveness of OSCC and lung adenocarcinoma cell lines.

Immunostaining four pairs of OSCC tissue also revealed that Gal-1 was strongly immunoreactive in metastatic lesions in lymph nodes (Fig. 1C, right) but weakly immunoreactive in primary tumor sites (Fig. 1C, left), which is consistent with our previous finding (21) that Gal-1 expression in primary OSCC tumors with regional lymph node metastasis was significantly higher than in those without regional lymph node metastasis, which suggested that Gal-1 expression is associated with tumor invasion and metastasis.

The expression of different members in the galectin family is associated with cancer progression (30). Galectin-3 (Gal-3) expression is either positively correlated with cancer progression, as in gastric (31) and thyroid (32) cancers, or negatively associated with tumor metastasis, as in breast (33) and prostate (34) cancers. Although increased Gal-3 expression is associated with reduced disease-free survival in persons with tongue cancers (35), the effects of Gal-3 on tumor invasiveness are still unclear. To compare the roles of Gal-1 and Gal-3 in oral cancer invasion and metastasis, we immunohistochemically analyzed their expression in three pairs of matched primary oral tumor and lymph node metastases. All three tissue pairs showed stronger Gal-1 staining in the cancer cells of metastatic lesions in lymph nodes than in those of the primary tumor (Supplementary Fig. S1A and B). However, two of three tissue pairs showed that Gal-3 expression was lower in the lymph nodes than in the primary sites (Supplementary Fig. S1C and D). The other pair showed no difference in the intensity of the immunostaining of Gal-3. Western blot analysis of the three oral cancer cell lines showed that, although the expression of Gal-3 was high in the least invasive cell line, OEC-M1, there was no apparent difference in Gal-3 expression between OC-2 and HSC-3 cells (Supplementary Fig. S1E). Taken together, these findings suggest that the influence of Gal-3 on OSCC invasion and metastasis may be relatively minor and that Gal-1 mediates oral cancer invasion and metastasis.

Gal-1 Modulated Tumor Cell Invasiveness

To determine whether Gal-1 increased tumor invasion, highly invasive cancer cells, CL1-5 and HSC-3, were treated with three different Gal-1 small interfering RNAs (Gal-1/siRNA) and then evaluated for tumor invasion. Gal-1 expression in CL1-5 and HSC-3 was reduced after transfection.
Gal-1 in Tumor Invasion and Metastasis

Expression of Gal-1 was positively correlated with cancer cell invasiveness. A and B. Two highly invasive cancer cell lines, HSC-3 and CL1-5, were transfected with Gal-1 siRNA or control siRNA. After transfection for 24 h, cells were lysed for Western blotting (top) or trypsinized for cell invasion assays (bottom). Relative invasion ability was normalized to that of cells transfected with control siRNA. C and D. To access the effect of Gal-1 on the invasiveness of less invasive tumor cells, OC-2 and OEC-M1 were transduced by lentivirus carrying GFP or Gal-1 genes. Top, Western blot analyses of Gal-1 expression in GFP and Gal-1 gene-transduced cells; bottom, overexpression of Gal-1 in OC-2 and OEC-M1 cells can increase the invasion ability. Relative invasion ability was normalized with that of cells transfected with GFP-lentivirus.

MMPs are a family of zinc-dependent enzymes that degrade extracellular matrix, such as gelatin and collagen. The gelatinases (MMP-2 and MMP-9) are associated with tumor metastasis and progression (4, 36). For example, MMP-2 and MMP-9 were highly expressed in OSCC patients with lymph node metastasis, and MMP-9 expression was related to poor survival rates of OSCC patients without regional metastasis (37). Therefore, we used reverse transcription-PCR and gelatin zymography to further examine whether Gal-1 affects MMP-2 and MMP-9 expression and enzymatic activity. Gal-1 overexpression in OC-2 cells elevated the mRNA expression and gelatinolytic activity of both MMP-2 and MMP-9 (Fig. 2A and B), but inhibiting Gal-1 reduced them (Fig. 2C). Furthermore, treating OC-2 cells with MMP inhibitor significantly reduced Gal-1 cell invasiveness (Fig. 2D). This showed that Gal-1 promoted tumor invasion in part by augmenting MMP-2 and MMP-9 expression and enzymatic activities.

Gal-1 Induced Actin Cytoskeleton Rearrangement

Increased cell motility is a key step in cancer invasion and metastasis; actin cytoskeleton remodeling is an important mechanism of altering cell motility. Changes in the actin cytoskeleton structure, such as increased filopodia formation, promote cancer cell migration and invasiveness (38). To determine whether Gal-1 affects actin cytoskeleton reorganization, cells were stained with rhodamine-conjugated phalloidin to visualize F-actin distributions. OC-2/Gal-1 cells showed not only an increase in the number of filopodia bundles but also a reduction in protrusive lamellipodia structures compared with OC-2/GFP cells (Fig. 4A). In contrast to Gal-1-overexpressing cells, si-Gal-1-treated cells (HSC-3/si-Gal-1) had markedly fewer and shorter filopodia (Fig. 4B). Compared with control cells, the filopodia were, on average, 45% longer (n = 15; P < 0.001, paired t test) in OC-2/Gal-1 cells and 18% lower (n = 15; P < 0.05) in HSC-3/si-Gal-1 cells (Fig. 4D). Taken together, these findings showed that Gal-1 strongly affected actin filament localization. Gal-1 expression modulated filopodia formation and increased tumor cell adhesion, migration, and invasiveness.

Gal-1 Regulates Rho Family Small GTPase Activity

The Rho family of small GTPases, Cdc42, Rac1, and RhoA, is a critical regulator of actin cytoskeleton dynamics. Activated Cdc42 and Rac1 induce actin polymerization to extend the cell membrane in finger-like structures, known as filopodia, and in sheet-like structures, known as lamellipodia (39). RhoA activation triggers the formation of both stress fiber and focal adhesion complex with integrins and associated proteins. In addition, Rho family activity affects the invasiveness of different kinds of tumor cells (38, 40). We hypothesized that Gal-1 regulates the activities of Rho family GTPases to increase cell motility, filopodia formation, and invasiveness. GTP-bound Rho, Rac, and Cdc42 were affinity purified from cell lysates using a glutathione S-transferase fusion construct of the
Rho-binding domain of rhotekin and the p21-binding domain of the target PAK1. We found that Cdc42 activity was significantly increased in OC-2/Gal-1 cells and decreased in HSC-3/si-Gal-1 cells (Fig. 4E), which showed that increased Cdc42 activity was involved in the Gal-1-induced actin cytoskeleton rearrangement and increased tumor invasiveness.

**Gal-1 Overexpression Increased Oral Cancer Cell Lung Metastasis In vivo**

Using an experimental metastasis assay, we next investigated whether Gal-1 increases tumor metastasis in vivo. OC-2/GFP or OC-2/Gal-1 cells ($10^6$) were intravenously injected into severe combined immunodeficient mice in each experimental group ($n = 15$). The number of lung metastatic nodules in individual mice was counted, under a dissection microscope, 35 days post-treatment. Elevated Gal-1 expression in OC-2 cells significantly increased pulmonary metastatic nodule development (Fig. 5A). Mice injected with OC-2/Gal-1 cells developed 2.3 times as many pulmonary metastatic nodules as mice injected with OC-2/GFP cells did (Fig. 5B).

**Discussion**

Aberrant gene expression changes or gene mutations in tumor cells or carcinoma-associated stromal cells increases their malignancy and accelerates cancer progression. We found that endogenous Gal-1 expression in OSCC and lung adenocarcinoma cell lines correlated with their levels of invasion. Moreover, Gal-1 overexpression in low metastatic cancer cells enables them to invade and metastasize in vitro and in vivo. Conversely, abrogating Gal-1 expression in highly invasive cancer cells decreases their levels of invasion. Our findings suggest that Gal-1 expression increases as cancer cells progress toward a more malignant phenotype and that Gal-1 expression levels affect the invasiveness of cancer cells. Gal-1 expression in glioblastoma was preferentially higher in more invasive parts of a xenograft, which suggests that Gal-1 expression is closely associated with cancer cell invasiveness (27). In addition, Gal-1 expression in cancer cells is a prognosis marker of glioma (16) and oral squamous carcinoma (21), which further supports the hypothesis that Gal-1 is pivotal in tumor invasion and metastasis.

A change in the proteolytic degradation of adjacent tissue is required during tumor invasion. MMP-2 and MMP-9 are major contributors to the degradation of gelatin and collagen extracellular matrix (4). Besides, MMP-2 and MMP-9 expression levels are correlated with lymph node metastasis and poor outcome in head and neck cancer patients (41, 42) and directly affect tumor invasiveness and metastasis (43). Galectin-7 promotes T-cell lymphoma progression through extracellular stimulation of MMP-9 transcription (44, 45). We showed that Gal-1 expression was involved in regulating the production and activities of MMP-2 and MMP-9 in OSCC cells and that Gal-1-induced increases in the expression of these MMPs may be one major factor that causes oral cancer cells to become highly invasive and metastatic. It is not clear how Gal-1 regulates MMPs. Investigations of the detailed mechanisms of Gal-1-induced MMP-2 and MMP-9 production and activities are necessary.

Rho family small GTPases, Rho, Rac, and Cdc42, have long been recognized as important modulators of actin cytoskeleton dynamics. When tumors invade the surrounding tissue, the morphologic characteristics and motility of the invading cancer cells must change. We found that Gal-1 overexpression in...
cancer cells increased the frequency and length of filopodia, which may be related to elevated Cdc42 activity. Cdc42 is the major contributor to filopodia actin structure, and the activation of Cdc42 promotes cancer cell migration and invasion (39, 46). However, it is unclear how Gal-1 modulates Cdc42 activity. Extracellular Gal-1 stimulation increases glioblastoma cell motility by modifying actin cytoskeleton and up-regulating Rho A expression, but its effect on Rho A activity has not been examined, and we found no significant increase in the expression of Rho family small GTPases (27). Our findings on the modulation of Cdc42 activity and actin cytoskeleton reorganization highlight the role of Gal-1 as an upstream regulator of the Rho family GTPase activity that promotes tumor invasion and metastasis. Endogenous Gal-1 expression

FIGURE 4. Expression of Gal-1 was positively correlated with the length and density of filopodia. A and B, Representative fluorescence photomicrographs reveal the different filopodial length and number in Gal-1-transfected (OC-2/Gal-1) or Gal-1 silencing (HSC-3/si-Gal-1) oral cancer cells compared with control cells. C and D, Quantitation of the number of filopodia per cell and the length of filopodia in oral cancer cells. Density and length of filopodia were analyzed by MetaMorph imaging software (Universal Imaging). Mean ± SD of 15 individual cells. * , P < 0.05; ** , P < 0.005; *** , P < 0.001. E, Analysis of the activation and expression of Rho family small GTPases by Rho family pull-down assay and Western blotting. Rho family small GTPases pull-down assay was described in Materials and Methods. Intensities of band were quantified by densitometry using GeneTools (Syngene). Fold change was the intensity of the bands corresponding to GTP-Rho family proteins in treated samples (OC-2/Gal-1 or HSC-3/si-Gal-1) normalized to the respective total Rho family protein levels and relative to the control samples (OC-2/GFP or HSC-3/si-C).
under a dissection microscope.

lung metastatic nodules in individual mice was counted after 35 days

immunodeficient mice in each experimental group (48). Cancer cells (1 \times 10^5 - 2 \times 10^5) were plated in the upper chamber well and incubated at 37°C for 24 h. Nonpenetrating cells are removed from the upper surface of the filter with a cotton swab. Penetrating cells were fixed and stained with a DiffQuick stain kit (International Reagents). For quantification, the assays were done triplicates and all of the cells invaded into the lower surface were stained and counted under a light microscope.

**siRNA Transfection**

Gal-1 expression was silenced by transfection of siRNA with RNAiFect transfection reagent (Qiagen). Gal-1 siRNAs were synthesized by Invitrogen. AllStar Negative Control siRNA were from Qiagen. The sequences were 5'-UGAUUGCAAC-CUCUGCAACACUCUCC (Gal-1 siRNA1), 5'-CACUCUC-CAGGUUUGAGAUUGAGCU (Gal-1 siRNA2), and 5'-UUCGUGUUCACGACUGUGUUGG (Gal-1 siRNA3).

**Reverse Transcription-PCR**

Total RNA was isolated using Trizol reagent (Invitrogen). Total RNA (1 μg) was reverse transcribed by using IMProm-II Reverse Transcripase (Promega). The following primers were used: MMP-9 forward 5'-CAACATCACCATTGGATCC-3' and reverse 5'-CCGGTGTAAGTCTCTGCT-3', MMP-2 forward 5'-GTCTGAAAGGACACACTAAAGAAGA-3' and reverse 5'-TTGCCATCTTTCTCAAGTTTAGG-3', and β-actin forward 5'-AGCCGAAATGTTGCGT-3' and reverse 5'-CAGGTACATGTTGTTGTTGCC-3'. All PCRs included β-actin primers to quantify PCR products.

**Production of Lentivirus**

The sequence encoding human Gal-1 was cloned into pWPXL replacing the GFP marker. All recombinant lentiviruses were produced by transient transfection of 293T cells according to standard protocols (51). Briefly, subconfluent 293T cells were cotransfected with 20 μg of a plasmid vector,
15 μg psPAX2, and 5 μg pMD2.G by calcium phosphate precipitation. After 16 h, medium was changed, and recombinant lentivirus vectors were harvested 48 h later and filtered through 0.45 μm filters. Virus titers were calculated at 72 h after virus infection by counting the number of GFP-expressing foci divided by the dilution factor. For virus infection, culture cells were incubated with culture medium-diluted virus supernant supplemented with polybrene (8 μg/mL) for 6 h. To achieve >95% infection efficiency, virus titers of 20 to 40 transduction unit/cells were used.

**Immunofluorescence Staining**

Immunofluorescence staining for F-actin was done as described previously (40). Briefly, cells were seeded on the cover slide coated with poly-L-lysine and incubated for 24 h. Cells were washed twice with PBS and fixed in 3.7% paraformaldehyde for 20 min on ice. After washing three times with PBS, cells were permeabilized in 0.05% Triton X-100 for 5 min on ice, washed with PBS, and incubated with rhodamine-conjugated phalloidin for 30 min at room temperature. Following another wash with PBS, the cells were examined by confocal fluorescence microscopy.

**Filopodia Measurements**

To determine the number of filopodia, all filopodial extensions >2 μm were counted on 15 individual cells, and the average ± SD per cell was calculated. To calculate the average filopodial length, the distance from the cell base to the tip was determined for all filopodia (>2 μm) of 15 individual cells in each sample, and the average ± SD was calculated per filopodium.

**Rho Family Small GTPases Pull-Down Assay**

The Rho family small GTPases pull-down assay was done as described previously (40). Briefly, OC-2 cells were lysed in 50 mmol/L Tris-HCl (pH 7.2), 500 mmol/L NaCl, 1% (v/v) Triton X-100, 5 mmol/L MgCl₂, 1 mmol/L DTT, and protease inhibitors and mixed with bacterially expressed glutathione S-transferase fusing Rho-binding protein, for RhoA-GTP, and glutathione S-transferase PAK3 p21-binding domain, for Rac1-GTP and Cdc42-GTP, and then bound to glutathione Sepharose in inhibitors and mixed with bacterially expressed glutathione S-transferase fusing Rho-binding protein, for RhoA-GTP, and glutathione S-transferase PAK3 p21-binding domain, for Rac1-GTP and Cdc42-GTP, and then bound to glutathione Sepharose at 4°C for 30 min. Beads were collected by centrifugation and prepared for Western blotting analysis with anti-RhoA, anti-Rac1, and anti-Cdc42 antibodies.

**Experimental Metastasis In vivo**

Cells were washed and resuspended in PBS. Subsequently, a single-cell suspension containing 10⁶ cells in 0.1 mL PBS was injected into the lateral tail vein of 6-week-old severe combined immunodeficient mice. Mice were killed after 5 weeks. Lung was examined for metastasis formation. The lungs were removed, weighed, and fixed in 10% formalin. The number of lung tumor colonies was counted under a dissecting microscope. All animal experiments were done in accordance with the animal guidelines at the National Cheng Kung University Laboratory Animal Center.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank Didier Trono (Department of Genetics and Microbiology, University of Geneva) for providing lentiviral vectors.

**References**

12. van den Brule F, Califice S, Garnier F, Fernandez PL, Berchuck A, Castronovo V. Galectin-1 accumulation in the ovary carcinoma peritumoral stroma is induced by ovary carcinoma cells and affects both cancer cell proliferation and adhesion to laminin-1 and fibronectin. Lab Invest 2003;83:377–86.
Specific inhibition of T-cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. Immunology 1999;97:100–6.


Molecular Cancer Research

Galectin-1-Mediated Tumor Invasion and Metastasis, Up-Regulated Matrix Metalloproteinase Expression, and Reorganized Actin Cytoskeletons

Ming-Heng Wu, Tse-Ming Hong, Hui-Wen Cheng, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-08-0297

Supplementary Material
Access the most recent supplemental material at:
http://mcr.aacrjournals.org/content/suppl/2009/03/04/1541-7786.MCR-08-0297.DC1

Cited articles
This article cites 51 articles, 10 of which you can access for free at:
http://mcr.aacrjournals.org/content/7/3/311.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/7/3/311.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.