A Novel Function for Galectin-7: Promoting Tumorigenesis by Up-regulating MMP-9 Gene Expression

Mélanie Demers, Thierry Magnaldo, and Yves St-Pierre

Abstract
Metastasis is a multistep process by which cancer cells, after acquiring several capabilities, spread to distinct sites in the body. It is the major cause of death in individuals suffering from cancer. We have recently identified galectin-7 as a new gene associated with the progression of T cell lymphoma toward a metastatic phenotype, suggesting a possible causal relationship. The present study was designed to investigate the role of galectin-7 in lymphoma. We found that the development of thymic lymphoma was accelerated when induced by lymphoma cells overexpressing galectin-7. Moreover, transfection of an expression vector containing the galectin-7 gene in low metastatic lymphoma cells increased their metastatic behavior and confers these cells with the new ability to overcome the resistance of intercellular adhesion molecule-1-deficient mice to lymphoma dissemination. Finally, we provide data suggesting that galectin-7 modulates the aggressive behavior of lymphoma cells by controlling the expression of metastatic genes, such as MMP-9. This hypothesis is based on the following evidence: (a) galectin-7 transfectants have higher levels of MMP-9 expression, (b) addition of β-lactose completely inhibits expression of MMP-9 by galectin-7 transfectants, and (c) recombinant forms of galectin-7 induces the expression of MMP-9 in both mouse and human lymphoma cells. Our results have uncovered the existence of a previously undescribed activity, the promotion of cancer cell malignancy, to galectin-7. (Cancer Res 2005; 65(12): 5205-10)

Introduction
Metastasis is the most critical step of the oncogenic process. During this process, cancer cells interact with the extracellular matrix, endothelial and peritumoral cells, as well as with microenvironmental factors, thereby modulating gene expression that impact on several regulatory pathways, migration, and proliferation. In fact, many aberrant gene expressions for oncogenes (1), adhesion molecules (2, 3), and matrix metalloproteinases (MMP; ref. 4) have been described in the metastatic process. For example, elevated expression of MMP-9 correlates with a poor clinical outcome in patients within non–Hodgkin’s lymphoma (5, 6).

Using an experimental lymphoma model, we have previously shown that aggressive lymphoma cells can be disseminating in the otherwise lymphoma-resistant intercellular adhesion molecule-1 (ICAM-1)-deficient mice (7). Because aggressive and nonaggressive lymphoma cells home with the same efficiency to target organs in normal and ICAM-1-deficient mice, this indicated that, at late stages of the disease, tumor cells with high metastatic efficiency are encoded with a repertoire of selected genes (8). However, the identity of the tumor-promoting genes allowing aggressive lymphoma cells to bypass the traditionally required exchange of intercellular signals during the establishment of a tumor in its target organs remains unknown.

To define the cascade of genetic alterations, which underlie progression to T cell malignancy, we have recently employed an approach based on genomic analysis of an aggressive lymphoma variant and its nonaggressive parental cells. We found that the most prominent change among the genes tested was the strong up-regulation of galectin-7 (9). Specifically, we reported that galectin-7 was constitutively expressed in aggressive T lymphoma cells at both mRNA and protein levels. Galectins are a family of β-galactoside-binding animal lectins with diverse biological activities, functioning both extracellularly and intracellularly by interacting with cytoplasmic and nuclear proteins. Their functions are considered to be related to immunity, development, apoptosis, cell-cell and cell-matrix interactions, and neoplastic transformation (10). In the case of galectin-7, its expression has been shown to be restricted to the stratified epithelium where it is associated to cytoplasmic and nuclear proteins. Its functions are considered to be related to immunity, development, apoptosis, cell-cell and cell-matrix interactions, and neoplastic transformation (10). Here, we report the unanticipated finding that galectin-7 up-regulates the growth and dissemination of lymphoma cells in vivo.

Materials and Methods
Mice. Breeder pairs for C57BL/6 mouse colony were purchased from Jackson Laboratory (Bar Harbox, ME). Mutant ICAM-1-deficient mice were generated by gene-targeting in 129-derived (H-2b) J1 embryonic stem cells that had been injected into C57BL/6 (H-2b) blastocysts (15). This strain carries a mutation in the fourth exon of the ICAM-1 gene. Male and female mice were bred in our animal facility and maintained under specific pathogen-free conditions and in accordance with institutional guidelines.

Culture of cell lines and reagents. The mouse T lymphoma cell line 267 was established in our laboratory from radiation-induced primary T cell lymphomas in C57BL/Ka (8). The diffuse large B cell line Ocy-2 has been described previously (16). For treatment with recombinant mouse or human galectin-7 (R&D Systems, Minneapolis, MN) or β-lactose, 106 lymphoma cells were resuspended in 200 μL of complete medium containing the indicated concentrations and incubated for 16 hours at 37°C in a humidified 5% CO2 atmosphere. All cells were maintained in RPMI 1640 complete medium supplemented with 10% (v/v) FCS, 2 mmol/L L-glutamine, 10 mmol/L HEPES buffer, 0.1 unit/mL penicillin, 50 μg/mL streptomycin, and 55 μmol/L β-mercaptoethanol. All products were from Life Technologies (Burlington, Ontario, Canada). Horseradish peroxidase–conjugated donkey anti-rabbit or anti-mouse secondary antibodies were obtained from Amersham (Amersham Pharmacia, Quebec, Canada). The rabbit polyclonal antibody specific for galectin-7 has been previously described (13). All other reagents were purchased from Sigma Chemicals (St. Louis, MO), unless otherwise indicated.
Western blot analysis. Cells were washed with PBS and resuspended in lysis buffer [10 mmol/L Tris (pH 7.5), 2.5% Triton X-100] containing protease inhibitors (Complete tablets, Roche, Laval, Quebec, Canada). Equal amounts of whole-cell extracts were separated on SDS-PAGE gel and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Mississauga, Ontario, Canada). The membranes were blocked with 10% milk in PBS/0.5% Tween 20 overnight, subsequently blotted for 2 hours with primary antibodies. Antibodies were probed with polyclonal rabbit anti-galectin-7 (1:1,000; ref. 13) or anti-β-actin (1:1,000). Secondary antibodies consisted of horseradish peroxidase–conjugated donkey anti-rabbit or antimouse IgG. Detection was done by the enhanced chemiluminescence method (Amersham).

RNA isolation and semiquantitative PCR. Total RNA was isolated from lymphoma cells using Trizol reagent according to the manufacturer’s instructions (Invitrogen Canada Inc., Burlington, Ontario, Canada). Two micrograms of total RNA was reverse-transcribed using the Omniscript instructions (Invitrogen Canada, Inc., Burlington, Ontario, Canada). The primers used for PCR amplification were (5′-CCATGTCTTCATCCATC-3′) for sense murine galectin-7 and (5′-GCTTGAAGAATTTTCAATGATGTC-3′) for antisense, (5′-CGAGTTGACCGACCAGTTGTGG-3′) for sense murine MMP-9 and (5′-CAGGCTGACGGCCCATTACAG-3′) for antisense, (5′-CATGGATGACGATATCGCTGCGC-3′) for sense β-actin and (5′-GCTTGCGGCGCGCTGCTGAGATGATC-3′) for antisense, (5′-CAACATTGCAATGTTGATCTGTC-3′) for sense human MMP-9 and (5′-CCGGTGTACTGCGCTGATC-3′) for sense glyceraldehyde-3-phosphate dehydrogenase and (5′-AGCCCTTCTCATGTTGGAAAGC-3′) for antisense. Thirty-five cycles of amplification were done in a thermal cycler (model PTC-100, MJ Research, Watertown, MA). PCR assays using equal amounts of RNA that were reverse-transcribed and amplified by PCR for 25 to 40 cycles with gene-specific primers confirmed that the amplification was in the linear range for each gene. Furthermore, each assay was repeated two to four times to verify the accuracy of the results. As an internal control, amplification of glyceraldehyde-3-phosphate dehydrogenase or β-actin mRNA was carried out by RT-PCR using specific primers. Amplified products were analyzed by electrophoresis on 1.2% agarose gels using ethidium bromide staining and UV illumination. Loading was equalized to the internal control mRNA.

Generation of stable transfectants expressing galectin-7. The cDNA encoding the murine galectin-7 gene (Genbank accession no. AF331640) was obtained from S19 lymphoma cells by PCR amplification using specific primers containing the XhoI and EcoRI restriction sites (a) sense, 5′-GACTATGTCATGCTGCTACCC-3′ and (b) antisense, 5′-TTGGCGGCGGCAATTTAGAGATTC-3′. The integrity of the DNA sequence was confirmed by DNA sequencing. To obtain stable 267 transfectants expressing galectin-7 constitutively, the cDNA encoding the murine galectin-7 was subcloned in Sro eukaryotic expression vector (kindly provided by Dr. François Denis, INRS-Institut Armand-Frappier, Quebec, Canada). Controls were generated using 267 lymphoma cells transfected with the empty Sro vector alone. Transfection was carried out by electroporation (Gene Pulser, Bio-Rad Laboratories) and were done using the following variables: 20 μg of DNA per 1 × 106 cells in Ham’s F-10 on ice; 960 μF; 240 V. After 48 hours of culture in complete medium, transfected cells were allowed to grow in complete medium containing 4 μg/mL of puromycin (Sigma) before individual colonies were selected and expanded. Galectin-7 expression was assessed by RT-PCR and Western blot analysis. As positive control, S19 lymphoma cells were used to confirm the expression of galectin-7 in stable transfectants.

Induction of thymic lymphoma upon intrathymic injection of lymphoma cells. Lymphoma cells (5 × 105) transfected with the empty vector (Sro) or with vector encoding galectin-7 were injected intrathymically in each lobe of 5- to 6-week-old C57BL/6 mice (at least 5-10 mice per group). Animals were carefully monitored periodically for clinical signs of thymic lymphoma: i.e., runting, swelling of the thorax, and dyspnea. When moribund, mice were sacrificed, and thymic lymphoma was confirmed and collected at necropsy.

Peripheral lymphoid tumor growth. Lymphoma cells (105) transfected with the empty vector (Sro) or with vector encoding galectin-7 were inoculated i.v. via the tail vein of 6- to 8-week-old C57BL/6 mice (5-10 mice per group). Animals were carefully monitored periodically for clinical signs of lymphoid tumor growth: dyspnea, running, and splenomegaly. When moribund, mice were sacrificed, and spleen, liver, and kidneys were examined and collected at necropsy. Each biopsy fragment was fixed in formaldehyde solution and stained for histology.

Statistical analysis. Comparisons between different groups for tumor uptake and survival distribution (mean survival time) were made using a log-rank test. The level of significance was determined at P < 0.05.

Results

Galectin-7 modulates growth of T lymphoma in vivo. We have previously shown that aggressive T lymphoma cells, but not their nonaggressive parental cells, expressed galectin-7 at the protein level (9), suggesting that galectin-7 may have a role in tumorigenesis. To test this hypothesis, we thus transduced the nonaggressive 267 T lymphoma cells with murine galectin-7 cDNA. Our transfectants overexpressed galectin-7 at levels similar to those expressed in aggressive lymphoma cells (Fig. 1A). We thus compared the growth of thymic lymphoma induced by intrathymic injection of galectin-7 transfectants with that of control lymphoma cells transfected with the control vector. Frequency and mean survival time were determined for several clones of each group. Our results showed that expression of galectin-7 significantly (P = 0.017) increased the lymphoma growth rate as the mean survival time of mice injected with galectin-7 expressing clones was 25 ± 5 days compared with 33 ± 12 days for control mice (Fig. 1B and C). No difference was detected between galectin-7-transduced cells and control cells in terms of in vitro growth when cells were grown in either 10% FCS or under conditions of lowered serum concentration (data not shown). These data show that overexpression of galectin-7 causes an increased malignant growth of lymphoma cells in vivo.

Galectin-7 modulates aggressiveness of T lymphoma cells. Because the development of metastatic tumors is a multistep process, we next tested whether galectin-7 could promote lymphoma metastasis. Lymphoma transfectants overexpressing galectin-7 and control transfectants were thus injected i.v. via the tail vein of syngeneic adult C57BL/6 mice and the development of lymphoid tumors in peripheral organs measured by assessing the percentage of survival of mice (7, 8). We found that galectin-7 accelerated the development of lymphoma as the mean survival time of mice injected with galectin-7 transfectants was significantly shorter (32 ± 10 versus 52 ± 12 days) than that of mice injected with control transfectants (P = 0.005; Fig. 2). To investigate the effects of galectin-7 expression on metastasis in more details, histopathologic analysis was done. We found that mice injected with lymphoma cells expressing galectin-7 developed large metastatic tumors in the liver and the kidneys with massive infiltration of tumor cells in the parenchyma (Fig. 3). Large areas of lymphoma growth were observed in the perivascular areas in liver sections. In contrast, only few scattered foci of tumor cells with limited infiltration were observed in mice injected with lymphoma cells transfected with the control vector. Similar results were observed in the spleen (data not shown). Collectively, these results show that expression of galectin-7 strongly stimulates the metastatic capacity of lymphoma cells for lymphoid as well as nonlymphoid organs.

Overexpression of galectin-7 overcomes the resistance of ICAM-1-deficient mice to lymphoma dissemination. We have
Galectin-7 in T cell Lymphoma

Galectin-7 induction of MMP-9 expression in lymphoma cells. The dissemination of lymphoid tumors in peripheral tissues is dictated by the emergence of aggressive lymphomas that constitutively express mmp-9, a gene previously associated with high-grade lymphomas (7). To determine whether there was a direct relationship between galectin-7 and mmp-9, we have compared the expression level of MMP-9 between control transfectants and those expressing galectin-7. We found that transfectants expressing galectin-7 expressed higher levels of MMP-9 than control transfectants (Fig. 5A). Moreover, incubation of galectin-7 transfectants with β-lactose, which inhibits binding of galectin-7 to its natural ligands (22, 23), inhibited MMP-9 in a dose-dependent manner (Fig. 5B), indicating that secretion of galectin-7 is responsible for the increased expression of MMP-9 in galectin-7 transfectants. To test this hypothesis, mouse and human recombinant galectin-7 were used to stimulate lymphoma cells to induce MMP-9 expression. For this purpose, the murine 267 T lymphoma and the human OCY-2 B lymphoma cells were incubated with soluble recombinant galectin-7 for ~16 hours at 37°C and the expression of MMP-9 was assessed by RT-PCR. Our results showed that galectin-7 induced MMP-9 expression in both cell lines (Fig. 5C). The induction was specific, as addition of β-lactose inhibited the expression of MMP-9. Moreover, addition of actinomycin D together with galectin-7 prevented the expression of MMP-9 (Fig. 5D), suggesting that induction of MMP-9 by galectin-7 involves de novo mRNA synthesis.

Previously shown that mice bearing mutations in both alleles of the icam-1 gene were resistant to the dissemination of nonaggressive lymphoma cells to peripheral organs upon iv. injection (17, 18). This gene encodes a cell adhesion molecule (ICAM-1, CD54) expressed on the vascular endothelium. Although ICAM-1 has been shown to be involved in the recruitment of circulating leukocytes at inflammation sites (19–21), resistance of ICAM-1-deficient mice is not due to the inability of lymphoma cells to "home" to the target organ, but rather at stages subsequent to migration. In fact, we have found that only aggressive lymphoma cells could overcome the resistance of such mice (7). Because these aggressive lymphoma cells constitutively express galectin-7, we thus tested whether galectin-7 could overcome the resistance of ICAM-1-deficient mice to lymphoma metastasis. We determined if nonaggressive lymphoma cells, such as 267 could develop lymphoma in ICAM-1-deficient mice if transduced with galectin-7. We indeed found that metastatic spread of 267 expressing galectin-7 lymphoma cells was increased (P = 0.016) in ICAM-1-deficient mice (Fig. 4) with a mean survival time of 61 ± 21 days as compared with cells transfected with the empty vector (75 ± 14 days). These results provide evidence that galectin-7 is a new gene that promotes the metastatic spread of tumor cells in vivo.

Galectin-7 expression increases lymphoma dissemination. Survival analysis of 6- to 8-week-old syngeneic C57BL/6 mice injected i.v. via the tail vein with (10^6) galectin-7 transfectants (●) or controls (○). When clinical signs of lymphoma became evident (dyspnea, running, and splenomegaly), the animals were sacrificed and spleen, kidneys, and liver harvested and fixed in 10% formalin for histologic examination. Data are representative of two independent experiments with several different clones expressing galectin-7 and controls.
Discussion

Galectin-7 has been originally identified as a keratinocyte marker (12, 24). In a recent study, we used microarrays to identify this gene whose expression was significantly increased in highly metastatic lymphoma cells as compared with their low tumorigenic parental cells (9). To determine whether galectin-7 plays a critical role in lymphoma progression, we have compared lymphoma development in mice injected with low tumorigenic cells transfected with an expression vector containing the gene encoding for murine galectin-7. Our results showed that: (a) the development of thymic lymphoma is accelerated when induced by cells expressing galectin-7, (b) lymphoma cells that express galectin-7 disseminate more rapidly in peripheral tissues that control lymphoma cells; immunohistologic analysis also reveals a more invasive phenotype of these cells, and (c) overexpression of galectin-7 by lymphoma cells is sufficient to overcome, at least partially, the resistance of ICAM-1-deficient mice to lymphoma. Finally, we propose that the increased tumorigenic behavior conferred by the expression of galectin-7 is mediated by its ability to induce MMP-9 expression. This hypothesis is based on the following evidence: (a) galectin-7 transfectants have higher levels of MMP-9 expression, (b) addition of β-lactose completely inhibits expression of MMP-9, and (c) murine or human recombinant galectin-7 induces the expression of MMP-9 in both mouse and human lymphoma cells. Our results thus provide the molecular basis sustaining the overexpression of this gene during the progression of lymphoma cells toward an aggressive phenotype.

Galectins are carbohydrate-binding proteins with an affinity for β-galactosides (10). To date, 14 mammalian galectins have been identified and several have been shown to be secreted and capable of altering cellular functions, such as cell-cell or cell-matrix adhesion. Together with our previous results showing that galectin-7 was associated with lymphoma aggressiveness (9), the results of our study were unanticipated in that galectin-7 has been thus far mostly considered as a marker for epithelial stratification during development and associated with proapoptotic functions in keratinocytes (12–14). Moreover, recent studies have shown that overexpression of galectin-7 in human DLD-1 colon carcinoma cells inhibits their growth when injected s.c. in the immunodeficient SCID mouse model (25). The effect was
attributed to the suppression of cell growth and possibly to reduced angiogenesis. Although the difference between the effect of galectin-7 observed in the colon carcinoma and lymphoma models could be at least in part explained by the fact that we used a syngeneic immunocompetent mouse model, it is also likely that the effect of galectin-7 depends on the type of tumor and/or the peritumoral environment.

The ability of galectin-7 to modulate MMP-9 expression is of prime importance in the context of lymphoma progression. Several studies have shown that MMP-9 is associated with lymphoma aggressiveness, including non-Hodgkin’s lymphoma (26, 27). Moreover, we have also shown that transfection of MMP-9 increased the aggressive behavior of lymphoma cells, consistent with the data we have obtained with our galectin-7 transfectants (28). Interestingly, we have found that galectin-7 induced the expression of MMP-9 but that incubation with β-lactose completely inhibited the induced expression of MMP-9 by galectin-7, suggesting that galectin-7 was acting at the extracellular level. Although the identity of the galectin-7 ligand is currently unknown, one could envisage that other galectins could also induce MMP genes. Future investigations will be necessary to examine this possibility by examining the repertoire of galectins. Because members of the galectin family have been shown to be cleaved by members of the MMP family or to modulate their proteolytic activation (29–31), our results open a new window from which we can view the functional relationship between the galectins and MMPs during different biological processes, such as neoplastic progression.

In conclusion, our study has uncovered the existence of a previously undescribed activity, the promotion of cancer cell malignancy, to galectin-7. In addition, we showed that the promalignant activity of galectin-7 is related to its capacity to induce MMP-9 expression. Further experiments will reveal whether the promalignant activity of galectin-7 we observed in lymphoma can be generalized for other tumors.
References

A Novel Function for Galectin-7: Promoting Tumorigenesis
by Up-regulating MMP-9 Gene Expression

Mélanie Demers, Thierry Magnaldo and Yves St-Pierre


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/12/5205

Cited articles
This article cites 32 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/12/5205.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/12/5205.full#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, use this link
http://cancerres.aacrjournals.org/content/65/12/5205. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.