

# Increased Galectin-1 Expression in Thymic Epithelial Tumors

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## Abstract

**Thymic malignancies are rare tumors where lack of preclinical models adversely affect development of new therapies. Galectin-1 is an important protein in cancer involved in maintaining an immunosuppressive environment. This study examined galectin-1 expression in a large thymic epithelial tumor issue microarray and found elevated expression compared to benign thymus controls. Galectin-1 is a potential therapeutic target in thymic malignancies.**

**Introduction:** Thymic epithelial tumors (TET) are rare malignancies with a paucity of data on biology and therapeutics. Galectin-1 is a member of the  $\beta$ -galactoside binding protein family and has been shown to mediate tumor growth via modulation of immune cell function. This study examined galectin-1 expression in TET. **Patients and Methods:** A tissue microarray of 68 patients with TET and 8 benign thymus controls were stained for galectin-1 expression and scored by a pathologist blinded to patient clinical and pathologic data. Galectin-1 expression +1 or greater staining intensity was considered positive. Clinical and pathologic data were abstracted from institutional databases. Expression of galectin-1 in thymic tumor was compared to benign thymus controls and correlated with pertinent clinical and pathologic data. **Results:** Galectin-1 expression was higher in TET compared to benign thymus controls (65% vs. 0%). No significant association between galectin-1 expression and the development of recurrent disease, paraneoplastic syndromes, or overall survival was noted. **Conclusion:** Galectin-1 is overexpressed in the majority of TET. Detection of galectin-1 may differentiate benign from neoplastic thymic processes. Additional studies are needed to assess the role of galectin-1 in the development of TET.

*Clinical Lung Cancer*, Vol. 20, No. 3, e356-61 © 2018 Elsevier Inc. All rights reserved.

**Keywords:** Thymic malignancy, Tumor microenvironment

## Introduction

Thymoma and thymic carcinoma are rare tumors, but they represent the third most common type of primary mediastinal tumor.<sup>1</sup> There is a paucity of data on the biology as well as therapeutics, especially in patients with refractory disease requiring treatment in the second line or on a subsequent basis.<sup>2</sup> The rarity of the tumor and the paucity of preclinical models in thymic

malignancies hinder research and development of potential therapeutic targets. Often thymic tumor is admixed with lymphocytes because the development of T cells occurs in the thymus.<sup>3</sup> Immune checkpoint inhibitors have demonstrated activity in thymic malignancies. However, high rates of serious paraneoplastic syndromes and immune related adverse events, particularly in thymoma, restrict its utility as an anticancer agent in this neoplasm.<sup>4,5</sup> Nevertheless, this proof of principle suggests that immunotherapy strategies may be beneficial in TET.

Galectin-1 is an evolutionarily conserved member of the family of  $\beta$ -galactoside binding proteins that has important functions in regulating inflammation and is increasingly recognized as an important protein mediating an immunosuppressive microenvironment allowing for tumor growth.<sup>6</sup> Its main roles in cancer pathogenesis are promoting angiogenesis and local immunosuppression.<sup>7,8</sup>

Galectin-1 is thought to mediate tumor growth and metastases through regulation of T-cell apoptosis.<sup>9</sup> Galectin-1 knockout tumors showed marked decrease in tumor growth and nodal

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Submitted: Apr 24, 2018; Revised: Nov 25, 2018; Accepted: Dec 8, 2018; Epub: Dec 21, 2018

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**Table 1** Clinical and Pathologic Characteristics of 68 Patients Included in Thymic Malignancy Tissue Microarray

Characteristic	Value
Age at diagnosis, median (range)	55 (2-86)
<b>Gender</b>	
Male	36 (53%)
Female	32 (47%)
<b>WHO Histology</b>	
<b>Thymoma</b>	
A	7 (10%)
AB	16 (24%)
B	39 (57%)
<b>B Subtypes</b>	
B1	8 (12%)
B2	9 (13%)
B3	18 (26%)
B (NOS)	4 (6%)
Thymic carcinoma	3 (4.5%)
Thymic epithelial tumor NOS	3 (4.5%)
<b>Masaoka Stage at Diagnosis</b>	
I	31 (46%)
IIa	12 (18%)
IIb	3 (5%)
III	12 (18%)
IVa	6 (9%)
IVb	4 (6%)
Presence of paraneoplastic disease	22 (32%)
Recurrent or metastatic disease	18 (26%)

Abbreviations: NOS = not otherwise specified; WHO = World Health Organization.

metastases from enhanced infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells.<sup>10</sup> In multiple tumor types, increased galectin-1 expression is associated with increased invasion, metastases, and poorer prognosis.<sup>11-14</sup> In lung cancer cell lines, galectin-1 promotes disease progression and chemotherapy resistance.<sup>15</sup> Targeting galectin-1 in preclinical mouse breast cancer models reversed cancer-associated immunosuppression and prevented breast cancer metastases.<sup>16</sup> Humoral immunity to galectin-1 has been associated with improved clinical outcomes to anti-VEGF and anti-CTLA-4 combination therapy in metastatic melanoma.<sup>17</sup> Galectin-1 secretion by tumors has been shown to induce CD8<sup>+</sup> T-cell apoptosis and lymphopenia and synergize with chemoradiotherapy.<sup>18</sup>

Because the thymus plays a critical role in T-cell development and many thymic tumors have substantial admixture of lymphocytes, we hypothesized that galectin-1 may be present in TET and that its expression could help TET evade immune surveillance. Therefore, galectin-1 could potentially be an important prognostic indicator and target in TET. Galectin-1 has been shown to induce apoptosis in human thymocytes.<sup>19</sup> Galectin-1 inhibitors are currently in development, and targeting thymic cancers by galectin-1 inhibition may be a promising therapeutic strategy in thymic malignancies.<sup>18,20</sup>

## Patients and Methods

### Tissue Microarray Construction

After obtaining approval from the institutional review board of Stanford University School of Medicine and the Scientific Research Committee at Stanford Cancer Institute, 68 patients with a pathologic diagnosis of thymic carcinoma or thymoma and 8 benign thymus controls with pathology were identified. Clinical and pathologic data were abstracted from the medical record by institutional databases.

Formalin-fixed, paraffin-embedded tissue was retrieved from the surgical pathology archives at Stanford University Hospital and Clinics. A tissue microarray (TMA) was constructed using a tissue arrayer (Beecher Instruments) to create one new paraffin block from representative 0.6 mm cores taken in triplicate from the blocks of TET and benign thymus controls as published in previous studies.<sup>21,22</sup>

### Galectin-1 Staining and Statistical Analyses

Immunohistochemical stains for galectin-1 (1:200 dilution; citrate pretreatment; mouse monoclonal antibody; Novocastra) and CK5/6 (1:50 dilution, Leica; ER2 [pH 9.0] retrieval, clone D5/16B4, Cell Marque) was performed on 4 μm thick TMA sections. CK5/6 staining was used to confirm the presence of epithelial cells within the TMA cores.

Galectin-1 expression was graded in intensity by a research pathologist at Stanford University School of Medicine who was unaware of the clinical and pathologic data. Galectin-1 was graded 0, 1+, 2+, or 3+ a priori by staining intensity, with staining of 1+ or greater considered positive. Because the TMA contained each patient's biopsy sample in triplicate, grading scores were averaged for each patient's tissue sample.

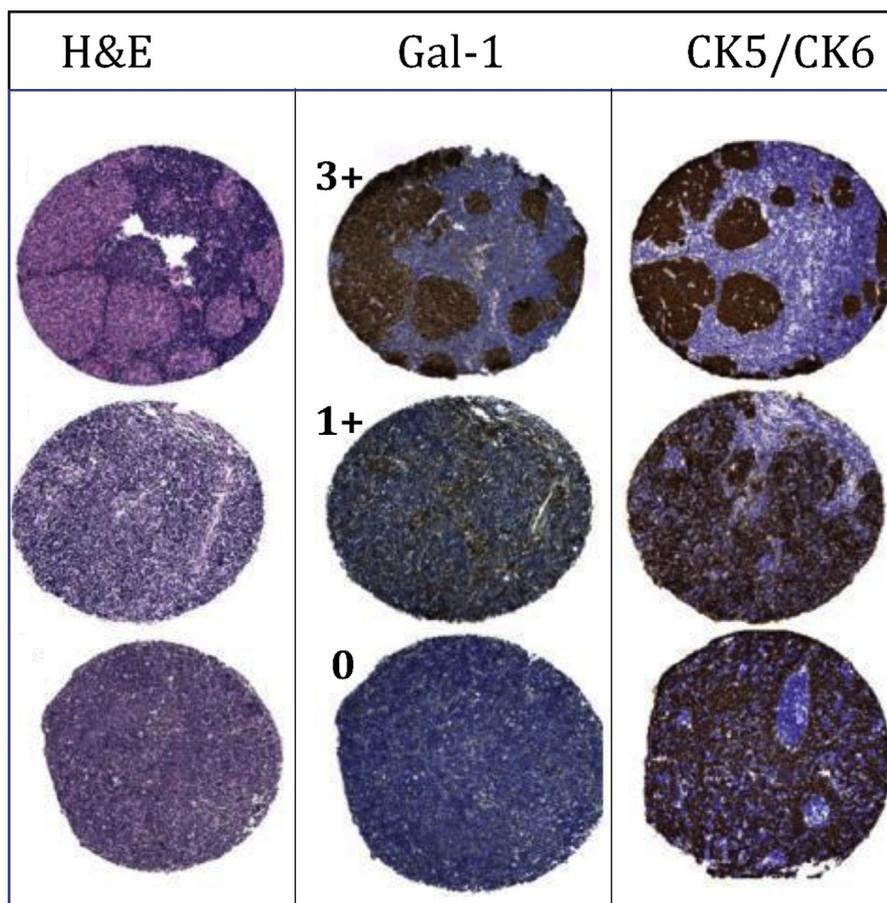
To detect a difference in galectin-1 expression, the average galectin-1 score was compared between cases and unpaired controls by the Wilcoxon rank sum test. In the 11 patient tumor samples that had paired adjacent benign resected tissue, a signed-rank test of the difference in average galectin-1 staining between tumor tissue and adjacent paired resected benign thymus was used.

To detect if there was a difference between histologic subsets of TET compared to unpaired benign thymic controls and account for multiplicity of testing, a nonparametric ANOVA was performed comparing ranked average galectin-1 expression by World Health Organization (WHO) histologic subtypes and unpaired controls. A post hoc Dunnett *t* test of ranked average galectin-1 expression was used to compare galectin-1 expression by WHO histologic criteria with unpaired thymic control galectin-1 expression as the reference value.

Logistic regression based on univariate analysis of mean galectin-1 score was performed examining the presence or absence of paraneoplastic syndromes, presence or absence of recurrent and metastatic disease, and pathologic Masaoka stage (ordinal). To determine the predictive value of galectin-1 discriminating between tumor and benign thymic controls, logistic regression was also performed examining mean galectin-1 expression associated with control versus thymic cancer tissue. Univariate Cox regression analysis was performed to examine the hazard ration for mean galectin-1 score with the time to recurrence and overall survival. All

## Increased Galectin-1 Expression

**Figure 1** Galectin-1 Expression in Thymic Epithelial Tumors. Representative Thymic Epithelial Tumors From Thymic Malignancy Tissue Microarray Demonstrating Galectin-1 Expression and Colocalization With CK5/CK6 Expression



statistical analyses were performed by SAS Enterprise Guide 5.0 (SAS Institute).

### Results

Demographic, clinical, histologic, and staging characteristics of patients included in the TET TMA are presented in Table 1. Galectin-1 expression was restricted to areas of tumor epithelial cells, as demonstrated by costaining with CK5/6 (Figure 1). Average galectin-1 expression of each patient's thymic tumor was compared to that of unpaired benign thymic controls (Wilcoxon test). Average galectin-1 expression (mean 1.5,  $n = 68$ ) in thymic tumors was significantly higher than unpaired benign thymic controls from patients with resected thymus for nonmalignant conditions (mean 0.125,  $n = 8$ ;  $P = .001$ , Kruskal-Wallis test). Among the 11 patients with paired adjacent benign thymus tissue, tumor average galectin-1 staining intensity was 1.82 versus 0.35 in paired adjacent surgically removed benign thymus (signed-rank test of mean difference in expression,  $P = .004$ ).

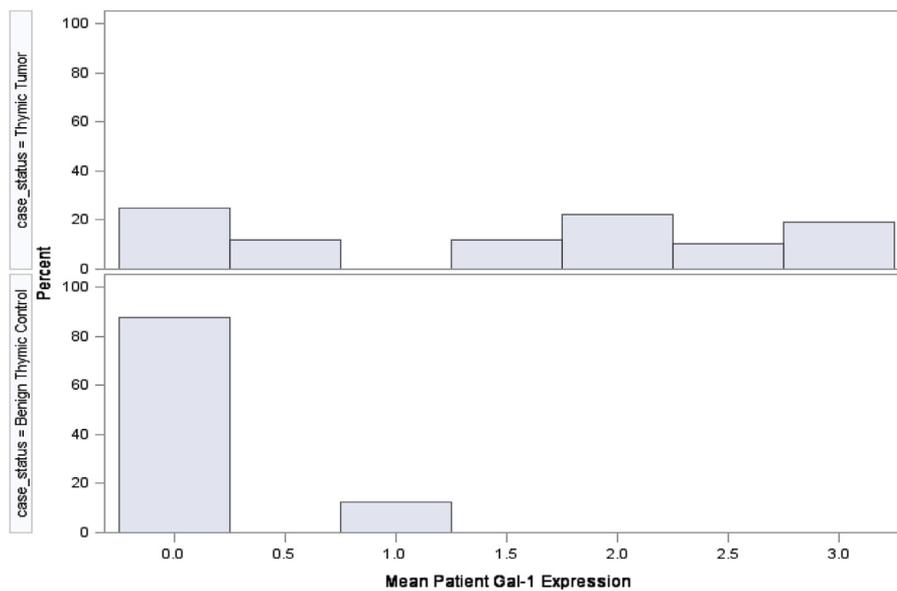
Sixty-five percent of TET had a mean galectin-1 score of  $> 1$  compared to zero benign thymic controls (Figure 2) with an odds

ratio point estimate of 8.197 (95% confidence interval, 1.158-57.889;  $P = .035$ ;  $c$  statistic = 0.845).

Significant differences were detected for galectin-1 expression by histologic subtype (ANOVA  $F = 29$ ,  $P < .0001$ ) between WHO A, AB, B thymoma, and thymoma not otherwise specified compared to benign thymus control (Dunnett  $t$  test of ranked mean differences,  $P < .05$ ). No statistically significant differences were detected between thymic carcinoma and benign thymic controls as a result of the small sample size of thymic carcinoma in the TMA ( $n = 3$ ).

Galectin-1 expression was not associated with the presence or absence of paraneoplastic syndromes or recurrent and/or metastatic disease in our analysis, but it was associated with a decrease in pathologic Masaoka stage. The hazard ratio obtained using Cox regression to examine galectin-1 expression as a prognostic marker for recurrence and overall survival was not significant (recurrence hazard ratio = 1.043,  $P = .88$ ; and overall survival hazard ratio = 1.45,  $P = .13$ ).

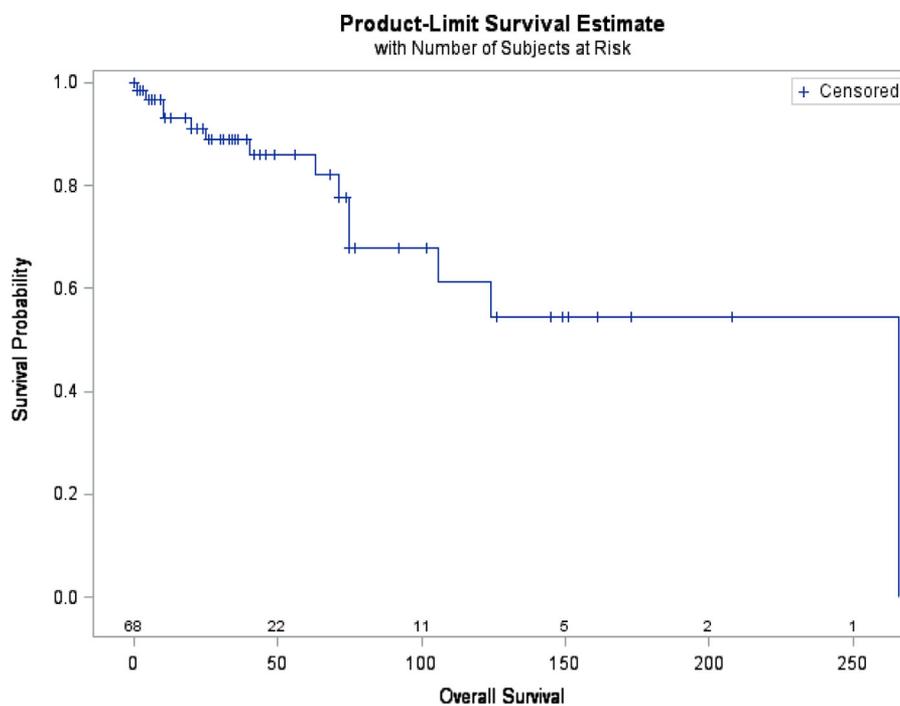
Overall survival was lengthy, at  $> 22$  years, consistent with the excellent outcomes and high rate of curability rate in many early-stage thymoma patients (Figure 3). Of the patients included in

**Figure 2** Histogram of Frequency of Patient Average Galectin-1 Staining of Thymic Tumor (Top) Versus Benign Thymic Control (Bottom)

the TMA, median recurrence-free survival was also lengthy, at 22 years, consistent with the long time to relapse for TET, as the majority of patients in our cohort had thymoma and not thymic carcinoma that has a worse prognosis (Figure 4).

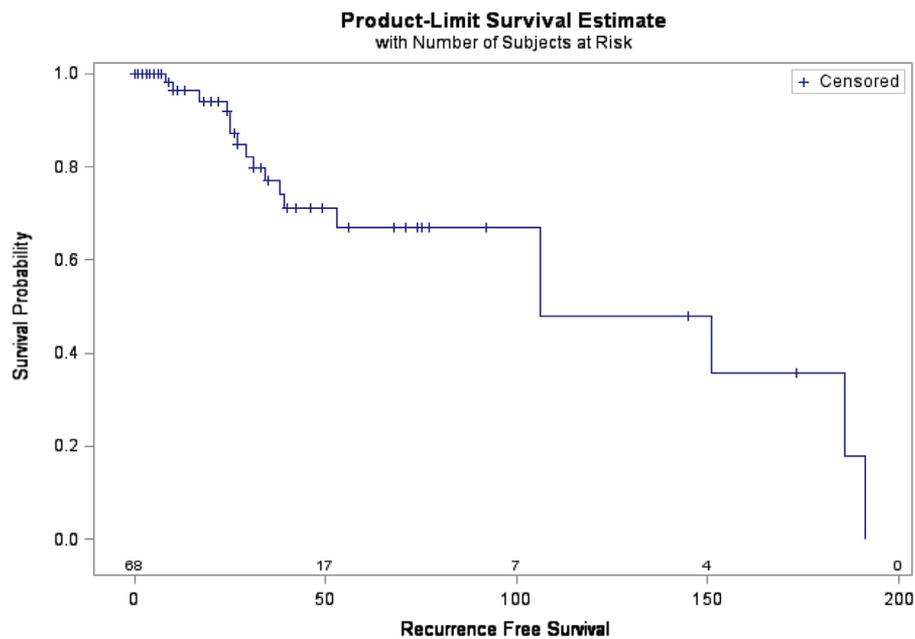
## Discussion

To our knowledge, this is the first report of galectin-1 expression in TET. In a 68-patient TMA, we found that galectin-1 is expressed in the majority of TET, and there was a statistically significant

**Figure 3** Kaplan-Meier Survival Curve for Overall Survival in Months of Patients Included in Tissue Microarray

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**Figure 4** Kaplan-Meier Survival Curve for RFS in Months of Patients Included in Tissue Microarray. RFS Was Defined as Time of Thymoma Diagnosis to Time of Recurrence or Death in Patients Who Were Not Initially Diagnosed With Metastatic Disease



Abbreviation: RFS = recurrence-free survival.

difference in the average galectin-1 expression between thymic tumors and benign thymus in both paired and unpaired controls.

In the 11 samples of surgical thymic specimens that contained tumor and benign adjacent controls, there were also significant differences in average galectin-1 expression, thus providing further evidence that thymic tumors have increased galectin-1 expression compared to nonmalignant thymus.

In using average galectin-1 staining as a predictor to differentiate between TET and controls, a receiver operating characteristic curve demonstrated a robust *c* statistic of 0.84. Many other tumor types also express galectin-1, including other tumors of the mediastinum such as thyroid carcinoma and Hodgkin lymphoma.<sup>12,17,18</sup>

Increased galectin-1 expression compared to benign thymus was conserved across WHO histologic subtypes except for thymic carcinoma, where there were too few samples to appropriately evaluate.

Patients with TET have a higher incidence of autoimmune and paraneoplastic syndromes.<sup>20</sup> As an important immunomodulatory protein, we examined whether average galectin-1 expression was associated with increased odds of paraneoplastic syndromes, but we found no association (Table 2).

Logistic regression examining the odds of metastatic disease or recurrence of disease by average galectin-1 expression showed a trend toward significance, with decreased recurrence/metastases ( $P = .11$ ) (Table 2). This is unusual because in other tumor types, such as Hodgkin lymphoma, galectin-1 expression is associated with recurrent disease. This is hypothesized to be due to galectin-1 creating tumor immune privilege. Our analysis is limited by the small sample size inherent in analyzing this rare tumor, by the high rate of censoring (18 events out of 68 patients), and by the fact that many patients were lost to follow-up.

Though we collected data on time to progression and overall survival and considered survival analysis of these end points with galectin-1 expression, the high curability and the lengthy time to recurrence that is characteristic of thymoma meant that many patients were eventually lost to follow-up. Therefore, we used pathologic Masaoka stage at diagnosis as a surrogate for poor outcome because it is a major prognostic factor for overall survival in these patients.<sup>23</sup> Galectin-1 expression correlated with a decrease in pathologic Masaoka stage (odds ratio = 0.55,  $P = .005$ ) (Table 2). Interestingly, this is the opposite effect of what might have been hypothesized, because galectin-1 expression correlates with poorer prognosis in multiple other tumor types.

**Table 2** Logistic Regression of Galectin-1 Expression in Thymoma With Relevant Clinical End Points Based On Univariate Analysis of Galectin-1 Score

End Point	Odds Ratio	95% Confidence Interval	<i>P</i>
Paraneoplastic syndromes	0.79	0.5-1.2	.31
Recurrence or metastases	0.67	0.41-1.1	.11
Pathologic Masaoka stage	0.55	0.37-0.83	.005

Cox regression analysis examining galectin-1 expression with overall survival and time to recurrence was not significant. There were a limited number of events for recurrence and for overall survival, given the high number of patients lost to follow-up and the comparatively better outcomes of patients with thymic neoplasms (particularly early stage) compared to other tumor types (Figures 3 and 4).

## Conclusion

This is to our knowledge the first large series showing galectin-1 expression in TET. Galectin-1 expression is increased in TET and may help differentiate thymoma from benign thymus. The functional significance of galectin-1 should continue to be investigated as a potential therapeutic target in thymic epithelial tumors.

## Clinical Practice Points

- TET are rare tumors with a lack of approved systemic therapies. Galectin-1 is an important protein in cancer involved in maintaining an immunosuppressive environment.
- Galectin-1 is overexpressed in a majority of thymic epithelial neoplasms.

## Acknowledgments

We acknowledge Steven Goodman, MD, PhD, and Ray Balise, PhD, at Stanford University School of Medicine, for assistance with statistical analyses. Supported in part by National Institutes of Health grant 5 KL2 RR025743 and a Stanford University School of Medicine Translational and Applied Medicine Pilot Award (to J.W.R.).

## Disclosure

The authors have stated that they have no conflict of interest.

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