Urol Res (1999) 27:362–367

ORIGINAL PAPER

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Galectin-1 and galectin-3 expression in human prostate tissue and prostate cancer

Received: 24 May 1998 / Accepted: 8 April 1999

Abstract Galectin-1 and galectin-3, two β-galactoside-binding proteins, have been suggested to play a role in the development and progression of cancer. We have studied the expression of these molecules in normal human prostate tissue and prostate adenocarcinoma. Immunohistochemistry was used to examine formalin-fixed, paraffin-embedded sections of seven normal human prostates, eight cases of prostatic intraepithelial neoplasia (PIN), 20 primary adenocarcinomas of the prostate, and 12 prostate cancer metastases. Galectin-1 was expressed in most cases of all four histologic types. In contrast, galectin-3 expression was significantly decreased in primary carcinoma and metastatic disease compared with normal and premalignant tissue. Galectin-3 expression in primary tumors tended to be less than that of surrounding normal glands. We conclude that loss of galectin-3 expression may be associated with the progression of prostate cancer.

Key words Galectin-1 · Galectin-3 · Human prostate cancer · Immunohistochemistry

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Introduction

Galectins are a family vertebrate lectins which appear to play diverse roles in cellular function including adhesion, growth, and differentiation [3]. The two best studied members of this family are galectin-1 and galectin-3, small proteins with molecular masses of approximately 14.5 and 29 kDa respectively. Significant interest has been generated in the possible role of these molecules in the development and progression of malignancies [21, 26]. We have previously reported that three of four prostate cancer cell lines examined express both galectins in the cytoplasm; only galectin-1, however, was found on the surface [8]. Transfection experiments demonstrated that surface galectin-1 could participate in cell–extracellular matrix interactions, particularly with laminin and fibronectin [8]. We now report our findings of immunohistochemical localization of galectin-1 and galectin-3 in normal prostate tissue, the premalignant lesion prostatic intraepithelial neoplasia (PIN), primary adenocarcinoma of the prostate, and metastases from prostate cancer.

Materials and methods

Antibodies

Polyclonal antibodies to galectin-1 were produced in rabbits by inoculation with galectin-1 isolated from human placenta, as previously described [18]. Rat monoclonal antibodies to galectin-3 (anti-Mac-2) were produced by the hybridoma clone M3/38 (Boehringer Mannheim, Indianapolis, Ind.). ^{125}I-labeled goat antibodies to rat and rabbit IgG were purchased from ICN (Irvine, Calif.).

Protein extraction from human tissue

Fresh frozen tissue was obtained from the Division of Pathology, M.D. Anderson Cancer Center. For protein extraction, the method described by Crittenden et al. [7] was used with a few modifications. Briefly, approximately 0.5 g of frozen tissue was immersed in an ice-cold detergent solution containing 0.9% NaCl, 1% sodium

deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 0.05% iodoacetamide, 1 mM PMSF, and 10 mM Tris-HCl buffer, pH 7.2. The specimens were then homogenized and centrifuged at 1500 *g* for 10 min at room temperature. The supernatants were collected and diluted with 1/2 volume of buffer containing 1% SDS, 4% β-mercaptoethanol, and 60 mM Tris-HCl buffer, pH 6.8, and heated to 95°C for 20 min. After cooling to room temperature, the mixtures were centrifuged at 1500 *g* for 10 min, and 1/2 volume of 1× SDS gel-loading buffer containing 50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol, and 10% glycerol was added to the supernatants. They were then boiled for 3 min and stored at −20°C.

Western blotting

For immunoblotting, proteins were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was incubated with rabbit polyclonal antibodies to galectin-1 or rat monoclonal antibodies to galectin-3, washed, incubated with ^{125}I-labeled goat anti-rabbit (galectin-1) or goat anti-rat (galectin-3) IgG antibodies, and air-dried. The nitrocellulose was then placed against X-ray film and kept at −70°C for autoradiography.

Immunohistochemical analysis of human prostate tissue

Formalin-fixed, paraffin-embedded tissue was obtained from the Division of Pathology, M.D. Anderson Cancer Center. Normal prostates were obtained from cystoprostatectomy specimens from patients with bladder cancer. These prostates were submitted in their entirety for histologic examination and only those without incidental malignancy or PIN were included in the group of normal prostates. Samples of primary prostatic adenocarcinoma and PIN were taken from radical prostatectomies, transurethral prostate resections, or transrectal needle biopsies. Tumors of both transition and peripheral zonal origin were included. Tumors were graded according to the Gleason grading system [12] with scores ranging from 5 to 9. Samples of metastatic disease were taken by needle or excisional biopsy.

Galectins were localized in tissue sections by the avidin–biotin peroxidase complex immunohistochemical technique. Briefly, the sections were deparaffinized in xylene and rehydrated in graded ethanol concentrations (100% to 50%). Putative endogenous peroxidase activity was inhibited by immersing the sections in 1% methanolic hydrogen peroxide for 30 min, and nonspecific binding of the secondary antibody was blocked by preincubation with 20% normal goat serum. The sections were then incubated at 23°C for 3 h with either rabbit anti-galectin-1 (1:200 dilution) or rat anti-galectin-3 (undiluted hybridoma M3/38-conditioned medium). After three washings in phosphate-buffered saline, the sections were incubated for 30 min at 23°C with biotinylated goat anti-rabbit IgG(H + L) for galectin-1 detection or biotinylated goat anti-rat IgG(H + L) for galectin-3 detection (Vector Laboratories, Burlingame, Calif.). The sections were subsequently incubated with avidin–biotinylated peroxidase complex (ABC-kit, Vector) for 30 min in the dark, followed by incubation in 3-amino-9-ethylcarbazole solution (Sigma, St Louis, Mo.) for 10–15 min to visualize the bound antibody by the colored peroxidase reaction product. Sections were counterstained for 30 s with hematoxylin, mounted with coverslips in Aqua mounting medium (Baxter, Houston, Tex.) and examined under a microscope. For negative controls, incubation with the primary antibody was omitted.

Staining for galectin-1 and galectin-3 was evaluated by two observers. Samples were graded from 0 to 3+, with 3+ representing the intensity of the stroma for galectin-1 and of macrophages for galectin-3. Sections that displayed weak staining in sparse cells were considered negative.

Results

Specificity of antibodies in tissue extracts

To ascertain the specificity of our antibodies for galectins in human prostate tissue, immunoblotting was performed using protein extracts from surgical specimens and biopsies of normal and malignant prostates. The presence of varying levels of both galectin-1 and galectin-3 was demonstrated in normal prostate tissues and prostate adenocarcinomas (Fig. 1). The interpre-

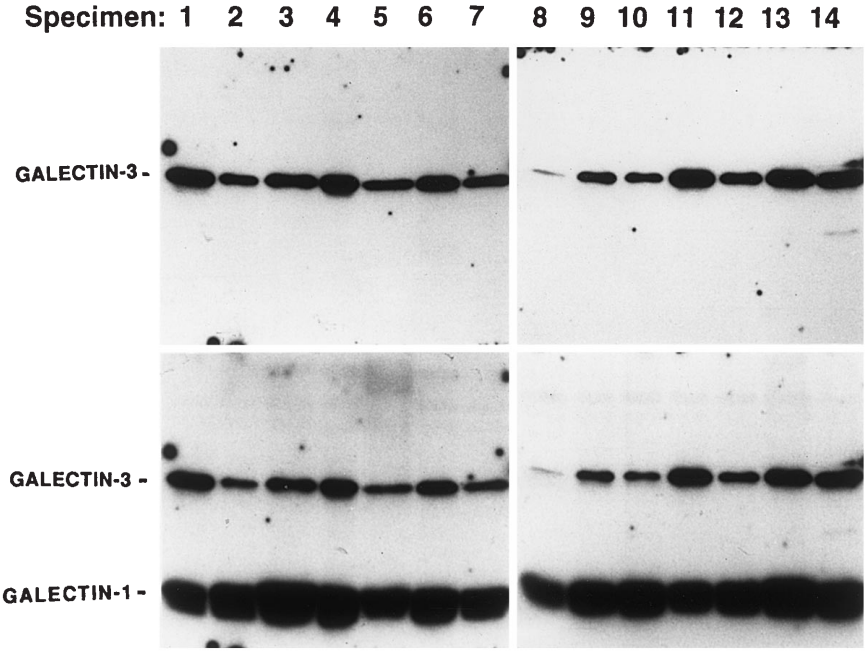


Fig. 1 Specificity of galectin antibodies in extracts of human prostate tissue. Protein extracts from frozen specimens were subjected to electrophoresis in 14% polyacrylamide gels, transferred to nitrocellulose membranes, and probed with antibodies to galectin-3 (*upper panels*) and, subsequently, antibodies to galectin-1 (*lower panels*). The *numbers* above each lane represent the following specimens: *1*, *8–10*: no evidence of malignancy; 2–7, 11–14: adenocarcinoma of the prostate

tation of these data is limited due to the multiple cell types in each sample (epithelial cells, stromal cells, endothelial cells, macrophages, etc.). This experiment did, however, demonstrate specificity of the antibodies for the appropriate lectins and lack of cross-reactivity with other proteins. This specificity is supported by our previous work in which cDNA for galectin-1 and galectin-3 was transfected into a nonexpressing cell line. Western blots of protein extracts from these transfected lines, using the same antibodies and conditions described here, revealed the identical bands [8, 9, and unpublished data]. These findings suggest that human samples could be analyzed accurately by immunohistochemistry.

Localization of galectin-1 and galectin-3 in benign and malignant prostate tissue

Formalin-fixed, paraffin-embedded sections of normal prostates, PIN, primary prostate adenocarcinomas, and prostate cancer metastases were analyzed by immunohistochemistry to determine the presence of galectin-1 and galectin-3. In all, we examined seven normal prostates, eight cases of PIN, 20 primary tumors, and 12 metastases. Metastatic sites included bone marrow (six), lymph nodes (two), liver (one), brain (one), skin (one), and subcutaneous nodule (one).

All normal prostates expressed both galectins in luminal cells, albeit in a heterogeneous pattern with positive and negative areas detected throughout the glands (Fig. 2a, b). There was intense and uniform expression of both galectins in the basal cells of normal glands (arrows). Galectin-1 was detected throughout the stroma and galectin-3 was found in macrophages, as is routinely seen. Both galectins were found in basal and luminal cells in all cases of PIN. Luminal cell galectin-3 expression in PIN was generally quite intense, although both galectins again appeared to be expressed in a heterogeneous fashion (Fig. 2c, d). In contrast to normal prostates and PIN, only 32% (6/19) of the primary adenocarcinomas expressed galectin-3 and 80% (16/20) expressed galectin-1 (Fig. 2e,f). Expression of both molecules was again heterogeneous when present. Decreased expression of galectin-3 was even more notable in samples of metastatic sites, with only one of 11 (9%) staining positive (Fig. 2h). All samples were positive for galectin-1 and expression was more uniform than that seen in normal prostates, PIN, or primary tumors (Fig. 2g). Results of the immunohistochemical analysis are summarized in Table 1.

Expression of the galectins was examined relative to the Gleason score. Of the 20 primary tumors examined, four were well differentiated (Gleason 5 or 6), eight were moderately differentiated (Gleason 7), and eight were poorly differentiated (Gleason 8 or 9). There was no apparent pattern of expression relative to differentiation, with positive samples evenly distributed.

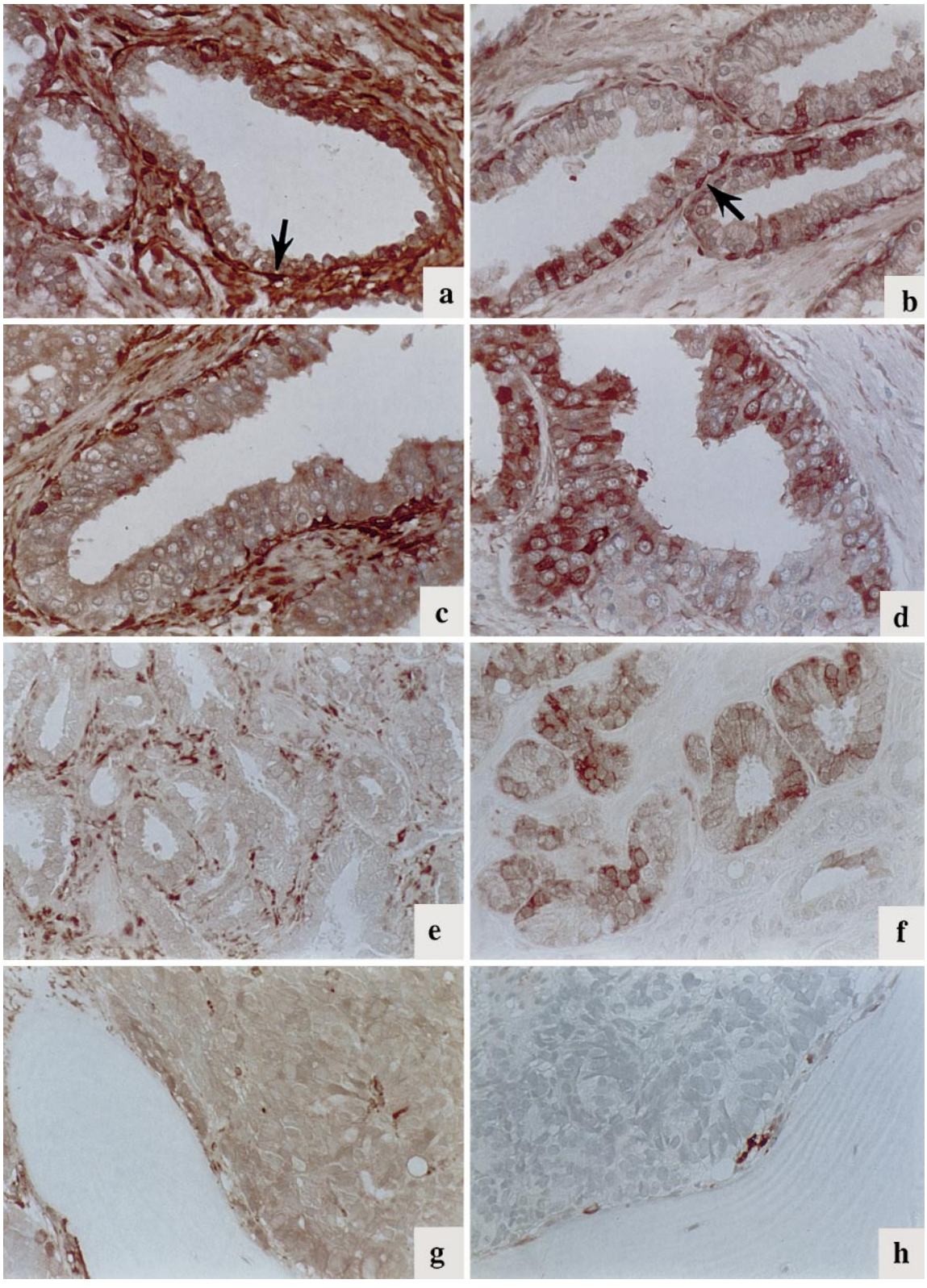
Fig. 2a–h Detection of galectin-1 and galectin-3 in archival material by immunohistochemistry.Samples of formalin-fixed, paraffin-embedded tissue from normal prostates, prostatic intraepithelial neoplasia (PIN), primary prostate adenocarcinomas, and metastatic disease were examined for expression of galectin-1 (**a**, **c**, **e**, **g**) and galectin-3 (**b**, **d**, **f**, **h**) by immunohistochemistry. **a**, **b** Normal prostate. Both galectins are detected in the basal cells (*arrows*). The luminal cells show heterogeneous expression with negative and positive cells noted within a given gland, more prominently shown for galectin-3. **c**, **d** PIN. Both galectins are present in the basal cells and luminal cells. As in normal glands, negative and positive cells of varying intensity are observed in the same gland. **e**, **f** Primary prostatic adenocarcinoma. Basal cells are absent. Staining is again heterogeneous, more pronounced with galectin-3. **g**, **h** Prostate cancer metastasis in bone marrow. Tumor cells are positive for galectin-1 and negative for galectin-3. Cells at the tumor-bone interface staining intensely for galectin-3 are interpreted as osteoblasts or macrophages

Among the four histologic types (normal, PIN, primary tumor, and metastasis), no statistical significance was noted in the percent expressing galectin-1 (Cochran-Armitage Trend test, $P = 0.65$) [1]. For galectin-3, however, the Cochran-Armitage Trend test was highly statistically significant ($P < 0.0001$). The differences between normal or PIN versus primary carcinoma ($P = 0.005$) and metastatic carcinoma ($P < 0.001$) were also significant by Fisher's Exact test. There was no significant difference between the primary and metastatic carcinomas ($P = 0.22$).

Among 44 samples that have both galectin-1 and galectin-3 assayed, we found one sample that did not express either galectin-1 or galectin-3 and 19 samples that expressed both. There were two samples that expressed galectin-3 but not galectin-1 and 22 samples that expressed galectin-1 but not galectin-3. Spearman's Rank Correlation was −0.10 with a standard error of 0.15. No statistically significant correlation was found between expression of galectin-1 and galectin-3. The result held in all histology types.

Expression of galectin-1 and galectin-3 in primary tumors versus surrounding normal glands

Using the samples of primary tumors, we addressed the question of difference in expression of either galectin in tumor compared with normal tissue in the same specimen. Among the transurethral resections and needle biopsies, there were not enough normal glands to make this comparison. However, using the prostatectomy specimens, 13 cases were adequate for evaluation of galectin-1 and 11 cases for galectin-3. As shown in Table 2, there was a trend for increased galectin-1 in tumor compared with normal tissue. The opposite trend was observed for galectin-3, with only three of 11 cases demonstrating greater expression in tumor relative to normal tissue; in six of 11 cases, normal tissues displayed higher levels of galectin-3, and in the other two cases expression was equal.



Discussion

Although the physiologic functions of the various members of the galectin family have not been clearly defined, these molecules have been implicated in critical processes such as cell adhesion to extracellular matrix proteins and regulation of cell proliferation [3]. Consequently, there has been considerable interest in the potential role of galectins in tumor development and

progression. Much attention has been given to galectin-3 in this regard as in vitro data have suggested that increased galectin-3 levels may be associated with the transformed and/or metastatic phenotypes [21].

There now exists a growing body of literature describing changes in galectin expression in human tumors. A consensus, however, has not been reached as many investigators have come to different conclusions. High levels of galectin-3 have been detected in papillary carcinoma of the thyroid [10, 25], Ki-1^+ anaplastic large cell lymphoma [16], squamous cell carcinoma of the head and neck [11], and primary and metastatic gastric carcinoma [17]. Bresalier et al. [4] analyzed galectin-3 expression in primary brain tumors and found that expression was increased in high-grade tumors relative to low-grade tumors. Similarly, Irimura et al. [15] reported that galectin-3 expression in colon cancer was increased in tumors of higher clinical stage. In contrast, Castronovo et al. [6] found that colon cancer cells produce less galectin-3 mRNA than adjacent normal colon; those findings were confirmed by Lotz et al. [19] who studied galectin-3 at both the protein and message level and made comparisons with normal mucosa. Castronovo et al. [5] also reported that in breast cancer galectin-3 expression decreased progressively from normal tissue and benign tumors to in situ carcinomas and finally to invasive carcinomas, with the lowest levels detected in tumors which had metastasized to regional lymph nodes; low or negative expression was also found in the nodal metastatic deposits. In keeping with these findings, van den Brule et al. [22, 24] observed decreased galectin-3 relative to normal tissue in cancer of the uterus and ovary.

Taken together with the data which we present, galectin-3 downregulation has now been found to occur with progression of cancers of the colon, breast, uterus, ovary, and prostate. If colon cancer is excluded because of the conflicting reports described above, one finds that all tumors on this list derive from tissues that are responsive to gonadal steroids or gonadotropins. One could postulate that galectin-3 expression is regulated in some way by these hormones and that decreased expression in malignancies reflects alterations in the normal hormone sensitivity and regulatory processes for the involved tissues. To our knowledge there have been no reports of a unique role for galectin-3 in these tissues beyond alterations of levels in the pregnant uterus [20, 23]. Regulation of expression by estrogens, testosterone, or gonadotropins has not been described, although modulation by the synthetic corticosteroid dexamethasone has been reported [2]. The upstream regulatory elements for the galectin-3 gene have only been partially elucidated and to date steroid hormone response elements have not been described [13, 14]. Also conflicting with this hypothesis is our observation that the "hormone-sensitive" prostate cancer cell line LNCaP does not express galectin-3, in contrast to the "hormone refractory" lines DU145 and PC3 in which galectin-3 is detected [8]. Furthermore, LNCaP cannot be induced to express this molecule by incubation with dihydrotestosterone at various concentrations [9]. An alternate hypothesis would be that these tissue types, when stimulated by the appropriate hormone, produce a unique ligand or binding partner for galectin-3; bound galectin-3 may then modify proliferation of that cell type, perhaps in favor of differentiation. In developing carcinomas, cells that either downregulate or lose expression of galectin-3 would be able to escape this antiproliferative effect and ultimately develop the capability to invade and metastasize. This theory may also explain the rather intense galectin-3 expression seen in premalignant PIN lesions. The enhanced galectin-3 expression may actually represent an attempt to counteract the ongoing proliferative changes within the transforming cells; those cells which gradually lose their ability to overexpress galectin-3 are able to proceed through the transformation process.

In conclusion, we have found that galectin-3 expression is frequently decreased in primary prostate adenocarcinomas compared with normal tissue from the same prostate gland, and that expression undergoes a statistically significant decline during progression from normal tissue and PIN, to primary adenocarcinoma and metastatic disease. Our data and those of others suggest a role for galectin-3 in suppression of tumor progression for some types of cancer. However, there continues to exist a discrepancy in the literature as the opposite effect has been described in other tumor types. We are currently conducting experiments to examine the effects of galectin-3 cDNA transfection into LNCaP cells in order to detect potential alterations in the behavior of this cell line and perhaps shed some light on this perplexing issue.

Table 1 Summary of the immunohistochemistry results

	Galectin-1		Galectin-3	
	n positive	%	*n* positive	%
Normal prostate	7/7	100	7/7	100
PIN	8/8	100	7/7	100
Primary tumor	16/20	80	6/19	32*
Metastasis	12/12	100	1/11	9†

*$P = 0.005$ compared with normal prostate and prostatic intraepithelial neoplasia (PIN)
†$P < 0.0001$ compared with normal prostate and PIN

Table 2 Galectin-1 and galectin-3 expression in primary tumors compared with normal tissue in the same specimen

	Galectin-1 ($n = 13$)	Galectin-3 ($n = 11$)
	n (%)	*n* (%)
Tumor > normal	7 (54)	3 (27)
Tumor = normal	4 (31)	2 (18)
Tumor < normal	2 (15)	6 (55)

Acknowledgement The authors would like to thank Mrs. Pat Gabler for her assistance in the preparation of this manuscript.

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