Decreased expression of galectin-3 is associated with the progression of cervical neoplasia

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Objective: Galectin-3, a member of the β-galactoside-binding proteins, is an intracellular and extracellular lectin that interacts with intracellular glycoproteins, cell surface molecules and extracellular matrix proteins. Galectin-3 is expressed widely in epithelial and immune cells and the level of expression varies in various cancer cells relative to the normal tissue from which they arise. We investigated whether the expression of galectin-3 is associated with the progression of cervical neoplasia.

Methods: The galectin-3 expression was evaluated by immunohistochemistry in 90 formalin-fixed paraffin-embedded cervical tissues: 10 normal cervical epithelia, 20 low-grade squamous intraepithelial lesions (LSILs), 20 high-grade squamous intraepithelial lesions (HSILs), and 40 invasive squamous cell carcinomas (ISCs).

Results: The immunohistochemical staining showed that the expression of galectin-3 was strong in all normal cervical epithelial regions. Staining gradually decreased in accordance with the histopathologic grades from an LSIL to an HSIL and an ISCC (P<0.001). In particular, the expression of galectin-3 was significantly decreased in HSIL (P<0.001) and the down-regulation was more pronounced in ISCCs than normal tissues (P<0.01).

Conclusion: These data constitute the first observation that the expression of galectin-3 is down-regulated in cervical cancer tissues and suggest the decreased expression of this galectoside-binding lectin is associated with the progression of cervical neoplasia.

Key Words: Cervix neoplasms, Cervical intraepithelial neoplasia, Galectin-3, Carcinoma, Squamous cell

INTRODUCTION

Galectins constitute a gene family of widely distributed carbohydrate-binding proteins characterized by their affinity for β-galactoside-containing glycans. Galectin-3, previously described as IgB binding-protein, CBP95, CBP30, Mac-2, L-29, L-31 and L34, is isolated as a monomer with a molecular weight of 31-kDa.¹ Its structure is composed of a C-terminal end, encompassing a carbohydrate binding domain, an intervening proline, glycine and tyrosine domain, and a short NH2-terminal domain.¹ The multifunctionality of this glycoprotein is reflected by its wide range of ligands, for example laminin,² carcinoembryonic antigen,³ and mucin.⁴ Galectin-3 is mainly a cytosolic molecule, but can easily transverse the intracellular and the plasma membranes to visit the nucleus and mitochondria, it can also be externalized despite its lack of classical localization signals at the amino terminal end of the molecule.⁵⁻⁶ Galectin-3 has been associated with the splicing apparatus in the nucleus and so is thought to be involved in this process and perhaps the proliferative potential of the cell.⁷

The biological functions of galectin-3 remain elusive. Studies from several groups suggest that galectin-3 may have a role in a variety of physiological and pathological processes. Different studies have linked the galectin-3 expression to cell growth,⁸ inflammation,⁹ apoptosis,¹⁰ metastasis,¹¹,¹² neoplastic transformation,¹³ and angiogenesis.¹²

The significance of the galectin-3 expression has been
evaluated in many neoplasms. Studies of pancreatic, gastric, thyroid, head and neck squamous cell, and renal cell carcinomas found galectin-3 to be upregulated in these tissues compared to normal tissues. However, some controversial results have been reported concerning different types of tumors. A decreased expression of galectin-3 was noted in some tumors as breast and ovarian carcinomas and uterine adenocarcinomas. Although these contradictory results cannot be completely explained, the levels of the galectin-3 expression depend on the organ or tissue, suggesting that tumor or tissue specific factors may modulate the galectin-3 expression; moreover, the heterogeneity of tumor cells, composed of different clones, might be of importance.

Cervical cancer occurs in a multi-step process, a sequential transition from a cervix with a normal epithelium to preneoplastic cervical intraepithelial neoplasia (CIN) and invasive cervical cancer. Although it is well accepted that high-risk human papillomaviruses (HPVs) are associated with cervical cancer, an HPV infection alone is insufficient for the malignant transformation of HPV-infected cells. Hence other cellular gene alterations are required in addition to an infection with HPVs for the development of cervical cancer. Identification of such gene alterations may be very important to cervical cancer screening and treatment.

In this study, we evaluated the expression of galectin-3 in cervical carcinoma tissues, in order to investigate the role of galectin-3 during the progression of cervical neoplasia. Here we present, for the first time, data showing that expressions of galectin-3 decrease gradually according to the progression of cervical neoplasia. Decreased expressions of galectin-3 may have a potential role in cervical neoplasia progression.

MATERIALS AND METHODS

1. Tissues samples

A total of 90 tissue specimens including 20 low-grade squamous intraepithelial lesion (LSIL), 20 high-grade squamous intraepithelial lesion (HSIL), 20 ISCC without lymph node (LN) metastasis, and 20 ISCC with LN metastasis in addition to 10 normal cervix were obtained from the Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine. Five μm-thick sections were cut from formalin-fixed, paraffin-embedded blocks and applied to routine histopathology slides and immunohistochemistry slides. Representative tumor sections including adjacent non-tumor mucous were studied by Immunohistochemical examinations. Two dedicated gynecological pathologists blindly reviewed all cases with regard to the histological type, histological grading, and LN status.

2. Immunohistochemical studies

Immunohistochemical staining was performed under the standard avidin-biotin complex peroxidase method (DAKO Corp., Carpinteria, CA) using formalin-fixed, paraffin-embedded tissue sections. Five-μm thick tissue sections were mounted on poly-L-lysine coated glass slides and dried at 37°C overnight. The sections were deparaffinized in xylene and sequentially washed in graded ethanol and finally in phosphate-buffered saline (PBS, pH 7.4). The samples were pretreated with 10 mmol/L sodium citrate, at pH 7.0, in a microwave for 15 min. The endogenous peroxidase activity was blocked with 3% H2O2 for 15 min and the samples were preincubated with a protein blocking solution for 10 min. Slides were incubated with primary mouse monoclonal anti-galectin-3 (Abcam, Cambridge, UK) at a 1:50 dilution for 60 min in a room temperature humid chamber. Slides were washed three times in PBS and then incubated with a biotinylated goat anti-rabbit secondary antibody for 30 min at room temperature. Antigen-antibody complexes were detected with the avidin-biotin-peroxidase method using diaminobenzidine as a chromogen substrate (Vectorstain ABC-Link, Vector Laboratories, Burlingame, CA) by the manufacturer’s protocol. Tissue sections were lightly counter-stained with hematoxylin and then examined by light microscopy. Normal prostate tissues served as a
positive control of galectin-3. As a negative control, mouse serum was used in place of the primary antibody.

Two experienced (specialized in gynecological pathology) pathologists blindly reviewed slides and evaluated the immunohistochemical data. The distribution and intensity of cell staining were assessed with observation of entire tumor areas, at least 10 high-power field (HPF) areas. The percentage of cells expressing galectin-3 was estimated by dividing the number of positively stained tumor cells by the total number of tumor cells per HPF. The staining was scored on a scale from 0 to 2+ as follows: 0, less than 10% with weak intensity; 1+, 10% to 50% with weak to moderate intensity; and 2+, more than 50% with moderate to strong intensity.

3. Statistical analysis

Statistical calculations were carried out using SPSS for Windows version 11.0. The Jonckheere-Terpstra test, to correlate decreased expressions of galectin-3 during progression from normal cervix to ISCC, and the least significance test using ranks for multiple comparisons among the five groups, were applied. A P value of < 0.05 was considered statistically significant.

RESULTS

Strong immunoreactivity (2+) was detected in the normal cervical squamous epithelium of all control cases (10/10, Table 1 and Fig. 1, 2). As expected, macrophages were always strongly positive, and they were used as an internal positive control. The pattern of immunostaining in each normal squamous epithelium was usually cytoplasmic, with some expression in the nucleus and on the plasma membrane. The endocervical gland and the basal layer of each normal endocervical gland exhibited strong cytoplasmic staining (Fig. 1). In contrast, 80% (32/40) of ISCCs, 70% (14/20) of HSILs, and 40% (8/20) of LSILs

![Image](image_url)

**Fig. 1. Immunohistochemical staining of cervical neoplasia for galectin-3.** No staining is noted in a normal squamous epithelium (A). Normal endocervical glands and endometrial glands in the proliferative phase showed positive staining (B and C, respectively). Low-grade squamous intraepithelial lesions showed very weak cytoplasmic staining, and high-grade squamous intraepithelial lesions showed weak to moderate cytoplasmic and membranous staining (D and E, respectively). Invasive squamous cell carcinomas showed strongly positive cytoplasmic and membranous staining (F). (Original magnification, × 100 (A-C, F) and ×200 (D-E)).

| Table 1. The expression of galectin-3 in normal cervical squamous epithelium, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), invasive squamous cell carcinomas (ISCC) without lymph node (LN) metastasis, and ISCC with LN metastasis |
|---------------------------------|-----------------|-----------------|-----------------|
| Cervical specimen               | Degree of Immunoreactivity |
| Normal**                       | D (0%)          | 1+ (0%)         | 2+ (10%)        |
| LSIL*                          | 1/20 (5%)       | 7/20 (35%)      | 12/20 (60%)     |
| HSIL*                          | 4/20 (20%)      | 10/20 (50%)     | 8/20 (30%)      |
| ISCC**                         | 10/40 (25%)     | 22/40 (55%)     | 9/40 (20%)      |

* = (p<0.005), ** = (p<0.001), *** = (p<0.01), **** = (p<0.15), ***** = (p<0.005),****** = (p<0.427) by multiple comparison
exhibited a weak or absent expression of galectin-3 (1+ or 0) (Table 1) (Fig. 2). The expression of galectin-3 in ISCCs was significantly decreased compared to that in normal cervical controls and LSILs (P<0.001 and P=0.005, respectively) (Table 1) (Fig. 2). But, there were no significant differences in the level of expression between HSILs and ISCCs (P=0.427), also no significant differences between LSILs and HSILs (P=0.115). When four histopathologic grades (normal, LSIL, HSIL, and ISCC) of specimens were compared in regard to the frequency of positive staining, the expression of galectin-3 gradually decreased in accordance with the progression from an LSIL to an HSIL and an ISCC (P<0.001; Fig. 2) (Table 1).

![Images of immunohistochemical galectin-3 staining](image)

Fig. 2. Percent scoring of immunohistochemical galectin-3 staining in normal cervical squamous epithelia, low-grade squamous intrapithelial lesions (LSILs), high-grade squamous intrapithelial lesions (HSILs), invasive squamous cell carcinomas (ISCCs) (P<0.001).

**DISCUSSION**

In this study, we demonstrated for the first time, that the expression of galectin-3 decreased according to the histopathologic grades of cervical neoplasia including CIN and cervical cancer. The expression of galectin-3 was strong in the squamous cell epithelia of all normal cervical tissues (Table 1) (Fig. 1). However, its expression gradually decreased from LSIL to HSIL, and in particular ISCC cases (P < 0.001, Table 1) (Fig. 2). Interestingly, the frequency of the strong (2+) expression of galectin-3 also gradually decreased from an LSIL (12/20, 60%) to an HSIL (6/20, 30%) and an ISCC (3/40, 8%) (Table 1) (Fig. 2). Thus, the decreased expression of galectin-3 was observed from an early stage, such as in an LSIL, in the progression of cervical neoplasia. These results indicate that the expression of galectin-3 may represent an early change during the disease progression and it appears to be associated with the malignant transformation of normal cervical squamous cells.

It is well known that LSILs form a heterogeneous group with respect to their natural history and viral content. Management of women with LSILs is presently limited by the inability to predict those women at risk of progression. A successful prediction of the risk by molecular markers, such as HPV variants, the expression of P16, HPV E6 and E7 mRNA, the expression of p16, loss of heterozygosity at specific chromosomal loci, and DNA ploidy, have been suggested. However, there is currently no marker identified that is close to clinical usefulness. In the current study, we found that the decreasing expression (none or weak) of galectin-3 was observed in 40% (8/20) of LSILs (Tables 1). In addition, 70% (14/20) of HSILs and (32/40) 80% of ISCCs exhibited the decreasing expression of galectin-3. However, a prospective observational study with long-term follow-up is necessary in order to identify the exact role of galectin-3 in the progression of an LSIL.

Although the relationship between the galectin-3 expression and cancer initiation or progression is not fully understood, the expression of galectin-3 has been preliminarily characterized in several cancer sites. Our observations of cervical carcinomas are consistent with previous reports showing a decreased expression of
galectin-3 in colonic, breast, endometrial, and ovarian cancer specimens. They are, however, in disagreement with reports from several other studies suggesting that an increased expression of galectin-3 is associated with a high metastatic capacity. Moreover regarding colon carcinoma, some conflicting data have been reported regarding the same type of tumors. Some studies showed higher levels of galectin-3 in colon neoplasm samples in comparison to normal mucosa specimens, furthermore, an overexpression is associated with advanced tumor stages and shorter survival. In contrast, other studies reported decreasing galectin-3 levels in colon cancer progression. Although these contradictory results cannot be completely explained, the heterogeneity of tumor cells, composed of different clones, might be of importance. Indeed, the ability of galectin-3 to promote or inhibit invasiveness may depend upon tumor-specific factors, such as the synthesis of metalloproteases or of specific counterreceptors.

Alternative explanations for the decreased expression of galectin-3 in the cervical neoplasia specimen in the current study can be explained as follows. First, galectin-3 may have different functions depending on the cell type in which it is expressed. In fact, cell surface galectin-3 can promote homotypic cell adhesion, whereas intracellular galectin-3 can play a role in transcription, through its serum response element-like domain or in mRNA splicing. The other possibility is that we investigated only one galectin family member. Interestingly, Lahr et al. conducted a large study of a panel of 61 tumor cell lines of different origin and they monitored the presence of mRNAs for human galectin-1, -2, -3, -4, -7, -8, and -9 by using the RT-PCR approach. Their main observation was that different human tumors exhibit a complex pattern of the galectin expression and they concluded, logically, that most studies exclusively focused on galectin-1 and -3 can confound any interpretation. The detailed fingerprinting of their work may contribute to elucidate the biological functions of distinct galectins.

The mechanisms by which galectins exert their effects remain largely unknown. Whatever they may be, are potentially attractive targets for the development of new therapeutic strategies in oncology. Since most glycosides are on the outer surface of cellular and secreted macromolecules, they are in a position to modulate and mediate a wide variety of events in cell-cell and cell-matrix interactions crucial to not only the development and normal function of a complex multicellular organism but also to give rise to a tumor. In addition, simple, highly dynamic protein-bound glycosides are plentiful in the nucleus and cytoplasm, where they appear to serve as regulatory switches.

In conclusion, this study demonstrates that the expression of galectin-3 gradually decreased with histopathologic grades from the stage of an LSIL to an HSIL. Although the precise effect of a decreased expression of galectin-3 awaits further investigation, our work suggests that galectin-3 may play a significant role in cervical carcinogenesis. Further studies, investigating galectin-3, will likely result in the development of novel approaches for the detection and therapy of this disease.

REFERENCES

1819-27.
Expression of galectin-3 in cervical neoplasia


Galectin-3

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β-galactoside -galactoside-

1, 2

Galectin-3

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