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Abstract. Vulvar cancer contributes to about 5% of all gynaecological cancers. Galectin-1, a member of an ubiquitous expressed β-galactoside-binding family that comprises over 140 members to date, has been shown to be involved in many physiological and pathological processes, such as tumour progression, by promoting cancer cell invasion and metastasis, in apoptosis, embryogenesis and immunobiology. As the result of these findings, galectin-1 has been described as a potential marker for tumour progression in some malignancies. In this study, the expression pattern of galectin-1 was determined in 73 formalin-fixed, paraffin-embedded vulvar tissues by a standard immunohistochemical method: 12 benign vulvar specimen, 41 vulvar intraepithelial lesions (VIN), according to their differentiation were subdivided into VIN I, II and III and 20 invasive squamous cell carcinomas (ISCC). The immunohistochemical analyses showed that the intensity of galectin-1 expression on stromal cells next to the neoplastic cells steadily increased according to the pathological grade: benign vulvar tissue <VIN I <VIN II <VIN III <ISCC (p<0.0001). In epithelial cells, negative or weak reactivity for galectin-1 was observed. These findings indicate that the expression of galectin-1 expression on stromal cells increases with the histopathological grade of vulvar tissues, and it can be suggested that these changes might be associated with the progression of vulvar neoplasia.

Vulvar cancer is the fourth most common gynaecological cancer and comprises 5% of malignancies of the female tract (1). In Germany, the incidence was 3.6 cases per 100,000 women in 2003, and in the United States there are an estimated 3490 new cases from this disease annually, with 880 estimated deaths per year (1). Although the rate of invasive vulvar carcinoma has remained stable over the past two decades, the incidence of in-situ disease (vulvar intraepithelial neoplasia) has more than doubled (2). Vulvar carcinoma is encountered most frequently in postmenopausal women. The mean age at diagnosis is 65 years, but may be falling, as indicated in a study of 78 women diagnosed with vulvar cancer between 1979 and 1993, during which the average age at presentation dropped from 69 to 55 during this interval (3). Risk factors for vulvar cancer include cigarette smoking, vulvar dystrophy (e.g. lichen sclerosus), vulvar or cervical intraepithelial neoplasia, human papillomavirus (HPV) infection, immunodeficiency syndromes, a prior history of cervical cancer, and northern European ancestry (4). The increasing incidence of vulvar intraepithelial neoplasia among young women may partially account for the fall in the mean age of diagnosis of vulvar cancer. Early detection and treatment of vulvar intraepithelial neoplasia may prevent development of cancer, explaining why the incidence of invasive vulvar cancer has remained stable even though the incidence of vulvar intraepithelial neoplasia has increased. Most vulvar cancers are squamous cell carcinomas (comprising over 90% of all vulvar malignancies). Other histologies include melanoma, Bartholin gland adenocarcinoma, sarcoma, Paget’s disease and basal cell carcinoma.

Galectins are well-conserved carbohydrate-binding proteins with a binding affinity to galactoside-containing glycoconjugates, and about 14 members of the Galectin family have been described so far.

These proteins are expressed and secreted in a wide range of species and, depending on the cell type or state of differentiation, they have been found in the nucleus, cytoplasm or extracellular matrix. They have attracted increasing attention because of their involvement in a large variety of physiological and pathological processes, such as cell growth, apoptosis, metastasis and immunology (5-10).

Galectin-1 belongs to a family of carbohydrate-binding proteins that share a conserved carbohydrate recognition domain of about 130 amino acids (11-13). There is direct evidence that galectin-1 expression is necessary for the initiation of the transformation into malign phenotypes and for immunobiology (4). Several studies have been reported that galectin-1 can inhibit T-cell activation and promote apoptosis of activated T-cells (14-17).
Different expression profiles have been observed in breast, colon, head and neck, and ovarian cancer (18-21). Increased expression of galectin-1 compared to normal cells has been seen in bladder, thyroid, endometrial and cervical carcinoma (22-26). In most cases, the different expression profiles correlate with the aggressiveness of tumours and the acquisition of the metastatic phenotype (27, 28).

No previous data are available regarding the expression of galectin-1 in vulvar neoplasia. This study examines the changes in expression in different vulvar tissues.

Materials and Methods

Tissue samples. All investigations were approved by the Ethics Committee of the Medical Faculty of the University of Wuerzburg, Germany. For immunohistochemical analysis, a total of 73 paraffin-embedded tissue specimens, including 10 vulvar intraepithelial neoplasia (VIN) grade I (VIN I), 11 VIN II and 20 VIN III lesions and 20 invasive squamous cell carcinoma (ISCC) samples, in addition to 12 normal vulvar samples, were obtained from the Department of Pathology, University of Wuerzburg. Tissue was chosen from routine biopsies of the vulva or vulvectomies. Representative immunohistochemically stained sections of epithelium including adjacent mucosa were studied by light microscopic examination.

Immunohistochemistry. Serial longitudinal sections were cut at 4 μm from paraffin-embedded cervical tissue specimens and placed onto slides coated with 3-aminopropyltriethoxy-silane (APES; Roth, Karlsruhe, Germany), de-waxed in xylene, rehydrated in graded ethanol and distilled water, and subjected to heat pretreatment by boiling in 0.2 mol/l sodium citrate buffer (pH 6.0) for 15 min. in a microwave oven (750 W/s). The sections were treated with H2O2 prior to immunostaining to block endogenous peroxidase activity. For the immunohistochemical staining procedure, the sections were incubated with the primary antibody (mouse anti-human galectin-1 monoclonal antibody; Novocastra, Newcastle, UK) at a dilution of 1:20, followed by the hors eradish peroxidase–labelled LSAB kit system (biotin–streptavidin system; DAKO, Hamburg, Germany). Binding of the antibodies was visualized with 3,3’-diaminobenzidine (Sigma, Deisenhofen, Germany), developed under light microscopic control to optimal contrast. The sections were then counterstained with haematoxylin, dehydrated through graded ethanol and xylene, and embedded in Entellan (Merck, Darmstadt, Germany).

Evaluation. Evaluation of the immunohistochemical staining of the slides was reviewed by two independent observers, experienced in gynaecological pathology and especially in the evaluation of immunostained tissue. The staining with antibodies against galectin-1 leads either to a nuclear or to a cytoplasmatic reaction. In some cases, both nuclear and cytoplasmatic staining was observed. No general scoring system for galectin staining is established yet. Therefore, a semi-quantitative method has been applied in accordance with other published works (26, 29).

The staining intensity on stromal cells was scored on a scale from 0 to 2. Score 0 was given to the tissue with no specific galectin-1 staining on stromal cells, score 1 with clear staining on stromal cells but weaker than on the inflammatory cells, such as macrophages, detected within the tissues. Score 2 was given, when the galectin-1 staining activity on stromal cells was comparable or even higher than on inflammatory cells.

Results

The results are summarized in Tables I and II and in Figures 1 and 2, respectively. The expression of galectin-1 was predominantly found to a high extent in macrophages (that therefore served as internal standard) and on stromal cells, such as fibrocytes and fibroblasts, adjacent to the tumour areas. A significant number of those cells were observed to be positive for galectin-1 in VIN II, VIN III and ISCC (9/11, 17/20 and 20/20, respectively). 80% of the ISCC tissues had an expression score of 2, while in the case of VIN III, half of the examined samples were scored as highly positive. Galectin-1 was found to be expressed in 5 out of 10 VIN I lesions with the expression intensity scored as 1. The majority (92%) of benign vulvar epithelia were observed without any galectin-1 reactivity on stromal cells.

The nonparametric Mann-Whitney U-test (Table II) reveals that the expression of galectin-1 was significantly higher in ISCC compared to normal control, VIN I and VIN II (both p<0.0001). Comparing the levels of galectin-1 expression between VIN I and VIN III, as well as between VIN II and VIN III, there was also a significant difference (p<0.05). No significant change was seen between the examined benign and VIN I, nor between the VIN I and VIN II tissues. Comparing the five different histopathological grades of the vulvar specimen regarding the frequency of...
Figure 2. Immunohistochemical staining of benign vulvar tissue and vulvar neoplasia for galectin-1. A, B: Benign vulvar tissue; C, D: VIN I; E, F: VIN II; G, H; VIN III; I, J: ISCC. Note the intense staining on immune cells (predominantly macrophages; black arrowheads at ×400 magnification) next to the galectin-1-positive stromal cells (white arrowheads at ×400 magnification).
positive staining for galectin-1, the number of positive samples gradually increased from benign to neoplastic tissues ($p<0.001$).

**Discussion**

To the best of our knowledge, this is the first study that shows an increased expression of galectin-1 in stromal cells adjacent to normal or neoplastic endothelia during the change of the phenotype of vulvar tissues from benign to neoplastic. Interestingly, Brustmann (31) reported an increased expression pattern of galectin-3 during the progression of vulvar neoplasia. However, the galectin-3 staining was detected exclusively in the examined epithelial cells. For low- and high-grade vulvar intraepithelial neoplasias cytoplasmic, nuclear or membranous staining with negative or weak and occasionally moderate reactivities. In ISCC samples, the staining pattern was cytoplasmic with a moderate or strong intensity. In contrast to Brustman (31), this study observed galectin-1 to be expressed mainly on stromal cells, such as inflammatory cells and fibroblasts or fibrocytes. Of the tissue sections examined, epithelial cells exhibited zero or weakly detectable levels of galectin-1 expression. The same staining pattern of galectin-1 was described in prostate and cervical cancer (26, 28, 30). Data from this study clearly demonstrate the pattern of the immunostaining changes during the progression from benign vulvar tissue through to vulvar cancer. In benign tissue and VIN I lesions, negative (score 0) or moderate (score 1) galectin-1 reactivity was found. The study also shows that the number of samples with strong (score 2) staining increased from VIN III to ISCC. Based on these results, it can be verified that changes in the expression intensity and pattern of galectin-1 expression on vulvar stromal cells are associated with the progression of vulvar neoplasia. Therefore, galectin-1 may be a suitable marker for the determination of the progression of benign vulvar tissue via intraepithelial lesions to vulvar cancer.

The results of these experiments on vulvar cancer confirm the observations from other studies, which describe galectin-1 expression to be restricted to stromal cells in other tumour entities. Gillenwater (21) demonstrated that galectin-1 was detected in the stroma around head and neck squamous cell carcinoma, and they suggested that the expression pattern of galectin-1 appears to be associated with the degree of squamous differentiation. An increased expression of galectin-1 in the tumour-associated stroma was observed for prostate carcinoma samples and this was correlated with the aggressiveness of the tumour (28). Strong galectin-1 immunopositivity of glioma cells and surrounded stroma was correlated to short patient survival (13). In contrast to our findings, some studies highlighted the expression of galectin-1 in cancer cells themselves. By immunohistochemistry, Xue (24) observed an increased expression of galectin-1 on thyroid carcinoma cells. Van den Brule (25) and Cindolo (22) showed an increased expression of galectin-1 in bladder and endometrial cancer cells compared to the corresponding normal cells. Different expression profiles have been detected for cancer cells and the surrounding host tissues in breast cancer (18). Low expression of galectin-1 and no modulation of staining intensity during progression has been demonstrated in cells of colon carcinoma (19, 20). An explanation for these distinct staining patterns and intensities of galectin-1 expression on different tumour entities has been proposed (42), suggesting that galectin-1 expression could be influenced by other tumour-specific factors such as metalloproteases or by specific, not yet known, counter receptors, which are specifically expressed in tumour. There are no common theories as to the origin of galectin-1 in tumour associated stroma cells. One study hypothesizes that galectin-1 could arise from the carcinoma cells and then be picked up by adjacent stroma cells (33). Another study suggested that galectin-1 could be secreted by tumour-associated fibroblasts itself upon stimulation by carcinoma cells (34). As previously demonstrated in prostate and ovarian cancer, as well as in melanoma (30, 35), galectin-1...
regulates the adhesion of tumour cells to the extracellular matrix by binding to several glycoconjugates such as laminin and fibronectin. Furthermore, galectin-1 inhibits full T-cell activation, induces cell arrest and apoptosis of activated T-cells, and suppresses the secretion of proinflammatory cytokines of T-cells in vitro (15, 16).

T-cells are believed to be the most effective players of the immune system to eliminate tumour cells. Galectin-1 expression in the stroma of vulvar neoplasia could act as a protective shield for cancer cells via the induction of apoptosis of activated T-cells.

This is the first study that showed an increased expression of galectin-1 in stromal cells throughout the progression of cervical neoplasia. Until now, many different tumour entities have been examined, however, the exact role of galectin-1 in the malignant transformation process is still vague. Additional investigations are necessary to clarify the relevance of galectin-1 in tumour initiation and tumour progression and to study the correlation between the expression pattern of galectins and tumour stage, disease recurrence and patience survival. Undoubtedly, vulvar cancer is a rare cancer type compared to colon, lung breast and prostate cancer (1); nevertheless, by collecting all the data and by its known protective shield for cancer cells, and suppresses the secretion of proinflammatory cytokines of T-cells, it neverthe-

References


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