Smyrna Tıp Dergisi **Araştırma Makalesi**

The Role and Importance of Galectin-3 in Gastric Carcinoma Metastasis
Galektin-3'ün Mide Karsinomu Metastazındaki Rolü ve Önemi

Melin Geçer[1], Nilsen Yıldırım Erdoğan[2], Nevra Dursun Kepkep[3]

[1] Bezmialem Foundation University, Faculty of Medicine Department of Medical Pathology, Istanbul, Turkey
[2] Department of Pathology, Unıversıty of Health Sciences, Istanbul Bagcılar Health Application and Research Center, Istanbul, Turkey
[3] Department of Pathology, University of Health Sciences, Başakşehir Cam and Sakura City Hospital, Istanbul, Turkey

Summary
Objective: Gastric cancer is the fourth most common malignant tumor worldwide and is the third common cause of cancer deaths. Despite the diagnostic and therapeutic advances in gastric carcinoma, the survival rate is very low. Targeted therapies have been developed to improve the response rate in these patients.
Material and Methods: HE stained slides of 60 gastrectomy materials from patients who were operated for gastric carcinoma were retrospectively examined. The tumor type, tumor depth, tumor size, grade, vascular invasion, inflammatory stromal response, surrounding lymph node metastasis, perinodal invasion, presence of perineural invasion were re-evaluated. The prevalence of staining was investigated.
Results: A slight increase was observed in the prevalence of Galectin-3 staining in cases with high pathological stage, vascular, perineural and perinodal invasion, and lymph node metastasis. It was found that as the severity of the inflammatory stromal response increased, the prevalence of Galectin-3 staining increased significantly. It was observed that well-differentiated tumors were stained slightly more commonly than less differentiated tumors
Conclusion: The increase in Galectin-3 expression has been found to be compatible with tumor aggressiveness. It was believed that the detection of Galectin-3 in early stage tumors can provide information about the approach to the tumor and can be used as a prognostic marker.
Key words: Galectin-3, gastric carcinoma, immuno-histochemistry.

Özet
Amaç: Gastrik kanser dünya çapında dördüncü en sık görülen malign tümör olup, kanser ölümlerinin üçüncü yaygın nedenidir. Gastrik karsinomdaki tanısal ve terapötik ilerlemelere rağmen sağ kalım oranı çok düşüktür. Bu hastalarda yanıt oranını iyileştirmek için hedefe yönelik tedaviler geliştirilmiştir.
Gereç ve Yöntem: Mide karsinomu nedeniyle opere edilmiş hastalara ait 60 gastrektomi materyalinin HE boyalı lamları; tümörün tipi, derinliği, büyüklüğü, evresi, vasküler invazyon, inflamatuar stromal yanıt, çevre lenf nodu metastazı, perinodal invazyon, perinöral invazyon varlığı açısından değerlendirildi. İmmüno-histokimyasal olarak boyanma yaygınlığı araştırıldı.
Bulgular: Tümörün patolojik evresi arttıkça vasküler, perinöral ve perinodal invazyon ve lenf nodu metastazı izlenen vakalarda galektin-3 boyanma yaygınlığında artış gözlenmiştir. İnflamatuar stromal yanıt şiddeti arttıkça galektin-3 boyanma yaygınlığı da istatistiksel olarak anlamlı bir şekilde artmıştır. İyi diferansiye tümörlerin az diferansiye tümörlere göre daha yaygın boyandığı görülmüştür.
Sonuç: Galektin-3 ekspresyonunun artışı tümör agresifliği ile uyumlu bulunmuştur. Erken dönem tümörlerde galektin-3'ün tespit edilmesi tümöre yaklaşım hakkında bilgi verebilir ve prognostik belirteç olarak kullanılabilir.
Anahtar kelimeler: Gastrik karsinom, galektin-3, immüno-histokimya

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Introduction

Gastric cancer ranks 4th among all cancers (7.8% of cancers) and 2nd in cancer-related death (9.7%) (1). The disease is observed in different incidences in different regions and is closely related to nutritional habits. Unhealthy eating habits, chemical exposure, infectious agents (such as H. pylori) and genetic causes are associated with gastric cancer (1).

The prognosis of early stage gastric cancers is quite good. However gasric cancers are usually asymptomatic and delays are frequent in the diagnosis and unfortunately, approximately 65% of the patients are detected in the locally advanced or metastatic stage at the time of diagnosis (2). As in other types of cancer, this leads to poor prognostic outcomes and directly affects the patient's survival. The most common symptoms in gastric cancer can also be observed in gastritis and peptic ulcer disease, hence patients are diagnosed late stage even if they are symptomatic (3).

The galectins are a family of mediators that have crucial roles for the immune response and thus they have the capacity to regulate inflammatory processes. This regulatory impact can be both inflammatory and anti-inflammatory depending on the localization (4,5). Galectin-3 is an endogenous β-galactosidase-binding protein with intracellular and extracellular localization that belongs to the S-type lectin family. Its' main functions can be elaborated as cell growth, adhesion, proliferation, differentiation, migration, and apoptosis (4,5,6). Galectin-3 is an IgE binding protein, also known as CBP35, CBP30, MAC-2, RL-29, L-29, hL-31, IL-34, or LBP. It is a member of the β-galactoside-binding protein family that recognizes N-acetylglucosamine structures of various glycoconjugates (7,8,9,10). Galectin-3 is expressed in various diseases such as diabetes, cardiac diseases, neurodegenerative diseases, and tumor formation (11,12,13).

Galectin-3 is expressed by the tumor cells and may contribute to the aggressiveness, progression and metastasis of tumor tissue (14,15,16). These are called tumor derived galectins and deteriorate the immune functions while enhancing inflammation. Tumor-derived galectins have bipotential consequences on both tumor and immune cells (17). In a tumor tissue the most prevalent immune cells are macrophages, and they are called tumor-associated macrophages (TAMs) (18,19,20). In previous literature the increased number of tumor-associated macrophages has been interpreted as an indicator of poor prognosis. These macrophages secrete galectin-3 into and leverage tumor tissue progression as galectin-3 facilitates tumor angiogenesis by regulating vascular endothelial growth factor (21). At this stage it should be mentioned that macrophages are not the only galectin-3 expressing cells but this expression is also performed by tumor stroma. This secretion ratio may be in favor of tumor cells in the progressed neoplasms.

Galectin-3 is abundant in the cell surface and in biological fluids such as serum and urine. It is also secreted by tumor cells, tumor-associated macrophages and inflammatory cells which make it both a diagnostic and prognostic biomarker. Galectin-3 has been utilized as a biomarker to detect glioma tumorigenesis (22,23,24,25).

The expression of galectin-3 is up-regulated in many types of cancers and new therapeutic strategies may be designed to facilitate the use of galectins as biological response modifiers to either tumor cells or immune cells. In this study it was aimed to elucidate the role of galectin-3 expression in metastasis biology and its role in predicting tumor metastasis in gastric cancers (26,27).

Materials and Method

This is a retrospective study. Hematoxylin-eosin-stained sections of 60 adenocarcinoma cases that were previously reported by pathology clinic were re-examined under light microscopy. The ethics committee approval has been granted on 22/12/2020 with protocol number 21/411. The study complied with the Declaration of Helsinki.

The sections were re-evaluated in terms of histological type, histological grade, stage, lymph node and distant metastasis, and vascular invasion. Grading was accomplished using the three values. TNM staging was re-evaluated according to American Joint Committee on Cancer (AJCC) 8th Edition. Histopathological tumor typing was based on the World Health Organization (WHO) and Lauren classifications.

A representative colon adenocarcinoma containing the transition of the adjacent normal mucosa-tumor tissue block from each tumor for immune-histochemical analysis have been chosen. Immune-histochemical study was also performed on one metastatic lymph node and distant metastasis section in all metastatic cases. We have utilized 4 μm section from each formalin-fixed, paraffin-embedded tissue block and mounted on to positively charged slides. The slides were stained with Galectin-3 (NCL-Gal3 Clone: 9C4) mice monoclonal antibody (Novo Castra Laboratories) with the streptavidin-avidin-biotin method. The immune-histochemical stained

sections were examined and scored by two different pathologists with light microscopy.

Both staining intensity and staining percentage were assessed for Galectin-3 in the center and invasive margin of the tumor, in the metastatic focus in the lymph node, and distant metastatic focus. According to the staining intensity, negative staining was evaluated as Category 0, mild staining as Category 1, moderate staining as Category 2 and strong staining as Category 3. The percentage of staining was evaluated as the ratio of the stained area to the total tumor area.

SPSS (Statistical Package for the Social Sciences) Windows 10.0 Software package was used for statistical analyses. Student's t-test, Mann Whitney U test, and Kruskal-Wallis test were utilized for comparisons. Pearson test was performed for correlation analysis. P-level less than 0.05 was considered statistically significant.

Results

The the mean age of patients with gastric tumor was 62.5 years (min:29 max:96). Of the patients, 42 (70%) were male and 18 (30%) were female. In the reconstructed microscopic examination; 40 tubular and tubulopapillary carcinomas, 16 signet ring cell carcinomas and 4 mucinous carcinomas have been observed.

Galectin-3 staining status has been segmented to three groups as 0-1, 2-3 and 4. In the immuno-histochemical examination, Galectin-3 expression was found to be more significant in tubular carcinomas than in signet ring cell carcinomas (Figure 1), (Figure 2).

Figure 1. *Galectin-3 expression in the score 2 group in signet ring cell carcinoma*

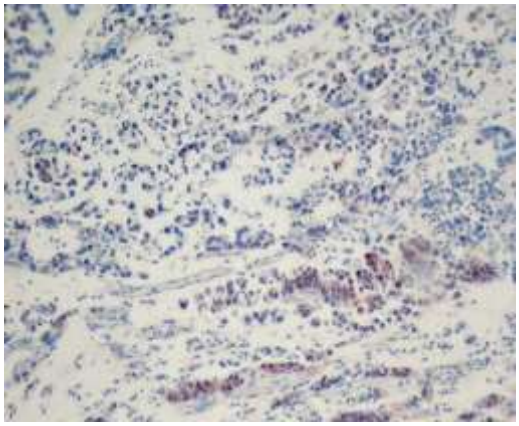
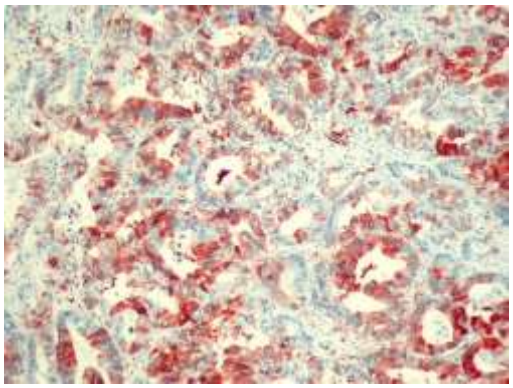
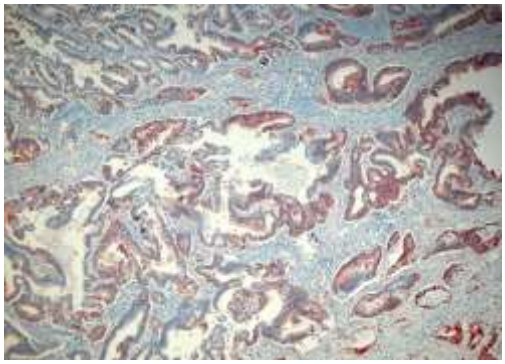


Figure 2. *Galectin-3 and score 4 staining in moderately differentiated tubular carcinoma.*



The mean prevalence of Galectin-3 staining in signet ring cell carcinoma cases was 1.44 and 9 cases showed little or no Galectin-3 staining, 10 cases showed moderate Galectin-3 staining and 1 case presented strong staining positivity. When the Galectin-3 expression of tubular adenocarcinoma and signet ring cell adenocarcinoma was examined, it was found that tubular carcinomas denoted higher Galectin-3 expression (p=0.005) (Figure 3).

Figure 3. *Galectin – 3 expression in the score 2+3 group in well-differentiated tubulo-papillary carcinoma*

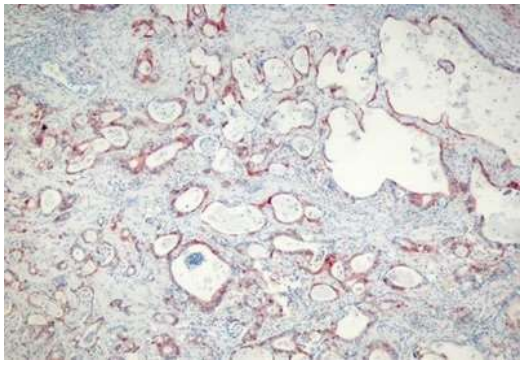


Inflammatory stromal response was evaluated in 3 groups as mild, moderate and severe. Twenty (33.9%) subjects had mild, 30 (50.8%) subjects moderate and 9 (15.3%) subjects had severe inflammatory stromal response.

In terms of scoring; 57.9% (n=11) of cases with mild inflammatory stromal response, 26.3% (n=5) of cases with moderate inflammatory stromal response, and 15.8% of cases with severe inflammatory stromal response (n=3) had Galectin-3 staining of 0-1. In addition, 28.1% (n=9) of cases with mild inflammatory stromal

response, 62.5% (n=20) of cases with moderate inflammatory stromal response, 9% of cases with severe inflammatory stromal response had Galectin-3 staining of 2-3. Among cases with mild infiltration, no Galectin-3 staining of 4 has been observed. Galectin-3 staining was found to be 4 in 62.5% (n=5) of cases with moderate inflammatory stromal response and 37.5% (n=3) of cases with severe inflammatory stromal response. In this study, Galectin-3 staining correlated with the inflammatory stromal response (p=0.012), (Figure 4).

Figure 4. *More than 50% staining with Galectin-3 in moderately differentiated tubular carcinoma with pronounced inflammatory stromal response*



According to the Lauren classification, 73.3% (n=44) of the cases were intestinal type and 26.7% (n=16) were diffuse type. In 57.9% (n=11) of the cases in the intestinal type group and 42.1% (n=8) of the cases in diffuse type had Galectin-3 score of 0-1. Additionally, 75.8% (n=25) of the cases in the intestinal type group and 24.2% (n=8) of the cases in diffuse type had Galectin-3 score of 2-3. Finally, 100% (n=8) of the cases in the intestinal type and had Galectin-3 score of 4. No staining matching Galectin-3 score of 4 was detected in any of the cases matching the diffuse type. A statistically significant correlation was found between the Lauren classification and the prevalence of Galectin-3 staining (p=0.005). Galectin-3 staining was not found statistically significant in gastric tumors showing vascular, perinodal, perineural and lymph node infiltration compared to gastric tumors without infiltration.

Discussion

Galectin-3 is a carbohydrate-binding protein that belongs to the S-type lectin group and has an affinity for β-galactoside sugars. Disorders on the cell surface or in glycoproteins to which galectin-3 is bound, such as mucins, play an important role in carcinogenesis in the gastrointestinal system (22). Increased galectin-3 levels have been reported in some neoplasms and this finding has been suggested to relate to disorders of cell growth, transformation, and metastasis (23). Although the biological functions of galectin-3 have not been fully elaborated, it has been found to induce tumor progression and metastasis by means of carbohydrate-mediated homotypic aggregation and inhibition of apoptosis (4,5).

Most colorectal adenocarcinomas develop on the basis of adenomas, rather than de novo development (27). Furthermore, only part of the cells in a tumor possesses metastatic phenotype and a heterogeneous cell population is formed within a tumor. Considering this, a score encompassing the whole tumor area may not reflect the actual galectin-3 expression. As a result, overall galectin-3 expression detected in a tumor did not show a significant correlation to its metastatic capacity, while an expression in the invasive component showed a positive correlation to the presence of metastases. This was considered to be a result of a heterogeneous population within the tumor (28).

In early stages of galectin research, promising data have been published by various investigators. Iramura and Lee have reported in separate studies that there existed a direct relation between galectin-3 expression and tumor stage (29,30). Similarly, Schoeppner et al. showed that galectin-3 expression was increased in proportion to an increase in stage and was correlated with reduced survival (31), hence, studies on some malignancies other than colorectal carcinomas have reported opposite results. Lotan et al. showed in gastric carcinomas (32) and Vandenbrule et al. showed in ovarian carcinomas (33) that metastatic properties and the clinical course did not correlate to galectin-3 levels. Unlike the studies on colon carcinomas, Lotz et al. found that reduced galectin 3 expressions were associated with tumor progression (34). The cases having no metastasis in the resection material (stage I and II) were included in the group with vascular invasion in the metastatic process to form a separate case group. When this group separately analyzed, a more significant relationship between galectin-3 and metastasis was observed.

The vascular invasion is taken as a single parameter. The significant relationship of vascular

invasion with tumor invasion and galectin scores could be explained by an increased cell population able to invade vessels during the metastatic process. This significant relationship, however, also suggests a role of Galectin-3 in the heterotypic-homotypic aggregation of tumor cells and thrombocytes during the entry into the vessel lumen and tumor embolization (35).

In another study (36), of 64 primary gastric cancer tissues and 10 normal gastric mucosa, a positive expression was observed in 85.9% of gastric tumor cases; and 46% of cases showed strong nuclear immunoreactivity. Serum Galectin-3 represents a potential diagnostic marker for patients with gastric carcinoma in determining the individual prognosis of these patients.

The enhancement of immunoreactivity was different in various histopathological subtypes in cancerous tissues. Significantly stronger expression of Galectin-3 was observed in gastric tumor tissues and they concluded that Galectin-3 may be a useful tumor marker for gastric carcinomas in terms of tumor metastasis, proliferation, cancer progression and tumor cell adhesion.

A strikingly diffuse staining in the tubular/tubule-papillary tumor group was found, which is one of the histological types of WHO classification, compared to the signet ring cell tumor group, and the result was statistically significant (p=0.005). This value was also supported by the detection of more widespread staining in the intestinal type compared to diffuse type tumors in the comparison made by considering the Lauren classification histological types, and this result was among the statistically significant results in the study (p=0.005). As the severity of the inflammatory stromal response increased, the prevalence of Galectin-3 staining increased statistically significantly (p=0.050).

Conclusion

Elevated Galectin-3 expression in various cancers was found to be compatible with tumor aggressiveness observed in gastric carcinomas. It was believed that the detection of Galectin-3 in early stage gastric tumors can provide information about the approach to the tumor and can be utilized as a prognostic marker.

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Corresponding Author
Melin Ozgun Gecer, MD
Bezmialem Foundation University Faculty of Medicine Department of Medical Pathology
E-mail: m.gecer85@gmail.com
Tel: +90.5325250470