

# The Prognostic Value of Fascin 1 and Galectin 3 in Laryngeal Carcinoma

Larenks Kanserinde Fascin-1 ve Galectin-3'ü n Prognostik Önemi

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## Abstract

The aim of this study was to evaluate the prognostic relevance of fascin-1 and galectin-3 that are actin proteins in laryngeal cancer. Informations and documents of 58 patients diagnosed with advanced laryngeal squamous cell carcinoma and treated surgically were included in the study. Surgical specimen slides were stained immunohistochemically at Benchmark staining platform by Ventana Universal DAB detection kit. For each marker, least 1000 cells were counted at light microscope. For galectin-3; more than 5% staining was considered as positive, %5 and less was accepted as negative. For fascin-1; staining intensity was categorized in 4 groups; 0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining, and the percentage of positive cells was grouped from 1 to 4 as follows; 1:  $\leq 10\%$ ; 2: 10-50%; 3: 50-75%; 4:  $\geq 75\%$ . Then, two scores gathered and final score 4 and less accepted as low staining; 5 and more accepted as high staining. Fascin-1 expression was found high in 41(70.68%); and galectin-3 expression was found positive in 36 (62.06%) patients. Fascin-1 and galectin-3 expressions showed no correlation with conventional prognostic factors, disease free survival and disease related death, statistically. Prognostic value of fascin-1 and galectin-3 in laryngeal cancer could not be demonstrated in this study. However, this study may contribute to the literature in this respect, as these two markers have not been studied together in any study of laryngeal cancer until now. Therefore, more studies are needed on this subject.

**Keywords:** fascin-1, galectin-3, actin protein, prognosis, laryngeal cancer

## Özet

Bu çalışmada birer aktin proteini olan fascin-1 ve galectin-3'ün larenks kanserinin prognozu ile ilişkilerinin değerlendirilmesi amaçlanmıştır. İleri evre larengeal skuamöz hücreli karsinom tanısı alan ve cerrahi olarak tedavi edilen 58 hastaya ait bilgi ve belgeler retrospektif olarak incelendi ve çalışmaya dahil edildi. Cerrahi spesmene ait patoloji preparatları Ventana Universal DAB tespit kiti ile Benchmark boyama platformunda immünohistokimyasal olarak boyandı. Her bir işaretleyici için ışık mikroskopunda en az 1000 hücre sayıldı. Galectin-3 için; % 5'in üzerinde boyanma pozitif, % 5 ve altı boyanma negatif olarak kabul edildi. Fascin-1 için; boyanma yoğunluğu 4 grupta kategorize edildi; 0: boyanma yok; 1: zayıf boyama; 2: orta derecede boyanma; 3: güçlü boyanma ve pozitif hücrelerin yüzdesi 1 ile 4 arasında gruplandı; 1:  $\leq 10\%$ ; 2: 10-50%; 3: 50-75%; 4:  $\geq 75\%$ . Ardından elde edilen iki puan toplandı ve 4 ve daha düşük nihai puan düşük boyanma olarak; 5 ve üzeri yüksek boyanma olarak kabul edildi. Fascin-1 ekspresyonu 41 (% 70.68) hastada yüksek bulundu; galectin-3 ekspresyonu 36 (% 62.06) hastada pozitif bulundu. Fascin-1 ve galectin-3, geleneksel prognostik faktörler, hastaliksız hayatta kalma ve hastalıkla ilişkili ölüm ile istatistiksel olarak hiçbir korelasyon göstermedi. Larenks kanserinde fascin-1 ve galectin-3'ün prognostik değeri bu çalışmada gösterilememiştir. Ancak bugüne kadar herhangi bir larenks kanseri çalışmasında bu iki belirteç birlikte çalışılmadığı için bu çalışma literatüre bu açıdan katkı sağlayabilir. Bu nedenle bu konuda daha fazla çalışmaya ihtiyaç vardır.

**Anahtar Kelimeler:** fascin-1; galectin-3; aktin proteini; prognoz; larenks kanseri

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## 1. Introduction

Early diagnosis increases the chance of survival in laryngeal cancer. However, especially in developing countries, the number of patients diagnosed at advanced stage is still rather high. Worldwide reported 5-year overall survival rate for patients with advanced laryngeal cancer is around 50 – 60 %, and despite all technological advances in diagnostic and treatment methods of oncology, satisfactory improvement at long-term survival rates couldn't be achieved in these patients. According to general oncologic view, the survival rate depends on characteristics of tumor, patient and type of applied treatment.<sup>1-3</sup> Still today, the most frequently and practically used prognostic factors are stage of the tumor and pathological characteristics such as size, grade of tumor, perineural and lymphovascular invasion, and nodal status.<sup>4,5</sup> However, it is possible to encounter patients with different course of disease having similar tumor characteristics. Therefore, a lot of study is carried out on new prognostic factors. Especially, studies on molecular biomarkers are more remarkable. Fascin-1 and galectin-3 are also examples for these molecular biomarkers.<sup>6-8</sup>

Fascin-1 and galectin-3 are proteins that are barely or never present in healthy epithelial tissues in human, but their expressions have been shown in various malignant epithelial tumors of body, and it is believed they have an important role in cancer progression and metastasis. Fascin-1 is an actin binding protein that is responsible for forming of bundles like lamellipodia and invadopodia make main skeleton of cellular protrusion of cancer cells to enhance cellular motility<sup>7,8</sup>. Galectin-3 is a carbohydrate binding protein that is thought to play role in tumor growth, cell growth, apoptosis inhibition, and cell adhesions.<sup>6,9-11</sup>

Many studies have shown prognostic relevance of fascin-1 in gastric, colorectal, breast, lung, and laryngeal carcinoma. However, the prognostic value of galectin-3 in epithelial malignancies has been studied only in a few studies, and the results are conflicting. To the best of our knowledge,

prognostic value of these 2 biomarkers have never been investigated together in laryngeal carcinoma. We aimed to address this subject in this study.

## 2. Methods

### *Study Design & Participants*

This study was carried out retrospectively. The study protocol was approved by the local ethics committee with the number 2011/05 and was supported with the number 201111035 by Project Development and Support Unit of our university.

The hospital files of patients diagnosed with advanced laryngeal squamous cell carcinoma and treated surgically between January 2003 and February 2011 were retrieved. After exclusion criteria (having a history of chemotherapy and/or radiotherapy before surgery, a following time shorter than one year, and a positive primary tumor margin) were met, 58 patients were included in the study.

Cigarette smoking was evaluated by using Brinkman index (the number of cigarettes smoked per day multiplied by the number of years of smoking).

### *Immunohistochemical Study*

All of surgical specimen slides of these 58 patients were stained immunohistochemically at Benchmark staining platform (Ventana Medical Systems Inc) by Ventana Universal DAB detection kit. As primary antibodies, Fascin-1 (clone FCN01, Neomarkers, Fremont, CA, USA; dilution 1:100) and Galectin-3 (clone 9C4, Leica Biosystems, Novocastra, Newcastle Upon Tyne, UK; dilution 1:100) were used. After staining, sections were dehydrated and closed. For each marker, least 1000 cells were counted at light microscope. For Galectin-3; more than 5% staining was considered as positive, %5 and less was accepted as negative. For fascin-1; staining intensity was categorized in 4 groups; 0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining. The

percentage of positive cells was grouped from 1 to 4 as follows; 1:  $\leq 10\%$ ; 2: 10-50%; 3: 50-75%; 4:  $\geq 75$ . Two scores gathered and final score 4 and less accepted as low staining; 5 and more accepted as high staining.

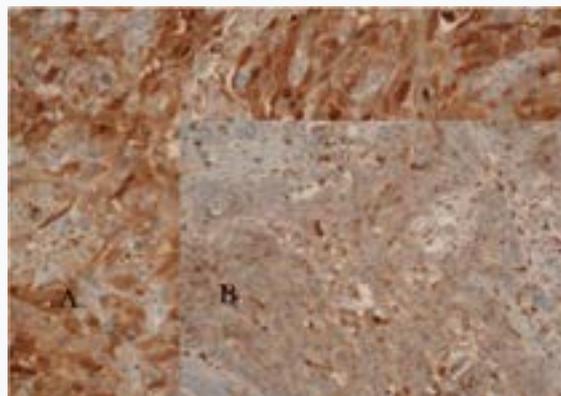
#### Statistical analysis

SPSS15 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Kaplan-Meier method used to form survival curves and log-rank test was used for analyzing survival curves. Disease free survival (DFS) was calculated from the date of curative surgery to that clinical or pathological recurrence. Disease related death (DRD) was calculated from the date of curative surgery to that death. Chi-square test was performed for the analysis of categorical variables. Univariate survival analyses and also multivariate survival analyses, with Cox proportional hazards models, were performed separately for DFS and DRD. Significance level was set as  $p < 0.05$ .

### 3. Results

A total of 58 patients were male with a mean age of 60.4. All patients underwent total laryngectomy and neck dissection. Thirty-nine patients received adjuvant radiotherapy. Median follow-up period was 46.6 months. Median time to recurrence was 17.1 (2-35) months, disease related death was 34.4 (4-91)

months. Primary tumor size varies between 0.7-7 cm (median 3.3 cm). Metastatic lymph nodes (at least 1, maximum 22) were found in 24 patients. Recurrence occurred in a total of 11 patients, 10 of whom died of disease. Table-1 presents clinic and pathologic characteristics of patients, results for fascin-1 and galectin-3 expressions, and prognostic implication of all these findings obtained by taking results of local recurrence and deaths related to disease. The correlation of fascin-1 & galectin-3 expression with pathologic characteristics is shown in Table-2. Fascin-1 expression was found high in 41 (70.68%) patients and galectin-3 expression was found positive in 36 (62.06%) patients. Positive and negative staining for galectin-3 and high and low staining for fascin-1 is shown in Figure 1 and 2. Fascin-1 and galectin-3 expressions showed no correlation with conventional clinic and pathologic prognostic factors, disease free survival and disease related death, statistically. Significant correlations were observed between 2 conventional pathologic prognostic factors (metastatic lymph node status, extracapsular extension at metastatic lymph node) and disease related death. Metastatic lymph node status, extracapsular extension at metastatic lymph node, and vascular invasion were significantly correlated with recurrence. There was no correlation between fascin-1 and galectin 3 expression.



**Figure 1.** A. Galectin-3 high expression (x200), B. Galectin-3 low expression (x200)

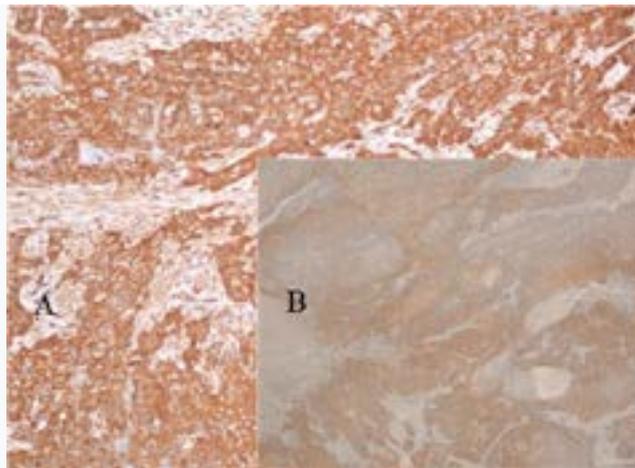


Figure 2. A.Fascin- 1 high expression (x200), B. Fascin-1 low expression (x200)

#### 4. Discussion

This study was conducted to explore prognostic value of fascin-1 and galectin-3 in patients with advanced laryngeal carcinoma. To interpret the results accurately and comparatively, conventional clinic and pathologic prognostic factors seen in Table 1 were evaluated additionally. As a result, fascin-1 and galectin-3 expressions showed no correlation with conventional prognostic factors, disease free survival and disease related death, statistically. Nevertheless, significant correlations were observed between 2 conventional pathologic prognostic factors (metastatic lymph node status, extracapsular invasion at metastatic lymph node) and disease related death. Besides, metastatic lymph node status, extracapsular extension at metastatic lymph node, and vascular invasion were significantly correlated with recurrence. The last 2 results were not surprising, because the idea indicates lymph node metastases is the main prognostic factor for patients with laryngeal cancer is nowadays already approved by literature.

Some results related to fascin-1 and galectin-3 expression in head and neck cancers reported in literature are summarized in Table 3. As seen in this table, the overall results mentioned for fascin-1 expression is that it is inversely correlated with survival in head and neck cancer.<sup>6,9,11-18</sup> However, the belief about galectin-3 expression is not clear in literature. Several different results have been reported about this marker. Miranda et al.<sup>16</sup>

reported insignificant relation between galectin-3 expression and disease free survival in laryngeal carcinoma, similar to us. The main reason for our statistically non-significant results of fascin-1 can be related to the insufficient number of patients, because it was remarkable that the p value ( $p = 0.076$ / Table 2) indicating the relationship between fascin-1 expression and metastatic lymph node was close to significant value statistically. Table 4 shows the comparison of our study and some of the studies we benchmarked in Table 3. As seen, all of these studies have quite high patient number compared to us. This is because these studies were made in country (China) has approximately 20% of the world's population and increased cancer incidence and mortality. Besides, our study only includes advanced cancer patients most of whom treated with adjuvant radiotherapy after surgery which resulted in a low recurrence rate and high survival rate. For this reason, statistically significant correlation may not be obtained between fascin-1 and disease free survival in our study. When we compared our study with other studies in this respect, we saw that most of studies in the literature including with low number patients did not give any information about adjuvant treatment and included both early and advanced stage patients. All these comments we have made up to now may also apply to galectin-3. Even so, to speak with evidence, of course we need more large series. Nonetheless, we think this study is

valuable, because fascin-1 and galectin-3 have not been evaluated together with any previous studies in laryngeal cancer.

## 5. Conclusion

Although this presented study did not reveal the prognostic value of fascin1 and galectin-3 in laryngeal cancer, there are some different

results in literature. To get rid of this topic, there is a need for multi-centered studies with large series.

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