Research Article

INTERNATIONAL JOURNAL of Indigenous Herbs and Drugs
ISSN 2456-7345

LAMININ-5 Γ2 CHAIN EXPRESSION CORRELATES WITHUNFAVORABLE PROGNOSIS IN SQUAMOUS CELL CARCINOMA OF THE TONGUE

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Article History:

Received on: 13-04-2019
Revised on : 25-06-2020
Accepted on : 29-06-2020

Keywords:

Immunohistochemistry (IHC), Laminin-5 γ2, squamous cell carcinoma of the tongue.

Abstract

Introduction**:** Oral cavity and oropharyngeal squamous cell carcinoma is the eighth most common cancer among men and fourteenth among women in the U.S. according to recent data. Oral and oropharyngeal cancers are the most common malignancies of the head and neck region and are well-known for their high rate of proliferation and nodal metastasis.

Aim of the work: To evaluate Laminin-5 γ2 chain expression as a prognostic marker in squamous cell carcinoma of the tongue in Tobruk-Libya

Patients, Materials, and Methods**:** The study group included 33 selected cases of squamous cell carcinoma of the tongue, diagnosed at the Pathology department of Tobruk Medical Center, Libya, between 2013 and 2019. All patients were surgically treated and underwent complete surgical excision with a safety margin. The selection process was based on the histological criteria for the diagnosis of squamous cell carcinoma of the tongue and was classified into well-differentiated (grade I), moderately differentiated (grade II).

Results: The details of 33 patients selected for analyses are as follows. The mean age of the patients at initial surgery was 65.5 years (range, 48–83 years), and 23 were (69.7%) males and 10 (30.3%) were females.

Conclusion: Laminin-5 γ2 could be used as useful markers to detect the invasiveness of squamous cell carcinoma of the tongue.

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INTRODUCTION

Oral cavity and oropharyngeal squamous cell carcinoma is the eighth most common cancer among men and fourteenth among women in the U.S. according to recent data [1].Oral and oropharyngeal cancers are the most common malignancies of the head and neck region, and are well-known for their high rate of proliferation and nodal metastasis [2,3]. Oral squamous cell carcinoma, the most frequent of all oral cancers, accounts for more than 90% of oral cancers [4].

Since most of the early-stage oral squamous cell carcinomas usually do not cause visible changes in the oral cavity, its early detection is very important for survival [5].

Nowadays there is much interest in novel molecular markers for predicting patient prognosis and

estimating overall survival rate in different cancers [6].

Recent progress in science has highlighted the importance of extracellular matrix (ECM) in regulation of cellular behavior and major developmental processes during cancer progression. Understanding how ECM composition and its deregulation influence the development and progression of diseases may help in an early cancer diagnosis [7].

Laminin is an important constitutional element of the basement membrane. Laminin-5, a member of the laminin family, forms hemidesmosomes with adhesion molecules called integrin [8].

Laminin-5, a heterotrimer consisting of α, β, and γ chains, not only functions in cell adhesion but also plays a role in signal transduction in association with cytokines and growth factors [9].

The laminin-5 γ2 chain is cleaved by MT1-MMP and active MMP-2 [10, 11]. The cleaved γ2 chains bind epidermal growth factor receptors (EGFR) on cancer cell surfaces and transmit intracellular signals that promote cell growth and mobility [12].

The most common methods used for laminin-5 detection and determination are immunohistochemistry and enzymelinked immunosorbent assay (ELISA), respectively [13].

The role of Laminin-5 γ2 chain expression in prognosis of squamous cell carcinoma of the tongue has not been thoroughly studied in Tobruk, Libya population. The aim of this study was to evaluate Laminin-5 γ2 chain expression as a prognostic marker in squamous cell carcinoma of the tongue.

PATIENTS, MATERIALS AND METHODS

The present study is a retrospective study. The study group included 33 selected cases ofsquamous cell carcinoma of the tongue, diagnosed at Pathology department of Tobruk Medical Center, Libya,between 2013 and 2019. All patients were surgicallytreated and underwentcomplete surgical excision with safety margin. The selection processwas based on the histological criteria for diagnosis of squamous cell carcinoma of the tongue and were classified into well differentiated (grade I), moderate differentiated (grade II) and poorly differentiated (grade III). Other clinicopathological data (gender, age, tumor size and lymph node metastasis) were extracted from medicalfiles.

All cases were studied by Laminin-5 γ2immunohistochemical stainingmonoclonal antibodiesexpression.

PROCESSING PROCEDURES

For each case, a representative paraffin-embedded tissue was chosen. The paraffin wax sections were cut at 4 microns and stained by:

a. Hematoxylin and eosin stain for routine histopathological examination.
b. Immunohistochemical staining by Laminin-5 γ2 monoclonal antibodies.

Each case of squamous cell carcinoma of the tonguewas studied for histopathological diagnosis and was classified into well differentiated (grade I), moderate differentiated (grade II) or poorly differentiated (grade III).

Each section obtained from the blocks was placed on positive charge slides, dewaxed in xylene, rehydrated in consecutive descending concentrations of ethanol (100%, 90%, 80%, and 70%), and rinsed in distillated water.

For antigen retrieval, slides were placed in a plastic container filled with sufficient citrate bufferpH 6 and heated in a microwave oven at 100ºC for three successive times, five minutes each. The amount of fluid in the container was checked and was added if necessary to prevent slides from drying out.

The slides were immersed in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase, and incubated with the primary antibody for Laminin-5 γ2 (mouse monoclonal, Novocastra), at 1:100 dilution, overnight, at 40C. Chromogen application by using DAB (3,3-diaminobenzidine tetrahydrochloride). The counterstaining of the sections was done with Mayer's Hematoxylin. Positive and internal negative controls were included for each staining procedure. Internal negative control sections were processed without the addition of primary antibodies. Basement membrane, known to express Laminin-5 γ2, were used as the positive controlin order to verify the accuracy of the technique.

Laminin-5 γ2immunostaining reactions were recognized as homogenous cytoplasmic expression.

IMAGE ANALYZER

Image Analyzer computer system Leica Q win 500 was used for accurately measuring the area and area

% as well as the intensity of reactions of Laminin-5 γ2 monoclonal antibodies.

MEASURING THE AREA PERCENTAGE OF REACTION

It was measured in the form of area and area percent inside a standard measuring frame of size 3105242 μm2 per 5 fields using magnification (X-200) by light microscope transferred to the monitor. Areas were masked by red binary color which could be measured using the computer system. Mean values were obtained for the whole specimens in each group.

MEASURING THE IMMUNOSTAINING INTENSITY (OPTICAL DENSITY)

Regarding the intensity of the reaction within the cells, the optical density was measured after transforming the image into grey mode. Areas with maximum gray were masked by blue binary color and then the intensity of grey was measured.

STATISTICAL ANALYSIS

Statistical analysis of variance (ANOVA) was used in order to explore the significant differences in the staining intensity of the positivity of Laminin-5 γ2 immunoreactions for each case. P-values equal to or less than 0.05 were considered statistically significant.

RESULTS

CLINICOPATHOLOGICAL FEATURES

The details of 33 patients selected for analyses are as follows. The mean age of the patients at initial surgery was 65.5 years (range, 48–83 years), and 23 were (69.7%) males and 10 (30.3%) were females. 18 cases of the tumours were well differentiated squamous cell carcinoma(54.5%); 8 cases were moderate differentiated squamous cell carcinoma(24.2%) and 7 cases were poorly differentiated squamous cell carcinoma (21.3%).Tumor size ranged between 6 and22 mm, with a mean of 14 mm. Lymph nodes metastasis were present in 5 cases (15.2%) and 28 cases (84.8%) shows no lymph nodes metastasis.

LAMIN-5 Γ2 EXPRESSION

[I] AREA PERCENTAGE

The expression of laminin-5 γ2 in well differentiated keratinized squamous cell carcinoma scored the highest levels (37.252 ± 16.870) followed by poorly differentiated squamous cell carcinoma (14.466 ± 3.408) and finally moderately differentiated squamous cell carcinoma (14.462 ± 3.827) as shown in (Table 01).

Table 01: Difference in mean Lamin-5 γ2 area percentage between squamous cell carcinoma grades lesions using ANOVA statistical test.

Squamous cell carcinoma	Area% (Lamin-5 γ2)		ANOVA	
	Range	Mean ± SD	F	P-value
Well differentiated	23.470 - 64.930	37.252 ± 16.870	4.22	0.012
Moderately differentiated	10.040 - 18.390	14.462 ± 3.827		
Poorly differentiated	11.820 - 20.170	14.466 ± 3.408		

[II] OPTICAL DENSITY

The expression of laminin-5 γ2 in poorly differentiated squamous cell carcinoma scored the highest levels (58.368 ± 4.027) followed by moderately differentiated squamous cell carcinoma (44.734 ± 0.386) and finally well differentiated keratinized squamous cell carcinoma (40.952 ± 8.844) as shown in (Table-2).

Table 02: Difference in mean Lamin-5 γ2 optical density between squamous cell carcinoma grades lesions using ANOVA statistical test.

Squamous cell carcinoma	Optical Density (Lamin-5 γ2)		ANOVA	
	Range	Mean ± SD	F	P-value
Well differentiated	25.760 - 47.420	40.952 ± 8.844	72.689	<0.001*
Moderately differentiated	44.370 - 45.330	44.734 ± 0.386		
Poorly differentiated	51.220 - 60.750	58.368 ± 4.027		

***p*-value <0.05 was considered to be statistically significant.**

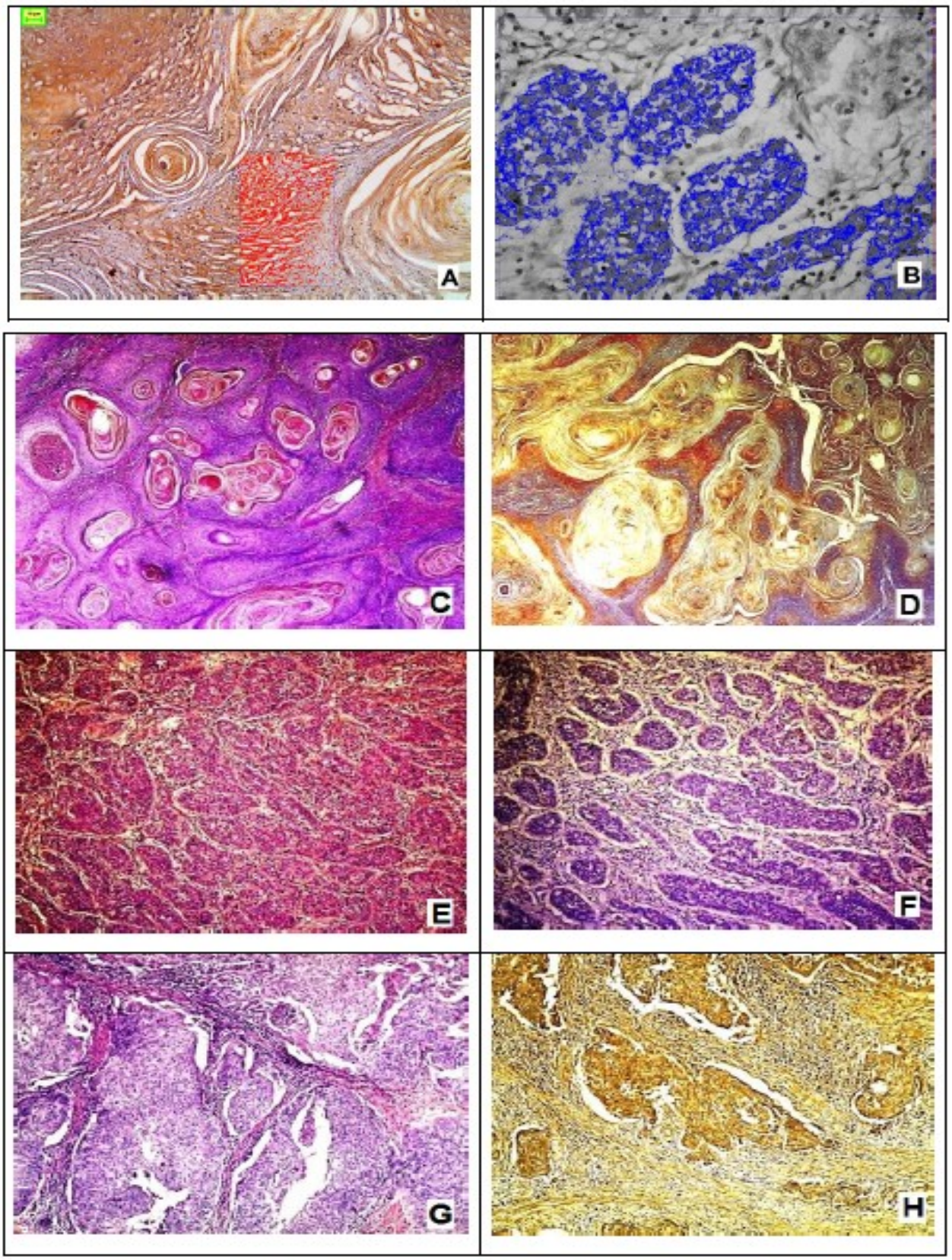

FIGURE 01

A- A copy display seen on the screen of the image analyzer system after masking the areas of positive reaction of squamous cell carcinoma by red binary color showing the way of measurement the area of the reaction.

B- A copy display seen on the screen of the image analyzer system after masking the areas of positive reaction of squamous cell carcinoma by blue binary color to measure the intensity of reaction.

C- Well differentiated keratinized squamous cell carcinoma (H&E, x100).

D- Well differentiated keratinized squamous cell carcinoma incubated with anti Laminin-5 γ2 antibody (Anti Laminin-5 γ2 antibody, X100).

E- Moderately differentiated squamous cell carcinoma (H&E, x100).

F- Moderately differentiated squamous cell carcinoma incubated with anti Laminin-5 γ2 antibody (Anti Laminin-5 γ2 antibody, X100).

G- Poorly differentiated squamous cell carcinoma (H&E, x100).

H- Poorly differentiated squamous cell carcinoma incubated with anti Laminin-5 γ2 antibody (Anti Laminin-5 γ2 antibody, X100).

DISCUSSION

Recent advances in cell biology have elucidated that invasion of cancer cells are accompanied by degradation of components of basement membranes which is not only degraded, but also newly synthesized and deposited at the tumor invasive front [14, 15]. Laminin-5 is one of these components that suggested to be relevant in invasion and metastasis of cervical carcinomas. Some authors suggested that increase expression of Laminin-5 γ2 chain in cancer cells indicates a highly malignant state and predicts a bad prognosis [16, 17]. The purpose of the present study was to demonstrate the immunohistochemical expression of Laminin-5 γ2 among different histological grades of invasive squamous cell carcinoma of the tongue, and to test the hypothesis that Laminin-5 γ2 is a useful marker for invasion and metastasis. The finding of differences in expression pattern of this marker depending on the degree of differentiation, the mode of growth and invasion of cancer cells is similar to the findings reported by Kenji and Shigetaka[18] and Kuratomi et al [19]. These patterns of expression can be classified into peripheral and diffuse patterns of expression. The peripheral pattern was used to describe the positive immunostaining of the cells in the periphery of cell nests and keratin pearls rather than central cells in well differentiated cases, while diffuse pattern was used to describe the positive immunostaining of most of infiltrating malignant cells in poorly differentiated cases according to Kenji and Shigetaka[18] and Kuratomi et al. [19]. These different patterns of expression were also explained by Kuratomi et al. [19] who mentioned that tumor nests showing expansive growth preserve cell–cell adhesiveness and preferentially show a polar differentiation toward cancer pearls. They added that the inner cells of the tumor nests are dormant and controlled with some differentiation mechanisms. These cells conserved some characters of normal squamous epithelium, therefore they are less invasive than the infiltrating cancer cells and only the peripheral cells can divide and degrade the surrounding stroma. On the other hands, cancer cells diminishing cell-cell adhesiveness strongly invaded through the stroma, and almost all of these cells diffusely expressed the Laminin-5 γ2 protein according toKuratomi et al. [19] and Gasparoni et al. [20]. The expression of Laminin-5 γ2 in stromal interface and at the invasive front of tumors agree with Franz et al. [21] and this confirm thatmesenchymal cells are capable of synthesis and deposition of the Laminin-5 γ2 chain in the tumor stroma. The intensity of immunoreactivity increases with decrease in histopathological differentiation agree with Ono et al. [22] who observed that intensity of expression in poorly differentiated squamous cell carcinoma scored the highest levels followed by moderately differentiated and then well differentiated cases. On the other hand Gasparoni et al. [20] found that there was no correlation between histopathological differentiation and intensity of expression. The observed progression of intensity of staining from weak pattern in well differentiated cases to strong pattern in poorly differentiated cases confirms that less differentiated cells were able to synthesize and express Laminin-5 γ2 more than well differentiated cells. The increased intensity of expression in high grade poorly differentiated cases than those with low grade well differentiated cases

agree with Gasparoni et al., [20], Ono et al. [22], Katoh et al. [23] and Nakayama et al. [24] and these findings confirmed the role of Laminin-5 γ2 chain in invasion and metastasis. This role was explained by Koshikawa et al. [11]; where they mentioned the role of matrix metalloproteinases (MMPs) in cleavage of Laminin-5 γ2 leading to the ability of laminin-5 to be deposited and assembled in the extra-cellular matrix. This finding agrees with Katayama et al. [25]who found that γ2 chain that released from cleavage of laminin-5 bind to specific integrins in surface of malignant cells inducing its migration. The findings that the intensity of expression increases with decrease differentiation are in agreement with Koshikawa and Giannelli[26], Lenander et al. [27], Nilsson et al. [28] and Baba et al. [29]; where they reported similar findings in colon, gastric, cervical, anal and oesophageal carcinomas.

LIMITATIONS OF THE STUDY

Our study has some limitations. First, a small sample size was used to identify the value of Laminin-5 γ2 expression in squamous cell carcinoma of the tonguebecause of the short study period. Second, there is no follow-up of the patients because this study is designed and performed recently.

CONCLUSION

From the results of the present study, it can conclude that The degree of histological differentiation of squamous cell carcinoma of the tongue is inversely correlated with the intensity of expression of Laminin-5 γ2. While the area percentage showed direct correlation. Expression of Laminin-5 γ2 in both invading cells and stroma may reflect a dynamic epithelial stromal interaction explaining its crucial role in tumor growth, invasion and metastasis. Laminin-5 γ2 could be used as useful markers to detect invasiveness of squamous cell carcinoma of the tongue. Measuring the intensity of reaction for the used marker is more reliable than measuring the area percentage. This because area percentage showed contradictory findings among the studied cases. A long-term follow-up study will be necessary to identify the clinical value of Laminin-5 γ2expression in squamous cell carcinoma of the tongue.

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