RESEARCH ARTICLE
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Evaluation of LRIG1 Expression in Larynx Pathologies

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ABSTRACT

Objective: Studies have been performed on many biomolecules to determine the prognosis of LSCC and predict the course of the disease. However, a molecular marker that can be used clinically has not yet been found. Therefore, in this study, we aimed to investigate the expression levels of LRIG 1 in laryngeal cancer.
Materials and Methods: In our study, 219 cases who underwent surgery due to LSCC and 88 randomly selected patients whose pathologic result were benign and premalignant lesions in Marmara University Pendik Education and Research Hospital between 2003 and 2018 were analyzed. Patients' data were obtained from the medical records. The tissue microarray method was used to evaluate specimens.
Results: There was a statistically significant difference between the tumor differentiation, diagnosis, and the expression of LRIG1 (respectively p=0.045, p<0.001). Also, an increase in the degree of dysplasia in premalignant lesions correlates with a decrease in LRIG1 expression (p=0.015).
Conclusion: Our findings suggest that LRIG1 plays a role in the early tumorigenesis of LSCC. Therefore, LRIG1 can be a target molecule for treatment approaches. However, LRIG1 was not correlated with overall survival of the LSCC.

Keywords: LRIG1, Laryngeal carcinoma, tumorigenesis

INTRODUCTION

Squamous cell carcinoma of the larynx (LSCC) accounts for 2.8 % of all cancers and is the second most common head and neck malignancy (1). Smoking and alcohol are the main known etiological factors for LSCC (2). In recent years, increasing exposure to toxic substances and smoking in women has reduced the male-to-female ratio to 6 (3). However, the decline in mortality rates parallels the reduction in the incidence of LSCC is not at the desired level; 5-year survival rates have not improved (1, 3). The expectation of an increase in estimated incidence and mortality rates in developing countries, particularly Turkey, indicates the need to develop more aggressive treatment methods. Therefore, elucidating the pathogenesis of LSCC is critical to this process.

3% of proteins in the human proteome consist of immunoglobulin (Ig)-like domain, and 0.9% contain a leucine-rich repeat (LRR) region (4). LRRs are proteins with repeating segments containing 11 aliphatic amino acids, including leucine. This repeating part is found in many proteins and is thought to be involved in interprotein interactions (5). The LRIG family are extracellular integral membrane proteins with 15 LRR and 3 Ig domains, a single-row transmembrane domain, and a cytoplasmic tail region.

The LRIG1 gene is located on the 3rd chromosome (3p14) and is expressed in many tissues (6). Deletion of the 3p14 region

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is frequently observed in various cancers (7). LRIG1 is a single transmembrane protein involved in growth factor signaling, cell proliferation, and tumor suppression mechanisms (7, 8). In addition, several recent studies suggest that the genes, transcripts, and proteins of leucine-rich repeats and immunoglobulin-like domains (LRIG1) have prognostic significance in various cancers including cutaneous squamous cell carcinoma, prostate cancer, glioma, and nasopharyngeal carcinoma (9-12).

Studies have been performed on many biomolecules to determine the prognosis of LSCC and predict the course of the disease. However, a molecular marker that can be used clinically has not yet been found. When we searched the literature, we did not find any study that investigated the effects of gene expression of LRIG 1 on LSSC. Therefore, in this study, we aimed to investigate the expression levels of LRIG 1 in laryngeal cancer.

MATERIALS AND METHODS

This study was approved by the Clinical Research Ethics Committee dated 07/04/2017, number 09.2017.283. In our study, the data of 219 cases who underwent surgery due to LSCC and 88 randomly selected patient whose pathologic result were benign and premalignant lesions in Marmara University Medical Faculty of Medicine Hospital between 2003 and 2018were obtained from the medical records.

The tissue microarray method, which allows the evaluation of more than one tissue at a time, was used. Hematoxylin-eosin (H&E) stained slides were examined, and areas where the tumor was seen were marked with a glass pen. For each patient, 2-3 tumor areas and one lymph node metastasis area were identified. The paraffin blocks of the marked slides were removed, and tissues were harvested from the regions that matched the marked sites using the 3-mm needle of the "quick-ray device." The removed tissues were embedded in 6x5 receiver blocks of the device. The sections obtained from these blocks were stained with H&E, and the accuracy of tissue removal was verified. The presence of tumor tissue was verified by reexamining the cases whose tumors could not be observed after blocking for technical reasons. Cases with small tumor tissue, premalignant and benign, were evaluated by examining sections taken directly from paraffin blocks.

The entire immunohistochemical staining process, including deparaffinization and antigen exposure, was performed with a fully automated immunohistochemistry stainer (Ventana BenchMark Ultra, Ventana Medical Systems, Tucson, AZ). From formalin-fixed, paraffin-embedded tissues, four µm-thick sections were prepared on positively charged slides. The slides were kept in an oven at 70°C for 1 hour. Antigen recovery was performed with ethylenediaminetetraacetic acid (EDTA) at pH:8. Incubation of the LRIG1 antibody at a dilution of 1/100 was performed for 1 hour. Harris Hematoxylin (Ventana Medical Systems) was used for background staining for 16 minutes. The bluing reagent (Ventana Medical Systems) was used for 4 minutes. The sections were completed with the hematoxylin and bluing solution, dehydrated, cleared with xylene, and covered with a coverslip, and the process was complete. The function of LRIG1 antibodies was confirmed by control staining. All slides were evaluated by the same two pathologists who were unaware of the patients' clinical data. The staining intensity after staining was classified into two different groups as follows; 0: not expressed and 1: expressed (Figure 1). If the scores were different in at least two other tissues of the same patient, the highest score was accepted. By evaluating the pathology reports of the cases, the tumor classifications were revised according to the 2017 revised American Joint Committee on Cancer (AJCC) TNM classification.

SPSS 25.0 program was used for statistical analysis, and $p<0.05$ was considered statistically significant.

RESULTS

Between 2003 and 2018, 219 patients had undergone total or partial laryngectomy and neck dissection for laryngeal pathology, and 88 patients who had undergone direct

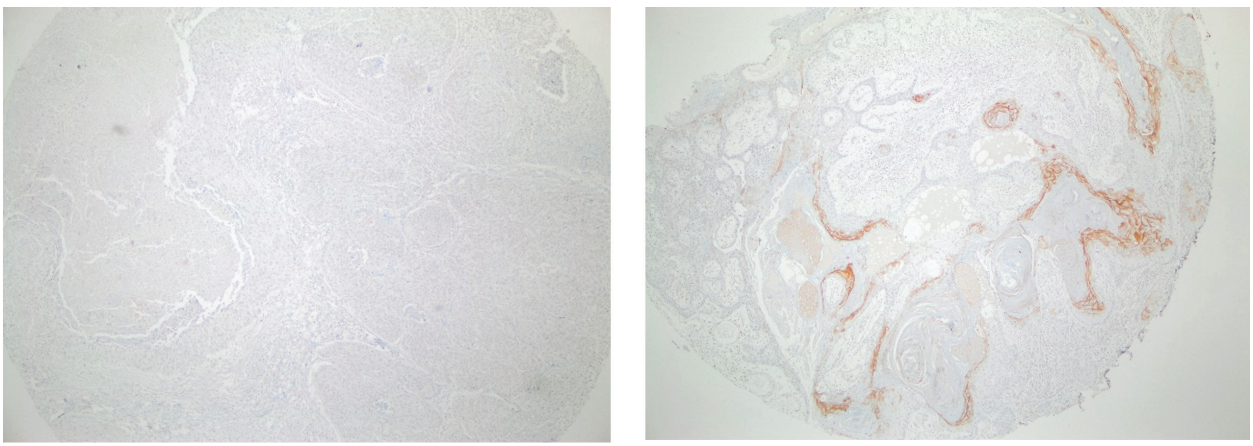

Figure 1: Expression of LRIG1 in tumoral tissue: On the left image, LRIG1 was not expressed, while LRIG1 expression was seen on the right image (X4 magnification)

laryngoscopy in the Department of Otolaryngology, Marmara University were included in the study. Twenty (12%) cases were female, and 283 (92.2%) were male. The mean age of the patients was 59.97±10.697. Of the patients, 258 (84%) had a smoking history, and 47 (15.3%) had an alcohol history. In addition, 91 patients received RT, and 70 patients received CT after primary surgery.

Comparison of LRIG1 expression and clinical and histopathological data

The relationship between the clinical and pathological data from the medical records and the expression of LRIG1 shown in Table 1.

There was a statistically significant difference between the tumor differentiation, diagnosis, and the expression of LRIG1 (respectively p=0.045, p<0.001). While LRIG 1 expression is more expressed in benign pathologies, it is significantly decreased in malignant cases. Furthermore, an increase in the degree of dysplasia in premalignant lesions correlates with a decrease in LRIG1 expression (p=0.015). Also, in the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

LRIG1 expression was not related to age, sex, stage, smoking, alcohol, tumor localization, perineural invasion, lymphovascular invasion, extranodal spread, postoperative CT, and RT requirement (p>0.05).

Survival Analysis

The clinical and histopathologic prognostic parameters affecting the survival of patients in the study group were evaluated using the Kaplan-Meier method and a Cox regression analysis. The survival of cases expressing LRIG1 in tumor tissue was

Table 1: Tumoral clinical and histopathological features according to LRIG1 expression

		Not expressed		Expressed			
		n	%	n	%	X2	p
Premalignant	**CIS**	12	66.7	6	33.3	8.419	0.015*
	HGD	12	54.5	10	45.5		
	LGD	4	21.1	15	78.9		
Diagnosis	**Malign**	161	80.9	38	19.1	27.58	<0.001**
	Premalign	28	47.5	31	52.5		
	Benign	16	59.3	11	40.7		
Stage	**Early**	45	78.6	12	21.1	0.387	0.547
	Advanced	115	82.7	24	17.3		
Differentiation	**Well**	28	68.3	13	31.7	6.207	0.045*
	Moderately	91	84.3	17	15.7		
	Poorly	40	87.0	6	13.0		
Lymph node metastasis	**no**	106	79.1	28	20.9	1.928	0.235
	yes	55	87.3	8	12.7		
Extranodal extension	**no**	28	84.8	5	15.2	0.376	0.710
	yes	27	90.0	3	10.0		
Cartilage invasion	**no**	102	79.2	27	20.8	0.188	0.026*
	yes	57	86.6	9	13.4		
Perineural invasion	**no**	128	84.2	24	15.8	0.720	0.463
	yes	29	78.4	8	21.6		
Lenfovascular invasion	**no**	107	81.7	24	18.3	0.058	0.998
	yes	54	83.1	11	16.9		
Chemotherapy	**no**	108	81.2	25	18.8	0.103	0.846
	yes	54	83.1	11	16.9		
Radiotherapy	**no**	94	81.0	22	19.0	0.116	0.852
	yes	68	82.9	14	17.9		
Smoking	**no**	21	67.7	10	32.3	0.465	0.522
	yes	175	73.5	63	26.5		

*p<0.05, **p<0.01 CIS: Carcinoma-in-situ, HGD: High grade dysplasia, LGD: Low grade dysplasia

significantly better than that of tumor cases lacking LRIG1. While the mean survival time for the LRIG1+ tumors was 2396 days, it was 1505 days in the LRIG1- group (p=0.01) (Figure 2). Disease stage, cartilage invasion, and lymphovascular invasion were also significantly related to LSCC survival in an univariate Cox regression analysis. In contrast, differentiation, perineural invasion, and extranodal spread were not associated with LSCC (Table 2). However, in a multivariate Cox regression analysis, only the stage of disease was associated with LSCC survival (95%CI, 1.055-28.895, p=0.043).

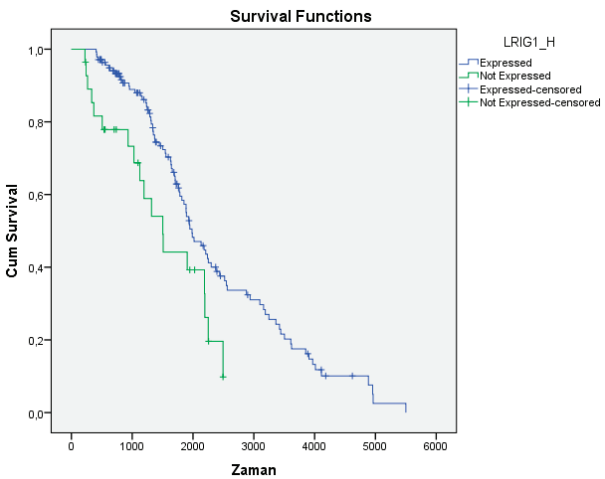

Figure 2: Survival of the patients according to LRIG1 expression

Therefore, LRIG1 is thought to be a tumor suppressor gene. Moreover, the 3p14 locus is deleted in many malignancies, supporting this view (7, 8, 12, 17).

LRIG1 has been shown to regulate contact inhibition in the lung cancer cell line. LRIG1 provides contact inhibition by forming a triple complex with E cadherin and EGFR. Induction of LRIG1 expression in these cells with weak endogenous LRIG1 expression significantly reduced tumor burden. Moreover, the reduced LRIG1 expression in early lesions compared with surrounding tissues suggests it is involved in the early stages of tumorigenesis (18). Also, LRIG1 is expressed in well- and moderately-differentiated tumors, whereas it is either weakly expressed or not expressed in undifferentiated tumors (19). Consistent with the literature, significant expression of LRIG1 was observed in benign pathologies in our study, whereas LRIG1 expression was decreased in premalignant and malignant cases. In addition, it was observed that LRIG expression decreased significantly when the degree of dysplasia increased in premalignant lesions (p=0.015). Also, in the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

As the severity of dysplasia increases in premalignant lesions, the decrease in LRIG1 expression suggests that LRIG1 plays a role in the early stages of LSCC tumorigenesis. In our study, the severity of LRIG1 expression was found to be significantly lower in malignant pathologies than in premalignant and benign pathologies. Since the severity of dysplasia increases in

Table 2: Univariate cox regression analysis

	Hazard ratio	95% Confidential interval	p
Stage	3.598	1.620-7.993	0.001**
Differentiation	1.375	0.731-2.586	0.340
Perineural invasion	1.472	0.869-2.492	0.151
Cartilage invasion	1.910	1.242-2.936	0.003**
Lymphovascular invasion	1.949	1.263-3.008	0.003**
Extra nodal Spread	1.025	0.506-2.075	0.945
LRIG1	1.918	1.697-2270	0.011*

*p<0.05, **p<0.01

DISCUSSION

LRIG1 interacts with many tyrosine kinase receptors and inhibits EGFR, RET, and MET receptor signaling pathways in different ways. Inhibition of these tyrosine kinases regulate cell proliferation. LRIG1 increases EGFR receptor ubiquitination, leading to receptor degradation via ligand-dependent negative feedback and by exhibiting a paracrine effect (13, 14). Dysregulation of EGFR has been shown to play a role in the pathogenesis of many epithelial malignancies. Also, LRIG1 interacts directly with MET receptors and induces lysosomal degradation of the receptors independently of ubiquitination (15). LRIG1 interacts with the RET receptor and prevents binding and activation of the ligand to the RET receptor (16).

premalignant lesions, the decrease in LRIG1 expression suggests that it plays a role in the early stages of LSCC tumorigenesis.

In our study, the presence of cartilage invasion, the intensity of immunostaining of LRIG1 decreases (p=0.026).

Decreased LRIG1 expression has been shown to be a poor prognostic marker for survival in skin cancer, cervical cancer, breast cancer, and bladder cancer (9, 20, 21). In addition, increased LRIG1 expression in oropharyngeal cancer, vaginal cancer, and cervical adenocarcinoma has been shown to indicate a good prognosis and correlate with prolonged survival (12, 22, 23). In our study, the mean survival time of LRIG+ cases were found to be longer in LSCC cases than in LRIG- cases.

However, it was not detected as an independent variable in multivariate Cox regression analyses.

Once antitumor effects were understood, LRIG1 was tested as a target molecule for therapy. LRIG1 gene transfer was performed with viral agents onto tumor tissue generated from 16 bladder cancer cell lines. It was found that tumor burden was significantly lower in cases who underwent LRIG1 gene transfer than in the control group (24). LRIG1 was transferred to implanted glioblastoma cells in a similar study, and patients with LRIG+ had more prolonged survival (25).

Recent studies have suggested that tumor tissues with increased LRIG1 expression are associated with a better response to platinum-based chemotherapeutic agents (26, 27). The demonstration that cancers showing increased LRIG1 expression respond well to platinum-based chemotherapy, which is commonly used for head and neck tumors such as LSCC, indicates that LRIG1 may be a target for new treatment regimens to be developed.

CONCLUSION

Low LRIG1 expression was significantly associated with prolonged survival in our study. Moreover, the expression of LRIG1 decreases as the degree of differentiation decreases. At the same time, it is more strongly expressed in benign cases, while its expression decreases in malignant cases. Our findings suggest that LRIG1 plays a role in the early tumorigenesis of LSCC. Therefore, LRIG1 can be a target molecule for treatment approaches.

Limitations

We used the tissue microarray method to evaluate malignant tissue, therefore the malignant tissue could not be evaluated as a whole. The relationship between the tumor and the surrounding tissue could not be evaluated. The limitations of our study are that it is retrospective and was only assessed at the protein level. Since RT has become more important in the treatment of early stage patients of LC in recent years, the number of early stage cases of LSCC was limited.

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Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee dated 07/04/2017, number 09.2017.283.

Informed Consent: Written informed consent was obtained.

Peer-Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Y.G., A.G., A.C.Y.; Data Acquisition- Y.G., A.G., S.Y.E., Z.L.C., T.M.; Data Analysis/ Interpretation- Y.G., O.A., A.C.Y.; Drafting Manuscript- Y.G., A.G., S.Y.E., T.M.; Critical Revision of Manuscript- Y.G., O.A., A.C.Y., Z.L.C.; Final Approval and Accountability- Y.G., A.G., O.A., A.C.Y., S.Y.E., T.M.

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