Overexpression of GLI1 and PTTG1 as Poor Prognostic Factor of Patients with Laryngeal Squamous Cell Carcinoma

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Abstract

Background: Laryngeal squamous cell carcinoma (LSCC) constitutes about 98% of all laryngeal carcinomas with high mortality rates. The transcription factors Glioma-associated oncogene homolog1 (GLI1) and pituitary tumor transforming gene-1 (PTTG1) were associated with poor prognosis in several carcinomas. To date, no previous studies addressed the role of GLI1 in LSCC and limited studies have examined the role of PTTG1 in LSCC.

Objectives: This study aims to evaluate the immunohistochemical (IHC) expression of GLI1 and PTTG1 in LSCC, to correlate their expression with clinicopathological features and patients' survival, and to assess the correlation between both proteins in LSCC.

Materials and methods: GLI1 and PTTG1 expression was immunohistochemically examined in 60 LSCC specimens and 50 benign vocal fold polyps (control group).

Results: GLI1and PTTG1 expression were significantly higher in LSCC than in the control group (p <0.0001 for each). Their expression was significantly higher in LSCC with larger size (p=0.001, p < 0.0001 respectively), higher grade (p <0.0001 for each), stage (p <0.0001 for each), lymph node metastasis (p<0.0001 for each), and lymphovascular emboli (p=0.003, p=0.004 respectively). High GLI1 and PTTG1 were the significant independent predictors for disease free survival (p=0.005, p=0.048 respectively). While higher tumor stage and higher GLI1 were the only significant independent prognostic factors for overall survival (p=0.049, p=0.041 respectively). Significant strong positive correlation was detected between GlI1 and PTTG1 in LSCC (p <0.0001).

Conclusion: These results suggest that GLI1 and PTTG1 may contribute to LSCC pathogenesis. Targeting both proteins may improve the clinical management in the near future.

Keywords: GLI1; PTTG1; Laryngeal squamous cell carcinoma; Survival.
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Introduction

Laryngeal carcinoma is the 2nd most common cancer among head and neck tumors, with more than 200,000 cases/year diagnosed worldwide **(Nocini et al.,2020)**. LSCC accounts for about 95-98% of laryngeal cancer, with high mortality and morbidity rates **(Ma et al.,2020).** Despite the greatest advances in the clinical management of LSCC, total laryngectomy may be the only therapeutic option in advanced stages **(Mo et al.,2021).** Post-surgical complications such as swallowing dysfunction and loss of speech greatly reduce the quality of life **(Mo et al.,2021)**. Given the limited therapeutic options in late stages and the complex molecular pathogenesis of LSCC that is still not fully understood **(Ma et al.,2020)**, there is an urgent need to discover novel molecules involved in LSCC pathogenesis. These can facilitate early diagnosis and predict patients' prognosis to allow the development of new therapeutic strategies with subsequent improvement of patients' quality of life **(Falco et al.,2022)**.

To date, accumulating evidence has revealed a greater similarity between normal fetal developmental mechanisms and malignant transformation, both of which share similar molecular and signaling pathways **(Cheng et al.,2016)**. The transcription factor Glioma-associated oncogene homolog1; GLI1 is a member of the GLI family of Krüppel-like zinc finger proteins and plays an important role in tissue growth during embryonic development **(Avery et al.,2021)**. However, it is inhibited in mature differentiated tissues to prevent abnormal cellular proliferation **(Avery et al.,2021)**. Aberrant activation and over-expression of GLI1 have been reported in several tumors such as breast **(Wang et al.,2017)**, colonic **(Po et al.,2020)**, and lung carcinoma **(Lei et al.,2022)**. GLI1 activation has been implicated in tumor initiation, progression, and relapse as a result of the up-regulation of certain oncogenes **(Avery et al.,2021)**. However, its prognostic impact and its correlation with survival remain controversial **(Cheng et al.,2016)**. Previous studies demonstrated that inhibition of GLI1 signaling resulted in anticancer activity **(Panneerselvam et al.,2019)**. Thus, it can be a potential therapeutic target in certain tumors **(Panneerselvam et al.,2019)**. To date, the IHC expression of GLI1 in LSCC and its correlation with patients' survival have not been previously investigated.

Securin is a protein encoded by a gene located on chromosome 5 and acts as a regulator of sister chromatid separation during mitosis **(Gong et al.,2022)**. It is also called pituitary tumor transforming gene-1; PTTG1 as it was first described in rat pituitary tumor cells **(Gong et al.,2022)**. It is normally expressed in tissues with proliferative activity such as spleen, testis, and thymus while it is rarely detectable in other mature differentiated cells **(Liu et al.,2022)**. Previous studies postulated that PTTG1 has been suggested to be an oncogene, PTTG1 up-regulation can promote tumor progression and metastasis in bladder **(Li et al.,2022)** and prostatic **(Fraune et al.,2020)** carcinomas while its down-regulation can produce the opposite effect **(Gong et al.,2022)**. So, it could be a promising target in cancer therapy **(Gong et al.,2022)**. However, the exact mechanisms by which PTTG1 contributes to tumor progression are still poorly understood **(Yoon et al.,2012)**. A recent study reported that PTTG1 overexpression resulted in activation of GLI1 in developing brain while PTTG1 silencing had a negative effect on GLI1expression. This interesting finding raises the question

of whether this correlation between PTTG1 and GLI1 can also be detected in carcinomas. To the best of our knowledge, such correlation has not been previously investigated in LSCC. A link between these proteins in LSCC may provide a deep knowledge about new signaling pathways that may be involved in LSCC pathogenesis and may have therapeutic importance.

To better understand the role of PTTG1 and GLI1 in LSCC, this study aims to assess the IHC expression of these proteins in LSCC specimens in comparison with the normal laryngeal tissue; and correlate their expression with the clinicopathological features of LSCC and patients' survival, and then assess the correlation between PTTG1 and GLI1expression in LSCC.

Materials and methods

This study was approved by the Institutional Ethics and Research Committee of Faculty of Medicine, Assiut University, Assiut, Egypt (IRB number: 17300879).

Specimens

One hundred ten (110) formalin-fixed paraffin embedded blocks were included and divided into; 60 LSCC specimens (14 specimens were obtained endoscopically and the remaining 46 specimens were laryngectomy specimens) and 50 benign vocal fold polyps as a control group. The blocks were obtained from the archives of the Surgical Pathology Laboratory, Assiut University Hospital, Faculty of Medicine (in the period between June 2017 and June 2022). The clinical data included patients' age, sex, type of specimen, tumor size, site, staging, lymph node metastasis, and follow-up data were obtained from the hospital medical records at Clinical Oncology Department, Otorhinolaryngology Department, Assiut University Hospital and also from Medical Oncology and Hematological malignancies Department at South Egypt Cancer Institute, Assiut University. Inclusion criteria include: patients with available clinical and follow-up data, and laryngeal specimens diagnosed as squamous cell carcinoma (SCC).

Hematoxylin & eosin (H&E) stained slides of LSCC were reexamined for detailed histopathological features including; histological grade according to the 2017 WHO grading **(Seethala,2017)**, tumor stage according to American Joint Committee on Cancer (AJCC), 8th Edition **(Amin et al.,2017)**, presence or absence of lymphovascular emboli and perineural invasion.

Immunohistochemical staining

Four µm thick sections were cut from the paraffin blocks. 3% H2O2 was applied to block the activity of endogenous peroxidase after deparaffinization and rehydration. Immersing and heating the sections in 10mmol/l citrate buffer (pH 6.0) at 90°C in a microwave for 12 min was performed for antigen retrieval. Then, tissue sections were incubated with the primary antibodies (GLI1 mouse monoclonal antibody (OTI4E2), Catalog number MA5-26638, Invitrogen, ThermoFisher Scientific, USA, dilution 1:200 and Securin (PTTG1) rabbit polyclonal antibody, Catalog number PA5-29399, Invitrogen, ThermoFisher Scientific, USA, dilution 1:300) overnight at room temperature. Secondary staining kits (Thermoscientific Corporation, Fremont, California, USA) were applied according to the manufacturer's instructions. Pancreatic islet cells **(Xu et al.,2010)** and testicular germ cells **(Rehfeld et al.,2006)** were used as positive control for GLI1 and PTTG1 respectively. Negative controls were obtained by

Table 1: Clinicopathological features of the studied groups.

Variables	No.	%	
LSCC (n=60)			
Age (years)			
≤ 60	35	58.3	
> 60	25	41.7	
Sex			
Male	52	86.7	
Female	8	13.3	
Size of tumor **(cm)**			
≤ 2.5	34	56.7	
> 2.5	26	43.3	
Site of tumor			
Supraglottic	22	36.7	
Glottic	32	53.3	
Subglottic	6	10	
Laterality			
Right	28	46.7	
Left	32	53.3	
Histologic grade			
Grade I, well differentiated	14	23.3	
Grade II, moderately differentiated	34	56.7	
Grade III, poorly differentiated	12	20	
T stage			
T1	8	13.3	
T2	30	50	
T3	18	30	
T4	4	6.7	
Lymph node metastasis			
Present	33	55	
Absent	27	45	
Lymphovascular emboli			
Present	37	61.7	
Absent	9	15	
Cannot be determined	14	23.3	
Perineural invasion			
Present	27	45	
Absent	19	31.7	
Cannot be determined	14	23.3	
Follow up data			
Recurrence (relapse)			
Present	20	33.3	
Absent	40	66.7	
Overall survival			
Alive	45	75	
Died	15	25	
Control group (n=50)			***p*** **value#**
Age (years)			
≤ 60	50	100	**<0.0001***
> 60	0	0	
Sex			
Male	35	70	**0.037***
Female	15	30	
Laterality			0.256
Right	29	58	
Left	21	42	

#

p value (LSCC versus control), *Significant (Chi-square test) (*p* <0.05).

processing the sections without adding the primary antibodies.

Immunohistochemical evaluation

Nuclear and/or cytoplasmic staining pattern was considered positive for GLI1 **(Feng et al.,2017; Parrack et al.,2023)** and PTTG1(**Xu et al.,2016; Feng et al.,2017; Teveroni et al.,2021**). The IHC stains of GLI1 and PTTG1 were evaluated using a histological score (H-score). The percentage of positive cells (0-100) was multiplied by the staining intensity (0 (negative), 1 (mild), 2 (moderate), 3 (strong)) to yield final scores of 0-300 **(You et al.,2020; Boruah et al.,2022)**. The IHC expression of both proteins in the tumor cells were independently assessed by two expert pathologists in a blinded manner to ensure the consistency of the results.

Statistical analysis

All analyses were done using statistical software package SPSS version16 (version16, Statistical Package for the Social Sciences; SPSS Inc., Chicago, Illinois, USA). Mann-Whitney test and Kruskalwallis (K-test) and Chi- square tests were used as appropriate. The Spearman Correlation Coefficient was used to assess the correlation between GLI1 and PTTG1 expression in LSCC. Kaplan-Meier survival curves and the log-rank test were used to perform outcome analysis including disease-free survival (DFS) and overall survival (OS). Significant parameters by univariate Kaplan–Meier analysis were further analyzed by multivariate Cox proportional hazards regression models to test for independence. Hazard ratios and 95% confidence intervals (CIs) were calculated. For all tests, *p* value of less than 0.05 was considered as statistically significant.

Results

Clinicopathological features of the studied groups:

The mean age of LSCC patients was 60.48±12.66 years while the mean age of the control group (vocal fold polyps) was 31.68 ± 8. Carcinoma was significantly associated with age more than 60 ($p<0.0001$) and male gender ($p= 0.037$) with no significant association with laterality (0.256). Most carcinomas were moderately differentiated (56.7%), occurred at the left side (53.3%) and in the glottic region (53.3%).The clinicopathological features of the studied cases are summarized in (**Table.1).**

Immunohistochemical results

GLI1 expression: Positive GLI1 expression was detected in tumor cells of 85% (51/60) LSCC specimens while most laryngeal specimens from the control group were negative (39/50;78%) **(Fig.1)**. The mean of GLI1 expression in LSCC (169.33±101.69) was significantly higher than that of the control group (25.60 ± 52.80) ($p<0.0001$) .

PTTG1 expression: Positive PTTG1expression was detected in tumor cells of most LSCC specimens (53/60; 88.3%) while it was expressed in only 7/50 (14%) laryngeal specimens from the control group (**Fig.1**). The mean of PTTG1 expression in LSCC (170.16 ± 98.57) was significantly higher than that of the control group (10.8 ± 30.76) ($p<0.0001$).

Relationship between GLI1 and PTTG1expression and the clinicopathological features of LSCC group

The mean of expression of both GLI1 and PTTG1 proteins was significantly higher in tumors with larger size ($p= 0.001$, $p <0.0001$ respectively), higher grade ($p <0.0001$ for each), higher stage ($p <0.0001$ for each), lymph node metastasis ($p <0.0001$ for each), presence of lymphovascular emboli (p

= 0.003, p = 0.004 respectively), and recurrence (p <0.0001 for each). Also, LSCC specimens from patients who died exhibited a significantly higher mean of GLI1 and PTTG1 than those from patients who are still alive (p <0.0001 for each).

No significant difference was detected in the mean of GLI1and PTTG1 expression regarding; patient age (p= 0.267, p= 0.695 respectively), gender (p= 0.504, p= 0.776 respectively), tumor site (p= 0.076, p= 0.074 respectively), laterality (p= 0.540, p= 0.771 respectively), and perineural invasion (p= 0.166, p= 0.181 respectively) **(Table. 2).**

Relationship between the clinicopathological parameters including GLI1 and PTTG1 expression and LSCC patients' survival

During the follow-up period, 20 (33.3%) of LSCC patients developed recurrence and 15 (25%) of the patients died. The mean DFS period was 37.58 ± 18.65 months, with a range of 7- 60 months. The mean OS period was 43.40 ±1.83 months (range; 14 - 60 months).

For statistical purposes in survival analysis, stages (I & II) were grouped as low stage, and stages (III and IV) were grouped as high stage. Also, histological grades were grouped as follows; low grade (I&II) and high grade (III). Moreover, The expression of GLI1 and PTTG1 was divided into low (≤180) and high expression (>180) according to the median expression value; of 180.

Univariate Kaplan-Meier-survival analysis demonstrated the following:

High GLI1 expression was associated with shorter DFS and OS in comparison to low GLI1 expression (DFS, 31.04 ± 4.16 vs. 58.87 ± 1.56 respectively, p <0.0001; **Fig. 2**), (OS, 43.76 ± 3.24 vs. 60, respectively, p <0.0001; **Fig. 2**). Also, higher PTTG1 expression was associated with shorter DFS and OS in comparison to lower PTTG1 expression (DFS, 32.75 ± 4.24 vs. 57.56 ± 2.05 respectively, p <0.0001; fig. 2), (OS, 44.06 ± 3.21 vs. 60, respectively, p <0.0001; **Fig. 2**).

A decline in DFS and OS was detected in tumors with higher histological grade (III) in comparison to lower histological grade (I &II) (DFS, 27.66 ± 5.73 vs. 50.56 ± 2.8 respectively,p <0.0001; **Fig. 2**), (OS, 40.41± 4.52 vs. 55.2±1.90, respectively, p <0.0001; **Fig. 2**). Also, advanced tumor stage (III &IV) was associated with shorter DFS and OS in comparison to early stage (I &II) (DFS, 31.5 ± 4.74 vs. 54.36 ± 2.58 respectively, p <0.0001; **Fig. 2**), (OS, 41.22 ± 3.64 vs. 59±1.39, respectively, p <0.0001; **Fig. 2**).

In addition, the presence of lymph node metastasis was associated with decreased DFS and OS in comparison to the absence of nodal metastasis (DFS, 36.76 ± 3.99 vs. 57.11 ± 2.41 respectively, p = 0.001; **Fig. 2**), (OS, 46.22 ± 2.93 vs. 60, respectively, p = 0.001; **Fig. 2**).

However, no significant association was found between DFS or OS and the other clinicopathological features examined as age (p = 0.419, p = 0.359 respectively), sex (p = 0.893, p = 0.365 respectively), tumor site (p = 0.094, p = 0.132 respectively) , laterality (p = 0.506, p = 0.675 respectively), tumor size (p = 0.133, p = 0.130 respectively), lymphovascular emboli (p = 0.156, p = 0.135 respectively), and perineural invasion (p = 0.344, p = 0.244 respectively).

After multivariate analysis using Cox proportional hazard model, only higher expression of GLI1 and PTTG1 was the significant independent predictor for DFS and an increased hazard of recurrence with p

values of 0.005 and 0.048 respectively. While higher tumor stage and higher GLI1 expression were the only significant independent prognostic factors for OS in LSCC with *p* values of 0.049 and 0.041 respectively **(Table. 3)**.

Correlation between GLI1 and PTTG1expression in LSCC

A significant strongly positive correlation was found between GLI1 and PTTG1 expression in LSCC (r=0.934, p <0.0001) **(Fig.3)**.

Table 2. Relationship between GLI1 and PTTG1expression with the clinicopathological Features of LSCC group.

Variables	**H score of GLI1 Mean ± SD**	***p* value**	**H score of PTTG1 Mean ± SD**	***p* value**
Age		0.267		0.695
≤ 60	177.42± 102.27		175.71± 100.21	
> 60	158± 101.85		162.40± 97.73	
Sex		0.504		0.776
Male	167.30± 100.31		169.03± 97.44	
female	182.50± 116.71	**0.001***	177.5± 112.47	**<0.0001***
***Size of tumor* (cm)**				
≤ 2.5	128.23± 104.92		129.11±98.88	
> 2.5	223.07±67.63		223.8±68.82	
Site of tumor		0.076		0.074
Supraglottic	202.27±103.32		203.63± 102.05	
Glottic	152.81± 96.19	0.540	155.31± 93.63	0.771
Subglottic	136.66± 109.66		126.66± 89.14	
Laterality		**<0.0001***		**<0.0001***
Right	182.14±89.08		179.28±91.04	
Left	158.12±111.77		162.18±105.51	
Histologic grade				
Grade I	41.42±35.70		53.57±45.67	
Grade II	193.52±75.95		189.11±74.31	
Grade III	250±83.23		252.50±85.29	
T stage				
T1	71.25±80.61	**<0.0001***	65±66.54	**<0.0001***
T2	142.66±91.79		143.33±82.68	
T3	231.66±75.78	**<0.0001***	234.44±76.32	**<0.0001***
T4	285±17.32		292.50±15	
Lymph node metastasis				
Present	228.78±69.94		228.78±66.50	
Absent	96.66±86.46		98.51±83.14	
Lymphovascular emboli		**0.003***		**0.004***
Present	219.45± 76.66		218.10± 75.27	
Absent	107.77± 88.56		115.55 ± 92.07	
Perineural invasion		0.166		0.181
Present	210.74± 87.65		212.59 ± 83.37	
Absent	178.94± 92.36		177.36± 92.30	
Recurrence		**<0.0001***		**<0.0001***
Present	252± 78.98		250.50± 81.46	
Absent	128± 85.73	**<0.0001***	130± 80.63	**<0.0001***
Overall survival				
Alive	136.88± 92.26		136.44±85.63	
Died	266.66±57.40		271.33± 57.30	

*Significant (Mann-Whitney and Kruskal-Wallis Test) (*p* <0.05).

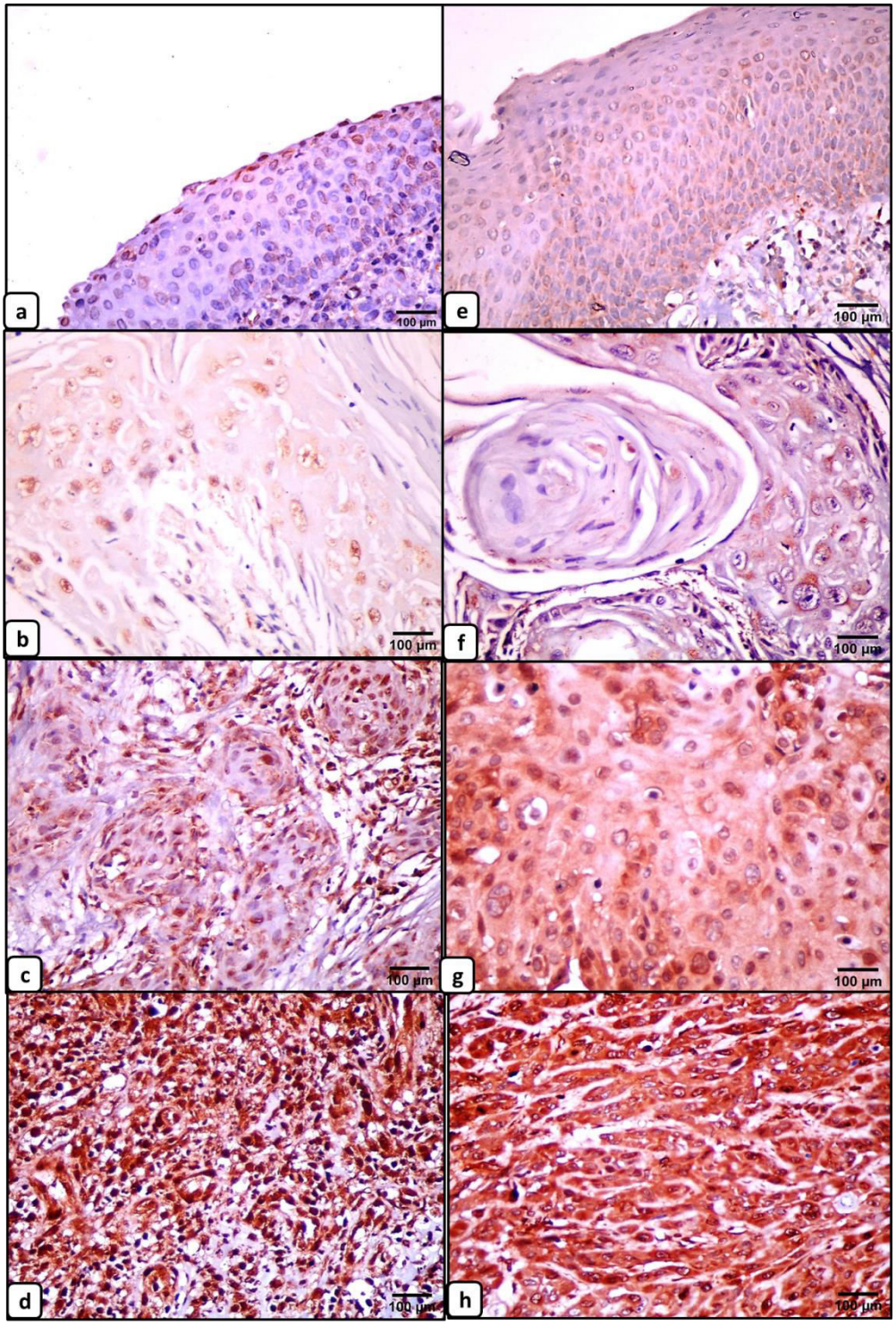

Fig.1. IHC expression of GLI1 and PTTG1. Focal weak GLI1 expression in the surface epithelium of benign vocal fold polyp (a,x400). Weak GLI1 expression in well differentiated LSCC (b,x400). Moderate GLI1 expression in moderately differentiated LSCC (c,x400). Strong GLI1 expression in poorly differentiated LSCC (d,x400). Focal weak PTTG1 expression in the surface epithelium of benign vocal fold polyp (e,x400). Weak PTTG1 expression in well differentiated LSCC (f,x400). Moderate PTTG1 expression in moderately differentiated LSCC (g,x400). Strong PTTG1 expression in poorly differentiated LSCC (h,x400).

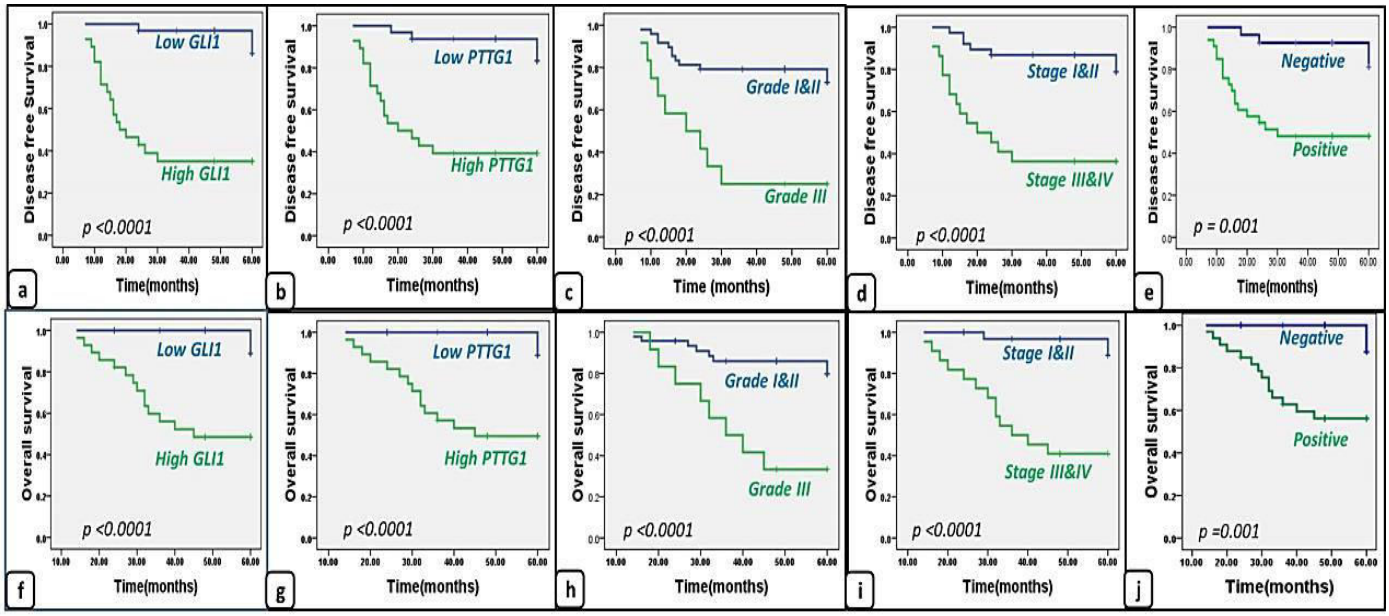

Fig. 2. Kaplan–Meier curves for survival analysis. Kaplan–Meier curves for disease-free survival; according to (a) GLI1 expression, (b) PTTG1 expression, (c) Histological grade, (d) tumor stage, (e) Lymph node metastasis. Kaplan–Meier curves for overall survival according to (f) GLI1 expression, (g) PTTG1 expression, (h) Histological grade, (i) tumor stage, (j) lymph node metastasis.

Table 3. Cox regression analysis of factors affecting DFS and OS in LSCC patients.

Variable analysis	Disease free survival (DFS)			Overall survival (OS)		
	Hazard ratio	95% confidence interval	*p* value	Hazard ratio	95% confidence interval	*p* value
GLI1	9.54	2- 45.4	**0.005***	9.49	1.10 - 81.89	**0.041***
PTTG1	4.02	1.01 - 16	**0.048***	5.56	0.63 - 48.52	0.120
Tumor stage	2.193	0.51 - 9.43	0.291	8.99	1- 80.12	**0.049***
Histologic grade	0.483	0.145 - 1.60	0.234	0.418	0.12- 1.43	0.164
Lymph node metastasis	0.771	0.14-3.58	0.680	0.418	0.06- 17.28	0.964

*significant (Cox regression analysis , p<0.05)

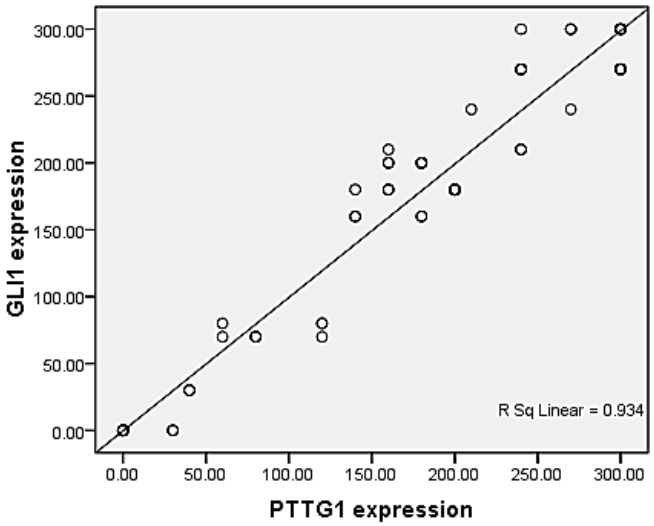

Fig.3. Correlation between GLI1 and PTTG1 expression in LSCC.

Discussion

LSCC is considered one of the most common carcinomas of the head and neck with reduced survival rates in the last two decades even after the current treatment strategies **(Zhou et al.,2022)**. LSCC showed male predominance **(Park et al.,2022)** with a mean age about 61 **(Liu et al.,2022)** which is consistent with the current results.

Investigating the different molecular mechanisms and signaling pathways underlying LSCC development is of significant interest for the discovery of innovative therapeutic options and better clinical management **(Zhou et al.,2022)**. This study aimed to investigate the role of IHCexpression of GLI1 and PTTG1 in LSCC.

GLI1 is a critical transcriptional factor and its activation has been linked to the stimulation of several hallmarks of cancer **(El Zaiat et al.,2023)**. GLI1 transcriptionally activates the target genes implicated in cell cycle progression and thus promotes tumor initiation **(El Zaiat et al.,2023)**. Previous reports have described GLI1 overexpression in SCC of esophagus **(Feng et al.,2017)** skin **(Tanese et al.,2018)** and lung **(Cui et al.,2017)** with limited or absent expression in the adjacent normal tissues. However, no previous studies reported its expression in LSCC. In agreement with previous reports **(Cui et al.,2017; Feng et al.,2017; Tanese et al.,2018)**, GLI1 was expressed in most of the LSCC specimens in the current study, while it was negative in most of the control specimens. This finding may suggest its role in LSCC development and promotion. However, the exact mechanisms regulating the activation and the expression of GLI1 in cancer especially LSCC remain poorly understood **(Wang et al.,2021)**.

The association between GLI1 expression and the clinicopathological features and patients' survival in cancer is controversial and has not been previously investigated in LSCC. Consistent with the current results, previous studies in several carcinomas reported that high GLI1 expression was associated with increased tumor size **(Yao et al.,2019)**, high grade **(Yao et al.,2019; Qi et al.,2020)**, advanced stage **(Cui et al.,2017; Lv et al.,2018; Shao et al.,2018; Yao et al.,2019; Qi et al.,2020)**, presence of nodal metastasis **(Cui et al.,2017; Lv et al.,2018; Shao et al.,2018; Yao et al.,2019)**, vascular emboli **(Yao et al.,2019)**, poor OS **(Jian-Hui et**

al.,2016; Cui et al.,2017; Lv et al.,2018; Shao et al.,2018; Qi et al.,2020) and shorter DFS **(Jian-Hui et al.,2016; Wang et al.,2017)**. Moreover, in agreement with the results of this study, previous reports postulated that GLI1 was a significant independent predictor for OS **(Jian-Hui et al.,2016; Lv et al.,2018; Shao et al.,2018; Qi et al.,2020)** and DFS **(Jian-Hui et al.,2016)**. In contrast, other studies failed to find an association between GLI1 expression and different clinicopathological features such as histological grade, clinical stage, lymph node metastasis, and lympho-vascular invasion **(Wang et al.,2017; Hashimoto et al.,2020)**.

Beside the role of GLI1as a transcription factor that can promote tumor progression and invasion, it can also stimulate tumor metastasis due to its role as a potential cancer stem cell (CSC) marker **(Cui et al.,2017)**. Its expression was closely linked to two CSC markers namely CD44 and LSD1 in lung SCC **(Cui et al.,2017)**. Thus, GLI1-positive cancer cells have the ability for unlimited self-renewal and proliferation with enhanced invasive properties **(Cui et al.,2017)**. In addition, it was found that GLI1 can initiate epithelial-mesenchymal transition (EMT) by increasing the expression of SNAIL1 and decreasing E-cadherin which in turn results in enhancing tumor cell migration, invasion, and resistance to apoptosis **(Lei et al.,2022)**. GANT-61 which is a GLI1 inhibitor has been proven to arrest cell proliferation, induce cell apoptosis, and attenuate EMT and cell migration in breast **(Neelakantan et al.,2017)** and non-small cell lung cancer **(Lei et al.,2022)**. However, whether GANT-61 suppresses the invasion and metastasis of LSCC remains largely unclear and requires further studies.

The IHC expression of PTTG1 in LSCC has not been widely investigated, there is only one previous study which examined PTTG1 expression in LSCC **(Ma et al.,2018)**. In accordance with the present study, **Ma et al.(2018)**, reported higher expression of PTTG1 in LSCC tissues than in the adjacent normal tissues and its expression was significantly higher in poorly differentiated tumors, higher stage, and those with lymph node metastasis. They also suggested that PTTG1 can be used as an early diagnostic marker for LSCC as they reported higher level of PTTG1 in serum of LSCC patients as compared to patients with vocal cord polyps (**Ma et al.,2020**). In addition, the current study revealed that higher PTTG1 was also correlated with larger tumor size, short OS, and DFS and it was a significant independent predictor for DFS. Similar results were described in hepatocellular carcinoma **(Fujii et al.,2006; Lin et al.,2019)** and clear cell renal cell carcinoma **(Gui et al.,2021)**.

Overexpression of PTTG1 in several carcinomas including LSCC with limited expression in normal tissue suggests its role in tumor initiation **(Ma et al.,2018)**. PTTG1 has been considered as an oncogene that promotes cancer development and progression in several ways **(Gong et al.,2022)**. It can produce chromosome instability by binding to separase and inhibiting sister chromosome separation during mitosis **(Gong et al.,2022)**. Also, it can interfere with DNA damage repair resulting in genomic instability by preventing Ku heterodimer formation **(Gong et al.,2022)**. Moreover, it can accelerate cancer metastasis through the up-regulation of matrix metalloproteinases; MMP **(Ma et al.,2018)** and vascular endothelial growth factor; VEGF **(Gong et al.,2022)**. Targeting PTTG1 reduces

the proliferative and invasiveness properties of malignant melanoma cells **(Caporali et al.,2017)**. Thus, It has been suggested to be a potential therapeutic target in several cancers **(Caporali et al.,2017)**.

On the contrary, **Hatcher et al.(2014)** found that PTTG1 loss in mice results in the up-regulation of certain genes involved in mammary gland proliferation as Cyclin D1 and progesterone receptor with subsequent tumor formation. They proposed that PTTG1 may act as a tumor suppressor gene at least in the mammary gland as is required for proper mammary gland morphogenesis in mice **(Hatcher et al.,2014)**. Additionally, they reported reduction of PTTG1 protein level in human breast tumors and this down-regulation was significantly related to tumor grade. This means that PTTG1 might be a "double-edged sword" in cancer which can act as an oncogene in most cancers but also as a tumor suppressor gene in others **(Gong et al.,2022)**.

The current study is the first to assess the correlation between GLI1 and PTTG1 expression in LSCC. A significant strong positive correlation was detected between the two proteins in LSCC specimens. In support, **Feng et al.(2017)** concluded that PTTG1 encouraged EMT and cancer metastasis in esophageal SCC cell lines via the activation of GLI1 by binding to its promoter. Moreover, down-regulation of PTTG1 levels inhibited the expression of GLI1 in vitro **(Feng et al.,2017)**. Thus, we can suggest that PTTG1 may activate GLI1 in the same manner previously described in esophageal SCC. However, the exact mechanism that explains the functional relationship between these proteins in LSCC requires further investigations.

Conclusion

The current results suggested that GLI1and PTTG1 may have a role in the initiation and progression of LSCC. Both GLI1 and PTTG1 are independent predictors for short DFS and recurrence while GLI1 is a significant independent predictor for poor OS. This may allow for the stratification of patients with a high risk for recurrence and poor prognosis. The positive correlation between the two proteins may open the way for a new field for LSCC treatment as targeted agents which act against both GLI1 and PTTG1 may achieve better inhibiting effects and lesser drug resistance than an agent which acts against only one protein. However, this study has some limitations as no molecular studies were performed to clarify the specific molecular interactions between GLI1and PTTG1proteins in LSCC and their possible role as a targeted therapy for LSCC patients. Therefore, further in-depth molecular studies on a larger sample size are still recommended to address the above deficiencies.

Conflict of interest

The authors have declared no conflicts of interest.

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List of abbreviations

CSC: Cancer stem cell.

EMT: Epithelial-mesenchymal transition.

IHC: Immunohistochemical

GLI1: Glioma-associated oncogene homolog1.

LSCC: Laryngeal squamous cell carcinoma.

PTTG1: Pituitary tumor transforming gene-1.

SCC: Squamous cell carcinoma

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