High expression of c-Met and EGFR is associated with poor survival of patients with glottic laryngeal squamous cell carcinoma

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Abstract. The present study was undertaken to explore the association between the expression of hepatocyte growth factor receptor (c-Met) and epidermal growth factor receptor (EGFR) with clinicopathological factors and survival status, to obtain prognostic biomarkers in patients with glottis laryngeal squamous cell carcinoma (GLSCC). The expression status of c-Met and EGFR protein was analyzed in 71 archival laryngeal cancer samples by immunohistochemistry. Statistical methods, including univariate and multivariate Cox regression analysis, were used to determine risk factors of progression. In addition, survival analysis was performed by the Kaplan-Meier method. The present study detected positive expression of c-Met and EGFR in 69.0 and 91.5% of GLSCC samples, respectively. The median disease-free survival (DFS) and overall survival (OS) times of all patients were 42.4 and 81.8 months, respectively, and the 2-year DFS and OS rates were 60.1 and 84.91%, respectively. Univariate Cox regression analysis revealed that patients with high expression of EGFR or c-Met had a predisposition for tumor recurrence. The expression of c-Met expression was significantly associated with that of EGFR (P=0.001). High expression of c-Met or EGFR was associated with shorter DFS and OS times. Findings of the multivariate Cox regression analysis indicated that c-Met-expression may be used as an independent predictor of DFS and OS (P=0.002 and P=0.008, respectively). However, EGFR expression was not an independent predictor for DFS and OS (P=0.352 and P=0.24, respectively). The high expression of c-Met and EGFR was associated with poor survival and are important predictors for prognosis of patients with GLSCC.

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Introduction

Laryngeal squamous cell carcinoma (LSCC) is the second most common type of head and neck squamous cell cancer (HNSCC), representing ~2.4% of all cancer cases and 2.1% of all cancer-associated mortalities worldwide in June 2009 (1). The latest Chinese cancer statistics indicated that an ~26,400 novel cancer cases and 14,500 cancer mortalities occurred in China in 2015 (2). Despite significant progress in surgery, radiotherapy and chemotherapy over the last few decades, there has been no improvement in the 5-year survival status of patients with laryngeal cancer (LC), which has remained steady at 70-80% (3). It was observed that the 3-year disease-free survival (DFS) rate post-surgical intervention was 71.2%, while there was a significant decrease in survival rates for patients with recurrent/metastatic (R/M) LC (4). One of the reasons for the poor survival rates in glottis LSCC (GLSCC) may be due to inadequate tumor profiling using conventional histopathology (4). Thus, it is necessary to elucidate the molecular basis of LC and to detect prognostic biomarkers, which may enable clinicians to improve the management of patients with LSCC.

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein. This receptor binds with different types of ligands, which induce receptor homo- and heterodimerization, leading to intrinsic tyrosine kinase activation, and resulting in cell proliferation, apoptosis, differentiation and survival (5). A total of <90% of patients with HNSCC express high levels of EGFR, which is associated with poor survival (6). EGFR inhibitors, including cetuximab, have been approved by the Food Drug Administration as monotherapy for R/M HNSCC (7). Several studies have explored the association between EGFR and prognosis. However, the role of EGFR overexpression remains controversial in HNSCCs, including LSCC (8-10).

Hepatocyte growth factor (HGF) receptor (c-Met) encodes a transmembrane tyrosine kinase. It is known to stimulate cell motility, dissociation of epithelial sheets, invasion of cellular matrix and induction of angiogenesis (11). This receptor was found to be overexpressed in the majority of types of solid tumors (12).

The incidences of overexpression of c-Met in lung, colorectal cancer and renal cell carcinoma are 13.7, 50.0 and 44.8%, respectively, and are strongly associated with poor prognosis (13-15). Overexpression of c-Met has been observed in 50-80% of HNSCC cases (16-18), with a mutation rate of 14% (19). Studies have demonstrated that overexpression of c-Met is associated with tumor progression, and is an important treatment target of HNSCC (16,20,21). However, the association between c-Met expression and survival status in glottic LSCC (GLSCC) has been rarely studied (22).

Since there is heterogeneity in results when cancer samples from different sites in the head and neck are used (23-26), analysis was restricted to only GLSCC samples. Association of c-Met and EGFR with clinical factors and survival status has not yet been studied in GLSCC. To the best of our knowledge, this is the first study that investigates the role of EGFR and c-Met in GLSCC. The expression of EGFR and c-Met was assessed in patients with GLSCC, and the expression of EGFR and c-Met was compared with clinical parameters and DFS and overall survival (OS) status. The present study found that high expression of c-Met or EGFR were associated with poor survival and are important predictors for prognosis of patients with GLSCC.

Materials and methods

Ethics. The experimental protocol was established, according to the ethical guidelines of the Declaration of Helsinki and was approved by the Human Ethics and Protocol Review Committee of the Third Military Medical University (Chongqing, China). Written informed consent was obtained from individual patients in the study. All patients or guardians, subsequent to reading, filled in and signed the consent form and agreed to be involved in the present study.

Patients. The present study included 71 male patients with a diagnosis of GLSCC, only male patients were included as the incidence of GLSCC tends to be higher in males than females (2). These patients were treated at the Third Affiliated Hospital, Third Military Medical University, Chongqing University (Chongqing, China) between December 2006 and December 2011. The median age was 60 years old (range, 39-79 years). The Tumor staging and grading was determined according to the American Joint Committee on Cancer tumor-node-metastasis classification system of 2002 (27) and histological grade was based on the World Health Organization system (Table I). Primary treatment for all 71 patients was surgery, which included 44 cases with post-operation radiotherapy, 7 cases with post-operation concurrent chemoradiotherapy and 20 patients without any post-surgery treatment. There were 39 recurrent cases. The primary endpoint of the study was DFS, and the secondary endpoint was OS.

Immunohistochemistry (IHC). All tissue samples were fixed in 4% formaldehyde solution at 4˚C for 24 h, dehydrated with 70, 80 and 95% alcohol, each for 5 min, followed by 100% alcohol 3 times for 5 min, embedded in paraffin and cut into 3-μm-thick sections for IHC. Human c-Met polyclonal antibody (catalog no. ZA-0636; Zhong Shan Golden Bridge Biological Technology, Beijing, China) and human EGFR monoclonal antibody (catalog no. ZA-0505; Zhong Shan Golden Bridge Biological Technology) primary antibodies were pre-diluted by the supplier. Immunostaining was achieved by SPlink Detection kit (Biotin-Streptavidin HRP Detection System; catalog no. SP-9001; Zhong Shan Golden Bridge Biological Technology) according to the manufacturer's protocol. Sections were dewaxed in xylene 2 times (5 min each) and rehydrated using a descending alcohol series (100% alcohol 2 times for 3 min each, followed by once with 95, 70 and 50% alcohol for 3 min each). Subsequently, endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in methanol at room temperature for 10 min to block endogenous peroxidase activity. Sections were incubated with the aforementioned primary antibody overnight at 4˚C and later sequentially incubated with biotin-labeled goat anti-rabbit IgG and HRP-conjugated streptavidin, pre-diluted by the supplier (Biotin-Streptavidin HRP Detection Systems; catalog no. SP-9001; Zhong Shan Golden Bridge Biological Technology) at room temperature for 1 h. The peroxidase reaction was visualized using 3,3'-Diaminobenzidine (DAB) substrate solution (0.05% DAB, 0.015% H_2O_2, PBS) for 5 min at room temperature and the sections were counterstained with hematoxylin. Negative controls were assessed by replacing the primary antibody with PBS.

The expression of EGFR and c-Met was evaluated using immunostaining. The slides were examined by two independent pathologists (Department of Pathology, Institute of Surgical Research, Daping Hospital, Third Military Medical University, Chongqing, China), who had no prior knowledge of the clinical and pathological parameters. The intensity of staining was classified into four grades: No staining, -; definite but weak staining, +; moderate staining, ++; and strong staining, +++ (28). This method has been used and validated previously (29). The proportion of positive cells was counted in five microscopic fields at x400 magnification (range, 0-100%). The percentage of cells with different staining intensities was determined by visual assessment with light microscopy. The H-score was calculated using the formula 1x (% of weak staining cells) + 2x (% of moderate staining cells) + 3x (% of strong staining cells; range, 0-300) (30).

Statistical analysis. Statistical analyses were performed using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). The χ^2 test and Fisher's exact test were used to analyze the association between expression of c-Met and EGFR, and clinicopathological factors. Univariate and multivariate Cox proportional hazards regression models were used to find prognostic factors of DFS and OS. The likelihood ratio test was used to determine if one covariate entered into the regression models is significant. Survival analysis was performed by the Kaplan-Meier method, and the log-rank test was used to compare the survival curves. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association between clinicopathological parameters and expression of EGFR and c-Met proteins in patients with

Table I. Association between clinicopathological factors and EGFR and c-Met expression.

		EGFR expression, n			c-Met expression, n		
Factors	Number, n (%)	Low	High	P-value[a]	Low	High	P-value[a]
Age				0.262			0.034[a]
≤50 years	11 (15.5)	7	4		9	2	
>50 years	60 (84.5)	24	36		25	35	
Smoking				0.841			0.634
No	13 (18.3)	6	7		7	6	
Yes	58 (81.7)	25	33		27	31	
Alcohol use				0.144			0.327
No	46 (64.8)	23	23		24	22	
Yes	25 (35.2)	8	17		10	15	
Tstage				0.200			0.060
T1	12 (16.9)	5	7		9	3	
T2	34 (47.9)	11	23		12	22	
T3	18 (25.2)	11	7		8	10	
T4	7 (10.0)	4	3		5	2	
N stage				0.368			1.000
N0	65 (91.5)	27	38		32	33	
N1	5 (7.0)	3	2		2	3	
N2	1 (1.5)	1	0		0	1	
Clinical stage				0.194			0.082
I	10 (14.1)	3	7		7	3	
II	30 (42.2)	10	20		10	20	
III	21 (29.6)	12	9		10	11	
IV	10 (14.1)	6	4		7	3	
Histological grade				0.525			0.213
G1	26 (36.7)	9	17		16	10	
G2	41 (57.7)	20	21		17	24	
G3	4 (5.6)	2	2		1	3	
Recurrence				0.001[a]			<0.001[a]
Yes	39 (55.0)	10	29		9	30	
No	32 (45.0)	21	11		25	7	

[a]P<0.05; EGFR, epidermal growth factor receptor; T stage, tumor stage; N stage, node stage.

GLSCC. Positive expression (either complete membranous staining or cytoplasmic staining near the cell membrane) of c-Met and EGFR was observed in 69.0 (49/71) and 91.5% (65/71) cases, respectively (Fig. 1). The median values of H-score of c-Met and EGFR expression were 160 (range, 0-270) and 240 (range, 0-270), respectively. When the H-score in individual subjects was greater than the median value, it was considered to be indicative of a high expression of c-Met and EGFR.

Table I presents the association between c-Met and EGFR expression and clinicopathological factors. High expression of EGFR or c-Met was significantly associated with tumor recurrence in GLSCC (P=0.001 and P<0.001, respectively). Factors, including old age, advanced T stages and tumor recurrence, were significantly associated with high expression of c-Met (P=0.034, P=0.06 and P<0.001, respectively). High expression of EGFR was strongly associated with only tumor recurrence (P=0.001) and not with other factors. In addition,

Table II. Association between EGFR expression and c-Met expression.

	EGFR, n		
Expression	Low	High	P-value
c-Met			0.001[a]
Low	22	12	
High	9	28	

[a]P<0.05; EGFR, epidermal growth factor receptor.

22 patients showed reduced expression of EGFR and c-Met, while high expression of EGFR and c-Met was detected in 28 patients (Table II). These findings suggested that there was

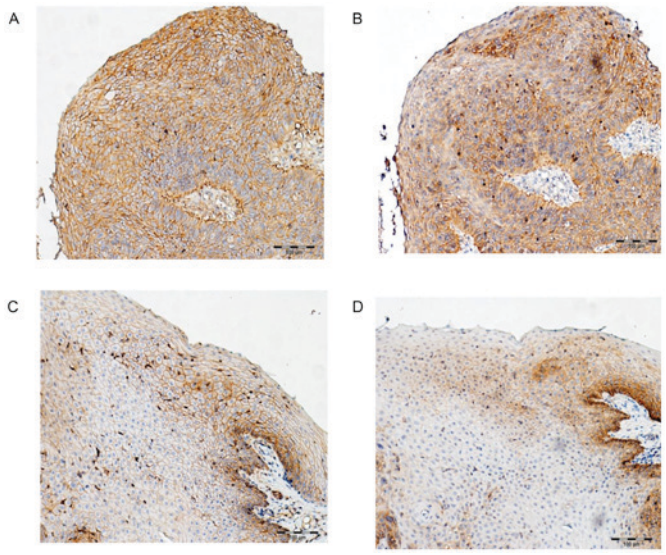

Figure 1. Expression of EGFR and c-Met in membrane and cytoplasm of laryngeal cancer sections; images were captured at x100 magnification. (A) EGFR high-expression with an H-score of 300, and 100% cells staining strongly (+++). (B) c-Met high-expression with an H-score of 270, and 90% cells staining strongly (+++). (C) EGFR low-expression with an H-score of 120, and 60% cells staining moderately (++). (D) c-Met low-expression with an H-score of 80, and 40% cells staining moderately (++). EGFR, epidermal growth factor receptor.

a significant positive association between the expression of EGFR and that of c-Met (P=0.001).

Survival analysis. All patients were followed up until mortality or until December 31, 2014. During the follow-up period, 20 patients succumbed due to GLSCC-associated disease, 6 patients were lost to follow-up. A total of 45 patients remain alive as of the last follow-up. The median follow-up time was 43 months (range, 12-96). The median DFS and OS times of all patients were 42.4 and 81.8 months, respectively. The DFS and OS for 1, 2 and 3-years were 75.9, 60.1 and 54.2%, and 95.22, 84.91 and 77.01%, respectively (Fig. 2A and B). Kaplan-Meier survival analysis for DFS revealed that patients with low levels of EGFR expression had a longer DFS time compared with patients with high expression, and the 2-year DFS rates were 69.5 and 51.7%, respectively, in these two groups of patients (log-rank χ^2=9.708; P=0.002; Fig. 2C). Similarly, the DFS time was longer in patients with low expression of c-Met compared with patients with high expression, and the 2-year DFS rates were 73.5 and 47.3%, respectively (log-rank χ^2=19.526; P<0.001; Fig. 2D). In addition, the 2-year OS rate was 89.8% in patients with low levels of EGFR vs. 79.4% in patients with high expression (log-rank χ^2=7.066; P=0.008; Fig. 2E), and the 2-year OS rate was 94.7% in patients with low level of c-Met vs. 73.1% in patients with high expression (log-rank χ^2=12.805, P<0.001; Fig. 2F).

The patients were categorized into three subgroups: A low-risk group, comprising of patients with low EGFR and c-Met expression; a high-risk group, comprising patients with high EGFR and c-Met expression; and a moderate-risk group, comprised of patients with only one highly-expressed protein (EGFR or c-Met). The 2-year DFS rates in low-, moderate- and high-risk groups were 81.8, 51.0 and 47.8%, respectively

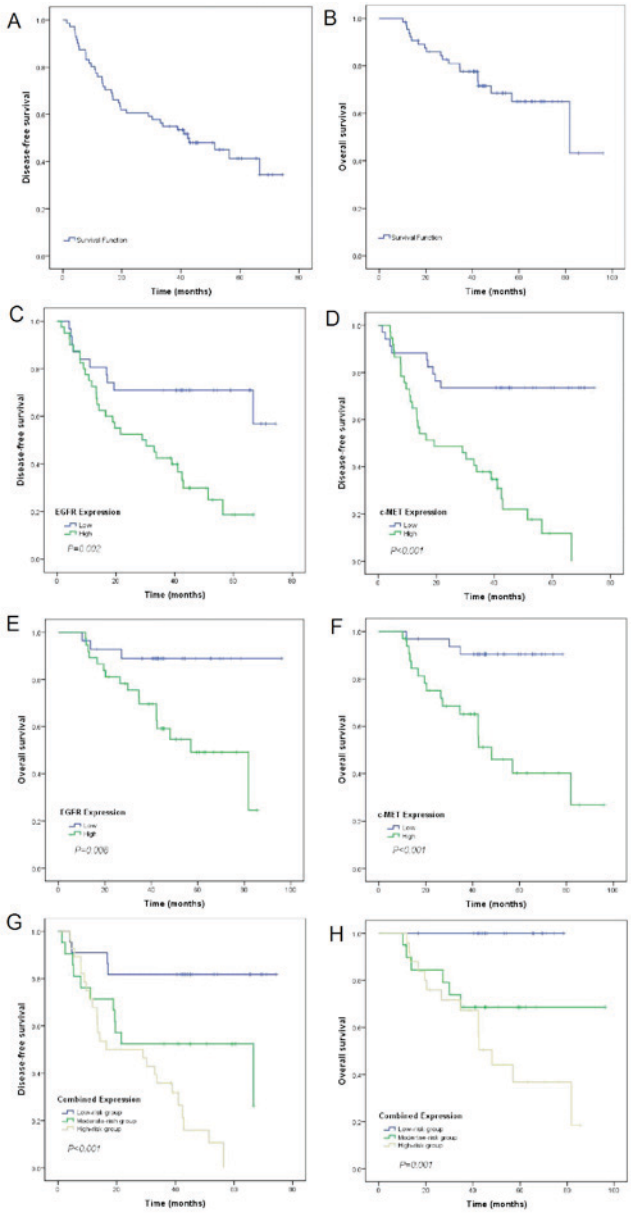

Figure 2. KM curves demonstrated different prognosis between subgroups. (A) Survival analysis of whole population for DFS, (B) Survival analysis of whole population for OS, (C) KM curves of DFS for EGFR low and high expression subgroups, (D) KM curves of FDS for c-MET low and high expression subgroups, (E) KM curves of OS for EGFR low and high expression subgroups, (F) KM curves of OS for c-MET low and high expression subgroups, (G) KM curves of DFS for low, moderate and high risk subgroups, (H) KM curves of OS for low, moderate and high risk subgroups. EGFR, epidermal growth factor receptor; DFS, disease-free survival; OS, overall survival; KM, Kaplan-Meier.

(log-rank, χ^2=20.214; P<0.001; Fig. 2G). The 2-year OS rates in low-, moderate- and high-risk groups were 100.0, 80.4 and 73.5%, respectively (log-rank, χ^2=13.570; P=0.001; Fig. 2H).

Univariate and multivariable analysis of prognostic factors for DFS and OS. Univariate Cox regression analysis showed a significant association of expression of EGFR- and c-Met- with DFS and OS (Tables III and IV). The risk of disease progression was significantly increased in patients with high expression of c-Met or EGFR when compared with those with low expression (HR=4.785, P<0.001; HR=3.028,

Table III. Univariate/multivariable analyses of prognosis factors for disease-free survival.

	Univariate			Multivariable		
Factors	95%CI	HR	P-value[a]	95%CI	HR	P-value
Age (≤50 vs. >50)	0.911-9.664	2.967	0.071	-	-	-
Smoking (no vs. yes)	0.343-1.528	0.724	0.396	-	-	-
Drinking (no vs. yes)	0.633-2.091	1.151	0.645	-	-	-
T stage (T1/2 vs. T3/4)	0.480-1.795	0.929	0.826	-	-	-
N stage (N1/2 vs. N0)	1.251-8.426	3.246	0.016[a]	0.993-19.773	4.432	0.051
Clinical stage (I/II vs. III/IV)	0.572-2.041	1.080	0.812	-	-	-
Histological grade (G1 vs. G2/3)	0.357-1.269	0.674	0.222	0.172-0.818	0.376	0.014
EGFR expression(high vs. low)	1.458-6.286	3.028	0.003[a]	0.631-3.650	1.518	0.352
c-Met expression(high vs. low)	2.239-10.225	4.785	<0.001[a]	1.695-9.678	4.050	0.002

[a]P<0.05; CI, confidence interval; HR, hazard ratio; EGFR, epidermal growth factor receptor; T stage, tumor stage; N stage, node stage.

Table IV. Univariate/multivariable analyses of prognosis factors for overall survival.

	Univariate			Multivariable		
Factors	95%CI	HR	P-value[a]	95%CI	HR	P-value
Age (≤50 vs. >50 years)	0.417-7.838	1.809	0.428	-	-	-
Smoking (no vs. yes)	0.159-1.029	0.405	0.058	0.163-1.654	0.520	0.268
Drinking (no vs. yes)	0.351-2.188	0.876	0.777	-	-	-
T stage (T1/2 vs. T3/4)	0.554-3.322	1.357	0.504	-	-	-
N stage (N1/2 vs. N0)	0.602-11.572	2.640	0.198	-	-	-
Clinical stage (I/II vs. III/IV)	0.784-4.756	1.895	0.156	-	-	-
Histological grade (G1 vs. G2/3)	0.288-1.682	0.697	0.421	-	-	-
EGFR expression (high vs. low)	1.333-15.562	4.554	0.016[a]	0.538-11.909	2.532	0.240
c-Met expression (high vs. low)	2.026-24.026	6.976	0.002	1.780-45.921	9.040	0.008

[a]P<0.05; CI, confidence interval; HR, hazard ratio; EGFR, epidermal growth factor receptor; T stage, tumor stage; N stage, node stage.

P=0.003, respectively). Additionally, the risk of mortality was significantly increased in patients with high expression of c-Met or EGFR when compared with those with low expression (HR=6.976, P=0.002; HR=4.554, P=0.016, respectively). Among all the other factors, only lymph node metastasis increased the risk of disease recurrence (HR=3.246, P=0.016).

The multivariate Cox proportional hazards model analysis revealed that c-Met expression is an independent prognostic factor for DFS and OS (HR=4.050, P=0.002; HR=9.040, P=0.008, respectively; Tables III and IV). By contrast, EGFR was not observed to be an independent prognostic factor for DFS and OS (P=0.352 and P=0.240, respectively). Histological grade was an independent prognostic factor for DFS and N stage had borderline significance (HR=0.376, P=0.014; HR=4.432, P=0.051, respectively; Table III).

Unlike results of c-Met, the assessment of the combined expression of c-Met and EGFR did not further improve prognostic capacity of c-Met for DFS and OS (likelihood ratio test, P=0.119 and P=0.160, respectively; Table V). These findings demonstrated that combined assessment of EGFR and c-Met expression did not have any more prognostic value superimposed effect in prognostic assessment.

Discussion

Tumor recurrence or metastasis following surgery is one of the main factors affecting prognosis (31). The known biomarkers of HNSCC, EGFR, cyclin D1, B-cell lymphoma 2, cyclin-dependent kinase inhibitor p27, vascular endothelial growth factor and p53 (32), are limited in their ability to predict prognosis, mainly due to the heterogeneity of cancers at different head and neck sites (32,33). This is evident from the inconsistent studies on the association of EGFR and c-Met with prognosis of HNSCC (Table VI). The present study indicated that high expression of c-Met and EGFR significantly reduced DFS and OS. These findings indicated that abnormal expression of c-Met or EGFR proteins serve as potential biological markers

Table V. Compared role of c-Met and combined assessment with both c-Met as well as EGFR for prognosis.

	HR (95% CI)		-2 Log likelihood		χ^2		P-value	
Expression	DFS	OS	DFS	OS	DFS	OS	DFS	OS
c-Met	4.785 (2.239-10.225)	6.976 (2.026-24.026)	277.754	135.494	2.426	1.978	0.119	0.160
Combination								
c-Met	3.816 (1.695-8.592)	4.941 (1.330-18.360)	275.328	133.516				
EGFR	1.832 (0.832-4.032)	2.372 (0.644-8.740)						

EGFR, epidermal growth factor receptor; HR, hazard ratio; CI, confidence interval; DFS, disease-free survival; OS, overall survival.

for GLSCC, and may have an improved predictive value when compared with clinicopathological factors.

EGFR is a member of the ErbB family, which promotes cell proliferation, invasion, metastasis and survival (5). Similar to findings from a previous study (34), the present study also revealed that >90% of patients with GLSCC expressed EGFR protein. However, EGFR was not demonstrated to be an independent prognostic factor of DFS and OS. The present findings are in contrast to previous studies, which have observed that EGFR overexpression, increases risk of recurrence and mortality in patients with LSCC (35-37). The reason for such discrepancies in findings may be attributed to small sample size (38), difference in scoring methods and studies conducted on HNSCC rather than only GLSCC samples. Additionally, the present study was a retrospective one. Furthermore, a meta-analysis revealed that EGFR is most appropriate as an independent predictor of DFS in oropharyngeal carcinoma and not in laryngeal cancer (33). This indicated that EGFR may not be a suitable prognostic marker for all types of head and neck cancers. However, considering the aforementioned study limitations, it may be prudent to study this in a larger sample size in a prospective study.

c-Met, with a molecular weight of 190 kDa, consists of an extracellular α-chain and transmembrane β-chain with tyrosine kinase activity (39). It has been observed that alterations of the c-Met gene in the form of amplification, deletion, mutation and overexpression are associated with tumor cell proliferation, migration, invasion and angiogenesis (40). Alterations of c-Met has been revealed to be associated with poor prognosis of numerous tumors, including breast, colorectal, liver, lung cancer and HNSCC (20,41). However, such an association has not been studied specifically in GLSCC. The present study demonstrated an association between high expression of c-Met and recurrence and mortality of patients with GLSCC. It was also observed that high expression of c-Met is frequent in GLSCC, and was directly associated with the relapse, age and T-stage, which are factors linked with poor prognosis (42). Previous studies have revealed associations between c-Met expression and lymph node metastasis (16,43-45). Since the present study consisted of very few patients (n=6) with nodal involvement, such an association could not be detected. It was observed that c-Met was an independent predictor of DFS and OS, which suggested that IHC evaluation of c-Met in primary tumors may contribute to identifying those patients with relapse and reduced chances of survival. Therefore, in the future, increased c-Met expression in patients with LSCC should be considered indicative of the requirement for good treatment modalities with consistent follow-up. In addition, univariate and multivariate analysis indicated that high expression of c-Met protein was significantly associated with a poorer prognosis when compared with that of EGFR protein. Thus, c-Met may perform a crucial function in the prognosis of LSCC; however, analysis in large numbers of LSCC samples is required.

c-Met and EGFR are frequently co-expressed in tumors, and act in synchrony to activate downstream signaling pathways, including Ras-Raf-extracellular signal-regulated kinase, signal transducer and activator of transcription 3 and phosphoinositide 3-kinase/Akt-mechanistic target of rapamycin cascades, to promote tumor progression (46). c-Met may be activated following EGFR activation, in the absence of HGF (47). c-Met activation by HGF was also shown to confer resistance to irreversible EGFR inhibitors (48,49). The possible reason for this may be that the downstream signaling pathways maybe activated by c-Met, which is independent of EGFRs, leading to EGFR inhibitor-resistance (49). Benedettini *et al* (50) also demonstrated that lung cancer cells with low response to EGFR inhibitors, including gefitinib and erlotinib, exhibited high levels of c-Met. c-Met inhibitors may be used to circumvent the problem of drug-resistance to EGFR therapies (51). Thus, combined therapy with c-Met as well as EGFR inhibitors may improve the control of tumor cell proliferation (51). Based on these findings, the association between c-Met and EGFR was analyzed, and it was revealed that there was a significant positive association between c-Met and EGFR expression. In addition, subgroup analysis revealed that the DFS and OS times were extended within the subgroups of low EGFR and c-Met expression compared with at least one highly-expressed protein. However, combination of c-MET and EGFR did not provide more prognostic information, compared with c-MET alone for DFS and OS. This may be due to the fact that EGFR was not an independent prognostic factor of DFS and OS. Therefore, additional studies with a larger sample size are required to investigate the combined role of EGFR and c-Met.

To conclude, c-Met and EGFR are important predictors of survival in patients with GLSCC. Therefore, IHC analysis of primary tumors with the biological marker c-Met may provide greater potential to identify the prognosis. However, evaluation

Table VI. Studies on c-Met-and EGFR-expression in HNSCC.

Gene	Author, year	Tumor sites	Method	Positive expression, n	Association between c-Met/EGFR expression and clinicopathological factors and survival	(Refs. no.)
EGFR	Almadori *et al* (2010)	Larynx	IHC	23/67	Metastases-free survival (P=0.0001); OS (P=0.0002)	(8)
	Kontic *et al* (2015)	Larynx	IHC	127/185	Histopathological grade (P<0.001); stage (P<0.001); metastasis (P<0.001); relapse (P<0.001); survival (P<0.001).	(9)
	Young *et al* (2011)	Multiple	IHC	81/93	Failure-free survival (P=0.35); OS (P=0.22)	(10)
	Carballeira *et al* (2014)	Lip	IHC	50/55	Tumor ulceration (P=0.022); tumor thickness (P=0.002); tumor width (P=0.021).	(17)
	Wei *et al* (2008)	Larynx	IHC	35/40	There was a good agreement between the primary tumors and the paired metastases regarding EGFR expression (P<0.05)	(34)
	Ma *et al* (2014)	Multiple	IHC	30/43	HPV infection (P=0.009); 3-year OS (P=0.037)	(36)
	Cao *et al* (2013)	Nasopharynx	IHC	102/127	Primary lesion stage (P=0.001); clinical stage (P=0.002); relapse (P=0.015); DFS (P=0.013); OS (P= 0.015).	(37)
	Won *et al* (2012)	Oropharynx/ Oral cavity	IHC	78/121	EGFR expression was higher in oral cavity cancers compared with oropharyngeal cancers (P=0.005)	(51)
	Jiang *et al* (2009)	Larynx	IHC	31/75	DFS (P=0.199); OS (P=0.293).	(52)
c-Met	Choe *et al* (2012)	Multiple	IHC	34/82	Lymph node metastasis (P<0.05); primary location of the tumor (P<0.05)	(16)
	Baschnagel *et al* (2014)	Multiple	IHC	100/107	Locoregional control (P=0.031); distant metastasis (P=0.005); DFS (P<0.001); OS (P<0.001)	(18)
	Kim *et al* (2010)	Oral	IHC	33/61	Lymph node metastasis (P=0.005), tumor classification (P=0.004); recurrence (P=0.018); survival (P=0.003)	(20)
	Zhang *et al* (2014)	Larynx	IHC	33/52	TNM stage (P<0.05); lymph node metastasis (P<0.05)	(42)
	Luan *et al* (2014)	Nasopharynx	IHC	74/106	TNM stage (P<0.01); cervical lymph node metastasis (P<0.05)	(43)
	Lim *et al* (2012)	Oral tongue	IHC	39/71	Neck metastasis (P<0.05); >4 mm depth of tumor invasion (P<0.05); survival rates (P<0.05)	(44)
	Zhao *et al* (2011)	Oral	IHC	44/86	DFS (P=0.010); OS (P=0.010)	(53)
	Kim *et al* (2006)	Hypopharynx	IHC	28/40	Lymph node metastasis (P<0.05)	(54)

IHC, immunohistochemistry; DFS, disease-free survival; OS, overall survival; EGFR, epidermal growth factor receptor; TNM, tumor-node-metastasis.

of c-Met and EGFR expression status should be performed on a larger sample population to obtain more reliable and consistent results. A more accurate prediction of outcomes with specific therapies, particularly molecular-targeted therapies, remains a worthy area of investigation.

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