Prognostic value of glioma-associated oncogene homolog 1 and SET domain-containing protein 2 immunohistochemical scores in locally advanced cervical cancer

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Abstract. The prognosis of patients with locally advanced cervical cancer (LACC) is often poor due to high treatment resistance. The present study aimed to investigate the association between the levels and localization of SET domain-containing protein 2 (SETD2) and glioma-associated oncogene homolog 1 (GLI1) proteins, which are implicated in CC, and their association with clinical outcomes in patients with LACC. In total, 84 patients with LACC diagnosed at Instituto Nacional de Cancerología (México City, México) between January 2016 and December 2018 were analyzed. Immunohistochemical staining was performed to evaluate the expression and localization of SETD2 and GLI1 proteins and immunoreactivity score (IS) was calculated. The association between IS and protein localization and clinicopathological factors and clinical outcomes was examined using the log-rank test and Cox regression model to investigate the disease-free survival (DFS) and overall survival (OS). The median age of the cohort was 46 years (range, 25-81 years). Analysis of SETD2 and IS values according to the sociodemographic and histopathological characteristics of the patients demonstrated that increased nuclear GLI1 (nGLI1) levels were significantly associated with a history of hormonal contraceptive use (P=0.029). Patients with nGLI1 IS >6 exhibited significantly worse OS (P=0.004) and DFS rates (P=0.013) compared with those with nGLI1 (IS <6). The univariate analysis revealed that lower OS was associated with nGLI IS >6 [hazard ratio (HR), 1.50; 95% CI, 1.44-14.13; P=0.010] and ncGLI1 IS <6 (HR, 1.86; 95% CI, 1.42-29.5; P=0.016), whereas a lower probability of DFS was significantly correlated with nGLI IS >6 (HR, 1.53; 95% CI, 1.23-17.49; P=0.023). The present study results demonstrated the utility of IS in evaluating the prognostic impact of SETD2 and GLI1 expression in patients with LACC.

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Introduction

Cervical cancer (CC) is the fourth most common cancer in female patients worldwide, with an estimated incidence of 662,301 novel cases and 348,874 deaths in 2022 (1,2). It has been reported that ~90% of CC deaths occur in low- and middle-income countries and are primarily associated with delayed diagnosis and failure to deliver guideline-concordant treatment in the advanced stages of CC (3).

According to the International Federation of Gynecology and Obstetrics classification system, the treatment of invasive CC depends on clinical evaluation and histopathological characteristics of tumors (4). However, some patients do not respond to the treatment chosen based on these criteria, highlighting the need to find predictive biomarkers to lower the risk of disease recurrence or metastasis (5). Furthermore, the identification of genetic and epigenetic alterations associated

with therapy resistance may increase survival in patients with locally advanced (LA)CC (5).

SET domain-containing protein 2 (SETD2) is a tumor suppressor associated with the trimethylation of lysine 36 on histone 3 (H3K36me3), a histone mark primarily associated with actively transcribed regions. Loss of function of SETD2 due to deletion or mutation is frequently detected in hematological and solid tumors, such as renal, lung, colon and breast cancer and CC (6). Furthermore, SETD2 mutations are associated with acquired chemoradiotherapy (CRT) resistance and poor survival due to alterations in mechanisms associated with DNA damage repair and tumor heterogeneity mediated by chromatin instability (7).

The increased risk of CRT resistance associated with the loss of SETD2 is associated with the increased activity of the hedgehog (Hh) and Wnt/β-catenin pathways in patients with brain cancer and osteosarcoma, respectively (7,8). Additionally, abnormal Hh signaling is detected in multiple human malignant tumors (9). In CC, alterations in Hh signaling are frequently associated with an increased risk of developing CRT, which may be associated with tumor hypoxia (10-13). Furthermore, increased expression of the glioma-associated oncogene homolog 1 (GLI1) protein, a transcriptional activator of the Hh pathway, is associated with chemoresistance in gynecological cancer (14). To the best of our knowledge, however, the relationship between SETD2 and GLI1 protein levels has not been investigated and their potential role as prognostic markers in patients with LACC remains unknown.

The present study explored the relationship between SETD2 and GLI1 based on their localization and protein levels using immunohistochemical assays. In addition, the immunoreactivity scores (IS) of SETD2 and GLI1, as well as the pathological characteristics of tumors and survival indicators, were evaluated to determine their potential utility as prognostic biomarkers in patients with LACC.

Materials and methods

Patients and sample collection. The present retrospective study included 84 patients, aged 25-81 years, with histological results of squamous cell cervical carcinoma (SCC), adenocarcinoma (ADC) and adenosquamous cell carcinoma (ASC) who received treatment between January 2016 and December 2018 at the National Cancer Institute (México City, México). The inclusion criteria were as follows: i) Patients with a diagnosis of LACC [stage IB1-IVA; International Federation of Gynecology and Obstetrics (FIGO) 2009] (15); ii) patients who underwent biopsy; iii) patients who did not receive treatment before biopsy (chemotherapy, radiotherapy, immunotherapy or hormonal therapy) and iv) patients who received concurrent treatment with cisplatin 40 mg/m^2 weekly with external beam radiation therapy of 45 Gy. Patients with incomplete treatment schemes or other types of primary cancer were excluded from the present study.

Treatment response was evaluated according to the Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1) (16). Patients with complete and partial responses were defined as responders, whereas those with stable or progressive disease were designated as non-responders.

Sociodemographic and clinicopathological data were collected from the medical files. Smoking was defined as consumption of ≥2 cigarettes/week for ≥1 year at any point in their lifetime; alcohol consumption was defined as alcohol intake >1 drink/day on average.

Immunohistochemistry (IHC). IHC staining was performed using formalin-fixed tissue as described previously (17). Formalin-fixed paraffin-embedded tumor blocks were obtained from the Institutional Pathology Tissue Bank between January 2024 and March 2024. Polyclonal antibodies directed at the C-terminus of SETD2 (cat. no. HPA042451; 1:50; Millipore Sigma) and GLI-1/GLI1 (cat no. C1; 1:50; Santa Cruz Biotechnology, Inc.) were used for the IHC assays. The conditions and antibody concentrations were previously validated in colon adenocarcinoma tissue for GLI1 and in the human small intestine for the SETD2 protein.

Two observers specializing in gynecological oncology at the National Institute of Cancer independently evaluated and scored immunoreactivity in a blinded manner. SETD2 and GLI1 expression were analyzed according to a previously described IS (18). The percentage of tumor-positive cells was graded as follows: 0, <5%; 1, 6-25%; 2, 26-50%; 3, 51-75% and 4, 76-100%. Positive cells (5%) were used as the cut-off to define negative tumors. The intensity of the immunoreactivity was scored as follows: 1, weak; 2, moderate and 3, strong. The percentage of positive cells and intensity values were multiplied to obtain the IS. Localization was categorized as membranous, cytoplasmic, nuclear or a combination.

DNA extraction and human papillomavirus (HPV) genotyping. Genomic DNA was extracted from the tumor tissue using the QIAamp DNA FFPE tissue kit (cat. no. 56404, Qiagen GmbH) according to the manufacturer's instructions. The integrity of extracted DNA was evaluated by PCR amplification of the β-globin gene using specific primers (forward: 5'GAAGAG CCAAGGACAGGTAC3'; reverse: 5'CAACTTCATCCACGT TCACC3' as described previously (19). Primers were synthesized at Integrated DNA Technologies (San Diego, Ca, USA).

HPV sequences were detected and typed using the E6 nested multiplex PCR protocol (Table SI) (20). PCR products were analyzed by electrophoresis on 2% agarose gels stained with GelRed (Biotium, Inc.) and visualized using the iBright FL1500 documentation system (Thermo Fisher Scientific, Inc.). The HPV type was determined by assessing the size of the amplified fragments. DNA samples from HPV-positive HeLa and CaSki cell lines (American Type Culture Collection), cultured in DMEM-F12 medium (GIBCO-BRL) with 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37˚C in a humidified environment with 5% CO_2 was used as positive controls, while a mixture without DNA was used as a negative control.

Statistical analysis. All statistical analyses were performed using SPSS software (version 22; IBM Corp.). Descriptive statistics are presented as mean ± SD of ≥2 independent experimental repeats for normally distributed values and median (25th and 75th percentiles) for skewed variables. The unpaired Student's t-test, Mann-Whitney U and Kruskal-Wallis tests were used to assess quantitative variables. Pearson's

correlation coefficient was used to evaluate the correlation between SETD2 and GLI1 levels. Fisher's exact test was used to evaluate differences between categorical variables. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method according to IS categories. Cox proportional hazard regression models were used to estimate the hazard ratios (HR) and 95% CIs of the variables with OS and DFS. P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the study population. The overall sociodemographic characteristics of the patients are shown in Table SII. The median age was 46 years (range, 25-81 years). According to the BMI, 52.4% (44/84) of participants were overweight or obese. Alcohol and tobacco consumption were reported in 15.5% (13/84) and 27.4% (23/84) of patients, respectively. Hormonal contraception was reported in 20.2% (17/84) of patients. According to histopathological classification, 89.3% (75/84) of patients had SCC, 7.1% (6/84) had ADC and 3.6% (3/84) had ASC (Table SIII). Based on the RECIST criteria, the overall response rate was 92.9% (78/84). Additional clinicopathological characteristics of the patients, such as the FIGO classification, lymph node status and tumor differentiation, are provided in Table SIII.

The distribution of the sociodemographic and histopathological characteristics, categorized by SCC and non-SCC (ADC + ASC) groups, revealed a significant difference in tumor differentiation (Table SIV). Most patients in the SCC group had moderately differentiated tumors (68.0%; 51/75), whereas those in the non-SCC group primarily had poorly differentiated tumors (55.6%; 5/9).

Expression and localization of SETD2 and GLI1. The overall positivity of SETD2 and GLI1 proteins was detected in 90.5% (76/84) and 82.1% (69/84) of tumors, respectively (Table SV; Fig. S1). Samples positive for SETD2 revealed a consistent nuclear (n) staining pattern, whereas GLI1 protein localization indicated nGLI1 and both n and cytoplasmic (c)GLI1 staining in 59.4% (41/69) and 40.6% (28/69) of samples, respectively. According to the histological classification, the positivity and localization of SETD2 and GLI1 were not significantly different between the SCC and non-SCC groups (Table SV).

Expression of SETD2 and GLI1 was compared using IS values. Analysis of SETD2 and GLI1 IS values according to the sociodemographic and histopathological characteristics of the patients revealed that increased nGLI1 levels were significantly associated with a history of hormonal contraceptive use (Table I). Tumors positive for HPV18 or other genotypes demonstrated significantly increased levels of nSETD2 compared with those positive for HPV16 (Table I). Differences in clinical outcomes were not significantly associated with changes in nGLI1, ncGLI1 or nSETD2 levels. However, an increasing trend was observed in ncGLI1 levels in overweight/obese patients (Table I).

Relationship between SETD2 and GLI1 expression levels. The relationship between SETD2 and GLI1 expression was evaluated based on tumor positivity and IS values. The distribution of samples positive for nSETD2 revealed no statistically significant differences between the nGLI1 (50.0%; 38/76) and ncGLI1 (31.6%; 24/76) groups (Table SVI). However, a positive correlation was detected between nSETD2 and nGLI1 levels based on IS values (R=0.428; Fig. 1A). No significant association was found between nSETD2 and ncGLI1 expression (Fig. 1B).

Prognostic utility of SETD2 and GLI1 IS values. The median follow-up period was 53 months (range, 1-120 months). Patients positive for GLI1 or SETD2 demonstrated no significant differences in OS and DFS rates compared with those negative for these proteins (Fig. S2). The predictive values of SETD2 and GLI1 for OS and DFS were evaluated by categorizing the IS values according to cell positivity >50% in combination with moderate or strong immunoreactivity (IS >6; Figs S3 and S4). Increased GLI1 and SETD2 expression (IS >6) was observed in 65.2% (45/69) and 26.3% (20/76) of cases, respectively. Patients with nGLI1 IS >6 had significantly worse OS and DFS rates compared with those with nGLI1 IS ≤6 (Fig. 2). Conversely, patients with ncGLI1 IS >6 exhibited improved OS compared with those with ncGLI1 IS ≤6. No significant differences were observed in the OS or DFS between the categories for nSETD2.

The univariate analysis revealed an increased probability of lower OS associated with nGLI IS >6 (HR, 1.50; 95% CI, 1.44-14.13) and ncGLI1 IS ≤6 (HR, 1.86; 95% CI, 1.42-29.55), whereas a lower probability of DFS was significantly correlated with nGLI IS >6 (HR, 1.53; 95% CI, 1.23-17.49). However, no significant association between nGLI1 and ncGLI1 and clinicopathological characteristics was detected in the multivariate analysis (Table II).

Discussion

The present study demonstrated the utility of IS in evaluating the prognostic impact of SETD2 and GLI1 expression in patients with LACC. Evaluation of IS values according to cellular localization revealed a significant correlation between increased levels of nSETD2 and nGLI1 proteins. Furthermore, increased nGLI1 levels were associated with oral estrogen consumption. The present results demonstrated the prognostic value of changes in the expression of nGLI1 and ncGLI1 on OS and DFS and their clinical value in predicting a higher likelihood of mortality.

Previous studies have suggested that chemotherapy resistance associated with upregulation or mutation of SETD2 in several malignancies, such as prostate, renal cancer, and gastric cancer, may be associated with alterations in Wnt/β-catenin and Hh signaling pathways (21-23). Furthermore, abnormal function of SETD2 and GLI1 is associated with CRT resistance in advanced CC (10,13,24). Therefore, this interaction has been proposed as a potential candidate for synthetic lethality in patients with a high probability of recurrence following chemoradiation (5). However, the causal relationship between alterations in SETD2 and GLI1 during cervical carcinogenesis remains unknown. In the overall study cohort, a significant association between increased nSETD2 and nGLI1 was reported, which indicated that their interactions were associated with nuclear accumulation. Consistent with

Table I. GLI1 and SETD2 cellular localization in patients with locally advanced cervical cancer.

	Cellular localization					
Variable	nGLI1 (n=41)	P-value	ncGLI1 (n=29)	P-value	nSETD2 (n=76)	P-value
BMI[a], kg/m^2)						
Normal (<25.0)	8 (2-9)	0.793	8 (8-12)	0.098	4 (2-8)	0.838
Overweight (≥25.0)	8 (6-8)		12 (8-12)		2 (1-8)	
Tobacco consumption[a]						
No	7 (4-9)	0.772	12 (8-12)	0.533	4 (2-8)	0.286
Yes	7 (2-8)		8 (8-12)		4 (1-6)	
Alcohol consumption[a]						
No	8 (4-8)	0.957	8 (8-12)	0.842	4 (2-8)	0.681
Yes	6 (4-9)		10 (8-12)		3 (1-8)	
Hormonal contraception[a]						
No	8 (6-8)	0.029[c]	8 (4-12)	0.365	4 (3-5)	0.957
Yes	9 (8-9)		8 (8-12)		4 (1-8)	
FIGO classification[b]						
II	8 (6-8)	0.247	8 (8-12)	>0.999	4 (1-8)	0.400
III	8 (4-8)		10 (8-12)		4 (1-6)	
IV	5 (1-8)		-		4 (3-8)	
Tumor differentiation[b]						
Well	6 (4-12)	0.897	6 (4-8)	0.149	5 (2-11)	0.232
Moderately	8 (6-8)		12 (8-12)		3 (1-6)	
Poorly	7 (5-8)		8 (6-10)		5 (4-8)	
Lymph node status[a]						
N0	8 (6-9)	0.327	8 (8-12)	0.872	4 (1-8)	0.968
N1	6 (4-8)		10 (8-12)		4 (2-8)	
HPV genotype[b]						
16	8 (4-8)	0.734	10 (6-12)	0.587	3 (1-4)	0.031[c]
18	5 (4-8)		8 (4-8)		5 (3-8)	
Other	8 (6-9)		8 (8-8)		8 (1-9)	
Clinical response[a]						
Responder	6 (4-8)	0.289	12 (8-12)	0.416	4 (1-6)	0.694
Non-responder	8 (8-12)		10 (8-12)		7 (2-8)	

[a]Mann-Whitney U or [b]Kruskal-Wallis tests were used to compare the differences; [c]P<0.05. Data are presented as median and IQR. Other HPV includes types 6, 11, 31, 43, 42, 45 and 58. n, nuclear; c, cytoplasmic; GLI1, glioma-associated oncogene homolog 1; SETD2, SET domain-containing protein 2; LACC, locally advanced cervical cancer; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus.

previous studies (11,13), the frequency of increased GLI1 expression (65.2%; 45/69) was notably higher compared with SETD2 upregulation (26.3%; 20/76). Although increased GLI1 expression is associated with epidermoid tumors (25), no significant differences were detected in GLI1 positivity according to histological classification in the present study. These results suggested that abnormal SETD2 expression was associated with increased nuclear accumulation and transcriptional activity of GLI1. Further studies are warranted to determine the mechanisms underlying this interaction.

The abnormal functionality of SETD2 in CC is associated with the maintenance of the productive replicative cycle during HPV infection, particularly in high-risk genotypes (26). According to Gautam *et al* (26), upregulation of SETD2 in cervical epithelial cells is associated with the ability of E7 oncoproteins to prolong protein half-life, which is key to sustain H3K36me3 levels in the early region of the viral genome. The present results demonstrated that tumors positive for HPV18 and other HPV genotypes (HPV6, 11, 31, 43, 42, 45 and 58) significantly increased nSETD2 levels compared with samples positive for the HPV16 genotype. This is consistent with previous findings that cells harboring HPV31 maintain higher levels of SETD2 compared with those with HPV16 infection (26). Thus, these findings support the hypothesis that HPV oncoproteins differentially modify the activity of target proteins during malignant transformation depending on the

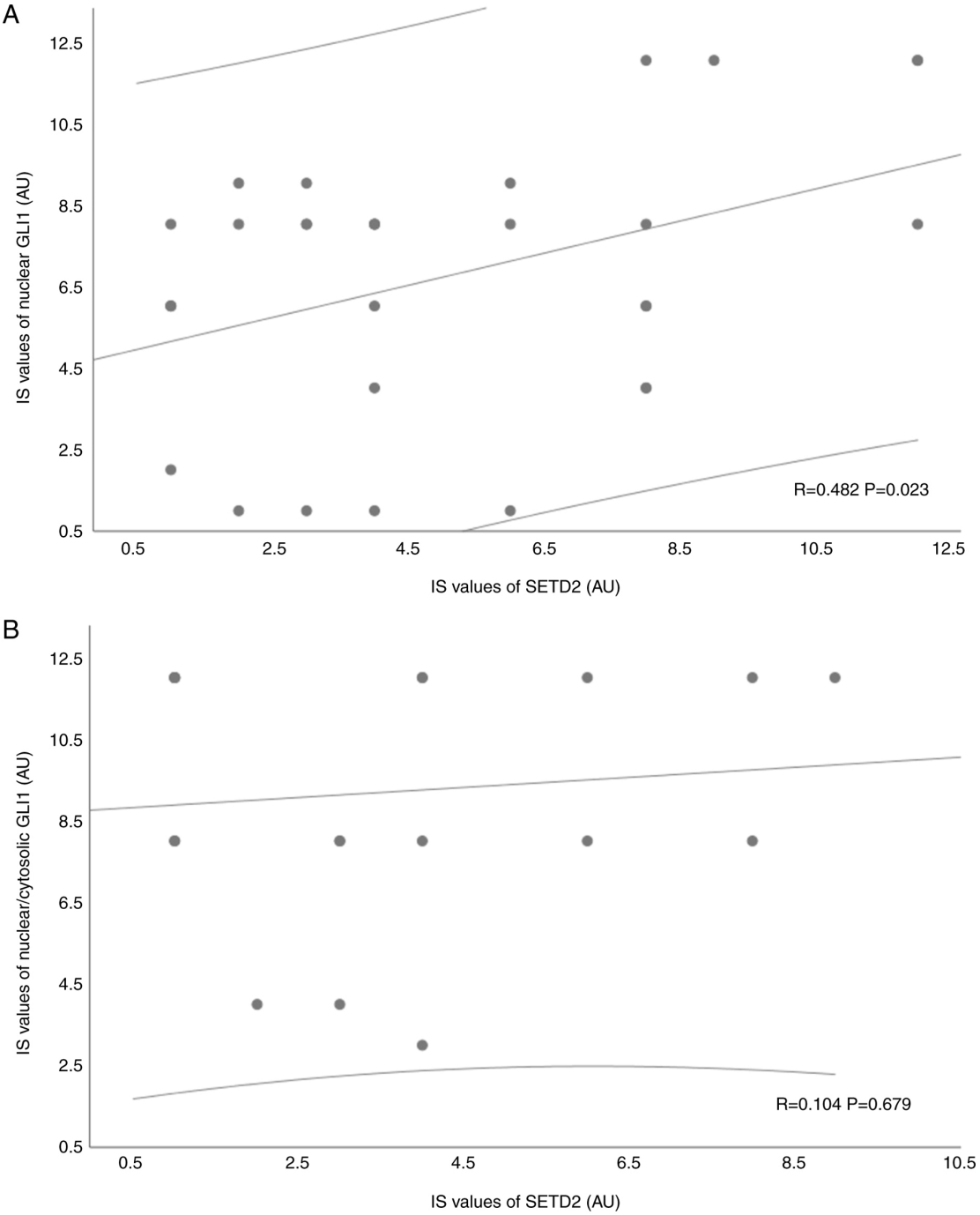

Figure 1. Association between GLI1 and SETD2 IS values according to subcellular localization The linear association between GLI1 and SETD2 expression was evaluated according to (A) nuclear and (B) nuclear/cytoplasmic localization of GLI1. GLI1, glioma-associated oncogene homolog 1; SETD2, SET domain-containing protein 2; IS, immunoreactivity score; AU, arbitrary unit.

cellular environment. Further studies are required to elucidate whether differences in SETD2 protein levels associated with HPV genotypes affect therapeutic strategies that target SETD2 in cancer.

The long-term consumption of oral contraceptives (OCs) is associated with an increased risk of developing CC, particularly glandular origin and HPV-positive tumors (HR, 1.77-3.3; CI 95%, 1.4-2.24) (27,28). Although findings regarding the role of steroid hormones in CC are controversial, the deleterious effects are primarily associated with enhanced transcriptional activity of HPV oncogenes (28,29). Similarly, the risk of invasive CC increases according to the duration of OC consumption (odds ratio, 1.90; 95% CI, 1.69-2.13), in addition to being associated with a lower OS time (30,31). The role of OCs in regulating non-HPV proteins implicated in cervical carcinogenesis and treatment responses remains poorly understood. In the present study, significantly higher nGLI1 levels were observed in patients who used OCs. This finding is consistent with those of previous studies reporting crosstalk between androgen stimulation and Hh signaling in prostate cancer, particularly the association between epithelial-mesenchymal transition and GLI1

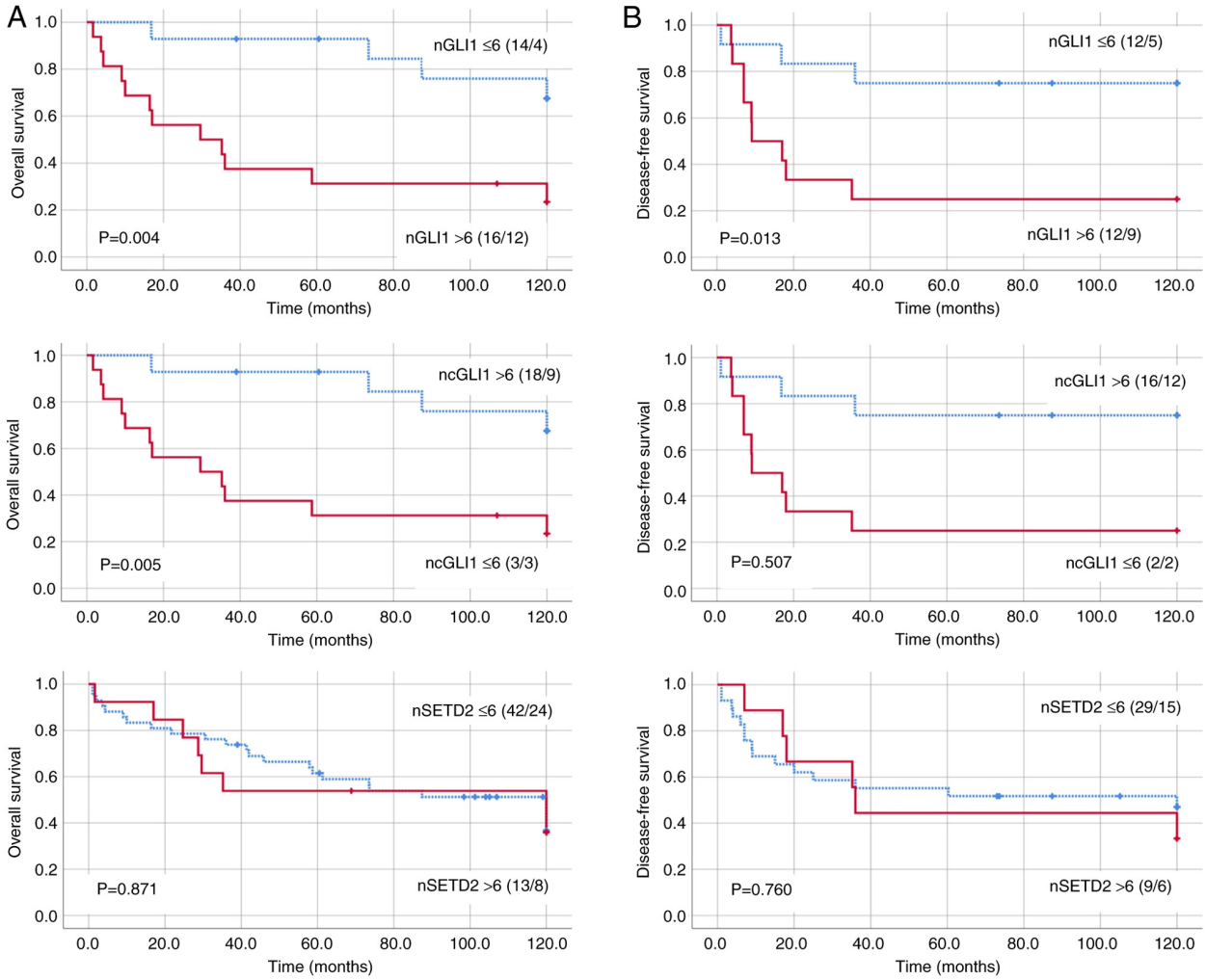

Figure 2. (A) Overall and (B) disease-free survival in patients with locally advanced cervical cancer according to nGLI1, ncGLI1 and nSETD2 IS values. Expression levels were categorized according to the IS value (IS >6 represents moderate or intense immunoreactivity in >50% area). P-values are provided by the log-rank (Mantel-Cox) test. Numbers in parentheses indicate the total number of patients/events. GLI1, glioma-associated oncogene homolog 1; SETD2, SET domain-containing protein 2; IS, immunoreactivity score; nc, nuclear and cytoplasmic.

upregulation (32,33). Furthermore, estrogenic stimulation in gynecological cancer is associated with the upregulation and nuclear translocation of GLI1 via the inhibition of glycogen synthase kinase-3β (34). Thus, the present results supported the hypothesis that long-term OC use contributes to CC progression by stimulating upregulation and nuclear translocation of GLI1.

Although 90.5% (76/84) of patients with LACC were SETD2-positive, exhibiting increased expression in 26.3% of cases, no notable associations were found between SETD2 and OS and DFS. In malignant neoplasia such as lung and endometrial cancer and CC, the abnormal function of SETD2 is associated with mutations that modify gene expression patterns, increasing the risk of CRT resistance and decreasing survival rates (4,5,19). According to The Cancer Genome Atlas Program, endocervical ADC presents the highest rate of SETD2 mutations (7%) (6). In the present study, SETD2 expression exhibited no difference between histological groups, which may be associated with the lower proportion of tumors of glandular origin. Further studies are warranted to clarify the association between the expression and the mutation rate of SETD2. Rate of SETD2 mutations may increase following treatment with neoadjuvant chemotherapy (platinum + paclitaxel) in patients with LACC and is associated with a lack of response (19). This suggests it is necessary to integrate both expression and mutational analyses when determining the prognostic and predictive value of SETD2 in LACC.

The Hh signaling cascade serves a key role in the proliferation, metastasis, recurrence, invasion and CRT resistance of CC (10). Nevertheless, the prognostic and predictive value depend on the target protein analyzed and the criteria employed for evaluation (13,18,35). Consistent with previous studies, the present study analyzed the prognostic utility of GLI1 by categorizing the IS; by contrast with other studies, the present study evaluated the differences according to cellular localization (18,36). This revealed that increased nGLI levels may predict a higher probability of mortality, associated with lower OS and DFS. By contrast, an increased probability of OS was associated with increased ncGLI1 levels. These results suggested that the prognostic and predictive utility of GLI1 depends on the analysis of proteins according to their cellular localization. Furthermore, this supports the hypothesis that the

Table II. Uni- and multivariate Cox regression analysis of survival rates in patients with locally advanced cervical cancer.

A, Overall survival

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
nGLI IS ≤6	Reference					0.437
nGLI IS >6	1.507	1.44-14.13	0.010	0.953	0.23-28.70	
ncGLI IS >6	Reference					0.988
ncGLI1 IS ≤6	1.869	1.42-29.55	0.016	7.15	0.00-16.27	
Hormonal contraception						
No	Reference					
Yes	0.431	0.57-4.14	0.394			
BMI, kg/m^2)						
Normal (<25.0)	Reference					
Overweight/obese (≥25.0)	0.155	0.60-2.25	0.645			
Histological subtype						
SCC	Reference					
Non-SCC	-0.089	0.32-2.56	0.866			
FIGO classification						
II	Reference					
III	0.344	0.72-2.74	0.311			
IV	0.129	0.47-2.70	0.771			
Lymph node status						
N0	Reference					
N1	-0.145	0.42-1.78	0.694			

B, Disease-free survival

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
nGLI IS ≤6	Reference					0.514
nGLI IS >6	1.535	1.23-17.49	0.023	1.032	0.12-61.92	
ncGLI1 IS >6	Reference					
ncGLI1 IS ≤6	0.750	0.21-20.49	0.517			
Hormonal contraception						
No	Reference					
Yes	0.017	0.34-3.02	0.976			
BMI, kg/m^2						
Normal (<25.0)	Reference					
Overweight/obese (≥25.0)	-0.061	0.44-2.01	0.874			
Histological subtype						
SCC	Reference					
Non-SCC	0.496	0.62-4.28	0.311			
FIGO classification						
II	Reference					
III	0.152	0.52-2.60	0.710			
IV	0.405	0.57-3.87	0.404			
Lymph node status						
N0	Reference					
N1	0.395	0.67-3.28	0.330			

GLI1, glioma-associated oncogene homolog 1; SETD2, SET domain-containing protein 2; SCC, squamous cell carcinoma; HR, hazard ratio; FIGO, FIGO, International Federation of Gynecology and Obstetrics; n, nuclear; c, cytoplasmic.

role of GLI1 in CRT resistance is determined by its nuclear translocation, which may markedly affect the probability of survival (11).

To the best of our knowledge, the present study is the first to investigate the association between the expression and localization of SETD2 and GLI1 proteins and their role in the outcome of patients with LACC. A prior study on members of the Hh pathway in CC established an increase in their expression using IHC. However, the aforementioned analyses did not include associations with pathological characteristics or clinical outcomes (37). Furthermore, epigenetic dysregulation is associated with several types of cancer including breast, liver cancer, prostate cancer, and small-cell bladder cancer (38). Histone methyltransferases are frequently mutated or deleted in human tumors (39,40); however, whether alterations in histone methyltransferases are associated with disease progression and response to treatment remains unknown. Despite the positive outcomes, the present study had limitations. The retrospective study design, with a limited sample size, based on FFPE samples, limited access to fresh biological material for functional assays of GLI1 and SETD2 proteins. However, the consistency of sensitivity analysis results suggested that sample size was not a notable limitation.

The outcomes in patients with LACC are poor because they present high rates of resistance to treatment (41). Therefore, the identification of patients at increased risk of recurrence who may benefit from more aggressive treatment strategies and the identification of novel therapeutic targets are key. The interactions of the Hh pathway in tumor cells are complex and the present study provided insight into the positive correlation between the expression of SETD2 and the transcription factor GLI1, which may represent a novel mechanism of epigenetic regulation involved in the clinical outcome of patients with LACC. The present data suggested that GLI1 was a valid and effective therapeutic target in patients with LACC who undergo chemoradiation.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ML conceived the study. ACo and EC confirm the authenticity of all the raw data. ACo, EC and ML wrote the manuscript. ACo and ML acquired funding. ACo conceptualized the study. GM, LC and ACa designed the methodology. AA and MR constructed figures. JC and LC supervised the study. JC, AA, EC and MR analyzed data. ACo, EC and ML edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics and Scientific Institutional Review Board (approval no. INCAN/CEI577/15) of the National Cancer Institute (Mexico City, México), and written informed consent was obtained from all participating patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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