SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 29: 22, 2025

A potential target of GXYLT2 affecting the prognosis of gastric cancer by enhancing the EMT process: A clinical retrospective study

YUNXIA ZHAO[1], LI LI[1,2] and ZHAOGEN CAI[3]

[1]Department of Pathophysiology, Bengbu Medical University, Bengbu, Anhui 233030, P.R. China; [2]Department of Pathology, Anqing 116 Hospital, Anqing, Anhui 246000, P.R. China; [3]Department of Pathology, The First Hospital of Bengbu Medical University, Bengbu, Anhui 233030, P.R. China

Received June 7, 2024; Accepted September 23, 2024

DOI: 10.3892/ol.2024.14767

Abstract. The present study aimed to investigate glucoside xylosyltransferase 2 (GXYLT2) as a potential prognostic marker for gastric cancer (GC). The expression levels of GXYLT2, Notch1, E-cadherin and vimentin in GC and adjacent tissues were detected by Elivision™ Plus immunohistochemistry. The relationship between GXYLT2, Notch1, E-cadherin and vimentin, and clinicopathological parameters was also analyzed. Univariate and multivariate Cox regression analyses were conducted to evaluate the effect of GXYLT2 on survival. The results revealed that GC tissues exhibited a marked increase in GXYLT2, Notch1 and vimentin, and a marked reduction in E-cadherin. The expression of GXYLT2 was related to the differentiation degree of GC and the pathological tumor-node-metastasis (pTNM) stage; the expression of Notch1 was related to the vascular and nerve infiltration of GC; and E-cadherin and vimentin expression were related to patient age, tumor size, tumor differentiation degree, depth of infiltration, lymph node metastasis and pTNM stage. Positive associations existed between GXYLT2 and Notch1 expression, GXYLT2 and vimentin expression, and Notch1 and vimentin expression. Furthermore, the Kaplan-Meier analysis showed that survival and prognosis were associated with factors such as GXYLT2 protein levels, pTNM stage, tumor dimensions, depth of infiltration and lymph node metastasis. Through Cox regression analysis, GXYLT2 was identified as an independent predictor for GC. In conclusion, GXYLT2 may be related to the pathogenesis of GC, and its abnormal expression could be associated with epithelial-mesenchymal transition. Consequently, GXYLT2 may be considered a promising prognostic marker in the context of GC.

Correspondence to: Professor Zhaogen Cai, Department of Pathology, The First Hospital of Bengbu Medical University, 801 Zhihuai Road, Longzihu, Bengbu, Anhui 233030, P.R. China
E-mail: chzgen@163.com

Key words: glucoside xylosyltransferase 2, gastric cancer, epithelial-mesenchymal transition, prognostic

Introduction

Gastric cancer (GC) presents a major risk to human health, which is a highly ranked tumor in terms of occurrence and death rates. Notably, the incidence and mortality rates of gastric cancer rank fifth among all tumors worldwide (1). Globally, >50% of new cases and fatalities as a result of GC are documented in East Asia. Compared with other types of cancer, the outlook for GC is typically unfavorable, exhibiting notable differences across regions. Globally, GC mortality is highest in East Asia, followed by central and South Asia and Eastern Europe, and lowest in South Africa (2-4). The rising trend of aging populations is expected to contribute to elevated incidences and deaths due to GC in the coming years. The unfavorable prognosis associated with GC primarily stems from its propensity for invasion and metastasis upon diagnosis (5). Identifying potential molecular targets implicated in the onset and advancement of GC is crucial for informing treatment strategies and evaluating prognosis.

Glycosyltransferases are ubiquitously present in nature and are vital for preserving the structural variability of natural compounds. These enzymes facilitate the transfer of glycosyl groups to proteins or lipids, thereby altering their properties, modulating protein function and participating in various biological processes (6). The enzyme glucoside xylosyltransferase 2 (GXYLT2), which belongs to the human glycosyltransferase 8 group, is responsible for producing a protein comprising 443 amino acids that operates as a xylosyltransferase. This enzyme facilitates the incorporation of xylose into the O-glucose (O-Glc) segment of epidermal growth factor (EGF) and the repeat sequences of various proteins, leading to the extension of the structure through xylose addition and resulting in a xylose-xylose-glucose trisaccharide (7,8).

The Notch gene encodes a group of evolutionarily conserved cell surface receptors. The pathway mediated by Notch is crucial in controlling cell differentiation, cell proliferation and programmed cell death, as well as the creation of cell boundaries. Intercellular communication is facilitated

by adjacent cells transmitting signals through the interaction between Notch receptors and ligands, which enhances and stabilizes molecular distinctions between cells, ultimately influencing cell fate (9). Notably, the extracellular region of the Notch receptor consists of repeats similar to EGF (10,11). Post-translation, glycosyltransferases within the endoplasmic reticulum and Golgi apparatus alter the EGF repeat sequence in the Notch protein along with glycans. The initial addition of xylose is facilitated by GXYLT enzymes GXYLT1 and GXYLT2.

Epithelial-mesenchymal transition (EMT) involves the dedifferentiation of epithelial cells, resulting in polarity loss, decreased cell-cell and cell-matrix interactions, and increased motility and migration. The process involves a steady decrease or disappearance of epithelial indicators, such as E-cadherin, coupled with an increase in mesenchymal markers, such as vimentin, N-cadherin, α-SMA, Snail and Slug (12). Numerous studies have demonstrated the presence of EMT in the physiological context of embryonic development, and the pathological context of tumor invasion and metastasis (13-15). EMT entails the differentiation of epithelial-derived tumor cells, originating from the endoderm and ectoderm, into mesenchymal-like cells from the mesoderm, causing enhanced metastatic and invasive capabilities.

It was hypothesized that GXYLT2 may act as a controlling factor in the spread and metastasis of GC. As a glycosyltransferase, GXYLT2 has been shown to exhibit an aberrant expression in GC, leading to atypical glycosylation of the Notch1 protein (16). This phenomenon, in turn, can modulate the Notch signaling pathway, facilitate the EMT process, diminish cell-stroma adhesion, and enhance the metastatic and invasive capabilities of cancer cells. Consequently, the infiltration and metastasis of GC ensue, ultimately contributing to a poor prognosis for affected individuals.

The present study aimed to explore the role of GXYLT2 in the development of GC by examining the levels of GXYLT2, Notch1 and EMT-associated indicators, E-cadherin and vimentin, in GC tissues. The study aimed to offer new perspectives in identifying possible molecular targets for diagnosing and treating GC.

Materials and methods

Specimens. Specimens, including 338 GC tissues and 30 paracancerous tissues, were randomly chosen from paraffin-embedded samples from Department of Pathology, The First Affiliated Hospital of Bengbu Medical University (Bengbu, China) obtained between January 2018 and December 2019. Samples for participation in this study were obtained with patient consent. The patients whose samples were used in the present study had not received any other treatment before surgery, and excluded minors, pregnant women and patients with serious underlying diseases, such as severe hypertension and diabetes. Paracancerous tissues were collected from the surgical margins of 30 of the 338 patients with GC, at a distance of ≥3 cm from the lesion. The pathological assessments for all cases were collaboratively conducted by two senior pathologists. Comprehensive clinical information was accessible for all patients, with 230 individuals being monitored until September 2023 or their death. Before undergoing surgery, no patient received either radiotherapy or chemotherapy. The present study was approved by the Ethics Committee of Bengbu Medical University [approval no. (2023)245].

Immunohistochemistry. GC and paracancerous samples were fixed in 10% neutral formalin at room temperature for 24 h and embedded in paraffin, and the paraffin-embedded specimens were sliced into 4-μm sections. Antigen retrieval was performed by heating the paraffin-embedded sections after deparaffinization and hydration; the heating temperature of antigen retrieval was 60-70°C and the reagents used were citric acid antigen repair solution and EDTA antigen repair solution. To block endogenous peroxidase, the sections were placed in 3% hydrogen peroxide solution and incubated at room temperature in the dark for 20 min. Subsequently, 5% BSA (Wuhan Servicebio Technology Co., Ltd.) blocking solution was applied to the tissue sections dropwise and was incubated at room temperature for 30 min. The sections were then incubated with a primary antibody solution consisting of 50 μl anti-GXYLT2 (rabbit polyclonal; 1:250; cat. no. bs-16377R; BIOSS), anti-Notch1 (rabbit polyclonal; 1:200; cat. no. bs-11976R; BIOSS), anti-E-cadherin (mouse monoclonal; ready-to-use; cat. no. MAB-0738; Fuzhou Maixin Biotechnology Development Co., Ltd.) or anti-vimentin (mouse monoclonal; ready-to-use; cat. no. RMA-0547; Fuzhou Maixin Biotechnology Development Co., Ltd.) overnight at 4°C. Subsequently, the samples were washed three times with PBS, incubated with 50 μl polymer reinforcement (Reagent A, ready-to-use; cat. no. KIT-9902; Fuzhou Maixin Biotechnology Development Co., Ltd.) at room temperature for 20 min, and washed again three times with PBS. After removal of the PBS solution, each section was incubated with 50 μl enzyme-labeled anti-mouse/rabbit polymer (Reagent B, ready-to-use; cat. no. KIT-9902; Fuzhou Maixin Biotechnology Development Co., Ltd.) for 30 min at room temperature. Subsequently, the samples were washed three times with PBS and were stained at room temperature for 3-5 min with 3,3'-diaminobenzidine for microscopic examination. Hematoxylin was used for counterstaining at room temperature for 3 min. PBS functioned as the negative control, while recognized positive sections treated with primary antibodies (from the companies of the reagents) were employed as the positive control. The procedures were executed according to the guidelines outlined in the Elivision™ Plus detection kit (Cat. no. KIT-9902; Fuzhou Maixin Biotechnology Development Co., Ltd.).

Criteria for determining positive results. Light yellow to brownish-yellow particles within the cytoplasm or cell membrane suggested the existence of positive staining, under a light microscope. The immunohistochemical results were assessed using a semi-quantitative scoring method that integrated the percentage of positive cells and the intensity of staining. Five high-power fields (magnification, x400) were randomly selected from each section, and 200 tumor cells were counted. The scoring system for positive cell proportion was as follows: 0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; 4, >75%. The staining intensity of positively stained cells was semi-quantified using a scoring system ranging from 0 to

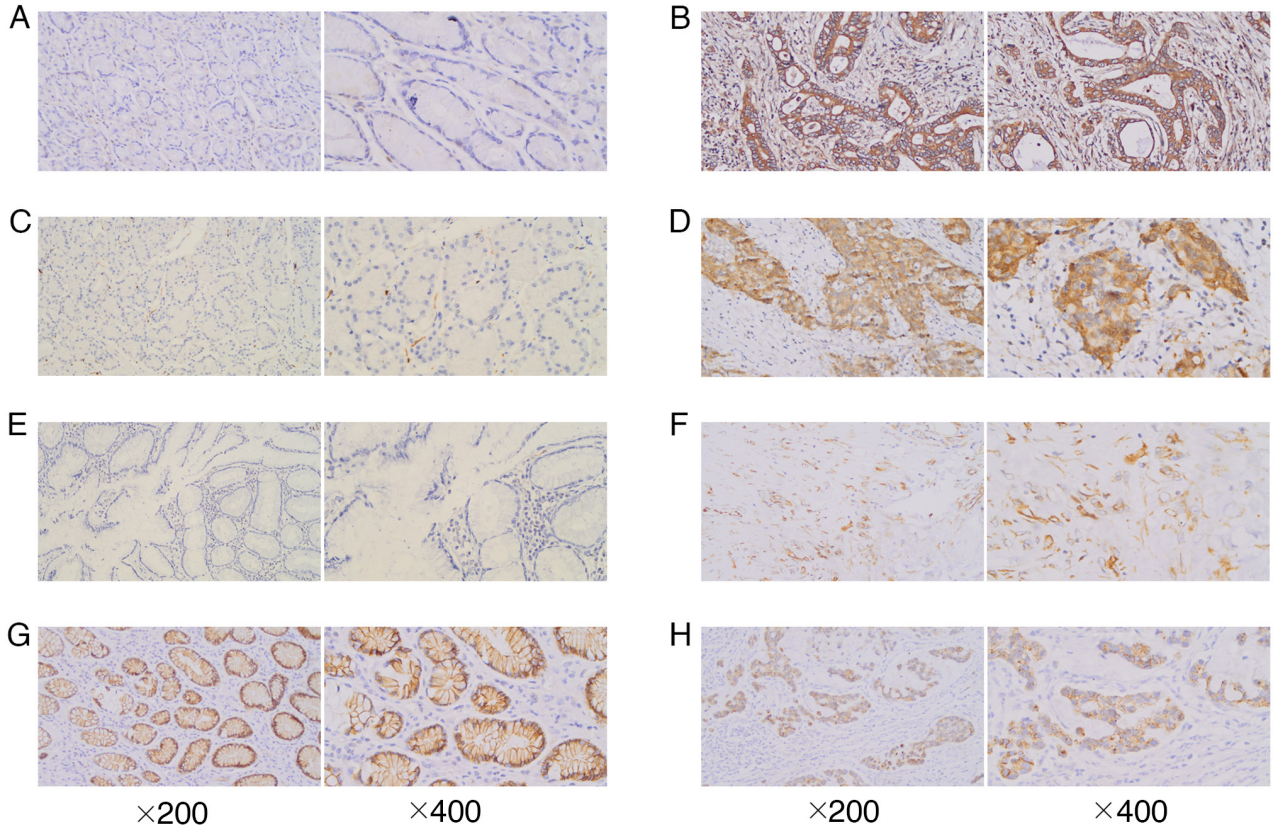

Figure 1. Immunohistochemical expression of each protein in paracancerous and gastric cancer tissues. Immunohistochemical expression of GXYLT2 in (A) paracancerous and (B) GC tissues. Immunohistochemical expression of Notch1 in (C) paracancerous and (D) GC tissues. Immunohistochemical expression of vimentin in (E) paracancerous and (F) GC tissues. Immunohistochemical expression of E-cadherin in (G) paracancerous and (H) GC tissues. GC, gastric cancer; GXYLT2, glucoside xylosyltransferase 2.

3: 0, no staining; 1, light yellow; 2, brown yellow 2; 3, tan. Multiplying the scores for staining intensity and the number of positive cells in each case allowed for the classification of negative expression (<3 points) and positive expression (≥3 points). Every outcome was evaluated using a double-blind approach, conducted three times.

Statistical analysis. The data were analyzed using SPSS 26.0 statistical software (IBM Corp.). The χ^2 test was used to analyze count data among the various groups, and this test was also used to investigate the association between two factors. The coefficient of contingency (C) was used to show the association between categorical variables in a contingency table for χ^2 tests. When the contingency table is a 2x2 table, C can be represented by the symbol φ, which represents the degree of association between two variables, $\varphi = \sqrt{\frac{x^2}{n+x^2}}$ The Kaplan-Meier technique was used for univariate survival analysis and the log-rank test was used to assess the effect of different factors on survival. Univariate cox regression was used to analyze the effect of each factor on survival. Furthermore, the Cox regression analysis was employed for multivariate survival analysis. P<0.05, falling within a 95% confidence interval, was considered to indicate a statistically significant difference.

Results

Fundamental details of the patients. The patients with GC included in the present study were aged between 26 and 89 years (median age, 65 years; mean ± SD, 63±9.98 years). Among the cases, 109 individuals were <60 years old and 229 individuals were ≥60 years old. Of the cases, 206 had tumor diameters <5 cm, whereas 132 cases had tumor diameters ≥5 cm. Regarding tumor differentiation, 7 cases exhibited high differentiation, 194 cases showed medium differentiation and 137 cases had low differentiation.

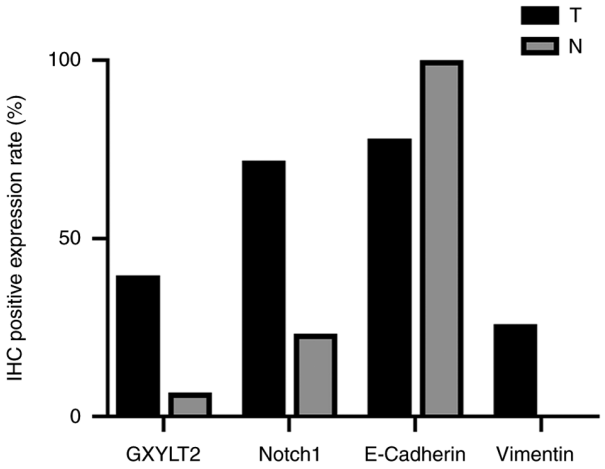

Figure 2. Percentage of cases with positive expression of GXYLT2, Notch1, vimentin and E-cadherin proteins in gastric cancer and paracancerous tissues among the total number of samples. GXYLT2, glucoside xylosyltransferase 2; IHC, immunohistochemistry; N, normal; T, tumor.

Table I. Association between the expression of GXYLT2, Notch1, E-cadherin and vimentin in gastric cancer tissues and various clinicopathological factors.

	GXYLT2			Notch1			E-cadherin			Vimentin		
Variable	+	-	P-value	+	-	P-value	+	-	P-value	+	-	P-value
Sex												
Male	102	148	0.464	185	65	0.146	198	52	0.431	64	186	0.758
Female	32	56		58	30		66	22		24	64	
Age, years												
≥60	91	138	0.960	169	60	0.259	189	40	0.004	50	179	0.011
<60	43	66		74	35		75	34		38	71	
Diameter, cm												
≥5	58	74	0.196	92	40	0.472	95	37	0.029	46	86	0.003
<5	76	130		151	55		169	37		42	164	
Differentiation												
Well/Moderate	90	111	0.019	147	54	0.539	178	23	<0.001	27	174	<0.001
Poor	44	93		96	41		86	51		61	76	
Depth of infiltration												
T1	5	16	0.463	14	7	0.598	20	1	0.001	3	18	0.004
T2	28	44		49	23		65	7		10	62	
T3	91	128		159	60		160	59		63	156	
T4	10	16		21	5		19	7		12	14	
Lymph node metastasis												
Yes	87	123	0.391	147	63	0.321	151	59	<0.001	69	141	<0.001
No	47	81		96	32		113	15		19	109	
pTNM												
I+II	68	129	0.023	137	60	0.256	171	26	<0.001	31	166	<0.001
III+IV	66	75		106	35		93	48		57	84	
Vascular and nerve infiltration												
Yes	18	24	0.649	36	6	0.033	30	12	0.263	11	31	0.980
No	116	180		207	89		234	62		77	219	

GXYLT2, glucoside xylosyltransferase 2; pTNM, pathological tumor-node-metastasis.

In terms of infiltration depth, cancer cells were limited to the mucosa or submucosa in 21 cases, extended into the lamina propria in 72 cases, reached the serous membrane in 219 cases, and breached the serous membrane or disseminated to adjacent tissues in 26 cases. A total of 210 cases exhibited lymph node metastasis, whereas 128 were devoid of such metastases. Concerning vascular and nerve infiltration, 42 cases demonstrated such infiltration, while 296 cases did not. Following the 8th American Joint Committee on Cancer/Union for International Cancer Control (14) pathological tumor-node-metastasis (pTNM) classification for gastric carcinoma, 63 cases were identified as stage I, 134 as stage II, 139 as stage III and 2 as stage IV.

Levels of GXYLT2, Notch1, E-cadherin and vimentin expression in GC and paracancerous tissues. GXYLT2 staining exhibited localization to the cell membrane, characterized by a brown-yellow hue, with a positive expression rate of 39.64% (134/338) in GC samples. These outcomes are presented in Figs. 1 and 2. By contrast, only 2 cases in the paracancerous group showed this staining trend (2/30), leading to a statistically notable disparity between the groups (χ^2=12.862; P<0.05). Notch1 expression was observed in the cytoplasm, presenting as brown-yellow particles. The positive rate of Notch1 was significantly higher in GC tissues (71.89%; 243/338) than in adjacent tissues (23.33%; 7/30) (χ^2=29.828; P<0.05). In GC tissues, 26.04% (88/338) showed positive vimentin staining, whereas no vimentin staining was observed in the paracancerous tissues (0/30), highlighting a notable disparity between the two groups (χ^2=10.265; P<0.05). E-cadherin staining was predominantly localized toward the cell membrane but could also be expressed in the cytoplasm. In the control group, E-cadherin exhibited a strong positive expression (30/30), whereas in GC cells, its expression was notably diminished, displaying a light yellow hue. The positive expression rate of E-cadherin in GC tissues was 78.11% (264/338), and a notable difference was noted between the control and GC tissues (χ^2=8.221; P<0.05).

Table II. Relationship between the expression of GXYLT2, Notch1 and vimentin in gastric cancer tissues.

	GXYLT2				
Variable	+	-	χ^2	P-value	φ
Notch1			8.323	0.004	0.155
+	108	135			
-	26	69			
Vimentin			7.929	0.005	0.151
+	46	42			
-	88	162			

GXYLT2, glucoside xylosyltransferase 2.

Table III. Relationship between the expression levels of Notch1, E-cadherin and vimentin in gastric cancer tissues.

	Vimentin				
Variable	+	-	χ^2	P-value	φ
Notch1			4.548	0.033	0.115
+	71	172			
-	17	78			
E-cadherin			114.722	<0.001	-0.503
+	33	231			
-	55	19			

Association between the expression levels of GXYLT2, Notch1, E-cadherin and vimentin in GC tissues, and clinicopathological factors. The findings indicated a significant association between the upregulation of GXYLT2 in GC tissues, and tumor differentiation degree and pTNM stage (P<0.05). The results are shown in Table I. Specifically, a positive expression of GXYLT2 was observed in 34.52% (68/197) of patients with pTNM stages I and II, which was lower than the 46.81% (66/141) positive expression detected in patients with pTNM stages III and IV; this disparity between the two groups was statistically significant. Furthermore, the positive expression rate of GXYLT2 was 44.78% (90/201) in well/moderately differentiated cases, compared with 32.12% (44/137) poorly differentiated cases. No notable link was determined between GXYLT2 expression and factors such as sex, age, tumor dimension, depth of infiltration, lymph node metastasis and vascular and nerve infiltration (P>0.05). Nonetheless, the expression of Notch1 was positively associated with the vascular and nerve infiltration (P<0.05). In instances of vascular and nerve invasion, the rate of positive expression stood at 85.71% (36/42), markedly surpassing the 69.93% (207/296) rate in situations lacking vascular and nerve invasion. Notch1 expression did not exhibit any association with the other clinicopathological parameters. Notably, positive E-cadherin expression was associated with factors such as age, tumor dimension, differentiation, depth of infiltration, lymph node metastasis and pTNM stage (P<0.05), but not vascular and nerve infiltration. A notable link was also identified between the positive expression of vimentin in GC tissues and several clinicopathological factors, such as age, tumor dimension, differentiation level, depth of infiltration, lymph node metastasis and pTNM stage (P<0.05), although vimentin was not associated with tumor vascular and nerve infiltration.

Relationship between the expression levels of GXYLT2, Notch1, E-cadherin and vimentin in GC tissues. In the group exhibiting a positive GXYLT2 expression, the incidence of Notch1-positive expression stood at 80.60% (108/134), compared with 66.18% (135/204) in the group with negative GXYLT2 expression, as shown in Table II. A statistically significant difference was observed between the two groups, indicating an association between GXYLT2 and Notch1 expression in GC (P=0.004, φ=0.155). In addition, a notable positive association was detected between GXYLT2 and vimentin expression in GC tissues. In the group with positive GXYLT2 expression, the rate of positive vimentin expression was 34.33% (46/134), markedly surpassing the 20.59% rate (42/204) observed in the group with negative GXYLT2 expression. Notably, GXYLT2 expression was positively associated with vimentin expression in GC tissues (P=0.005, φ=0.151). A positive association was also observed between Notch1 and vimentin expression in GC tissues (P=0.033, φ=0.115) , as shown in Table III. The prevalence of vimentin positivity in the Notch1-positive group was 29.22% (71/243), implying an increase compared with that in the Notch1-negative group (17.89%; 17/95). Conversely, the incidence of vimentin positivity in the E-cadherin-positive expression group was 12.50% (33/264), which was markedly lower than that in the E-cadherin-negative expression group (74.32%; 55/74). Notably, a significant negative association existed between E-cadherin expression and vimentin expression (P<0.001, φ=-0.503).

GXYLT2 as an important prognostic factor. A follow-up study was carried out on 231 patients to explore the predictive effects of GXYLT2, Notch1, E-cadherin and vimentin on patients with GC. Kaplan-Meier survival and multivariate Cox regression survival analyses were conducted. The findings demonstrated a mean survival time of 36.04±17.43 months, with a postoperative survival rate of 39.40%. Among the 231 patients with follow-up data, 112 patients exhibited positive GXYLT2 expression. The overall survival rate was 27.68% for GXYLT2-positive patients and 50.42% for GXYLT2-negative patients. The Kaplan-Meier analysis revealed a statistically significant disparity in survival rates between the two groups (log-rank=13.419; P<0.001) (Fig. 3A). There was no significant difference between the expression of Notch1 protein and survival of patients with GC (log-rank=1.551; P>0.05) (Fig. 3B). By contrast, a significant association was identified between E-cadherin expression and survival (log-rank=3.94; P<0.05) (Fig. 3C). However, the expression of vimentin protein was not associated with the survival of patients with GC (log-rank=2.813; P>0.05) (Fig. 3D). Furthermore, the univariate Cox regression analysis indicated that tumor diameter, depth of infiltration, lymph node metastasis, pTNM stage and GXYLT2 expression were significant factors influencing the survival duration of patients with GC (P<0.05) (Fig. 3E).

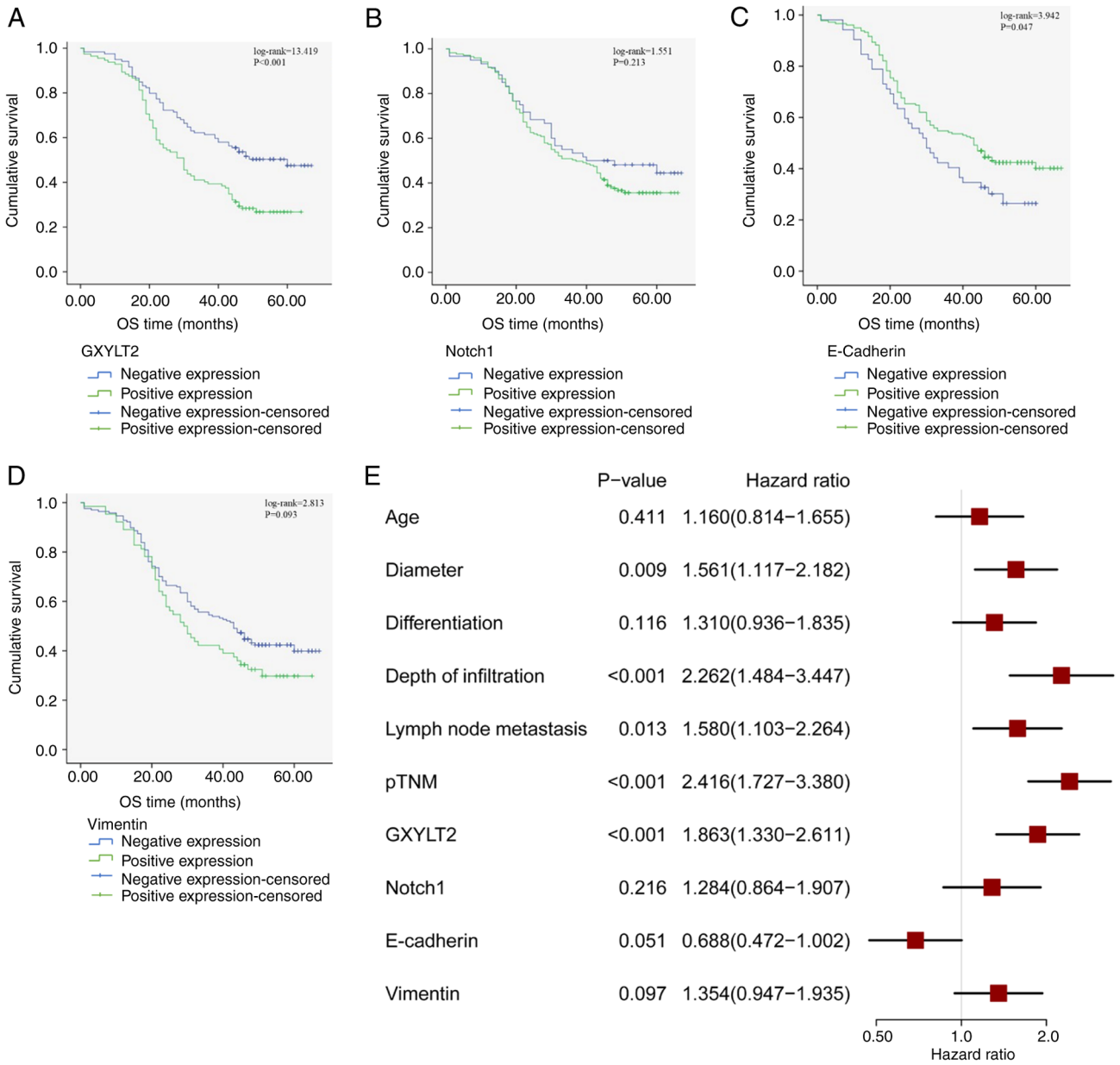

Figure 3. Kaplan-Meier analysis of the survival of patients with GC. (A) Survival of patients was significantly lower in the GXYLT2 positive expression group than that in the GXYLT2 negative expression group. (B) No significant association was detected between the expression levels of Notch1 and prognosis. (C) E-cadherin expression was associated with the prognosis of patients with GC. (D) No association was detected between vimentin expression and the prognosis of patients with GC. (E) Results of the univariate Cox regression analysis showed that tumor diameter, depth of infiltration, lymph node metastasis, pTNM stage and GXYLT2 expression significantly influenced the survival duration of individuals diagnosed with GC. GC, gastric cancer; GXYLT2, glucoside xylosyltransferase 2; OS, overall survival; pTNM, pathological tumor-node-metastasis.

Findings from the multivariate Cox regression analysis indicated that pTNM (P<0.001; Table IV; Fig. 4) and GXYLT2 (P<0.001) independently and significantly predicted the prognosis of patients with GC, whereas the other factors analyzed showed no notable association with prognosis.

Discussion

GC is a frequent type of malignant tumor. Recently, advancements in all-encompassing surgical treatment techniques have led to a reduction in mortality rates (17); however, s GC mortality rates are still high. A primary cause of the unfavorable outlook for GC lies in its discovery at a late stage. Furthermore, GC has significant heterogeneity and a complex pathogenesis, characterized by a multifaceted process of multiple gene alterations and aberrant expression (18). The investigation of molecular mechanisms associated with GC is crucial for enhancing the specificity of diagnosis and treatment targets, with the aim of facilitating the advancement of personalized and comprehensive clinical interventions for patients with this disease.

The process of glycosylation, a vital post-translational protein alteration, is crucial for preserving the functionality of proteins and aiding their participation in numerous physiological activities, such as growth, development and immune protection (19-22). N-glycosylation and O-glycosylation exist

Table IV. Comprehensive Cox regression analysis of overall survival.

Variable	B	SE	Wald	df	P-value	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Age	-0.296	0.194	2.327	1	0127	0.744	0.509	1.088
Diameter	0.042	0.193	0.048	1	0.827	1.043	0.714	1.524
Differentiation	-0.246	0.187	1.733	1	0.188	0.782	0.542	1.128
Depth of infiltration	-0.408	0.256	2.541	1	0.111	0.665	0.403	1.098
Lymph node metastasis	0.149	0.246	0.367	1	0.544	1.161	0.717	1.878
pTNM	-0.702	0.253	7.676	1	0.006	0.496	0.302	0.814
GXYLT2	-0.500	0.189	7.015	1	0.008	0.607	0.419	0.878
Notch1	-0.213	0.212	1.008	1	0.315	0.808	0.534	1.225
E-cadherin	0.310	0.254	1.490	1	0.222	1.363	0.829	2.241
Vimentin	0.257	0.255	1.019	1	0.313	1.293	0.785	2.141

GXYLT2, glucoside xylosyltransferase 2; pTNM, pathological tumor-node-metastasis; B, β, regression coefficient; SE, standard error; df, degree of freedom; Exp(B), exponentiation of the B coefficient.

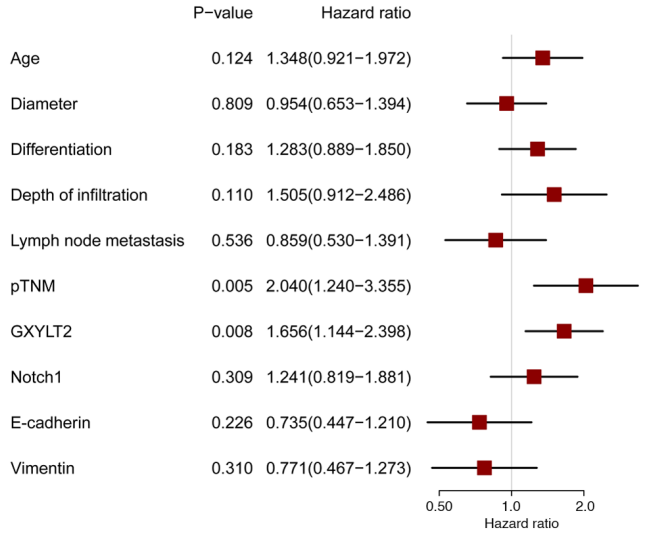

Figure 4. Results of the multivariate Cox regression analysis. pTNM stage and GXYLT2 expression were significant prognostic factors for patients with gastric cancer. GXYLT2, glucoside xylosyltransferase 2; pTNM, pathological tumor-node-metastasis.

in human cells, and O-glycosylation is completed by various glycosyltransferases, including GXYLT2. Research has demonstrated that abnormal glycosylation can significantly influence the onset and progression of cancer by modulating tumor proliferation, invasion, metastasis and angiogenesis (23-26). However, limited research exists on the role of GXYLT2 as a regulator in GC. Our previous bioinformatics analysis confirmed that GXYLT2 may be an important target for the diagnosis and treatment of GC (27).

The present study detected a notable upregulation in the expression of GXYLT2 within GC tissues, indicating a potentially pivotal role of GXYLT2 in the pathogenesis and progression of GC. *In vitro* experimental studies have documented a reduced expression of GXYLT2 in colorectal cancer and an increased expression in renal cell carcinoma (28,29). Abnormal expression of GXYLT has been shown to trigger anomalous glycosylation of intracellular proteins in triple-negative breast cancer, particularly the O-Glc trisaccharide modification of EGF repeats, and the xylose extension of O-Glc glycans on Notch1 is crucial for their transport (16,30). Therefore, the present study further detected the expression of Notch1 in GC tissues; the results revealed that it was significantly increased and that it was positively associated with the expression of GXYLT2. This finding suggested that GXYLT2 may lead to abnormal glycosylation of Notch1, and affect the occurrence and development of tumors. Research has indicated that the Notch signaling pathway exhibits abnormal activation in numerous malignant tumors and is of paramount importance in regulating the EMT process (31), suggesting its potential as a novel target for tumor therapy.

To clarify the possible involvement of GXYLT2 and Notch1 in the EMT process of GC, the expression of E-cadherin and vimentin was measured, both of which are crucial factors linked to EMT, in GC tissues. The results of the present study showed a notable reduction in E-cadherin levels and a substantial increase in vimentin levels in GC tissues. A negative association existed between the levels of E-cadherin and vimentin, whereas a positive association existed between GXYLT2, Notch1 and vimentin, indicating the significant role of GXYLT2 and Notch1 in the EMT mechanism of GC. Research has shown that reducing GXYLT2 levels hinders the proliferation, spread and metastasis of cancer cells, whereas increasing GXYLT2 expression intensifies these effects (32). Suppressing GXYLT2 signaling has been reported to result in a significant decrease in innate Notch intracellular domain levels and a substantial increase in Notch1 protein levels, suggesting that GXYLT2 serves a role in activating the Notch1 signaling pathway in human cells (32). Subsequent administration of the Notch1 inhibitor DAPT may result in the inhibition of cell proliferation and migration, as well as the complete reversal of the EMT process (32). This previous study supported the present

hypothesis that GXYLT2 may facilitate tumor cell proliferation, migration and EMT through the Notch signaling pathway. Moreover, the present study, to the best of our knowledge, is the first to examine the effect of GXYLT2 on prognosis and survival from a histological standpoint. A link was identified between the survival rate of patients and GXYLT2 protein expression, in which elevated GXYLT2 protein levels were associated with a poor prognosis for patients with GC. Therefore, the presence of GXYLT2 could act as an independent predictive indicator for patients with GC. The present study further validated the association between GXYLT2 expression and pTNM, as well as the association between Notch1 protein expression and nerve vessel invasion in tumors. The heightened expression of GXYLT2 and Notch1 could potentially increase the invasive and metastatic capabilities of GC cells.

A previous study suggested that GXYLT2 expression was mostly negatively correlated with tumor mutational burden (TMB) and microsatellite instability (MSI) in 33 tumor tissues from a public database (33), and TMB and MSI are considered genomic biomarkers for identifying patients with cancer who may benefit from treatment with immune checkpoint inhibitors (34-36). Therefore, it could be hypothesized that GXYLT2 may serve as a prognostic marker and potential immunotherapeutic target for GC, providing new directions for future studies.

Despite the valuable findings of the present study, its limitations include the restricted number of clinical specimens and the inadequate sample size. Literature examining the direct effect of GXYLT2 on GC is also lacking; we aim to explore this topic in our forthcoming studies. In addition, the present study only demonstrated an association between the protein levels, but did not assess how GXYLT2 regulates Notch1 signaling and EMT at the molecular level. In the future, we aim to explore how GXYLT2 regulates Notch1 signaling and EMT at the molecular level by knocking down or overexpressing GXYLT2.

In conclusion, the present results indicated that GXYLT2 may affect the EMT of GC through abnormal alteration of Notch1 EGF sequences, suggesting that GXYLT2 may be an effective prognostic marker for GC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Education Fund Item of Anhui Province (grant no. 2022AH051525) and the Natural Science Foundation of Bengbu Medical University (grant no. 2020byzd035).

Availability of data and materials

The data generated in this study can be requested from the corresponding author.

Authors' contributions

YZ was involved in conceptualization, methodology and first draft writing, and acquired funding. LL wrote, reviewed and edited the manuscript, analyzed and interpretated the data, and processed the images. ZC was involved in conceptualization, writing, reviewing and editing, and data collection and processing. YZ and LL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Bengbu Medical College (approval no. [2023]245). All research participants or their legal representatives provided written informed consent. All methods were conducted in accordance with The Declaration of Helsinki (37).

Patient consent for publication

All research participants or their legal representatives provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74: 229-263, 2024.
2. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, Bonaventure A, Valkov M, Johnson CJ, Estève J, *et al*: Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): Analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet 391: 1023-1075, 2018.
3. Bray F, Laversanne M, Weiderpass E and Soerjomataram I: The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer 127: 3029-3030, 2021.
4. Siegel RL, Miller KD, Wagle NS and Jemal A: Cancer statistics, 2023. CA Cancer J Clin 73: 17-48, 2023.
5. Miao ZF, Chen H, WangZN, Jia-Fu JI, Liang H, Xu HM and Wang J: Progress and remaining challenges in comprehensive gastric cancer treatment. Holistic Integrative Oncology 1: 2731-4529, 2022.
6. Lairson LL, Henrissat B, Davies GJ and Withers SG: Glycosyltransferases: Structures, functions, and mechanisms. Annu Rev Biochem 77: 521-555, 2008.
7. Lee TV, Sethi MK, Leonardi J, Rana NA, Buettner FF, Haltiwanger RS, Bakker H and Jafar-Nejad H: Negative regulation of notch signaling by xylose. PLoS Genet 9: e1003547, 2013.
8. Sethi MK, Buettner FFR, Krylov VB, Takeuchi H, Nifantiev NE, Haltiwanger RS, Gerardy-Schahn R and Bakker H: Identification of glycosyltransferase 8 family members as xylosyltransferases acting on O-glucosylated notch epidermal growth factor repeats. J Biol Chem 285: 1582-1586, 2010.
9. Shi Q, Xue C, Zeng Y, Yuan X, Chu Q, Jiang S, Wang J, Zhang Y, Zhu D and Li L: Notch signaling pathway in cancer: From mechanistic insights to targeted therapies. Signal Transduct Target Ther 27: 128, 2024.
10. Bakker H and Gerardy-Schahn R: A sweet development in Notch regulation. J Biol Chem 292: 15974-15975, 2017.
11. Wharton KA, Johansen KM, Xu T and Artavanis-Tsakonas S: Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats. Cell 43: 567-581, 1985.
12. Blavier L, Lazaryev A, Shi X-H, Dorey FJ, Shackleford GM and DeClerck YA: Stromelysin-1 (MMP-3) is a target and a regulator of Wnt1-induced epithelial-mesenchymal transition (EMT). Cancer Biol Ther 10: 198-208, 2010.

13. Chen T, You Y, Jiang H and Wang ZZ: Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. J Cell Physiol 232: 3261-3272, 2017.
14. Kim DH, Xing T, Yang Z, Dudek R, Lu Q and Chen YH: Epithelial mesenchymal transition in embryonic development, tissue repair and cancer: A comprehensive overview. J Clin Med 7: 1, 2017.
15. Amack JD: Cellular dynamics of EMT: Lessons from live in vivo imaging of embryonic development. Cell Commun Signal 19: 79, 2021.
16. Urata Y, Saiki W, Tsukamoto Y, Sago H, Hibi H, Okajima T and Takeuchi H: Xylosyl Extension of O-Glucose glycans on the extracellular domain of NOTCH1 and NOTCH2 regulates notch cell surface trafficking. Cells 9: 1220, 2020.
17. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR and Winchester DP: The eighth edition AJCC Cancer Staging Manual: Continuing to build a bridge from a Population-based to a more 'personalized' approach to cancer staging. CA Cancer J Clin 67: 93-99, 2017.
18. Weiser TG, Haynes AB, Molina G, Lipsitz SR, Esquivel MM, Uribe-Leitz T, Fu R, Azad T, Chao TE, Berry WR and Gawande AA: Size and distribution of the global volume of surgery in 2012. Bull World Health Organ 94: 201-209F, 2016.
19. Cisło M, Filip AA, Arnold Offerhaus GJ, Ciseł B, Rawicz-Pruszyński K, Skierucha M and Polkowski WP: Distinct molecular subtypes of gastric cancer: From Laurén to molecular pathology. Oncotarget 9: 19427-19442, 2018.
20. Hancock CN, Kent L and McClure BA: The stylar 120 kDa glycoprotein is required for S-specific pollen rejection in Nicotiana. Plant J 43: 716-723, 2005.
21. Josè-Estanyol M and Puigdomènech P: Plant cell wall glycoproteins and their genes. Plant Physiol Biochem 38: 97-108, 2000.
22. Pearce G, Siems WF, Bhattacharya R, Chen YC and Ryan CA: Three hydroxyproline-rich glycopeptides derived from a single petunia polyprotein precursor activate defensin I, a pathogen defense response gene. J Biol Chem 282: 17777-17784, 2007.
23. Tan Z, Lu W, Li X, Yang G, Guo J, Yu H, Li Z and Guan F: Altered N-Glycan expression profile in epithelial-to-mesenchymal transition of NMuMG cells revealed by an integrated strategy using mass spectrometry and glycogene and lectin microarray analysis. J Proteome Res 13: 2783-2795, 2014.
24. Fuster MM and Esko JD: The sweet and sour of cancer: Glycans as novel therapeutic targets. Nat Rev Cancer 5: 526-542, 2005.
25. Munkley J and Elliott DJ: Hallmarks of glycosylation in cancer. Oncotarget 7: 35478-35489, 2016.
26. Pinho SS and Reis CA: Glycosylation in cancer: Mechanisms and clinical implications. Nat Rev Cancer 15: 540-555, 2015.
27. Zhao Y, Hu S, Zhang J, Cai Z, Wang S, Liu M, Dai J and Gao Y: Glucoside xylosyltransferase 2 as a diagnostic and prognostic marker in gastric cancer via comprehensive analysis. Bioengineered 12: 5641-5654, 2021.
28. Wu Y and Liao Q: MicroRNA-204-5p hampers the malignant progression of clear cell renal cell carcinoma through GXYLT2 downregulation. Kidney Blood Press Res 47: 654-663, 2022.
29. Zhou ZH, Wang QL, Mao LH, Li XQ, Liu P, Song JW, Liu X, Xu F, Lei J and He S: Chromatin accessibility changes are associated with enhanced growth and liver metastasis capacity of Acid-adapted colorectal cancer cells. Cell Cycle 18: 511-522, 2019.
30. Rana NA, Nita-Lazar A, Takeuchi H, Kakuda S, Luther KB and Haltiwanger RS: O-glucose trisaccharide is present at high but variable stoichiometry at multiple sites on mouse Notch1. J Biol Chem 286: 31623-31637, 2011.
31. Dongre A and Weinberg RA: New insights into the mechanisms of Epithelial-mesenchymal transition and implications for cancer. Nat Rev Mol Cell Biol 20: 69-84, 2019.
32. Cui Q, Xing J, Gu Y, Nan X, Ma W, Chen Y and Zhao H: GXYLT2 accelerates cell growth and migration by regulating the Notch pathway in human cancer cells. Exp Cell Res 376: 1-10, 2019.
33. Song YB, Bao WG, Liu DH, Wei LQ, Yang ST, Miao XJ, Lin CY, Li HJ, Lan D and He HM: Pan-cancer analysis of the prognostic significance and oncogenic role of GXYLT2. Medicine (Baltimore) 102: e35664, 2023.
34. McNamara MG, Jacobs T, Lamarca A, Hubner RA, Valle JW and Amir E: Impact of high tumor mutational burden in solid tumors and challenges for biomarker application. Cancer Treat Rev 89: 102084, 2020.
35. Palmeri M, Mehnert J, Silk AW, Jabbour SK, Ganesan S, Popli P, Riedlinger G, Stephenson R, de Meritens AB, Leiser A, *et al*: Real-world application of tumor mutational burden-high (TMB-high) and microsatellite instability (MSI) confirms their utility as immunotherapy biomarkers. ESMO Open 7: 100336, 2022.
36. Picard E, Verschoor CP, Ma GW and Pawelec G: Relationships between immune landscapes, genetic subtypes and responses to immunotherapy in colorectal cancer. Front Immunol 11: 369, 2020.
37. World Medical Association: World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. Nov 310: 2191-2194, 2013.

Copyright © 2024 Zhao et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.