

# $\alpha$ 2,3 sialic acid processing enzymes expression in gastric cancer tissues reveals that ST3Gal3 but not Neu3 are associated with Lauren's classification, angiolymphatic invasion and histological grade

Michael W.L. Quirino,<sup>1\*</sup> Amanda P.B. Albuquerque,<sup>1\*</sup> Maria F.D. de Souza,<sup>1</sup> Antônio F. da Silva Filho,<sup>1</sup> Mário R. Martins,<sup>2</sup> Maira G. da Rocha Pitta,<sup>1</sup> Michelly C. Pereira,<sup>1</sup> Moacyr J.B. de Melo Rêgo<sup>1</sup>

<sup>1</sup>Laboratory of Immunomodulation and New Therapeutical Approaches, Research Centre for Therapeutic Innovation Suely Galdino (NUPIT-SG), Federal University of Pernambuco, Recife, PE

<sup>2</sup>Pernambuco Cancer Hospital-HCP, Recife, PE, Brazil

\*These authors contributed equally

## ABSTRACT

Gastric cancer (GC) is one of the leading causes of cancer-related deaths worldwide. Despite progress in the last decades, there are still no reliable biomarkers for the diagnosis of and prognosis for GC. Aberrant sialylation is a widespread critical event in the development of GC. Neuraminidases (Neu) and sialyltransferases (STs) regulate the ablation and addition of sialic acid during glycoconjugates biosynthesis, and they are a considerable source of biomarkers in various cancers. This study retrospectively characterized Neu3 and ST3Gal3 expression by immunohistochemistry in 71 paraffin-embedded GC tissue specimens and analyzed the relationship between their expression and the clinicopathological parameters. Neu3 expression was markedly increased in GC tissues compared with non-tumoral tissues ( $p < 0.0001$ ). Intratumoral ST3Gal3 staining was significantly associated with intestinal subtype ( $p = 0.0042$ ) and was negatively associated with angiolymphatic invasion ( $p = 0.0002$ ) and higher histological grade G3 ( $p = 0.0066$ ). Multivariate analysis revealed that ST3Gal3 positivity is able to predict Lauren's classification. No associations were found between Neu3 staining and clinical parameters. The *in silico* analysis of mRNA expression in GC validation cohorts corroborates the significant ST3Gal3 association with higher histological grade observed in our study. These findings suggest that ST3Gal3 expression may be an indicator for aggressiveness of primary GC.

**Key words:** Neuraminidase 3; primary gastric cancer; ST3 beta-galactoside alpha-2,3-sialyltransferase 3.

**Correspondence:** Moacyr Jesus Barreto de Melo Rêgo, Laboratory of Immunomodulation and New Therapeutical Approaches, Institute/University/Hospital: Research Centre for Therapeutic Innovation Suely Galdino (NUPIT-SG), Federal University of Pernambuco, Av. Prof. Moraes Rego 1235, Cidade Universitária, Recife, PE 50670-901, Brazil. E-mail: moacyr.rego@ufpe.br

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## Introduction

Gastric cancer (GC) is the sixth most common cancer worldwide.<sup>1</sup> The average five-year survival rate for advanced-stage patients is only 20%, and it is accompanied by tumor metastasis and drug resistance.<sup>2,3</sup> Despite progress in the last decades, factors such as late diagnosis, high molecular heterogeneity, and the absence of reliable biomarkers used in clinical practice for the prediction of patient outcome make GC the third leading cause of cancer-related deaths worldwide.<sup>1,4,5</sup>

Recent data to evidence promising new biomarkers in GC with basis on their clinical implications, diagnostic methods, and the efficacy of targeted agents. Among these, circRNAs and lncRNAs are suggested as minimally invasive biomarkers capable of acting in the diagnosis and prediction for disease-free and overall survival for GC patients.<sup>6-9</sup> Regarding the treatment effectiveness and usefulness prognostic, BMF, HAS2, SHB, AREG, EREG and HBEGF genes are suggested as predictive markers for response to anti-HER therapies,<sup>10</sup> while ITGAL, HLA-E and GLP2R expression are considered as poor prognostic biomarker for GC patients.<sup>11-13</sup>

Glycosylation modifications are usually associated with a poor prognosis in many cancer types.<sup>14-18</sup> In addition, the abnormal expression and activity of glycosyltransferases and glycosidase enzymes has been consistently linked to dismal prognosis in cancer patients.<sup>18-21</sup> In GC, the overexpression of sialylated glycans has been associated with tyrosine kinases hyperactivation receptors, resulting in pro-invasive phenotypes, chemoresistance, more aggressive tumors, and poor patient prognoses.<sup>22-26</sup> In the context of GC, the remarkable match between the transcriptomic profile of cancer-relevant glycosyltransferase-coding genes and the expression of their respective glycan products makes the analysis of the expression of glycosyltransferases and glycosidases an important path towards the discovery of new biomarkers.<sup>27</sup>

Sialyltransferases, known to catalyze the transfer of sialic acid residues to the oligosaccharide side chain of the glycoconjugates, have specific expression patterns in different cells and tissues, as well as differences in substrate specificities and types of linkages formed.<sup>28,29</sup> Among the 20 sialyltransferases described to date, ST3Gal3, 4, and 6 have been connected to sialyl-Lewis antigen (sLe) formation during malignant transformation.<sup>30,31</sup> In GC, there is a remarkable match between the transcriptomic profile of ST3Gal3 and ST3Gal4 genes and the expression of sialylated versions of the Lewis antigens, a fact that is related to the malignant phenotype of GC cells.<sup>23,27,30,32</sup>

Unlike sialyltransferases, the neuraminidases (Neu) - also known as sialidases - cleave sialic acid residues from glycol-conjugates and are also associated with cancer progression.<sup>33,34</sup> Among the four different mammalian sialidases (Neu1-Neu4) identified to date,<sup>35,36</sup> Neu 1, 2, and 4 are down-regulated, while Neu3 is significantly up-regulated in many human cancers such as colon, renal, prostate, and ovarian tumors.<sup>37-40</sup> Particularly by modifying the cellular ganglioside composition, Neu3 regulates different physiological phenomena such as proliferation, apoptosis, and tumor transformation.<sup>27,40</sup> Ganglioside expression is higher in GC than in non-cancerous corresponding tissues, and its staining has been associated with an augmented tumor infiltration, presence of distant metastasis, and reduction in the patient's overall survival rate after the tumor resection.

Taking into account that the irregular expression of alpha2,3-sialylation-related enzymes is a pathway implicated in GC development and chemoresistance,<sup>26,27,41</sup> analyzing these changes may be a significant means of recognizing more specific phenotype markers and therapeutic targets in order to better enhance the diagnosis and treatment of the disease. In this work, we characterized for the first time the immunostaining of Neu3 and ST3Gal3 in gas-

tric adenocarcinoma biopsies, and we also evaluated the association between the immunostaining pattern and clinicopathological features of patients.

## Materials and Methods

### Patients and samples

Biopsies previously fixed in buffered formalin and embedded in paraffin were obtained from 71 patients diagnosed with gastric adenocarcinoma who underwent surgical resection, from 2013-2016 years at the Pernambuco Cancer Hospital (HCP). Clinicopathological data, such as age, sex, lymph node involvement, histological grade, Lauren's classification, nodal status, *H. pylori* infection, surgical staging, radiotherapy, as well as relapse and outcome parameters (overall survival and disease-free survival) it was collected in medical charts (Supplementary Table 1). The flow of participants through the study is described in STARD diagram (Supplementary Figure 1).

### Immunohistochemistry

To evaluate of ST3Gal3 and Neu3 expression we followed the methods described for de Souza *et al.*<sup>42</sup> Briefly, biopsy slices were deparaffinized with xylol and rehydrated in graded ethanol. Antigen retrieval was done using citrate buffer in microwave for 15 min. Endogenous peroxidase blocker was performed with 3% hydrogen peroxide for 30 min at room temperature, followed by blocking the nonspecific binding with 1% phosphate-buffered saline for 30 min at room temperature. The sections were then incubated with rabbit polyclonal antibodies against human ST3Gal3 and Neu3 (CUSABIO, dilution 1: 100) at 8°C overnight. Next, sections were incubated with the amplification system (Easylink On, ImmPRESS™, and DAKO EnVision™) at 25°C for 1h and the reaction was visualized with diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO, USA). Nuclei was counterstained with Mayer's hematoxylin and specimens were dehydrated in graded alcohol and mounted. The positive control used was colon and prostate cancer tissues according to the antibody manufacturer's designation (Cusabio Technology LLC, Houston, TX, USA). Negative controls were produced in the samples by omitting the primary antibodies (Supplementary Figure 2).

### Image analysis

Histomorphological analysis considered the enzyme staining site (cytoplasmic, membrane, perinuclear and nuclear). We analyzed the entire representative extension of the histological slide, considered positive when more than 10% of tumor cells were stained in different degrees of intensity.<sup>36</sup> Semi-quantitative analysis of the stained cells was done using immunoreactive score (IRS) classification by analyzing 5 random fields in each slide. The score evaluation was done by two independent evaluators through the analysis of images at 200x magnification, and the results expressed as negative, weak, intermediate and strong staining. Samples with neoplastic cells staining less than 10% were denoted as negative. Analysis was performed in an integrated image system (BIOPTICA B20) microscope coupled to a CMOS camera (2584x1936 pixels resolution) with ISCapture image capture software. Expression profile was correlated with clinical-histopathological and outcome parameters.

### In silico analysis of validation cohorts

The validation cohort analyzed in this study was extracted from the cBioPortal PC genomic ([www.cbioportal.org](http://www.cbioportal.org)).<sup>43</sup> Data from mRNA expression in Stomach Adenocarcinoma was obtained

from TCGA Provisional, TCGA Nature and TCGA PanCancer cohorts, comprising for 415, 265 and 412 patients, respectively. Briefly, value normalized of ST3GAL3 and NEU3 mRNA expression was compared with clinical-pathological data (age, sex, lymph node involvement, histological grade, Lauren's classification, nodal status, *H. Pylori* infection, surgical staging, radiotherapy and relapse) and with outcome parameters (overall survival and disease-free survival). Statistical association was performed using the Fisher's Exact Test and Kaplan-Meier curves with long-rank test using GraphPad Prism version 7.0.

### Statistical analysis

Fisher's exact test was performed in GraphPad Prism version 7.0. A p-value  $\leq 0.05$  was considered statistically significant. Analysis of outcome was evaluated through Kaplan-Meier curves with a long-rank test. Multivariate logistic regression analysis it was performed using STATA9.1, with stepwise forward selection

## Results

### Neu3 expression it is associated with malignant transformation in GC

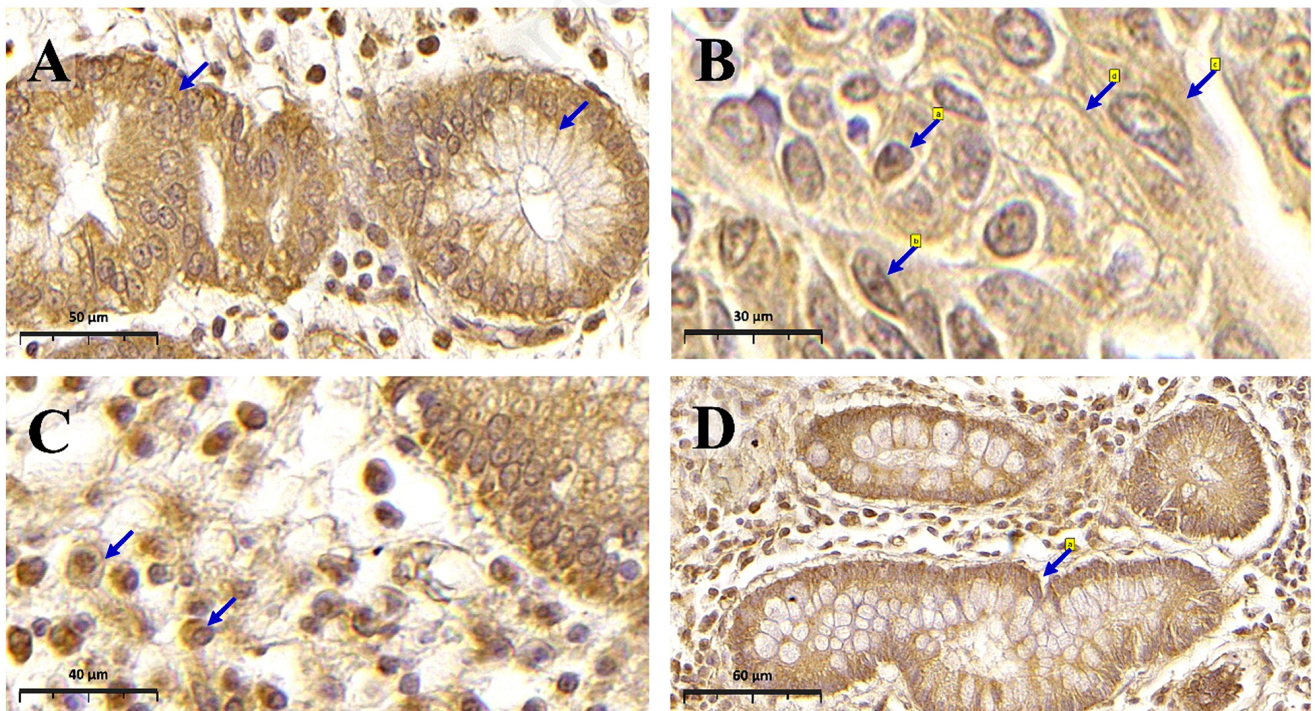
In order to characterize the expression in GC, we evaluated Neu3 expression through immunohistochemistry in tumors and normal adjacent gastric tissues from 71 patients. As shown in Figure 1, Neu3 immunoreactivity was observed in 66 gastric adenocarcinoma samples, with staining predominantly located in the cytoplasmic region in 34 samples (51.51%) (Figure 1A) and different profile combinations such as membranous, cytoplasmic, perinuclear,

and nuclear staining in 32 samples (48.48%) (Figure 1B). Sporadic positive staining on the stromal cells was also observed (Figure 1C). Furthermore, 15 areas of metaplasia were found in the specimens evaluated; in all of them the neoplastic tissue was also positive for Neu3 (Figure 1D), and 35 samples showed areas corresponding to normal tissue, in which only 12 were Neu3 positive. All normal Neu3-positive areas were in samples with positive neoplastic counterparts; the opposite did not happen. Compared with higher staining in gastric cells, Neu3 expression significantly decreased in metaplasias and normal mucosa tissues ( $p < 0.0001$ ) (Table 1). Collectively, these observations showed that Neu3 expression is increased in GC tissues compared with non-tumoral tissues. Association analyses revealed no significance differences between Neu3 expression and the clinicopathological parameters evaluated (Table 2).

No associations were found between Neu3 staining and overall survival. Additionally, in agreement with our immunohistochemistry results, there was no significant association between NEU3 mRNA expression and clinicopathological parameters, as shown in the validation cohort analysis (Table 3).

### ST3Gal3 expression is associated with angiolymphatic invasion, histological grade and Lauren's classification in GC

ST3Gal3 immunostaining was observed in cytoplasmic (17 samples, 94.44%) and membrane (1 sample, 5.56%) regions on GC cells (Figure 2A). In metaplasia areas, ST3Gal3 staining was positive in 20 samples (28.16%), including 7 samples (9.86%) with cytoplasmic staining (Figure 2B). Also, ST3Gal3 positivity was observed in 8 samples (22.87%) with areas corresponding to normal tissue; in two of them, the neoplasm was positive. Ducts and producer (faveolar) cells were positive in normal counterpart.



**Figure 1.** Immunohistochemistry of Neu3 on gastric adenocarcinomas. A) Neu3 staining predominantly located in the cytoplasmic region (blue arrows). B) Neu3 expression in membranous, cytoplasmic, perinuclear and nuclear localizations (blue arrows). C) Positive staining on the stromal cells (blue arrows). D) Metaplasia area positive for Neu3.

There was no significant difference in ST3Gal3 positivity among tumor tissues, metaplasia, and normal mucosa (Supplementary Table 2).

Association analysis between expression and clinicopathological parameters of GC patients revealed a positive association between ST3Gal3 expression and intestinal subtype Lauren's classification ( $p=0.0042$ ). As shown in Table 4, ST3Gal3 was negatively associated with angiolymphatic invasion ( $p=0.0002$ ) and

with higher histological grade G3 ( $p=0.0066$ ). Multivariate analysis confirmed that the positivity to ST3Gal3 was able to predict Lauren's classification (Table 5). Despite the ST3Gal3 expression indicating greater survival, there was no significant association (Supplementary Figure 3).

ST3GAL3 mRNA expression analysis in validation cohorts composed of gastric adenocarcinoma patients revealed a significant association of its expression in patients older than 60 years

**Table 1. Paired comparison of Neu3 staining in non-tumoral, neoplastic cells and metaplasia adjacent gastric tissue.**

	Non-tumoral	Neoplastic	Metaplasia	p-value
NEU3 <sup>(+)</sup>	10	66	20	<0.0001
NEU3 <sup>(-)</sup>	20	5	0	<0.0001

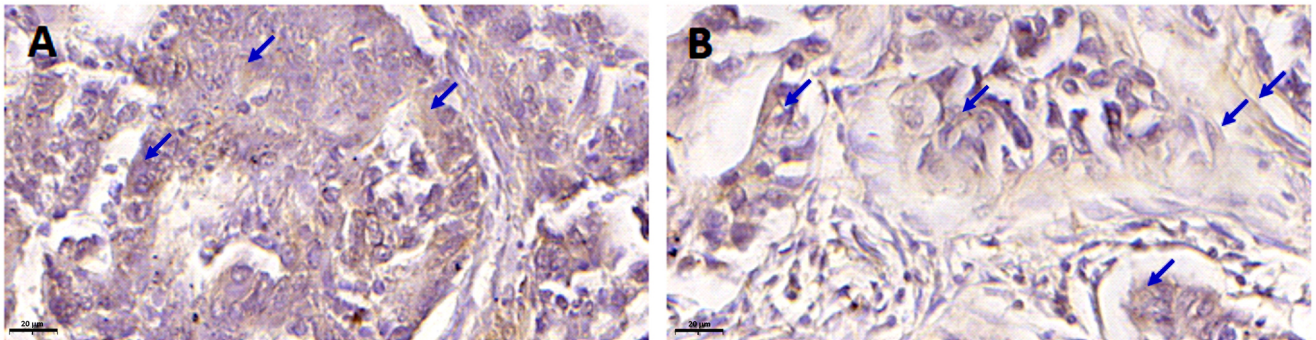
**Table 2. Association analysis of Neu3 expression with clinicopathological parameters of gastric cancer patients.**

Clinicopathological parameters	NEU3 <sup>(+)</sup> n (%)	NEU3 <sup>(-)</sup> n (%)	p Neu3
Age (years)			
≥60	33 (46.48)	3 (4.23)	>0.9999
<60	33 (46.48)	2 (2.82)	
Sex			
Female	21 (29.58)	3 (4.23)	0.3275
Male	45 (63.38)	2 (2.82)	
Surgery			>0.9999
Total gastrectomy	30 (42.25)	2 (2.82)	
Partial gastrectomy	36 (50.70)	3 (4.23)	
Neoadjuvant treatment			>0.9999
I	61 (85.92)	5 (7.04)	
III	5 (7.04)	0 (0.00)	
Surgical staging (TNM)			0.6049
(I and II)	17 (23.94)	2 (2.82)	
(III and IV)	49 (69.01)	3 (4.23)	
Lymph node involvement			0.3369
Yes	44 (61.97)	2 (2.82)	
No	22 (30.99)	3 (4.23)	
Histological grade			0.0539
GI + GII	35 (49.30)	0 (0.00)	
GIII	31 (43.66)	5 (7.04)	
Chemotherapy			0.6518
Yes	37 (52.11)	2 (2.82)	
No	29 (40.85)	3 (4.23)	
Radiotherapy			>0.9999
Yes	21 (29.58)	1 (1.41)	
No	45 (63.38)	4 (5.63)	
Recurrence			0.5809
Yes	16 (22.54)	0 (0.00)	
No	50 (70.42)	5 (7.04)	
Lauren's classification			>0.9999
Intestinal	32 (47.06%)	2 (2.94%)	
Diffuse	31 (45.59%)	3 (4.41%)	
Angiolymphatic invasion			0.0666
Detected	29 (50.00%)	0 (0.00%)	
Not detected	34 (42.65%)	5 (7.46%)	
<i>H. pylori</i> infection			>0.9999
Yes	9 (13.94%)	0 (0.00%)	
No	52 (78.79%)	5 (7.35%)	

Lauren's classification, N-68; Angiolymphatic invasion, N-67; *H. pylori* infection N-66.

(TCGA Nature:  $p=0.0045$ ; TCGA PanCancer:  $p=0.0003$ ; TCGA Provisional:  $p<0.0001$ ), with nodal invasion where it was less expressed in patients who had cancer-positive lymphnodes ( $p=0.00486$ ), with histological grade where ST3Gal3 expression increased in samples with higher grade G3 ( $p<0.0001$ ), and with

intestinal subtype Lauren's classification ( $p<0.0001$ ) (Table 6). In summary, these results showed that ST3Gal3 expression is significantly associated with angiolymphatic invasion, histological grade, and Lauren's classification in GC.



**Figure 2.** Immunohistochemistry of ST3Gal3 on gastric adenocarcinomas. A) ST3Gal3 staining located in the membrane and cytoplasmic regions (blue arrows). B) ST3Gal3 staining negative (blue arrows).

**Table 3.** Association analysis of NEU3 expression with clinicopathological features of gastric cancer patients *in silico* study.

Clinical data	TCGA Nature n=293			TCGA PanCancer n=412			TCGA Provisional n=415		
	NEU3(+) n (%)	NEU3(-) n (%)	p	NEU3(+) n (%)	NEU3(-) n (%)	p n (%)	NEU3(+) n (%)	NEU3(-) n (%)	p n (%)
Age (years)									
<60	36(13.74)	43(16.41)	0.0045	64(15.69)	58(14.22)	0.2793	58(13.98)	64(15.42)	>0.9999
≥60	88(33.59)	95(36.26)		132(32.35)	154(37.7)		138(33.25)	155(37.3)	
Sex									
Female	47(17.74)	55(20.75)	0.7049	71(17.23)	74(17.96)	0.6060	72(17.35)	75(18.07)	0.6087
Male	80(30.19)	83(31.32)		123(29.85)	124(34.9)		124(29.88)	144(34.7)	
Surgical staging (TNM)									
(I and II)			-			0.4785			0.3101
(III and IV)	-	-		79(20.05)	101(25.6)		78(20.00)	102(26.1)	
			102(25.89)	112(28.4)	102(26.15)	108(27.6)			
Nodal invasion									
0	-	-	-	9(2.28)	16(4.06)	0.3037	45(12.64)	61(17.13)	0.3528
>1				176(44.67)	193(48.9)		121(33.99)	129(36.2)	
Angiolymphatic invasion									
Not detected	-	-	-	-	-	-	-	-	-
Detected									
Histological grade									
GI + GII	-	-	-	-	-	-	69(17.00)	91(22.41)	0.2226
GIII				122(30.05)	124(30.5)		124(30.5)		
Lauren's classification									
Intestinal	88(34.64)	85(33.46)	0.2230			-			-
Diffuse	25(9.84)	40(15.74)		-	-		-	-	
Mixed	7(2.75)	9(3.54)							
Radiotherapy									
Yes	-	-	-	32(8.58)	39(10.46)	>0.9999	19(9.27)	29(14.15)	>0.9999
No				138(37.00)	164(43.9)		61(29.76)	96(46.83)	
Relapse									
Yes	-	-	-	-	-	-	-	-	-
No									
<i>H. pylori</i> infection									
Yes	-	-	-	-	-	-	7(3.95)	13(7.34)	0.4791
No							70(39.55)	87(49.15)	

## Discussion

Aberrant sialylation in glycoconjugates is a characteristic feature of malignancy.<sup>27</sup> Human sialidases have been implicated in cancer progression.<sup>34,44</sup> In this scenario, Neu3 expression is markedly upregulated in different human tumors when compared to non-tumor tissues, a fact that evidences its potential utility as a diagnosis biomarker.<sup>39,45-47</sup> Additionally, this increased Neu3 expression is associated with migration, invasion, tumor progression, and therapeutic resistance in many cancer types.<sup>44,48,49</sup>

In the present study, no associations were observed between Neu3 expression and clinical-pathological parameters, disease-free progression, or overall survival. So far, there are no studies linking significant Neu3 expression with survival outcomes. The immunohistochemistry analyses of Neu3 expression in 71 patients with clear cell adenocarcinoma of the ovary showed no significant difference in survival outcomes.<sup>30</sup> Other studies revealed that

increased NEU3 mRNA expression in ovarian, prostate, colorectal, and GC samples was not significantly correlated with clinicopathological parameters.<sup>4</sup> Even so, tissue availability and clinical-related information, as well as the state of conservation, were some of the factors limiting the sample size during the work period. Considering this context, we performed an *in silico* analysis to corroborate our findings. As expected, there was no significant association between NEU3 mRNA expression and clinicopathological data of validation cohorts in this evaluation.

Recently, many scientific papers have demonstrated the involvement of Neu3 in oncogenic transformation mediated by EGFR.<sup>40,47,50-52</sup> EGFR is overexpressed in 27-64% of gastric tumors; it is well known that EGRF signaling is directly associated with chemoresistance in this cancer type.<sup>53-56</sup> However, as enzyme activity is highly context-dependent, which severely limits the extrapolation of relevant findings from one pathological setting to another,<sup>18</sup> the detailed mechanism and consequences of Neu3 expression in malignant transformation of GC requires further elu-

**Table 4. Association analysis of ST3Gal3 expression with clinicopathological features of gastric cancer patients.**

Clinical and pathological parameters	ST3GAL3 <sup>(+)</sup> n (%)	ST3GAL3 <sup>(-)</sup> n (%)	p
Age (years)			
≥60	11 (15.49)	25 (35.21)	0.4148
<60	7 (9.86)	28 (39.44)	
Sex			
Female	4 (5.63)	20 (19.72)	0.2647
Male	14 (28.17)	33 (46.48)	
Surgery			>0.9999
Total gastrectomy	8 (11.27)	24 (33.80)	
Partial gastrectomy	10 (14.08)	29 (40.85)	
Initial treatment			>0.9999
I	17 (23.94)	49 (69.01)	
III	1 (1.41)	4 (5.63)	
Surgical staging (TNM)			
I and II	2 (2.82)	16 (22.54)	0.1297
III and IV	16 (22.54)	37 (52.11)	
Lymph node involvement			
Yes	13 (18.31)	33 (46.48)	0.5719
No	5 (7.04)	20 (28.17)	
Histological grade			
GI + GII	14 (19.72)	21 (29.58)	0.0066
GIII	4 (5.63)	32 (45.07)	
Chemotherapy			>0.9999
Yes	10 (14.08)	28 (39.44)	
No	8 (11.27)	25 (35.21)	
Radiotherapy			
Yes	8 (11.27)	13 (18.31)	0.1389
No	10 (14.08)	40 (56.34)	
Recurrence			
Yes	3 (4.23)	15 (21.13)	0.7450
No	13 (12.31)	40 (56.34)	
Lauren's classification			
Intestinal	14(20.59%)	20 (29.41%)	0.0042
Diffuse	3(4.41%)	31 (45.59%)	
Angiolymphatic invasion			
Detected	9(13.64%)	20(29.85)	0.0002
Not detected	8(12.12%)	30(44.78%)	
<i>H. pylori</i> infection			
Yes	1(1.56%)	7(10.49%)	0.6673
No	15(23.44%)	41(64.06%)	

cidation. In addition, a better understanding of the molecular mechanism that regulates NEU3 gene expression and enzyme activity could be important for developing novel, targeted treatments for GC.

An increase in sialic acid moieties on the cell surface is a shared characteristic of many tumors.<sup>57-5</sup> In GC, this increased cell sialylation is considered to be a potential mechanism for invasive phenotypes and differential efficacy of targeted therapy.<sup>26,6</sup> Previous work has disclosed that an increase of  $\alpha$ 2,3-sialylation by high expression of ST3Gal4 leads to SLe<sup>x</sup> expression and induces c-Met activation, invasive phenotypes, and higher therapeutic

resistance in GC.<sup>23,2</sup> Additionally, the overexpression of ST3Gal4 is associated with MET and RON signaling activation, which are frequently altered in GC, leading to a pro-invasive phenotype.<sup>23,24,61</sup>

In our study, we found an association between the absence of ST3Gal3 and no angiolymphatic invasion, and also with a higher histological grade. In GC patients, stages II-IV are associated with high recurrence rates, ranging from 25% to 40%, and with metastatic cases not amenable to re-resection.<sup>62-64</sup> Our *in-silico* analysis of ST3GAL3 mRNA expression corroborated the negative association with higher histological grade of gastric adenocarcinoma

**Table 5.** Univariate and multivariate regression analysis of Lauren classification in gastric cancer patients.

Variable	OR	Univariate			p	OR	Multivariate		
		95%	CI	p			95%	CI	p
ST3Gal3	7.23	1.84	28.4	0.005	0.14	0.03	0.57	0.006	
Chirurgical stage	1.00	0.34	2.92	1.000	1.08	0.61	1.91	0.777	
Age	1.25	0.49	3.21	0.633	1.14	0.40	3.23	0.804	
Gender	0.46	0.16	1.26	0.134	0.57	0.19	1.73	0.328	

**Table 6.** Association analysis of ST3GAL3 expression with clinicopathological parameters of gastric cancer patients in-silico study.

Clinical data	TCGA Nature n=293		p	TCGA PanCancer n=412		p	TCGA Provisional n=415	
	ST3GAL3 (+) n (%)	ST3GAL3 (-) n (%)		ST3GAL3 (+) n (%)	ST3GAL3 (-) n (%)		ST3GAL3 (+) n (%)	ST3GAL3 (-) n (%)
Age (years)								
<60	53(20.23)	130(49.62)	0.0045	78(19.12)	208(50.9)	0.0003	78(18.89)	219(53.0)
≥60	38(14.50)	41(15.65)		56(13.73)	66(16.18)		56(13.56)	60(14.53)
Sex								
Female	31 (11.70)	71 (29.79)	0.289	44(10.68)	101(24.5)	0.5098	44(10.60)	103(24.8)
Male	61 (23.02)	102 (38.49)		91(22.09)	176(42.7)		91(21.93)	177(42.6)
Surgical Staging (TNM) (I and II) (III and IV)	-	-	-	56(14.21)	124(31.4)	0.6659	56(14.36)	124(31.7)
			72(18.27)	142(36.0)	69(17.69)		141(36.1)	
Nodal invasion								
0	-	-	-	13(3.30)	12(3.05)	0.0486	31(8.54)	75(20.66)
>1				118(29.95)	251(63.7)		83(22.87)	174(47.9)
Angiolymphatic invasion								
No detected	-	-	-	-	-	-	-	-
Detected								
Histological grade								
GI + GII	-	-	-	-	-	-	31(7.64)	129(31.7)
GIII							99(24.38)	147(36.2)
Lauren's classification								
Intestinal	38(14.96)	135(53.14)	<0.0001	-	-	-	-	-
Diffuse	45 (17.71)	20 (7.87)		-	-	-	-	-
Mixed	6 (2.36)	10 (3.93)		-	-	-	-	-
Radiotherapy								
Yes	-	-	-	20(5.36)	51(13.67)	0.2681	14(6.83)	34(16.59)
No				107(28.69)	195(52.2)		34(16.59)	123(60.0)
Relapse								
Yes	-	-	-	-	-	-	-	-
No								
<i>H. pylori</i> infection								
Yes	-	-	-	-	-	-	5(2.82)	15(8.47)
No							31(17.51)	126(71.1)

observed in immunohistochemistry findings and evidence that molecules negatively related to higher histological grades have potential utility as indicators of good tumor phenotype. However, further studies addressing this hypothesis are warranted.

GC is a heterogeneous disease, and the different molecular subtypes have been linked to distinct patterns of disease progression, prognosis, survival outcome, and recurrence patterns after surgery. Diffuse-subtype tumors with a molecular classification of mesenchymal-like type have the worst prognosis, a tendency to occur at an earlier age, and the highest recurrence frequency, while intestinal-subtype tumors with a molecular classification of microsatellite-unstable type have the best overall prognosis and the lowest frequency of recurrence (22%).<sup>65</sup> Also, in GC, aberrant sialylation has been considered a source for biomarkers with potential consequences for patient stratification, survival outcomes, and chemoresistance.<sup>57,60,66,67</sup>

To the best of our knowledge, this is the first study in which ST3Gal3 expression is significantly related and can predict Lauren's classification of intestinal subtypes in GC. However, further investigations are needed to determine whether the relationship between ST3Gal3 expression and GC subtypes is significant, particularly in identifying new prognostic and diagnostic markers as well as therapeutic targets.

Given that immunostaining in non-tumoral tissues is lower than in metaplastic and tumoral tissues, we hypothesize that the increase in Neu3 expression may be an indicator of malignant transformation in gastric carcinogenesis. Furthermore, our findings revealed an intriguing link between ST3Gal3 expression and aggressiveness, laying the groundwork for future research using cell glycosylation as a biomarker in primary GC.

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