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Signal transduction proteins in tumors from Puerto Rican and Caucasian gastric adenocarcinoma patients: expression differences with potential for specific targeted therapies

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Abstract

Gastric cancer is the second cause of cancer death with approximately 800,000 annual new cases reported worldwide. The incidence of gastric adenocarcinoma has progressively increased, while 5-year survival rates of 20% have remained unchanged for several decades. New strategies for early detection and treatment remain a significant unmet medical need. Advances in our understanding of signal transduction mechanisms have led to novel treatment approaches in a variety of tumors. Over-expression of the *HER2/NEU* gene is associated with aggressive behavior and poor prognosis in breast cancer, and this has made the Her2/neu protein a target for directed therapy. Several reports indicate that there are ethnic differences in the clinical behavior of gastric cancer, but the reasons for this observation are not clearly understood. We have investigated potential differences in the expression of selected signal transduction proteins (Her2/neu, EGF-R, Cyclin D1 and p-Stat3) in gastric cancers from 100 Puerto Rican and 95 Caucasian patients. Puerto Rican patients had higher disease stages at presentation (Stage IV disease was observed in 44% of Puerto Ricans and in 18% of Caucasians). The majority of tumors in both populations showed over-expression of p-Stat3 but there were no statistical significant differences between the two groups. Her2/neu was over-expressed in the tumors of both patient populations but at higher

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levels in Puerto Ricans (13%) than in Caucasians (8%). Importantly, we have observed a more prominent intra-tumoral heterogeneity of Her2/neu expression than what is typically observed in breast cancer. Nevertheless, targeted therapies should be considered in the treatment of gastric cancer, and clinical trials with therapies targeting the EGFR pathway warrant evaluation.

Keywords

Stomach cancer; gastric adenocarcinoma; Her-2/neu; Epidermal growth factor receptor; Stat-3; Cyclin D1

Introduction

Adenocarcinoma of the stomach is the second leading cause of cancer death worldwide with 800,000 new cases reported annually (1). In the United States, approximately 21,900 new cases and 13,500 deaths are reported every year (2). The prognosis of patients with advanced cancer continues to be poor with a median survival of 6 months. The only potentially curative treatment is complete surgical resection. Unfortunately, post-surgical recurrence occurs in over 70% of patients.

According to the 2000 US Census Bureau report (4), Puerto Ricans account for 9.7% of the Hispanic population in the United States. Migration studies (5, 6) have shown that, from 1958 to 1971, Puerto Ricans living in New York City had lower mortality and higher survival rates than Puerto Ricans who resided in Puerto Rico (PR); similar results have been reported for age-adjusted mortality rates (7, 8). At the University Hospital of Puerto Rico, the largest tertiary hospital on the island, clinical parameters, mortality and survival were studied in 394 patients with gastric adenocarcinoma (9). The male to female ratio was 2.5:1, the median age at presentation was in the seventh decade, and the most frequent presenting symptoms were weight loss, vomiting and abdominal pain. At presentation, approximately 50% of the patients had localized disease, and 33% had distant metastases. The most common surgical procedure performed was a subtotal gastrectomy. The operative mortality, within one month, was 20% and the overall five-year survival 8.4%. These results are similar to those previously reported in other Hispanic populations (10, 11).

Responses to single-agent chemotherapy are usually not durable and the use of combination chemotherapy, slightly more effective than single-agent therapy in achieving objective radiographic responses, has not led to significant advances in survival. Thus, the North Central Cancer Treatment Group (NCCTG), in a prospective study comparing treatment with a single-agent (5-fluorouracil) and three multi-agent systemic chemotherapy regimens (5-fluorouracil, doxorubicin and methyl-lomustine [FAMe]; 5-fluorouracil, doxorubicin and Cisplatin [FAP]; and FAMe alternating with triazinate), reported no survival benefits (3). It is hoped that molecular targeted agents will lead to a significant improvement in our ability to control the outcomes in the treatment of gastric cancer, as has been the case for other tumors.

Since its identification more than twenty years ago, intracellular signaling mediated by the Epidermal Growth Factor Receptor (EGF-R) has proven to play an important role in cancer-

cell proliferation, angiogenesis and metastasis (12) and be responsible in part for adverse prognosis (13). The *HER2/NEU* oncogene, on chromosome 17q, shares significant homology with the *EGF-R* gene (14). Her-2/neu testing has become standard of care in the pre-treatment evaluation of breast cancer. There is also interest in Her-2/neu expression as a prognostic factor and a potential target of therapy in tumors of the gastrointestinal tract (15). In gastric adenocarcinoma, high levels of EGF-R and Her-2/neu expression are associated with low overall survival. The reported range of Her2/neu expression varies widely, however (16–21). This may be due to differences in patient selection, since Her-2/neu over-expression appears to be more common in advanced tumors rather than in early disease. The majority of studies, however, consider Her-2/neu status of significant prognostic value in gastric cancer (22–25).

Partial clinical success with Trastuzumab (Herceptin®) in two patients whose tumors over-expressed Her-2/neu stimulated us to evaluate Her-2/neu expression in a large number of Puerto Rican patients to investigate the potential pathogenic role of the EGF-R and Her-2/neu pathways in gastric adenocarcinoma. We also analyzed the expressions of Cyclin D1, as a proliferative marker, and of phospho-Stat3 (p-Stat3), since expression of p-Stat3 correlated with that of Her2/neu in the context of a breast carcinoma clinical trial performed by our group (27). In an effort to understand potential ethnic differences at the molecular level, we compared the results obtained in Puerto Rican patients, who resided on the island, with those of a similar group of Caucasian non-Hispanic patients from the H. Lee Moffitt Cancer Center and Research Institute.

Material and Methods

Case Study # 1

A 39 year-old male was diagnosed at 14 years of age with stage IIA Hodgkin's disease. He was treated with mantle radiation and remained asymptomatic for 25 years. He then began to lose weight and experience progressive dysphagia to solids. An abdominal computerized tomographic (CT) scan revealed thickening of the gastro-esophageal (GE) junction, antrum and posterior fundus compatible with a gastric neoplasm, as well as nodular densities at the gastro-hepatic ligament and peri-pancreatic region. An upper endoscopy confirmed the presence of a mass at the GE junction and a biopsy revealed a poorly differentiated adenocarcinoma with signet-ring cell features. An FDG positron emission tomographic (PET) scan demonstrated radiotracer avidity in the stomach and multiple regional gastric nodes but a chest CT revealed no thoracic metastases. Classified as stage T3N2M1, a total gastrectomy with Roux-en-Y pouch reconstruction and D2 lymphadenectomy was performed. During surgery, extensive disease was identified at the celiac axis, gastric lesser curvature and porta hepatis. Pathologic examination revealed a poorly differentiated invasive adenocarcinoma with signet-ring features, involving the upper third, middle, and lesser curvature of the stomach (cardia, fundus and corpus). The tumor invaded the esophageal adventitia and showed vascular invasion and involvement of the surgical esophageal margins. There were metastases in 12 of 27 lymph nodes with 1/3 of the retro-pancreatic lymph nodes positive. Immunohistochemistry showed that the tumor cells were positive (2 to 3+) for Her-2/neu and focally positive for EGF-R. He received postoperative

radiation to the tumor bed to a dose of 48 Gy, delivered in 27 fractions. One month following completion of radiation therapy, chemotherapy cycles of Docetaxel, Cisplatin and 5-Fluorouracil were administered in a manner described elsewhere (56). Given the expression of Her-2/neu in tumor cells, Trastuzumab (Herceptin) (4 mg/kg i.v. loading and 2 mg/kg i.v. weekly thereafter) was administered for four months. Three years later he was recurrence-free as evidenced by normal serum carcinoembryonic antigen, negative thoracic and abdomino-pelvic CT scans, and a normal PET scan.

Case Study # 2

A 32 year-old male marathon runner, with no history of prior illness, presented with phlebitis and bilateral deep vein thrombosis of the lower extremities during training prior to a marathon competition. Abdominal and thoracic CT scans revealed bilobar hepatic lesions and thickening of the gastric fundus at the level of the gastro-esophageal junction. An endoscopy revealed a polypoid mass at the distal esophagus that extended into the EG junction and cardia. Biopsy of the mass demonstrated a well-differentiated adenocarcinoma, intestinal type. He was treated with chemotherapy (5-FU 425mg/m² i.v. and leucovorin 20mg/m² i.v., daily for five days). His course was complicated by a pulmonary embolism treated with an inferior vena cava filter. Immunohistochemistry of the tumor showed positivity (2+) for Her-2/neu. Trastuzumab (Herceptin) was administered together with cycles of Cisplatin, Docetaxel and 5-FU every four weeks. A follow-up CT scan, two months later, showed interval improvement and partial radiographic remission by RECIST criteria. The patient remained asymptomatic and was able to run a marathon the following year. Eight months later, he presented with vestibular symptoms and ataxia. An MRI revealed brain metastases that were treated with palliative external whole-brain beam radiation with a total dose of 30 Gy in ten fractions. Four months later, he developed radiographic evidence of progression of his hepatic disease. He received Trastuzumab and Paclitaxel on a weekly basis for five cycles, in addition to Cisplatin and 5-FU every four weeks, but his condition deteriorated until death due to disease progression.

Selection of cases

One hundred cases of gastric adenocarcinoma were retrospectively selected both at the Universidad de Puerto Rico in San Juan (PR) (Hispanic population), and ninety-five at the H. Lee Moffitt Cancer Center and Research Institute (MCC), Tampa (FL) (Caucasian population). Clinico-pathological information was obtained for all cases after Institutional Review Board approval at both institutions and adherence to HIPAA guidelines. From the available material, paraffin blocks containing representative areas of both tumor and adjacent normal tissue were selected. Confirmation of the diagnosis and quantification of marker signal was performed independently, in the two patient groups, by two pathologists (BC and CMC) with experience in gastro-intestinal pathology.

Immunohistochemistry (IHC)

Immunohistochemistry conditions for all markers were optimized at the MCC Pathology Core. For Her-2/neu, antigen retrieval was performed in a microwave oven with HIER-citrate buffer. The rabbit polyclonal antibody (DakoCytomation) was used at a concentration of 1:300 for 30 minutes, and detection was performed using the EnVision+ detection kit. For

EGF-R, antigen retrieval was performed with proteinase K digestion (25 ug/ml for 17 minutes). The clone 111.6 mouse monoclonal antibody (Signet Laboratories) was used at a concentration of 1:50 for 30 minutes, and detection was performed using the DakoCytomation LSAB+ Detection Kit with avidin/biotin blocking (Figure 1). For Cyclin D1, antigen retrieval was performed in a microwave oven using a HIER-EDTA buffer. The clone AM29 mouse monoclonal antibody (Zymed Laboratories) was used at a concentration of 1:100 for 30 minutes, and detection was performed using the DakoCytomation LSAB2 Detection Kit with avidin/biotin blocking. For Phospho-Stat3, antigen retrieval was performed as previously described (27) in a microwave oven with HIER-citrate buffer and mild trypsinization. The Tyr705 rabbit polyclonal antibody (Cell Signaling) was used at a concentration of 1:400 overnight, and detection was performed using the Vector Laboratories Vectastain Elite Detection Kit with avidin/biotin blocking. Her-2/neu and EGF-R were located on the membrane, and Cyclin D1 and p-Stat3 in the nucleus.

Quantification of signals

A semi-quantitative method was used. For each of the markers, the percentage of positive cells, the signal intensity, and the tissue distribution of positive cells were scored independently by two investigators with comparable results. For each slide and for each marker, signal intensity was assigned to one of the four categories already familiar to the practicing surgical pathologist: negative (0), mild (1), moderate (2) and high (3). The percentage of positive cells in each of the four intensity categories was then estimated and this percentage was then multiplied by its corresponding intensity score (0 to 3). The sum of the four products was reported as the final score and used for statistical analysis. Her-2/neu expression was also quantified according to the Dako FDA-approved method. Following the approach recommended for breast cancer, a score of 1+ was considered negative and a score of 3+ positive. All cases with a score of 2+ were analyzed by Fluorescence *in situ* Hybridization (FISH) to detect amplification of the *HER2/NEU* gene, using the PathVysion® Her-2DNA Probe Kit at USLABS (Irvine, CA).

Statistical methods

The Wilcoxon Rant Sum Test was used to identify differences between the two groups of patients with regard to clinico-pathological parameters such as degree of differentiation and tumor stage. The t-test was used to detect differences in protein expression between tumor and adjacent non-neoplastic mucosa (statistical significance is a p value lower than 0.05). The Spearman Correlation Coefficient test was used to determine if there was a correlation between protein expression in the tumor and tumor grade, tumor stage or tumor size. Finally, t-tests were used to detect differences in protein expression between the two patient groups.

Results

At the University of Puerto Rico, the tumors of two Puerto Rican patients were found to over-express Her-2/neu and these patients were treated with trastuzumab-containing regimen. The resulting partial clinical responses (see description of the cases in Material and Methods) motivated us to evaluate Her-2/neu expression in a large number of Puerto Rican patients to investigate the potential pathogenic role of the EGF-R and Her-2/neu pathways in

gastric adenocarcinoma. We also analyzed the expressions of Cyclin D1, as a marker of proliferation, and of p-Stat3, since the expression of p-Stat3 was found to correlate with that of Her-2/neu in the context of a breast carcinoma clinical trial performed by our group (27). In an effort to understand potential ethnic differences at the molecular level, we compared the results obtained in the tumors of PR patients residing in the island with those of a similar group of tumors from Caucasian non-Hispanic patients obtained from the files of the H. Lee Moffitt Cancer Center and Research Institute.

Relevant descriptive statistics are depicted in Table 1. All Puerto Rican patients were treated at the Veterans Affairs Hospital and were all male, while both genders were represented in the Caucasian group in similar percentages (56% males vs 44% females). No significant differences were found in tumor size or degrees of differentiation between the two patient groups. With regard to location within the stomach, gastroesophageal tumors were more common in Caucasians than in Puerto Ricans (31% vs 9%), while antral and body tumors (anterior and posterior aspects) were more common in Puerto Ricans (58%) than in Caucasians (36%). A statistically significant difference ($p < 0.0001$) was also observed in disease stage at presentation with 44% of Puerto Ricans and 18% of Caucasians presenting with Stage IV.

Figure 1 shows representative images of p-Stat3, Cyclin D1 and EGF-R tissue expression. Figure 2 shows representative of Her-2/neu detected by immunohistochemistry and amplification of the *HER-2/NEU* gene detected by FISH. When compared with adjacent non-neoplastic mucosa, both p-Stat3 and Cyclin D1 were over-expressed in the tumors of both populations with a $p = 0.0001$ for p-Stat3 in both groups, and with a $p = 0.0031$ in Caucasians and a $p = 0.0030$ for Puerto Ricans for Cyclin D1 (t-Test) (data not shown). Furthermore, EGF-R and Her-2/neu were not observed in the non-neoplastic mucosa in either group. The percentage of tumors positive for Her-2/neu, EGF-R, p-Stat3 and Cyclin D1 were respectively 8%, 3%, 100% and 36%, in Caucasian patients, and 13%, 15%, 87% and 42% in Puerto Rican patients (Table 2). Significant differences between the two groups were observed for Her-2/neu ($p = 0.0047$) and EGF-R ($p = 0.0077$) expression, with higher levels for both proteins in tumors from Puerto Rican patients. For Her-2/neu expression, all cases with a 2+ score by immunohistochemistry were subsequently analyzed by Fluorescence *in situ* Hybridization (FISH) at USLabs (Figure 2). When the results of both immunohistochemistry and FISH were evaluated, Her-2/neu was considered positive in 8% of Caucasians and in 13% of Puerto Ricans, a difference that is statistically significant ($p = 0.0047$). Of interest is that, among the tumors with a 2+ Her-2/neu expression by IHC (12 in the Caucasian group and 14 in the Puerto Rican group), amplification of the Her-2/neu gene was detected by FISH in 42.86% in Puerto Rican tumors but only in 6.7% of Caucasian tumors. Since the frequency of tumors with a score of 3+ by IHC was similar in both groups (3% in Caucasians and 7% in Puerto Ricans), most of the differences in Her-2/neu expression is revealed only by FISH analysis performed on 2+ tumors, highlighting the importance of detecting gene amplification prior to therapy.

Table 3 shows correlations between protein expression and clinico-pathological parameters. In the Puerto Rican group, EGF-R correlated directly with degree of differentiation, indicating higher expression of protein as tumors become poorly differentiated. Her-2/neu,

however, correlated inversely with degree of differentiation, indicating a tendency to loss of expression as tumor become more aggressive. Also, in the tumors from Puerto Rican patients, expression of p-Stat3 correlated directly with both size and disease stage (size is a criterium for stage assignment), indicating higher levels of activated Stat3 in tumors with advanced disease.

Importantly, a significant degree of intra-tumoral heterogeneity was observed in Her-2/neu and EGF-R expression (Figure 2). Some tumors were diffusively positive while others were positive in some regions and entirely negative in others with no clear relationship between the superficial and invasive components of the tumor. Of interest, however, is that Her2/neu was uniformly over-expressed in tumors with signet-ring cell morphology. Not enough lymph node metastases were available for analysis to identify any relationship in expression between the primary tumor and its metastases.

Discussion

In Western countries, the overall incidence of gastric cancer is declining while that of proximal gastric cancer is increasing (28). Ethnic variations in disease features may be in part responsible for these results. Thus, native white Americans are at low risk, while American Indians, Blacks, and Hispanics, as well as immigrants from Russia, Scandinavia, Japan, and some Latin American countries are at high risk (10, 11, 28–31). From 1958 to 1979, the incidence of gastric cancer in Puerto Rican-born residents in New York City (PR-NYC) was slightly higher than in Puerto Ricans living in Puerto Rico (PR-PR), and comparable to that of other Hispanic groups that migrated to New Mexico and Los Angeles (5–9). This rising incidence of gastric cancer in Puerto Ricans has been accompanied by a decrease in overall mortality (1, 4). Although environmental factors (32) and favorable disease biology (33–36) have been implicated to explain this trend, our study shows that the disease is more advanced in Puerto Ricans than in Caucasians at presentation. Further investigation into the epidemiology, pathogenesis, and molecular biology of gastric cancer in Hispanic patients is warranted (35–39).

One factor that may affect the geographical and ethnic variation in gastric cancer epidemiology is infection by *H. pylori*, now categorized by the International Agency for Cancer Research as a group 1 carcinogen, an agent carcinogenic to humans (40). Several reports from the United States have found the highest frequencies of gastric cancer in geographic areas and populations with the highest rates of acquisition of *H. pylori* infection (41, 42), and a high prevalence of *H. pylori* infection has also been documented in blacks and Hispanics in general (43, 44). Since a relationship between *H. pylori* infection and Her-2/neu expression and other parameters has been previously reported (41) we are currently evaluating our cases to investigate the potential role of *H. pylori* infection in gastric oncogenesis in the two ethnic groups.

Recently, the development of targeted therapies has made the identification of potential molecular targets in tumors a necessity. The *HER2/NEU* gene, located on chromosome 17q21, is related to the *v-erbB* oncogene of the avian erythroblastosis virus. It encodes a trans-membrane glycoprotein receptor with intrinsic tyrosine kinase activity homologous to

EGF-R but unable to bind EGF. Amplification of the *EGF-R* and/or the *HER2/NEU* oncogenes, and over-expression of their proteins, have been reported at various levels in gastric adenocarcinoma (15–26). In fact, correlations between Her-2/neu protein over-expression and *HER2/NEU* gene amplification have been reported (49–52), and over-expression of Her-2/neu appears to be an independent prognostic factor in gastric cancer (18, 45–48).

In our study, both Her-2/neu and EGF-R were expressed at higher levels in Puerto Ricans than in Caucasians. Furthermore, in PR patients, tumor grade correlated directly with EGF-R expression but inversely with Her-2/neu expression. This may be explained by the fact that in poorly differentiated tumors of many different types, the expression of some markers is either lost or not consistently detected while other markers remain preserved. Of interest in our study is that a specific subtype of poorly differentiated adenocarcinoma, the signet-ring cell type, consistently expressed high levels of Her-2/neu, suggesting a growth dependency on this pathway in this tumor type and a potential application of Trastuzumab in this disease known to carry a dismal prognosis.

Studies using cell lines (51, 53) have revealed a potential therapeutic use in targeting the Her-2/neu signaling pathway. *Ex vivo* analysis of our tumors, however, reveal a high degree of intra-tumor heterogeneity in Her-2/neu expression. With the exception of signet-ring cell carcinomas, where Her-2/neu tends to be diffusively and strongly expressed, a very high degree of variation in the expression of Her-2/neu is observed in most cases. Thus, within a given tumor, areas of very high Her-2/neu expression are often seen adjacent to completely negative areas, and without obvious morphological differences between the two regions. This intra-tumor heterogeneity has been previously reported (49), and may be a significant impediment in achieving successful therapeutic response, since Her-2/neu-negative tumor cells are expected to be insensitive to the target therapy and with time will repopulate the tumor.

The prominent over-expression of p-Stat3 in the vast majority of tumors of both patient populations suggests a pathogenic role of the Stat3 pathway in gastric adenocarcinoma that is worth further investigation. Since there were no observable differences between the two patient groups, Stat 3 may play a more universal pathogenetic role than Her-2/neu. Stat3 inhibitors, however, are not yet available for therapy.

Given the intra-tumoral heterogeneity and the conflicting information reported in the literature regarding correlation between Her-2/neu expression in the primary tumor and prognosis (18, 20), it is important to emphasize that evaluation of Her-2/neu expression, not only in the primary tumor but also in its metastases, may add very valuable information with regard to prognosis and therapy selection. Furthermore, given the concomitant over-expression of EGF-R identified in our cases and in previous reports (14, 54, 55), improved survival in gastric adenocarcinoma could be achieved with the use of multiple targeted therapies, either simultaneously or sequentially.

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Figure 1.

Figure 2.

Table 1

Demographics and Tumor Characteristics by Ethnicity

	Caucasian (n= 95)	Puerto Rican (n=101)
Gender, N (%)		
Male	54 (56.8)	101 (100)
Female	41 (43.2)	0
Tumor Size, mean (SD), cm	4.0 (3.7)	3.8 (3.2)
Tumor Location *, N (%)		
Antrum	14 (14.7)	35 (35.4)
Fundus	10 (10.5)	27 (27.3)
GE Junction	30 (31.6)	12 (12.1)
Body	24 (25.3)	19 (19.2)
Other	16 (17.7)	6 (6.0)
Tumor Differentiation, N (%)		
Well differentiated *	8 (8.4)	27 (26.7)
Moderately differentiated *	34 (35.8)	15 (14.9)
Poorly differentiated	53 (55.8)	59 (58.4)
Disease Stage, N (%)		
I	22 (23.2)	10(9.9)
II	14 (15.1)	13 (13.1)
III	40(42.1)	23 (22.8)
IV *	17 (18.3)	53 (53.5)

P value calculations regarding disease stage were based in four categories (I, II, III, and IV).

* Statistically significant difference between Caucasians and Puerto Ricans ($p < 0.05$).

Table 2

Protein expression in tumors

Protein	MCC %	PRCC %
p-Stat3*	98	85
Cyclin D1	48	45
EGF-R*	3	15
Her2/neu	8	13

* Statistically significant difference between Caucasians and Puerto Ricans ($P < 0.05$)

Table 3

Levels of protein expression in tumors of both ethnic groups

	Caucasian			Puerto Rican		
	N	Mean (Std)	Min – Max	N	Mean (Std)	Min – Max
p-Stat3	93	1.86 (0.99)	0.0–3.00	98	1.71 (1.15)	0.0–3.00
Cyclin D1	94	0.19 (0.48)	0.0–2.97	98	0.29 (0.54)	0.0–2.40
EGF-R *	95	0.01 (0.06)	0.0–0.50	98	0.15 (0.52)	0.0–3.00
Her2/neu *	95	0.16 (0.57)	0.0–2.97	101	0.53 (0.94)	0.0–3.00

* Statistically significant difference between Caucasians and Puerto Ricans (P <0.05).
Data for some specimens were excluded due to difficulties with interpretation or staining quality

Mean difference in protein expression in relationship to location, grade and stage by groups (ANOVA)

Table 4

Protein	Characteristic		Caucasians		Puerto Ricans	
			Mean (Std)	P-value	Mean (Std)	P-value
Her-2/neu	Location	Antrum	0.42 (1.06)	.842	0.64 (1.02)	0.697
		Body	0.09 (0.27)		0.54 (0.83)	
		Fundus	0.22 (0.66)		0.46 (0.76)	
		G-E Junction	0.12 (0.54)		0.57 (1.05)	
		Other	0.07 (0.22)		0.18 (0.49)	
	Grade	MD	0.15 (0.55)	0.229	0.47 (0.68)	0.108
		PD	0.16 (0.62)		0.42 (0.87)	
		WD	0.14 (0.29)		0.82 (1.16)	
	Stage	I	0.28 (0.85)	0.807	0.67 (1.11)	0.767
		II	0.06 (0.18)		0.36 (0.54)	
		III	0.15 (0.52)		0.29 (0.59)	
		IV	0.13 (0.51)		0.67 (1.08)	
p-Stat3	Location	Antrum	1.87 (1.04)	0.477	1.71 (1.21)	0.323
		Body	1.94 (1.00)		1.62 (1.14)	
		Fundus	2.18 (1.03)		1.70 (1.17)	
		G-E Junction	1.60 (0.98)		2.13 (0.93)	
		Other	1.98 (0.93)		1.31 (1.21)	
	Grade	MD	1.68 (0.98)	0.384	1.76 (1.21)	0.027
		PD	1.93 (0.99)		1.97 (1.05)	
		WD	2.15 (1.01)		1.15 (1.16)	
	Stage	I	1.81 (1.03)	0.901	1.43 (1.20)	0.034
		II	2.06 (0.76)		1.08 (1.17)	
		III	1.77 (1.00)		1.51 (1.05)	
		IV	1.83 (1.13)		2.00 (1.13)	
Cyclin D1	Location	Antrum	0.33 (0.73)	0.789	0.18 (0.37)	0.653
		Body	0.13 (0.22)		0.26 (0.52)	

Protein	Characteristic	Caucasians		Puerto Ricans	
		Mean (Std)	P-value	Mean (Std)	P-value
	Fundus	0.21 (0.47)		0.35 (0.64)	
	G-E Junction	0.19 (0.59)		0.43 (0.69)	
	Other	0.12 (0.29)		0.43 (0.61)	
	Grade		0.926		0.596
	MD	0.13 (0.29)		0.21 (0.38)	
	PD	0.25 (0.60)		0.33 (0.57)	
	WD	0.05 (0.07)	0.654	0.26 (0.55)	0.299
	Stage				
	I	0.22 (0.63)		0.28 (0.60)	
	II	0.12 (0.25)		0.30 (0.42)	
	III	0.18 (0.34)		0.11 (0.31)	
	IV	0.22 (0.72)	0.469	0.36 (0.63)	0.062
EGF-R	Location				
	Antrum	0.01 (0.04)		0.13 (0.38)	
	Body	0.00 (0.00)		0.34 (0.95)	
	Fundus	0.00 (0.00)		0.05 (0.17)	
	G-E Junction	0.02 (0.09)		0.00 (0.00)	
	Other	0.00 (0.00)		0.44 (0.78)	
	Grade		0.502		0.123
	MD	0.02 (0.09)		0.09 (0.31)	
	PD	0.00 (0.02)		0.21 (0.62)	
	WD	0.00 (0.00)	0.709	0.07 (0.35)	0.371
	Stage				
	I	0.01 (0.03)		0.00 (0.00)	
	II	0.01 (0.04)		0.23 (0.83)	
	III	0.01 (0.08)		0.30 (0.71)	
	IV	0.00 (0.00)		0.10 (0.35)	