

Utility of Molecular Genetic Signatures in the Delineation of Gastric Neoplasia

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BACKGROUND. Current techniques to define gastric neoplasia are limited but molecular genetic signatures can categorize tumors and provide biological rationale for predicting clinical behavior. We identified three gene signatures: Chromogranin A (CgA), MAGE-D2 (adhesion), and MTA1 (metastasis) that define gastrointestinal (GI) carcinoids and hypothesize that their expression can delineate gastric neoplasia. This strategy provides a molecular basis to define neuroendocrine gastric carcinoids (GCs), neuronal stromal tumors (GISTs), or epithelial cell (gastric adenocarcinomas [GCAs])-derived tumors.

METHODS. Total RNA was isolated from 38 GCs: Type I/II ($n = 7$), Type III/IV ($n = 6$), GISTs ($n = 12$), GCAs ($n = 13$), and normal mucosa ($n = 12$). Quantitative reverse transcriptase polymerase chain reaction (Q RT-PCR) gene expression was quantified against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and CgA and MTA1 protein expression levels were analyzed by immunohistochemical analyses of a gastric neoplasia microarray.

RESULTS. CgA was elevated in Type I/II (10-fold; $P < .01$) and Type III/IV (100-fold, $P < .005$), decreased in GISTs (100-fold, $P < .03$), and unchanged in GCAs. MAGE-D2 was 5-10-fold elevated ($P < .05$) in Type III/IV, GISTs, and GCAs but not in Type I/II tumors. MTA1 (> 5 -fold, $P < .01$) was elevated in GCs (Type III/IV $>$ I/II, $P < .05$), in GISTs (> 4 -fold, $P < .05$), and GCAs. CgA protein levels were elevated in GCs ($P < .005$) but not in GISTs and GCAs. MTA1 levels were elevated in all tumors ($P < .02$) compared with normal, and especially with tumor invasion ($P < .05$).

CONCLUSION. CgA discriminates GCs from other gastric neoplasms; overexpression of MAGE-D2 and MTA1 differentiate Type III/IV from Type I/II GCs. GISTs share similar expression patterns with Type III/IV GCs but have decreased CgA. MTA1 is a marker of tumor invasion. *Cancer* 2006;106:1480-8.

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Malignant tumors of the stomach include adenocarcinomas, gastric carcinoids (GCs, Types I and II [gastrin-dependent], Type III [sporadic], and neuroendocrine carcinomas, Type IV)¹ and malignant gastrointestinal stromal tumors (GISTs). These tumors develop from at least three different cell types in the stomach. Gastric adenocarcinomas (GCAs) are epithelial-derived cancers, carcinoids developed from neuroendocrine enterochromaffin-like (ECL) cells, whereas GISTs originate from the interstitial cells of Cajal.^{2,3} Carcinoids and GISTs therefore generically share a neural genotype, although they are derived from different cell types. Gastric neoplasia has in the past been classified and differentiated on a histologic basis with some use of immunohistochemistry to delineate cell type and predict prolifer-

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ative rates.⁴⁻⁶ Recently, it has become apparent that molecular genetic signatures provide a sophisticated tool to categorize tumor types and, in addition, may be more useful than standard immunohistochemistry because gene expression can provide an accurate biologic rationale for predicting clinical behavior and outcome than pattern recognition. In particular, this strategy may be of importance because little is known at a molecular level with regard to the mechanisms involved in gastric carcinogenesis. The identification of genes with differential expression in specific gastric tumors may, in addition, provide insight into the initiating mechanisms predicting progression and facilitate precise diagnosis in lesions that are of mixed or indeterminate cellular origin.^{7,8} In this regard, recent gene classification studies have been able to precisely distinguish GISTs and GCAs from normal gastric mucosa.^{9,10} Little, however, is known about gastric carcinoids and their biologic relation to either GISTs or GCAs, and there is debate whether ECL cell tumors can develop into neuroendocrine carcinomas or even gastric adenocarcinomas.¹¹

In previous studies, using an Affymetrix (Santa Clara, CA) GeneChip approach,^{10,12} we identified overexpression of the neuroendocrine secretory gene product Chromogranin A (*CgA*: 6.2-8.3 $P < .012$), the adhesion gene, *MAGE-D2* (1-2.3 $P < .02$), and the breast cancer malignancy gene, *MTA1* (+2.6 $P < .01$) in malignant gastrointestinal carcinoids including Type III and Type IV gastric carcinoids. Chromogranin A is considered a neuroendocrine tumor marker because the majority of peptide-producing endocrine neoplasms including gastrointestinal carcinoids secrete *CgA*.¹³ *MAGE-D2* has been identified as a molecular marker predictive of colorectal liver metastases and is overexpressed in $> 75\%$ of primary colonic tumors with metastases.¹⁴ Overexpression of *MTA1* mRNA and protein correlates with tumor invasion and metastasis in a variety of tumors including breast, prostate, hepatocellular, esophageal, gastric, and colorectal carcinomas.¹⁵⁻²⁰ The utility of this panel of marker genes in delineating gastric neoplasia or identifying individual tumors with an invasive phenotype has not been previously examined.

We hypothesized that because gastric neoplasia (GCAs, GISTs, and gastric carcinoids) are the consequences of the transformation of different cell lineages (epithelial, interstitial cells, and ECL cells, respectively), the panel of selected gene markers would be able to differentiate the tumor types and provide a predictive biological characterization for each of these tumors at a molecular level. Whereas pathologic techniques can distinguish between these tumor types, the role of these molecular markers in the evaluation of

gastric neoplasia has not previously been undertaken. We utilized a quantitative real-time polymerase chain reaction PCR (Q RT-PCR) approach to identify the presence of the panel of marker genes selected in gastric tumors, and confirmed the expression, utility, and specificity of these markers against normal gastric mucosa. We then examined protein expression of *CgA* and *MTA1*, which have well-defined commercially available antibodies, to assess and compare the utility of an immunohistochemical approach in a novel gastric neoplasia tissue microarray (YTMA63) that we designed and developed.

MATERIALS AND METHODS

These studies were approved by the Human Investigations Committee at Yale University School of Medicine.

Patients and Samples

Tissue specimens

Tumor tissue was collected from 38 patients (M:F = 19:19; median age [range], 61 yrs [34-79]) with histologically proven gastric carcinoid tumors (Type I/II gastric carcinoid tumors [$n = 7$], Type III/IV [$n = 6$]), GISTs ($n = 12$) CD117-positive (considered to be derived from same stem cell as interstitial cells of Cajal; potentially responsive to tyrosine kinase inhibitors),²¹ mitotic figures (0-5/10 high power field [HPF]), or 13 gastric adenocarcinomas (GCAs: intestinal type: $n = 11$; diffuse type: $n = 2$) who had either undergone resection of the primary tumor between 1997 and 2004 at the Yale University Department of Surgery or were collected by the Cooperative Human Tissue Network, which is funded by the National Cancer Institute. Paired normal tissue samples were also obtained from adjacent, macroscopically normal gastric nontumor mucosa in eight patients.

Tissue microarray immunostaining, image acquisition, and data analysis

A gastric tumor tissue microarray containing 38 GCAs, 12 GISTs, 7 gastric carcinoids, as well as normal tissue samples from each of these sites (Yale Tumor Microarray [YTMA63]) was constructed as previously described.²² Briefly, formalin-fixed paraffin-embedded tissue blocks were retrieved, along with the corresponding hematoxylin and eosin (H&E)-stained slides, from the archives of the Yale University School of Medicine Department of Pathology. Blocks were stored under ambient conditions within a temperature range of 18-37 °C. Areas of tumor (adenocarcinoma, GIST, or carcinoid), distinct from normal tissue elements, were identified by a researcher (R.L.C.) and marked for subsequent retrieval and analysis. Core

biopsies (0.6 mm in diameter) were taken from each donor block and arrayed into a recipient paraffin block (45 × 20 mm) using a tissue puncher/arrayer (Beecher Instruments, Silver Spring, MD) as described by Xie et al.²³ The final microarray consisted of primary tumors and matched normal and was represented by two cores/case. It was previously determined that analysis of two cores concurs with analysis of an entire tissue section with > 95% accuracy.²⁴ In the current study, correlation analysis of CgA immunostaining was 96.98%, indicating that potential tumor heterogeneity was not an issue. This array was examined by AQUA (Automated Quantitative Analysis) after immunohistochemical staining.^{22,25} AQUA is a set of algorithms developed by R.L.C. at Yale University that allows for the rapid, automated analysis of large-scale cohorts of tumor samples on tissue microarrays and generates reproducible continuous scale data that have the advantage over traditional pathology of discriminating subtle, low-level staining differences as well as being able to accurately score staining in subcellular compartments.

Clinical characteristics including tumor grade, invasion, presence of lymph node metastases, mitotic figures, and tumor size were available for all patients.

Tissue Techniques

RNA isolation

Total RNA was isolated from frozen gastric neoplasia ($n = 38$) and normal tissues ($n = 12$) using TRIzol reagent (Invitrogen, Carlsbad, CA) as described previously.²⁵ RNA was dissolved in DEPC water, measured spectrophotometrically, and an aliquot analyzed on Bioanalyzer (Agilent Technologies, Palo Alto, CA) to assess the quality of RNA.

Quantitative RT-PCR

Thirty-eight primary gastric tumors and 12 control tissues were examined by Q RT-PCR. *CgA*, *MAGE-D2*, *MTA1*, and *GAPDH* message was quantitatively measured as previously described.²¹ Q RT-PCR was performed using the ABI 7900 Sequence Detection System. Total RNA from each sample was subjected to reverse transcription using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) following the manufacturer's directions. Briefly, 2 μg of total RNA in 50 μL of water was mixed with 50 μL of 2× RT mix containing Reverse Transcription Buffer, dNTPs, random primers, and Multiscribe Reverse Transcriptase. RT reaction was carried out in a thermal cycler for 10 minutes at 25 °C followed by 120 minutes at 37 °C. Real-time PCR analysis was then performed in triplicate. Briefly, cDNA in 7.2 μL of water was mixed with 0.8 μL of 20× Assay-on-

De-mand (Applied Biosystems) primer and probe mix and 8 μL of 2× TaqMan Universal Master mix in a 384-well optical reaction plate. Each preformulated Assay-on-Demand assay contains two unlabeled PCR primers and a FAM dye-labeled gene-specific TaqMan MGB probe. The target gene, assay probe ID, exon:exon boundary, and assay location relative to a reference sequence are: *CgA* = Hs00154441_m1, exons 4-5, base 468 of NM_001275; *MAGE-D2* = Hs00374760_m1, exons 3-4, base 638 of NM_014599; *MTA1* = Hs00183042_m1, exons 17-18, base 1823 of NM_004689; *GAPDH* = Hs99999905; exons 3-4, base 132 of NM_002046. The following PCR conditions were used: 50°C for 2 minutes, then 95 °C for 10 minutes, followed by 40 cycles at 95 °C/0.15 minutes and 60°C/1 minute. A standard curve was generated for each gene using serial dilution of a cDNA mixture obtained by pooling equal amounts from each sample. Before the study, a large pool of standard cDNA was prepared and used on each plate to provide consistent real-time PCR results after Applied Biosystems recommendations ("Performing Relative Quantitation of Gene Expression Using Real-Time Quantitative PCR," Applied Biosystems Application Note). The expression level of target genes was normalized to internal *GAPDH*. We previously also examined the utility of *β -actin*, *hypoxanthin*, and *18S RNA* as housekeeping genes and noted that *GAPDH* provided the least variability in expression across tumor types. Data were analyzed using Microsoft EXCEL and calculated using the relative standard curve method (ABI, User Bulletin #2).

Tissue microarray immunostaining, image acquisition, and data analysis

The immunohistochemical expression of CgA and MTA1 in gastric neoplasia was examined by immunohistochemistry and AQUA quantitation of staining intensity in YTMA63. Expression values of these proteins were correlated with tumor type.

Carcinoid tissue microarray slides were stained as described previously.^{22,25} For antigen retrieval purposes, sections were immersed in citrate buffer (10 mM sodium citrate, pH 6.0) and subjected to 1 × 10 minutes high temperature/high pressure treatment followed by treatment with 0.3% H₂O₂ in methanol for 30 minutes at 37 °C to inactivate endogenous peroxidase. Slides were incubated for 24 hours at 4 °C with either 1:1000 dilution of the anti-CgA mouse monoclonal antibody (DAKO, Carpinteria, CA) or 1:100 dilution of the anti-MTA1 (Santa Cruz Biotechnology, Santa Cruz, CA; A11) mouse antibody. Goat antimouse antibodies conjugated to a horseradish peroxidase-decorated dextran polymer backbone (Envision;

DAKO) were used as a secondary reagent. For automated analysis, gastric tumor cells or normal mucosal epithelia were identified by the use of a fluorescently tagged anticytokeratin antibody cocktail (AE1/AE3; DAKO), nuclei were visualized by 4',6-diamidino-2-phenylindole (DAPI), and targets visualized with a fluorescent chromagen (Cy-5-tyramide; NEN Life Science Products, Boston, MA).^{22,25} Briefly, monochromatic, high-resolution (1024 × 1024 pixel; 0.5- μ m) images were obtained of each histospot. Areas of tumor or normal epithelia from stromal elements were distinguished by creating a mask from the cytokeratin signal. Coalescence of cytokeratin at the cell surface localized the cell membranes and DAPI was used to identify nuclei. The CgA signal from the tumor cells or normal epithelial cells was scored and expressed as signal intensity divided by the cytokeratin mask area, whereas the MTA1 signal from the nucleus of tumor cells or epithelial cells was automatically scored and expressed as signal intensity divided by the nuclear area. Disc scores from the same tumor were averaged to produce a single score. Histospots containing < 10% tumor, as assessed by mask area (automated), were excluded from further analysis.

Statistical Analysis

The results are expressed as mean \pm SEM; *n* indicates the number of patients in each study group. Statistical significance was calculated using parametric (Student *t*-test) and nonparametric (Wilcoxon) tests for paired and unpaired values as appropriate, with a probability of < .05 representing significance.

RESULTS

Real-Time PCR

CgA

The expression of CgA was examined in two different types of gastric carcinoids—nonaggressive Type I and II tumors and malignant (invasive and metastatic) Type III/IV tumors, as well as GISTs and GCAs. Quantitative RT-PCR analysis demonstrated that mRNA levels of CgA were significantly elevated in both the Type I/II carcinoids ($P < .05$) and Type III/IV gastric carcinoid tumors ($P < .005$) compared with normal mucosa (Fig. 1). A significant difference was noted in expression levels between these types, with significantly higher levels recorded in the latter ($P < .05$). In contrast to the gastric carcinoids, message for CgA was significantly decreased ($P = .03$) in GISTs and was not significantly different from normal mucosa in GCAs.

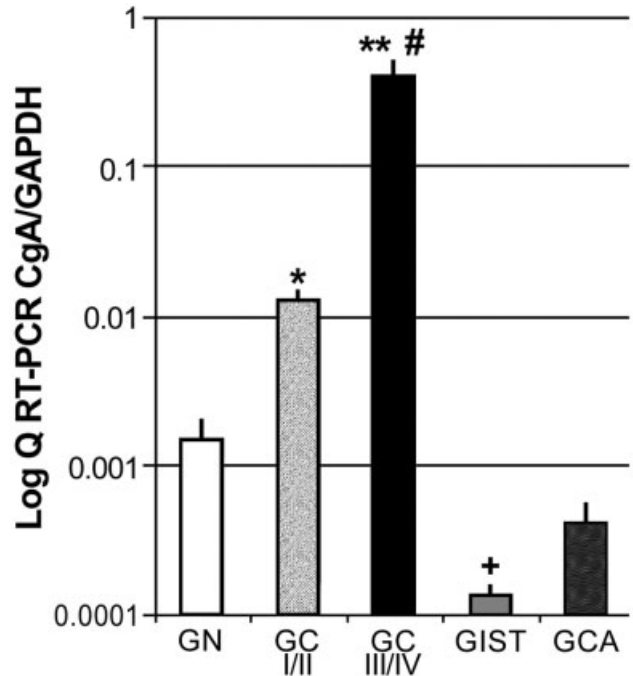


FIGURE 1. Message levels of CgA determined by Q RT-PCR. Levels of CgA were significantly overexpressed in Type I/II gastric carcinoids (GC I/II; 10 \times) and Type III/IV gastric carcinoids (GC III/IV; 100 \times) compared with normal mucosa (GN). Levels were overexpressed in Type III/IV tumors compared with Type I/II. Levels of CgA were significantly decreased in gastrointestinal stromal tumors (GISTs) (100 \times) compared with normal. Gastric adenocarcinomas (GCAs) expressed similar levels as normal mucosa. (# $P = .05$; + $P = .03$; * $P = .01$, ** $P < .005$). Mean \pm SEM.

MAGE-D2

MAGE-D2 is a putative adhesion gene and predictive marker of colorectal liver metastases.¹⁴ Levels of MAGE-D2 were elevated (5-fold, $P < .05$) in Type III/IV gastric carcinoids compared with normal mucosa, but were not elevated in Type I/II tumors (Fig. 2). Message levels were elevated (8-fold, $P < .005$) in GISTs and (10-fold, $P < .01$) in GCAs compared with normal tissue, respectively. This is broadly consistent with the known clinical and pathologic propensity of such lesions to invade and metastasize.^{3,6}

MTA1

MTA1 is an estrogen-antagonistic breast cancer malignancy gene that has been used to identify progressive (metastatic) disease in a wide range of tumors including breast, prostate, hepatocellular, esophageal, gastric, and colorectal.¹⁵⁻²⁰ It also plays a role in cytoplasmic rearrangement and is associated with an invasive phenotype in vitro.²⁶ Message levels were elevated in Type III/IV carcinoids (5-fold, $P < .005$), in

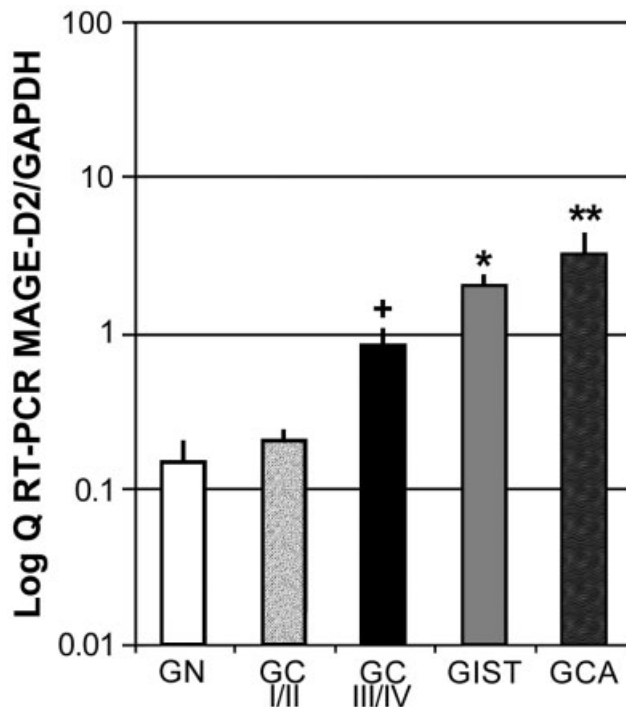


FIGURE 2. Message levels of *MAGE-D2* determined by Q RT-PCR. Levels of *MAGE-D2* were significantly overexpressed (~5×) in malignant gastric carcinoids (GC III/IV) samples, gastrointestinal stromal tumors (GISTs) (8×), and gastric adenocarcinomas (GCAs; 10×) compared with normal mucosa (GN). (+ $P = .05$; * $P < .005$; ** $P = .01$). Mean ± SEM.

GISTs (> 4-fold, $P < .05$), and in gastric adenocarcinomas (10-fold, $P = .01$) (Fig. 3).

Protein Immunohistochemistry of CgA and MTA1 in Gastric Tumors

The immunohistochemical expression of CgA and MTA1 was examined by immunohistochemistry and AQUA quantitation of staining intensity on YTMA63. Expression values of either CgA or MTA1 were correlated with tumor type.

Expression levels of cytoplasmic CgA were elevated in gastric carcinoid tumors compared with GISTs, GCAs, and normal mucosa ($P < .005$) (Fig. 4). Expression levels of CgA were indistinguishable in gastric adenocarcinomas and GISTs compared with normal mucosa. A subanalysis of the gastric carcinoids demonstrated that CgA was significantly elevated in the Type III/IV carcinoids (733 ± 34 , $P = .02$) compared with the Type I/II carcinoids (350 ± 153).

MTA1 was identified as previously described in a prostate tissue microarray (TMA).¹⁶ Expression levels of membrane and nuclear MTA1 on YTMA63 were elevated in all three gastric tumor types ($P < .02$) compared with normal mucosa (Fig. 5). Levels of

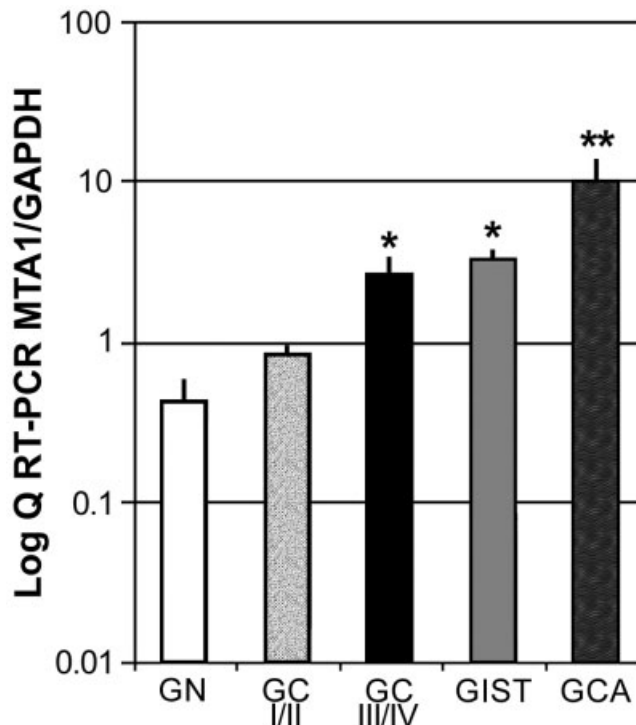


FIGURE 3. Message levels of *MTA1* determined by Q RT-PCR. Levels of *MTA1* were significantly overexpressed (5×) in malignant gastric carcinoids (GC III/IV), in gastrointestinal stromal tumors (GISTs) (>4×), and gastric adenocarcinomas (GCAs; 10×) compared with normal mucosa (GN). (+ $P < .01$; * $P < .005$; ** $P = .01$). Mean ± SEM.

MTA1 were significantly elevated in Type III/IV gastric carcinoids ($P < .05$) and GISTs ($P = .007$) compared with GCAs. GISTs with histologically documented invasion ($n = 3$) had significantly increased membrane MTA1 levels compared with noninvasive GISTs ($n = 8$) (174 ± 24 vs. 72 ± 27 , $P = .05$). No correlation was noted between MTA1 and tumor size, mitotic figures, or the presence of necrosis in these tumors. The majority of GCAs (> 96%) exhibited mucosal invasion, so the presence of metastases (lymph node) was examined with MTA1 expression. This demonstrated that tumors with lymph node metastasis had elevated membrane MTA1 levels compared with GCAs with no metastases (29 ± 8 vs. 3.1 ± 0.6 , $P = .003$). This relation was also identified between MTA1 and the TNM classification: tumors classified as T3 or higher (tumors surpassing the serosa) exhibited higher MTA1 levels than those classified as T1/T2 (43 ± 16 vs. 14 ± 6 , $P = .036$).

DISCUSSION

These data demonstrate that gastric neoplasia can be differentiated by a Q RT-PCR approach using a se-

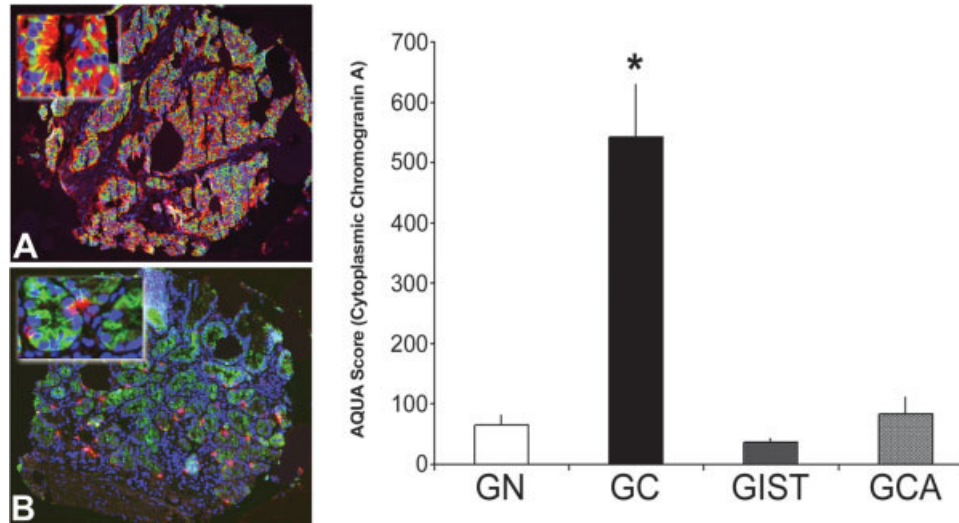


FIGURE 4. Expression levels of CgA determined by AQUA quantitation in gastric carcinoids ($n = 7$), gastrointestinal stromal tumors (GISTs) ($n = 12$), and gastric adenocarcinomas (GCAs) ($n = 38$) and in normal tissue ($n = 18$) on YTMA63. Left panel: Significant cytoplasmic CgA staining in (A) a gastric carcinoid with overlap between cytokeratin and cytoplasmic CgA (inset) compared with minimal CgA staining in (B) a GCA specimen. Triple color staining; blue: nuclei (DAPI), green: cytokeratin (Alexa488, used as the tumor mask for AQUA analysis), red: CgA (Cy5) (magnification $\times 100$). Right panel: Using AQUA analysis of the triple color secretions (A,B) levels of CgA were identified to be significantly overexpressed in gastric carcinoids (GC) compared with normal mucosa (GN), GISTs, and adenocarcinomas (GCA). CgA was increased in GCA and decreased in GISTs compared with normal, but this was not significant. (* $P < .005$). Mean \pm SEM.

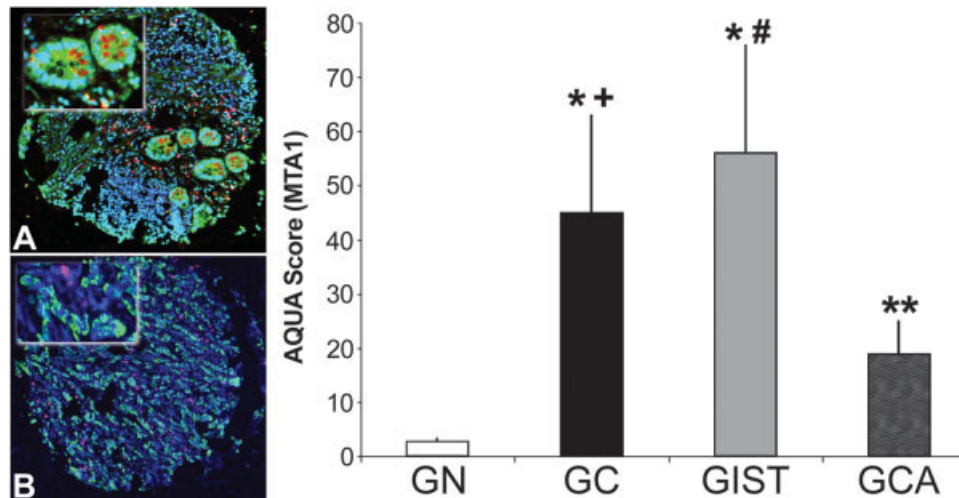


FIGURE 5. Expression levels of MTA1 determined by AQUA quantitation in gastric carcinoids ($n = 7$), GISTs ($n = 12$) and gastric adenocarcinomas ($n = 38$) and in normal tissue ($n = 18$) on YTMA63. Left panel: Significant cytoplasmic and membrane-bound MTA1 staining in a gastric carcinoid. (A inset) compared with minimal MTA1 staining in a normal gastric specimen (B inset). Triple color staining as described in Figure 4. Right panel: Levels of MTA1 were significantly overexpressed in gastric carcinoids (GC), GISTs, and gastric adenocarcinomas (GCA) compared with normal mucosa (GN). Both carcinoids and GISTs had significantly elevated MTA1 levels compared with adenocarcinomas. (+ $P < .05$; * $P < .02$; # $P < .007$; ** $P < .003$). Mean \pm SEM. GN: normal gastric mucosa; GC: gastric carcinoid; GIST: gastrointestinal stromal tumor; GCA: gastric adenocarcinoma.

lected panel of well-characterized candidate marker genes with known biological function: *CgA*, *MAGE-D2*, and *MTA1*. In addition, differences in malignant potential could be identified in gastric carcinoids using this gene panel. Elevated gene expression levels of *CgA* specifically identified gastric carcinoids, and Type

III/IV tumors could be differentiated from less aggressive Type I/II tumors by higher levels of *CgA*, and the expression of *MAGE-D2* and *MTA1*. In this study, *MAGE-D2* was ubiquitously expressed in the more aggressive (Type III/IV) group of carcinoids, GISTs, and GCAs. Cell membrane- and nuclear-localized

MTA1 protein expression was elevated in invasive GISTs and gastric carcinoids.

Chromogranin A is considered a useful marker for neuroendocrine tumors.¹³ Tumors derived from non-neuroendocrine cells, however, may also express neuroendocrine marker genes,²⁷⁻³¹ perhaps because of neuronal dedifferentiation of such lesions.³² We therefore examined message and protein levels of CgA in gastric neoplasia to identify whether this adequately differentiated neuroendocrine ECL cell tumors from neuronally derived (CD117-positive, presumably derived from the same stem cells as interstitial cells of Cajal) GISTs and epithelial cell-derived GCAs. Message levels of CgA were elevated in both types of gastric carcinoid tumors compared with GISTs and GCAs but were higher in the Type III/IV group compared with the less aggressive Type I/II group. This raises the possibility that levels of CgA expression relate to tumor aggression. Message levels in GCAs were not different from normal mucosa, whereas GISTs had a significantly lower CgA message. This relation was also noted at a protein level, where immunohistochemical levels of CgA were elevated in gastric carcinoids but were similar or lower compared with normal mucosa in GISTs and GCA tumor specimens. Immunohistochemically, CgA is variably expressed in gastric adenocarcinomas³³ and is absent in GISTs.³⁴ In the present study, 8% of both GCAs (3 of 38 samples) and GISTs (1 of 12 samples) expressed CgA at levels above that expressed in normal mucosa. All three of the GCAs with elevated CgA levels were intestinal-type tumors as opposed to diffuse tumors, suggesting that the cell of origin of these lesions may be different. The data in the current study are consistent with the proposal that CgA may be a useful marker to differentiate between the different gastric tumor types.

MAGE-D2 encodes one of the cancer testes (CT) families of genes,³⁵ but because it does not encode any of the known MAGE antigenic peptides in its sequence,³⁵ it cannot be considered a classic CT antigen. In addition, it has been identified as a molecular marker to predict liver metastases from colorectal tumors and is overexpressed in > 75% of primary colon tumors with metastases.¹⁴ Although the precise function of MAGE-D2 is unclear, it is currently accepted as involved in the process of cell adhesion.³⁶ The overexpression of *MAGE-D2* in more aggressive Type III/IV gastric carcinoids, GISTs, and GCAs indicates that malignancy and metastasis of gastric tumors, irrespective of the cell of origin, is associated with expression of this gene. The identification of up-regulation of this gene may therefore be predictive of malignant and metastatic behavior associated with an invasive gastric neoplastic phenotype.

Comparative analyses in other epithelial cell-derived tumors have noted that *MTA1* mRNA is differentially expressed in nonmetastatic and metastatic tumors, suggesting a direct role for this gene in enhancing the metastatic potential of malignant tumor cells.³⁷ In a study undertaken by Toh et al.,¹⁵ a relative overexpression of *MTA1* mRNA (tumor/normal ratio = 2) was observed in 13 of 34 (38.2%) gastric carcinomas. Clinicopathologic correlations in this study demonstrated that gastric carcinomas overexpressing *MTA1* mRNA exhibited significantly higher rates of serosal invasion and lymph node metastasis and tended to have a higher rate of vascular involvement. These data suggest that overexpression of the *MTA1* gene correlates with tumor invasion and the presence of metastases and that high expression of *MTA1* mRNA may be a potential indicator for assessing the malignant potential of GCAs.¹⁵ These observations are supported by a recent functional study using the pancreatic carcinoma-derived PANC-1 cell-line,²⁶ which demonstrated that enhanced expression of MTA1 protein triggered the development of motile, invasive cells by altering the organization of the cellular cytoskeleton. In addition, these transformed cells had a significant reduction in cell proliferation. In the current study, *MTA1* message was overexpressed in Type III/IV gastric carcinoids, in GISTs, and in adenocarcinomas. At a protein level, nuclear and membrane MTA1 levels were elevated in all gastric neoplasia. Interestingly, MTA1 levels were higher in GISTs and gastric carcinoids than in GCAs. The former two tumor types generally exhibit a lower proliferative potential than the epithelial-derived tumors.¹⁻³ These findings are thus consistent with the in vitro studies in PANC-1 cells, where MTA1 overexpression was associated with a low proliferation.²⁶ A subanalysis of GISTs and GCAs identified that invasion per se was associated with the highest levels of membrane MTA1 levels. Specifically, tumors with histologically documented invasion had significantly increased (twofold to 10-fold elevated, $P < .05$) MTA1 levels compared with noninvasive tumors. This was also reflected in the TNM classification, where tumors classified as T3 or higher exhibited higher MTA1 levels ($P < .04$) than those classified as T1/T2. These findings highlight that malignancy is probably related to gene activation of specific cellular events (e.g., cytoskeletal rearrangement, adhesion) as well as the cellular mitotic rate.

The prognostic significance of this molecular information can be inferred from studies that demonstrate poorer 5-year survival rates in patients with metastatic disease. Thus, for gastric carcinoids, patients that present with localized disease have a calculated survival of 67.5% compared with those pa-

tients with regional (3.1%) or distant (6.5%) disease.³⁸ A similar relation has been noted for GISTs, where the survival of patients with metastatic disease is ~20 months.³⁹ Molecular markers that play biologic roles in metastasis and are identified to be overexpressed in gastric neoplasia may therefore be of prognostic utility.

In the current study the histopathology of each tumor type was well defined. In marginal cases where either the cellular etiology of a neoplasia is unclear or the different types of gastric carcinoids cannot be distinguished, differential gene or protein expression of these marker genes may provide powerful discriminatory information.

In conclusion, our data demonstrate differential expression of *CgA*, *MAGE-D2*, and *MTA1* mRNA and *CgA* and *MTA1* protein in gastric neoplastic tissue, indicating the utility of these markers to identify gastric tumor malignancy. Overexpression of *MAGE-D2* and *MTA1* differentiates Type III/IV carcinoids from Type I/II carcinoids. The decreased spectrum of gene alterations in the generally less aggressive Type I/II gastric carcinoids is consistent with their indolent clinical status.¹ Neuronally derived (CD117-positive) GISTs share similar gene expression patterns with neuroendocrine cell Type III/IV carcinoids, but can be differentiated by decreased levels of *CgA*. Protein expression of *MTA1* was shown to be directly associated with clinical evidence of tumor invasion. This study demonstrates that a biomarker panel assessment of gastric tumors using four selected genes may help redefine diagnostic strategy and ultimately be of utility in amplifying prognostic assessment.

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