

# Exocrine and Endocrine Modulation in Common Gastric Carcinoma

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**Key Words:** Gastric carcinoma; Neuroendocrine differentiation; Endocrine differentiation; Exocrine phenotype; Endocrine phenotype; Prognosis

DOI: 10.1309/AJCPM13KVNQZQBUV

## Abstract

*Diagnostic and prognostic implications of endocrine differentiation were evaluated in 103 common gastric adenocarcinomas and undifferentiated carcinomas. Maturely differentiated exocrine and endocrine phenotypes were evaluated by using gastric exocrine and endocrine markers along with intestinal exocrine and endocrine markers.*

*Immunohistochemical analysis revealed that 66 tumors (64%) were positive for generic endocrine markers such as chromogranin A and/or synaptophysin. The 14 patients with more than 20% tumor cells positive for at least 1 endocrine marker experienced a poorer prognosis than patients with no (n = 37) or 1% to 20% (n = 52) positivity. The 16 carcinomas expressing the maturely differentiated exocrine gastric phenotype significantly correlated with poorer outcome compared with carcinomas with mature exocrine intestinal (n = 22) or mixed/gastrointestinal (n = 64) phenotypes. Among tumors expressing chromogranin A and/or synaptophysin, the maturely differentiated endocrine gastric phenotype (n = 26) was a negative prognostic factor compared with mature endocrine intestinal (n = 21) and mixed/gastrointestinal (n = 5) phenotypes.*

*Endocrine differentiation and maturely exocrine/endocrine gastric phenotypes are associated with an unfavorable prognosis and may identify subsets of patients for tailored therapy.*

Gastric cancer still represents a significant public health problem worldwide because of its poor prognosis and the aging of the world's population. Although its incidence and mortality rates have gradually decreased during past decades, it is second only to lung cancer as the leading cause of cancer death in both sexes worldwide. According to estimates by the World Health Organization, there were approximately 989,000 newly diagnosed gastric cancer cases and 737,000 deaths due to stomach cancer in 2008.<sup>1</sup> Eastern Asia (mainly China and Japan), Central and South America, and Central and Eastern Europe are the highest risk areas around the world, and men are affected about twice as often as women.<sup>1,2</sup> Currently, surgery represents the only potentially curative treatment, but, despite technical surgical advances and the use of adjuvant therapies, the 5-year overall survival rates of patients who have resectable gastric cancer are unsatisfactory, ranging from 20% to 30%.<sup>3</sup> The high mortality rate is mostly due to late diagnosis of disease. With such a poor prognosis, there is a pressing need to shape new strategies for developing therapies through a better definition of pathologic cancer subtypes.

Because most gastric tumors of the stomach are epithelial in origin, they are divided into 2 major categories, exocrine (adenomas and carcinomas) and endocrine (carcinoid tumors and endocrine cell carcinomas). The overwhelming majority of conventional gastric cancers are adenocarcinomas that are considered to originate from a progenitor cell specializing toward an exocrine cell lineage.<sup>4</sup>

However, there have been several reports showing that (neuro)endocrine markers chromogranin A (CgA) and/or synaptophysin (Syn) were found immunohistochemically in about 15% to 70% of conventional gastric carcinomas, depending on antibodies or the criteria for positivity used.<sup>5-9</sup>

Hence, stomach cancers with endocrine differentiation might have different types of histogenesis, implying for a stem or progenitor cell the ability to differentiate toward exocrine and endocrine lineages.<sup>10</sup> Dual differentiation has previously been described in conventional carcinomas of various organs, such as breast, prostate, lung, pancreas, and colon, with clinical behavior usually determined by the most aggressive cell population.<sup>11</sup>

Considering gastric adenocarcinomas, Jiang et al<sup>12</sup> reported that tumors with more than 20% of the tumor cells expressing CgA and/or Syn, defined by the authors as large cell neuroendocrine carcinomas, significantly correlated with a poorer overall survival rate than adenocarcinomas without endocrine differentiation or up to 20% of tumor cells expressing CgA and/or Syn. Accordingly, the poor prognosis of the large cell variant of gastric neuroendocrine carcinoma has been previously described.<sup>13-15</sup> It remains unclear how the presence of endocrine differentiation may confer an adverse prognosis, and little is known about specific therapies that might be effective in these cases.

In addition, common gastric cancers may express maturely differentiated exocrine markers that may be of gastric (MUC5AC and MUC6) or intestinal (MUC2, CD10, villin) derivation and are commonly used for phenotyping stomach cancers of the gastric, intestinal, or mixed/gastrointestinal type. From a prognostic viewpoint, the exocrine phenotype has been reported to be associated with a poorer outcome and greater malignant potential compared with other phenotypes, whereas no prognostic data on maturely differentiated endocrine phenotypes are available.<sup>16</sup>

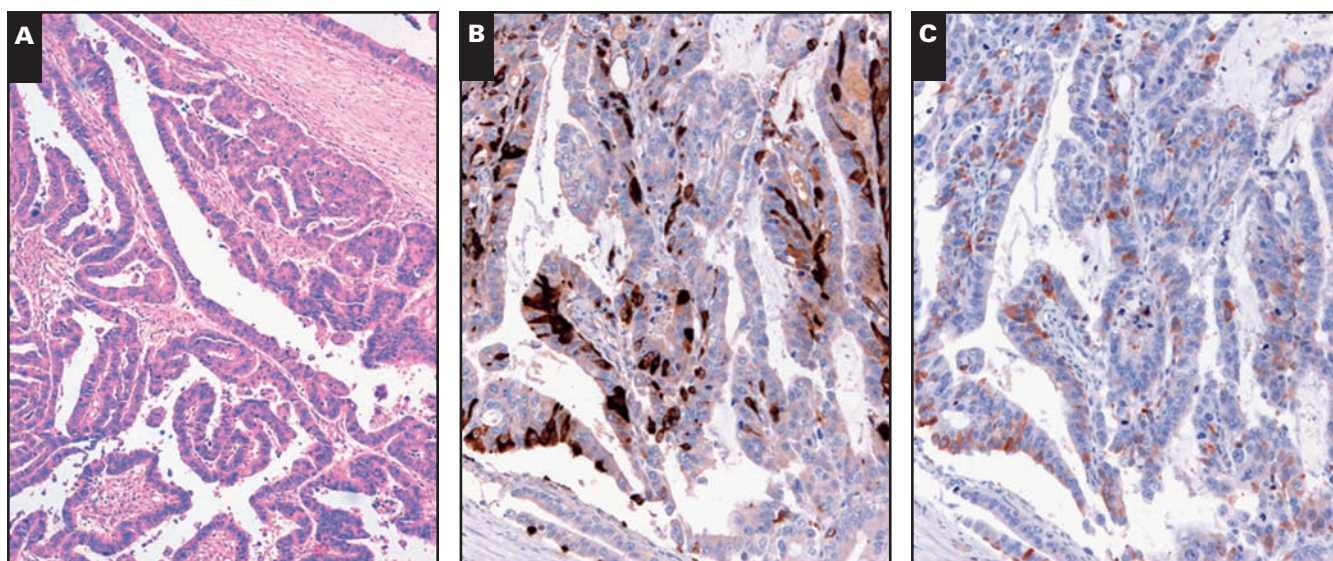
In the present study, we evaluated the diagnostic and prognostic implications of endocrine differentiation in conventional epithelial gastric carcinomas, as demonstrated by the presence of CgA+ and/or Syn+ tumor cells. We also investigated the diagnostic and prognostic relevance of maturely differentiated exocrine and endocrine phenotypes.

## Materials and Methods

### Cases

A total of 103 primary stomach cancers from patients who underwent surgical resection and had tumors diagnosed at the CRO-National Cancer Institute, Aviano, Italy; the Mediterranean Institute of Oncology, Viagrande, Italy; or the Oncopath Lab, Siracusa, Italy, between 1999 and 2007 were examined. Of the 103 patients, 63 were men and 40 were women, ranging in age from 40 to 94 years (mean  $\pm$  SD, 65.3  $\pm$  11.1 years). All specimens were routinely fixed in Bouin solution or 10% buffered formalin, embedded in paraffin, and stained with H&E for histologic examination.

Tumors were classified as 71 adenocarcinomas and 32 undifferentiated carcinomas according to the criteria established by the World Health Organization.<sup>17</sup> Examples of common gastric carcinoma are shown in **Image 1A**, **Image 2A**, **Image 2C**, **Image 2E**, and **Image 2G**. Staging was performed according to the International Union Against Cancer system for the extent of tumor spread.<sup>18</sup> There were 5 T1, 16 T2, 29 T3, and 53 T4 cases (T1, tumor invades lamina propria, muscularis mucosae, or submucosa; T2, tumor invades



**Image 1** Common gastric adenocarcinoma with endocrine differentiation (**A**, H&E,  $\times 25$ ). This tumor is diffusely immunoreactive for generic (neuro)endocrine markers chromogranin A (**B**, hematoxylin counterstain,  $\times 25$ ) and synaptophysin (**C**, hematoxylin counterstain,  $\times 25$ ).

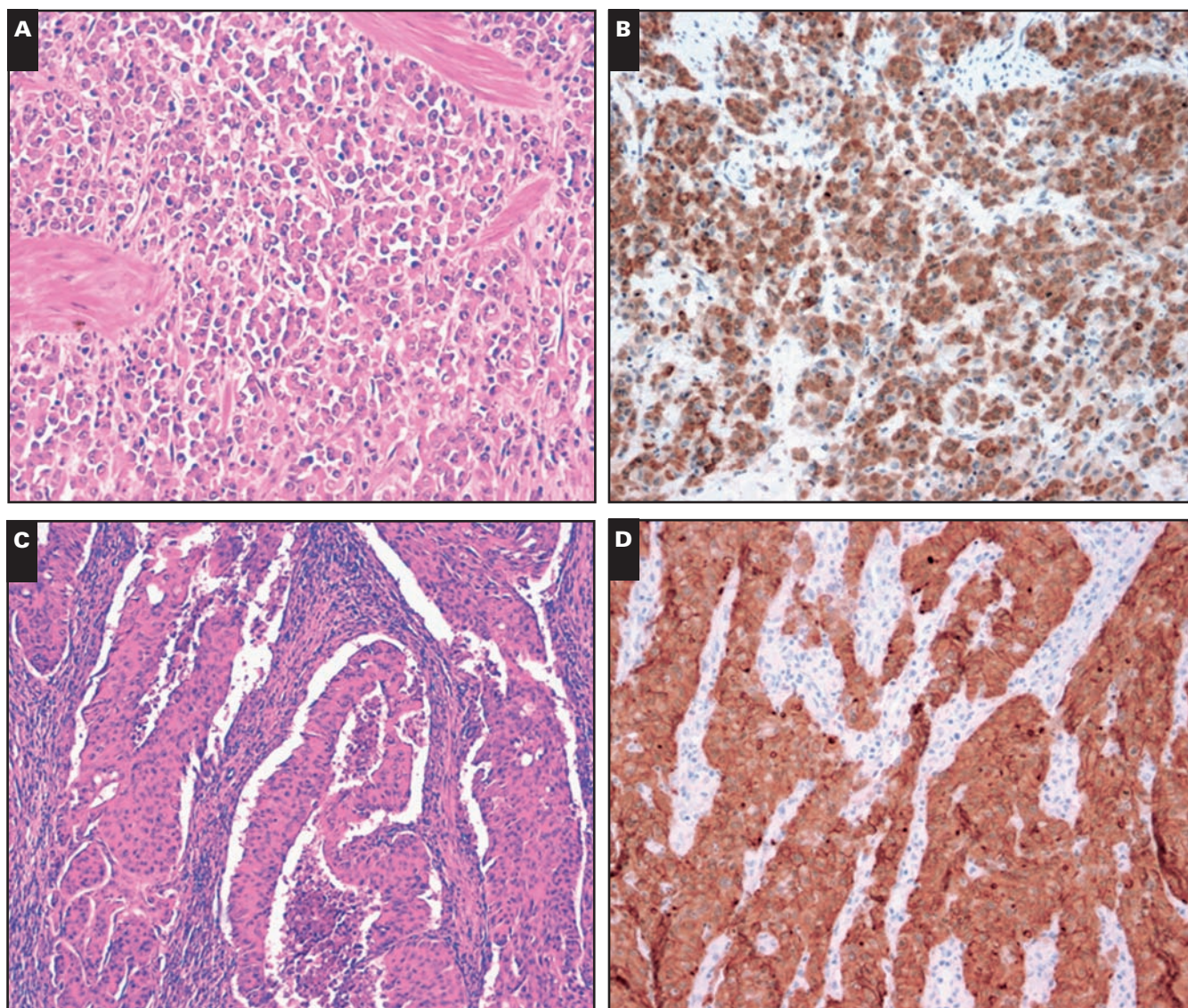


muscularis propria; T3, tumor invades subserosa; T4, tumor perforates serosa or invades adjacent structures). Nodal metastases were present in 69 patients, distributed as follows: 18 N1, 17 N2, and 34 N3 cases (N1, metastasis in 1-2 regional lymph nodes; N2, metastasis in 3-6 regional lymph nodes; N3, metastases in  $\geq 7$  regional lymph nodes). Distant metastasis (M1) was present in 12 patients. Tumor staging revealed 8 stage I (T1/T2 N0 M0), 36 stage II (T1 N2/N3 M0, T2 N1/N2 M0, T3 N0/N1 M0, T4 N0 M0), 47 stage III (T2 N3 M0, T3 N2/N3 M0, T4 N1/N2/N3 M0), and 12 stage IV (any T, any N, M1) cases.

Written informed consent was obtained from patients for publication of this clinical series and any accompanying images.

### Immunohistochemical Analysis

For most immunohistochemical analyses, 2- to 3- $\mu$ m thick consecutive sections of primary tumors were processed with the automated immunostainer, BenchMark XT (Ventana, Tucson, AZ). Immunohistochemical staining was done with antibodies against the following antigens: CgA (DAKO, Glostrup, Denmark), diluted 1:600; Syn (DAKO), diluted 1:150; MUC5AC (clone MRQ-19, Ventana), prediluted; MUC6 (clone MRQ-20, Ventana), prediluted; MUC2 (clone MRQ-18, Ventana), prediluted; villin (clone CWWB1, Novocastra Laboratories, Newcastle upon Tyne, England), diluted 1:150; CD10 (clone 56C6, DAKO), diluted 1:25; gastrin (Ventana), prediluted; somatostatin (Ventana), prediluted;



**Image 2** Examples of stomach carcinomas expressing maturely differentiated exocrine or endocrine markers. **A**, Diffuse type, infiltrating undifferentiated/poorly differentiated carcinoma (H&E,  $\times 10$ ). **B**, Mucin MUC6 is a mature exocrine marker of the gastric type ( $\times 20$ ). **C**, Sheets of neoplastic cells arranged in a solid pattern (H&E,  $\times 10$ ). **D**, CD10 is a mature exocrine marker of the intestinal type ( $\times 20$ ).



glucagon-like peptide-1 (GLP-1; clone 8G9, Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:100; and gastric inhibitory polypeptide (GIP; Bachem, Bubendorf, Switzerland), diluted 1:2,000. Nuclear counterstaining was accomplished with Harris hematoxylin. Omission of the primary antibody was used as a negative control.

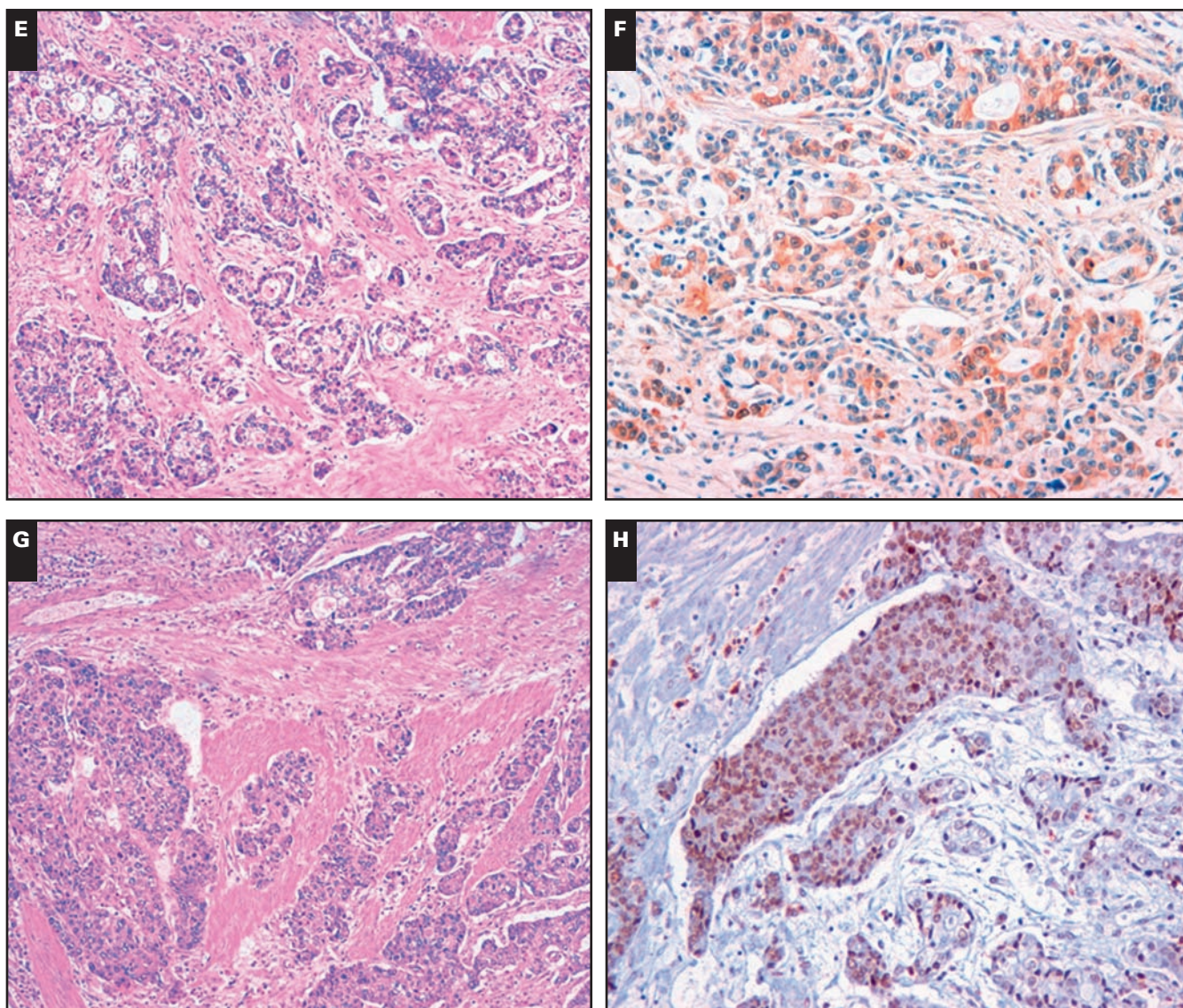
The results for staining were evaluated with reference to the percentage of positively stained tumor cells. We randomly selected 10 representative microscopic fields at  $\times 40$  original magnification and counted at least 100 tumor cells for each field. The cases were defined as positive when 1% or more of tumor cells were positively stained in each section.

CgA and Syn were examined as generic (neuro)endocrine markers.<sup>19</sup> Tumors with no or fewer than 1% of cells stained

for both CgA and Syn were classified as common gastric carcinomas; tumors with at least 1% tumor cells immunoreactive for CgA, Syn, or both markers were classified as common gastric carcinomas with endocrine differentiation. According to previous authors, gastric cancers with endocrine differentiation were stratified into 2 subgroups by using a cutoff value of more than 20% tumor cells positive for CgA, Syn, or both markers.<sup>12</sup>

### Classification of Phenotypic Expression

The mucins, MUC5AC and MUC6, were examined as gastric exocrine markers, whereas mucin MUC2, villin, and CD10 were used as intestinal exocrine markers. Gastric cancers were classified into 3 types based on the expression of maturely differentiated exocrine markers: (1) exocrine gastric



**E**, Tubular and solid pattern of adenocarcinoma (H&E,  $\times 10$ ). **F**, Gastrin is a mature endocrine marker of the gastric type ( $\times 20$ ). **G**, Prevalent solid pattern of poorly differentiated adenocarcinoma (H&E,  $\times 10$ ). **H**, Gastric inhibitory polypeptide is a mature endocrine marker of the intestinal type ( $\times 20$ ).

phenotype (Exo-G): MUC5AC+ and/or MUC6+ but MUC2–, villin–, and CD10–; (2) exocrine intestinal phenotype (Exo-I): MUC2+, villin+, and/or CD10+ but MUC5AC– and MUC6–; or (3) exocrine mixed/gastrointestinal phenotype (Exo-GI): at least 1 gastric and 1 intestinal exocrine phenotype marker simultaneously positive. Cancers negative for gastric and intestinal exocrine phenotype markers were classified as having an exocrine null phenotype.

Gastrin and somatostatin were investigated in CgA+ and/or Syn+ tumors as gastric endocrine markers, and GIP and GLP-1 were used as intestinal endocrine markers. Gastric cancers were classified into 3 types based on the expression of maturely differentiated endocrine markers: (1) endocrine gastric phenotype (Endo-G): positive for gastrin and/or somatostatin but GIP– and GLP-1–; (2) endocrine intestinal phenotype (Endo-I): GIP+ and/or GLP-1+ but negative for gastrin and somatostatin; or (3) endocrine mixed/gastrointestinal phenotype (Endo-GI): at least 1 gastric and 1 intestinal endocrine phenotype marker simultaneously positive. Cancers negative for both gastric and intestinal endocrine phenotype markers were classified as having an endocrine null phenotype.

### Statistical Analysis

Differences between groups were analyzed by using the  $\chi^2$  test or Fisher exact test, when appropriate.<sup>20</sup> Survival analyses were performed with the Kaplan-Meier method.<sup>21</sup> Differences between tumor categories were analyzed with the log-rank test. A probability value (*P*) less than or equal to .05 was considered statistically significant (2-sided).

## Results

### Prognostic Implications of Endocrine Differentiation

Among the 103 gastric adenocarcinomas and undifferentiated carcinomas, 45 tumors (44%) showed CgA immunoreactivity, 64 (62%) showed Syn immunoreactivity, and 66 (64%) were positive for at least 1 (neuro)endocrine marker **Image 1B** and **Image 1C**. The cases were divided in 3 groups according to the percentage of CgA+ and/or Syn+ tumoral cells: 0% (37 [36%]), 1% to 20% (52 [50%]), and more than 20% (14 [14%]). Clinicopathologic data for the cases are shown in **Table 1**. No significant correlations were recognized between CgA and/or Syn expression and age, type of diagnosis, histopathologic grading, pTNM, or clinical stage. There was a preponderance of men among cases with 1% to 20% positivity for CgA and/or Syn markers (*P* = .01). Endocrine differentiation in scattered tumor cells was also found in lymph node metastases from tumors expressing CgA and/or Syn in more than 20% of cells.

**Table 1**  
Clinicopathologic Characteristics of 103 Patients With Gastric Carcinoma and Correlation With Chromogranin A and/or Synaptophysin Tumoral Expression\*

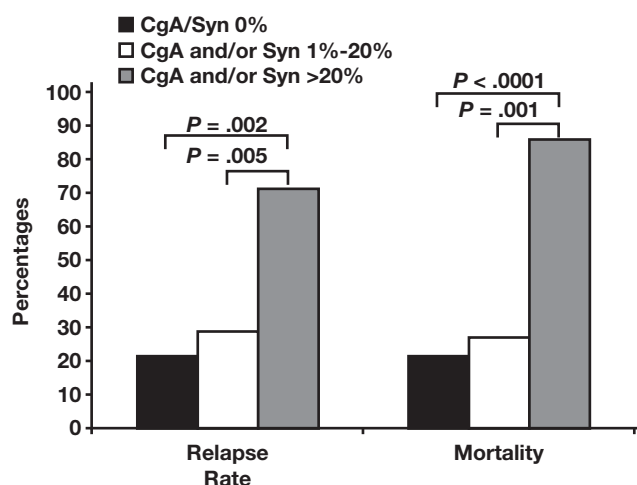
	Chromogranin A and Synaptophysin Tumoral Expression	Chromogranin A and/or Synaptophysin Tumoral Expression	
	0%	1%-20%	>20%
Age (y)			
Mean $\pm$ SD	67.8 $\pm$ 10.4	64.3 $\pm$ 11.4	62.3 $\pm$ 11.3
Range	50-84	40-94	49-85
Sex			
Male	17	39 <sup>†</sup>	7
Female	20	13	7
Diagnosis			
Adenocarcinoma	28	35	8
Undifferentiated carcinoma	9	17	6
Histopathologic grading			
G1 + G2	3	8	0
G3	25	27	8
G4	9	17	6
Primary tumor			
T1 + T2	6	13	2
T3 + T4	31	39	12
Regional lymph nodes			
N0	10	16	8
N+	27	36	6
Distant metastasis			
M0	33	45	13
M1	4	7	1
Clinical stage			
I	1	6	1
II	14	16	6
III	18	23	6
IV	4	7	1

\* Data are given as number of cases unless otherwise indicated. For histopathologic grading, primary tumor, regional lymph nodes, distant metastasis (pTNM) and clinical stage see Sobin et al.<sup>18</sup> N+ includes N1, N2, and N3.

<sup>†</sup> *P* = .01.

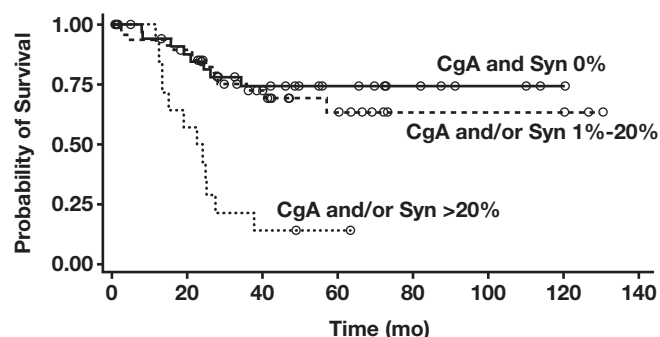
Follow-up data were available for all patients. At last follow-up, 51 patients had died (34 died of cancer, 17 died of other causes), and 52 were alive (50 alive without evidence of disease, 2 alive with progression of disease). The relapse rate and disease-specific mortality were evaluated in relation to tumoral expression of CgA and/or Syn markers. Patients with tumors with more than 20% of cells expressing CgA and/or Syn had a poorer prognosis compared with patients with tumors with 1% to 20% of cells expressing CgA and/or Syn and tumors without CgA/Syn expression (relapse rate, *P* = .005 and *P* = .002; mortality, *P* = .001 and *P* < .0001, respectively). Of 14 patients with more than 20% tumor cells positive for CgA and/or Syn, 10 (71%) had recurrence and 12 (86%) died of cancer. Of 51 patients with 1% to 20% tumor cells immunoreactive for CgA and/or Syn, 15 (29%) had recurrence and 14 (27%) died of cancer. Of 37 patients with neither CgA nor Syn tumoral expression, 8 (22%) had recurrence after surgical resection and 8 (22%) died of cancer **Figure 1**. These results were confirmed after adjustment by stage (stages I/II vs III/IV).



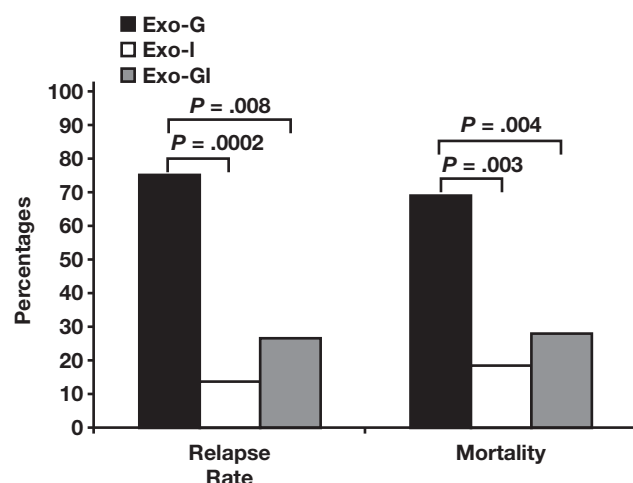


**Figure 1** The presence of endocrine differentiation in more than 20% of tumor cells in conventional gastric carcinomas is a negative prognostic factor. In these cases, the relapse rate and disease-specific mortality are higher than in other cases. Differences are statistically significant. CgA, chromogranin A; Syn, synaptophysin.

The 5-year survival rates were significantly different among patients with different percentages of tumor cells positive for the (neuro)endocrine markers CgA and/or Syn **Figure 2**. Patients with gastric carcinomas with more than 20% positivity for CgA and/or Syn had a significantly worse prognosis than did patients with gastric carcinomas showing no or 1% to 20% positivity for CgA and/or Syn markers ( $P < .0001$ ). The same results were confirmed by adjusting by clinical stage (stages I/II vs III/IV;  $P < .0001$ ).



**Figure 2** Kaplan-Meier disease-specific survival curves demonstrating a significant difference in survival among patients with gastric carcinoma showing 0%, 1% to 20%, and more than 20% of tumor cells positive for chromogranin A (CgA) and/or synaptophysin (Syn) markers ( $P < .0001$ ). Results were confirmed by adjusting by clinical stage ( $P < .0001$ ).



**Figure 3** The maturely differentiated exocrine gastric (Exo-G) phenotype in conventional gastric carcinomas is a negative prognostic factor. In these cases, the relapse rate and disease-specific mortality are higher than in cases with mature Exo-I (exocrine intestinal phenotype) and mature Exo-GI (exocrine mixed/gastrointestinal phenotype) carcinomas. Differences are statistically significant.

### Prognostic Implications of Maturely Differentiated Exocrine Phenotypes

In our series, immunoreactivity for MUC5AC, MUC6 **Image 2B**, MUC2, CD10 **Image 2D**, and villin was observed in 73 (70%), 35 (34%), 51 (50%), 40 (39%), and 67 (65%) of tumors, respectively. On the basis of the expression of these differentiated exocrine markers, we phenotypically classified the 103 gastric cancers as carcinomas with mature Exo-G phenotype (16 [16%]), mature Exo-I phenotype (22 [21%]), and mature Exo-GI phenotype (64 [62%]). Only 1 gastric carcinoma (1%) showed no mature exocrine marker expression and was defined as a carcinoma with an exocrine null phenotype.

Considering the main clinicopathologic variables listed in Table 1, there were no significant differences among tumors with Exo-G, Exo-I, and Exo-GI phenotypes (data not shown).

The relapse rate and disease-specific mortality were evaluated in relation to the expression of mature exocrine phenotypes. Differences among mature Exo-G tumors compared with mature Exo-I and among mature Exo-G

tumors compared with mature Exo-GI were statistically significant (relapse rate,  $P = .0002$  and  $P = .008$ ; mortality,  $P = .003$  and  $P = .004$ , respectively). In detail, 12 (75%) of 16, 3 (14%) of 22, and 17 (27%) of 64 patients with mature Exo-G, Exo-I, and Exo-GI carcinomas, respectively, had a relapse, whereas 11 (69%) of 16, 4 (18%) of 22, and 18 (28%) of 64 patients died of cancer **Figure 3**. These results were confirmed after adjustment by clinical stage (stages I/II vs III/IV).

Hence, cases of mature Exo-G carcinomas significantly correlated with a lower 5-year disease-specific survival rate than did cases of mature Exo-I and mature Exo-GI carcinomas ( $P < .0004$ ) **Figure 4**. Tumor stage and maturely differentiated exocrine phenotype were independently prognostic factors for survival.

### Prognostic Implications of Maturely Differentiated Endocrine Phenotypes in the Setting of CgA+ and/or Syn+ Tumors

Of 66 common gastric carcinomas with endocrine differentiation, 24 (36%), 14 (21%), 23 (35%), and 14 (21%) showed immunoreactivity for gastrin **Image 2F**, somatostatin (not shown), GIP **Image 2H**, and GLP-1 (not shown), respectively. On the basis of expression of these markers, tumors were classified as carcinomas with a mature Endo-G phenotype (26 [39%]), a mature Endo-I phenotype (21 [32%]), and a mature Endo-GI phenotype (5 [8%]). Moreover, 14 tumors (21%) showed no mature endocrine markers expression and were defined as carcinomas with an endocrine null phenotype.

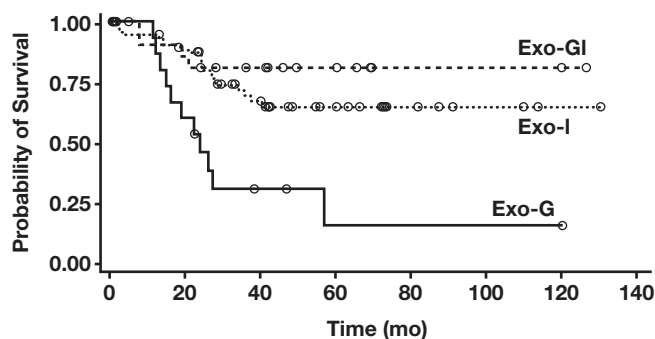
Considering the main clinicopathologic variables listed in Table 1, there were no significant differences between tumors with Endo-G, Endo-I, and Endo-GI phenotypes (data not shown).

The maturely differentiated Endo-G phenotype was significantly associated with a higher relapse rate and higher disease-specific mortality than Endo-I and Endo-GI maturely differentiated phenotypes (mature Endo-G tumors vs mature Endo-I and vs mature Endo-GI tumors, relapse rate,  $P < .0001$  and  $P = .01$ ; mortality,  $P = .001$  and  $P = .06$ , respectively). In detail, 17 (65%) of 26, 1 (5%) of 21, and 0 (0%) of 5 patients with mature Endo-G, Endo-I, and Endo-GI gastric carcinomas, respectively, had a relapse, while 18 (69%) of 26, 4 (19%) of 21, and 1 (20%) of 5 died of cancer **Figure 5**. These results were confirmed after adjustment by clinical stage (stages I/II vs III/IV).

Disease-specific survival in the Endo-G phenotype group was significantly worse than in the Endo-I and Endo-GI phenotype groups ( $P = .002$ ) **Figure 6**. Significant differences were also observed after adjustment for clinical stage (stages I/II vs III/IV;  $P = .003$ ).

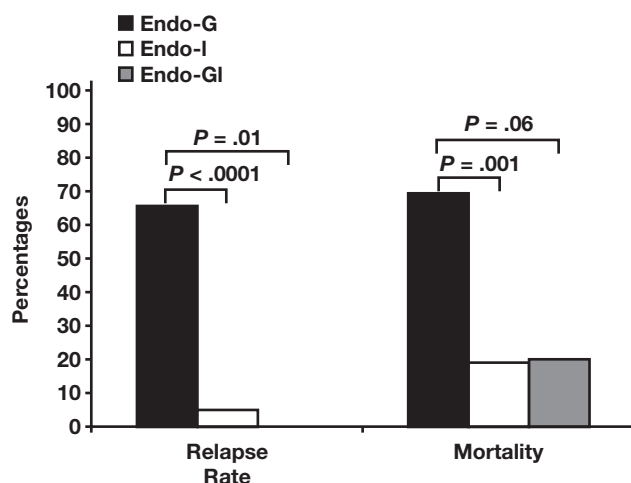
## Discussion

The presence of tumor cells with endocrine features, detected by immunohistochemical analysis, in common gastric carcinomas is a known phenomenon that has been described in about 15% to 70% of stomach tumors, depending mainly on different criteria used for evaluation or variable sensitivity of antibody clones used.<sup>5-7,10,12,22</sup> The expression



**Figure 4** Kaplan-Meier disease-specific survival curves showing a significant difference in survival among patients with maturely differentiated Exo-G (exocrine gastric phenotype), Exo-I (exocrine intestinal phenotype), and Exo-GI (exocrine mixed/gastrointestinal phenotype) common gastric carcinomas ( $P < .0004$ ). Results were confirmed by adjusting by clinical stage ( $P = .006$ ).

of CgA and Syn is used to assess endocrine differentiation in these tumors. Therefore, we evaluated the expression of both markers in a series of 103 common stomach carcinomas, namely 71 adenocarcinomas and 32 undifferentiated carcinomas. Among tumors expressing CgA, Syn, or both (64%), 52 and 14 cases showed 1% to 20% and more than 20% of cells with endocrine differentiation, respectively. Because



**Figure 5** The maturely differentiated endocrine gastric (Endo-G) phenotype in conventional gastric carcinomas with endocrine differentiation (positive for chromogranin A and/or synaptophysin) is a negative prognostic factor. In these cases, the relapse rate and disease-specific mortality are higher than in cases of the mature Endo-I (endocrine intestinal phenotype) and mature Endo-GI (endocrine mixed/gastrointestinal phenotype) stomach carcinomas. Differences are statistically significant.

23 tumors (22%) were immunoreactive for only 1 endocrine marker, testing both CgA and Syn markers proved useful to properly determine the presence of endocrine differentiation. We did not find a correlation between the presence of endocrine differentiation and pTNM or other common clinical parameters, even though a male preponderance was seen in the group characterized by 1% to 20% of tumor cells expressing CgA and/or Syn.

The histogenesis of mixed exocrine-endocrine stomach cancers remains to be fully understood. In tissues characterized by high rates of cellular turnover, ie, the epithelium of the gastrointestinal tract, stem or progenitor cells are probably the only cells that persist long enough to acquire and accumulate multiple genetic alterations and epigenetic changes leading to malignant phenotypes. In the stomach, the multipotent stem/progenitor cell compartment is believed to lie in the neck/isthmus region of gastric glands, giving rise to 5 functionally distinct epithelial cell types: parietal, surface mucous (pit), zymogenic, neck, and enteroendocrine cells.<sup>23</sup>

A lineage progenitor has typically been thought to be committed to the production of a mature cell type that performs a specific function. Thus, a preparietal cell gives rise to a parietal cell, not an enteroendocrine cell. An analysis of a transgenic mouse model of metastatic gastric cancer provided some evidence for more plasticity of progenitor cell commitment and differentiation than previously described.<sup>24</sup> In these mice, the transition from preparietal cell hyperplasia to neoplasia and metastasis is marked by increased expression of endocrine markers such as CgA and DOPA decarboxylase. Hence, the finding that an epithelial lineage progenitor that normally gives rise to a nonendocrine cell type can

transdifferentiate to metastatic cells with an endocrine phenotype may provide a possible explanation concerning the cellular origin of gastric carcinomas with endocrine differentiation. Other original histogenetic theories basically imply that (mutated) multipotent stem/progenitor cells harbor the ability of differentiating toward exocrine and endocrine lineages, though little pertinent evidence from gastric cancer stem cells has been reported.<sup>10,25,26</sup>

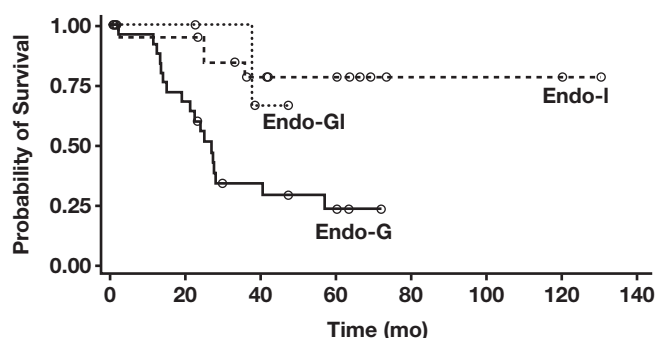
It is interesting that endocrine differentiation in common gastric cancer should be considered an intrinsic tumoral phenomenon, as supported by the presence of scattered tumoral cells expressing CgA and/or Syn in lymph node metastases from carcinomas with endocrine differentiation. The opposite theory of entrapment of normal endocrine cells in the primary tumor could be discharged considering that CgA+ and/or Syn+ cells had true neoplastic morphologic features with evident cytonuclear atypia and are detectable in infiltrating areas classically devoid of normal enteroendocrine cells.

From a clinicopathologic viewpoint, conventional gastric adenocarcinomas have been reported to be prognostically distinct from those variably immunoreactive for at least 1 generic endocrine marker, such as CgA and Syn.<sup>12</sup> In addition, when a cutoff value of 20% tumor cells was used to discriminate adenocarcinomas with endocrine differentiation from large cell neuroendocrine carcinomas, the latter were significantly associated with a worse prognosis and, therefore, have been considered a distinct clinical and histopathologic entity.

In general, endocrine differentiation may be important for pathologic classification and could also be clinically relevant. In fact, in our tumor series, the presence of endocrine differentiation with a cutoff of 20% of CgA+ and/or Syn+ tumor cells was significantly correlated with a higher relapse rate and higher disease-specific mortality compared with conventional tumors and tumors expressing CgA and/or Syn in 1% to 20% of cells.

From a clinical standpoint, a gastric cancer with more than 20% CgA and/or Syn expression may be a bona fide discrete entity as it may be associated with definite therapeutic and/or prognostic findings. Therefore, pathologists should identify and quantify possible endocrine differentiation in high-grade gastric carcinomas.

The second part of the study was focused on analyzing, in the same series, the differential expression of maturely differentiated exocrine and endocrine markers. Prognostically significant differences in relapse rate, disease-specific mortality, and overall survival emerged, indicating that patients with carcinomas expressing only mature exocrine markers of the gastric type had a worse prognosis than did patients with carcinomas expressing only intestinal-type markers or both gastric and intestinal markers. Hence, our data confirmed some previous observations on the negative prognostic impact of the gastric exocrine phenotype.<sup>16,27</sup>



**Figure 6** Kaplan-Meier disease-specific survival curves showing a significant difference in survival among patients with maturely differentiated Endo-G (endocrine gastric phenotype), Endo-I (endocrine intestinal phenotype), and Endo-GI (endocrine mixed/gastrointestinal phenotype) stomach carcinomas with generic endocrine differentiation ( $P = .002$ ). Results were confirmed by adjusting by clinical stage ( $P = .003$ ).



The biologic basis of these findings may rely on the enhanced ability of intramucosal stomach carcinomas with a gastric phenotype to degrade the extracellular matrix through overexpression of matrix metalloproteinases compared with those with an intestinal phenotype.<sup>28</sup> Furthermore, Shibata et al<sup>29</sup> demonstrated that the ratio between the apoptotic index and proliferative index was significantly lower in (early) stomach cancers with a gastric phenotype compared with the intestinal phenotype, and Yamazaki et al<sup>30</sup> reported that microsatellite instability was significantly associated with the gastric phenotype, whereas an APC mutation was associated with the intestinal phenotype.

Patients with tumors of the exocrine gastric phenotype have a better prognosis when treated with fluorouracil-based therapy compared with other chemotherapeutic regimens.<sup>31</sup> This may be due to lower intratumoral expression of thymidylate synthase in the gastric phenotype compared with intestinal phenotype tumors, a finding based on the association of high expression of thymidylate synthase with drug resistance.<sup>31,32</sup>

At present, however, information about a specific therapy based on tumor phenotyping is incomplete. Validated and standardized diagnostic criteria are currently missing even if we and other investigators propose that phenotyping is not only possible but also may be clinically relevant using relatively few markers.

Concurrently with exocrine phenotyping, we were able to document that tumors positive for generic endocrine markers such as CgA and Syn may also express mature endocrine markers. In our series, 79% of stomach carcinomas with endocrine differentiation were immunoreactive for maturely differentiated endocrine markers of the gastric type (gastrin, somatostatin) and/or of the intestinal type (GIP, GLP-1) (mature Endo-G phenotype [39%], mature Endo-I phenotype [32%], and mature Endo-GI phenotype [8%]). This further characterization of the endocrine phenotype not only has a speculative interest but may also provide a more refined prognostic stratification. Our data suggest that tumoral expression of maturely differentiated endocrine markers of the gastric type may be considered a negative prognostic factor because patients with such tumors had a higher relapse rate and higher disease-specific mortality, but these results need to be confirmed in a larger series.

Immunohistochemical evaluation of endocrine differentiation and assessment of maturely differentiated exocrine and endocrine phenotypes in conventional gastric carcinomas may supply useful prognostic information. The presence of endocrine differentiation is a significant prognostic marker when a cutoff value of more than 20% positive tumor cells is fixed. Mature Exo-G and mature Endo-G phenotypes are negative prognostic markers. Exocrine phenotypic characterization of common gastric

carcinomas may be, in some cases, predictive of a patient's therapeutic response.

Because selected patients are at high risk of relapse, appropriate postoperative follow-up may be indicated. It is unknown if gastric carcinomas with endocrine differentiation are more or less chemosensitive or radiosensitive than conventional nonendocrine carcinomas. Further studies are needed to shed light on this topic.

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*Supported in part by the research project "Integrazione delle attività di ricerca attraverso la costruzione di strutture e reti di collaborazione interistituzionali-Rete Nazionale Telepatologia (TESEO)" grant No. ACC/R8.5, Rome, Italy (Drs Canzonieri and Carbone, for immunohistochemistry evaluation); by the research project "Ruolo delle fosfoproteine nella chemioresistenza delle cellule staminali tumorali di colon e retto con analisi comparativa immunofenotipica," grant No. 527/B/3A/3 from the Istituto Superiore di Sanità, Rome (Dr Canzonieri, for immunohistochemistry analysis).*

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*Acknowledgments: We are grateful to Paola Ceolin, Barbara Canal, Antonella Selva, Laura Zannier, and Sandro Barbuscia for skilled technical assistance.*

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