Clinicopathologic and molecular characteristics of gastric cancer showing gastric and intestinal mucin phenotype

Naohide Oue, Kazuhiro Sentani, Naoya Sakamoto and Wataru Yasui

Department of Molecular Pathology, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

Gastric cancer (GC), one of the most common human cancers, can be classified into gastric or intestinal phenotype according to mucin expression. TP53 mutation, allelic deletion of the APC gene and nuclear staining of β-catenin are frequently detected in the intestinal phenotype of GC, whereas CDH1 gene mutation, microsatellite instability and DNA hypermethylation of MLH1 are common events in the gastric phenotype of GC. Our Serial Analysis of Gene Expression (SAGE) and *Escherichia coli* ampicillin secretion trap (CAST) analyses revealed that CDH17, REG4, OLFM4, HOXA10, DSC2, TSPAN8 and TM9SF3 are upregulated in GC and that CLDN18 is downregulated in GC. Expression of CDH17, REG4, HOXA10 and DSC2 and downregulation of CLDN18 are observed in the intestinal phenotype of GC. In contrast, OLFM4 is expressed in the gastric phenotype of GC. Expression of TSPAN8, TM9SF3 and HER2 are not associated with either gastric or intestinal phenotypes. Ectopic CDX2 expression plays a key function in the GC intestinal phenotype. MUC2, CDH17, REG4, DSC2 and ABCB1 are direct targets of CDX2. Importantly, these genes encode transmembrane/secretory proteins, indicating that the microenvironment as well as cancer cells are also different between gastric and intestinal phenotypes of GC.

**Definition of gastric and intestinal phenotypes of gastric cancer**

Previously, gastric/intestinal classification was determined by H&E staining. Egashira *et al.* provided the initial histologic characteristics of gastric/intestinal phenotypes of GC. The gastric phenotype of GC consists of cuboidal or columnar cells with clear cytoplasm that are arranged side-by-side like foveolar epithelial cells or pyloric gland cells. Their nuclei are round and situated in the basal cytoplasm. Mucin droplets are found in the apical cytoplasm. In contrast, the intestinal phenotype of GC resembles colorectal cancer, and is mainly composed of columnar cells with eosinophilic cytoplasm and goblet cell differentiation. The intraluminal surface of tubules has a striated border and surface coat mucin.

After 2000, the gastric/intestinal phenotypes of GC were analyzed by immunohistochemistry using MUC5AC (or Human gastric mucin (HGM)) and MUC6 as markers for the gastric phenotype, and MUC2 and CD10 (or villin) as markers for the intestinal phenotype. Based on expression of these markers, GC cases are classified into four phenotypes: gastric or foveolar (G type), intestinal (I type), gastric and intestinal mixed (GI type), and neither gastric nor intestinal (N type). Several addi-
Clinical characteristics of gastric cancer gastric/intestinal phenotypes

Substantial effort has been devoted to analyzing characteristics of gastric/intestinal phenotypes of GC. Although the data have been controversial and the characteristics of gastric/intestinal GC types are still ambiguous, several important points have been established. One important concept is that almost all intramucosal GC cases show a gastric phenotype, including gastric and intestinal mixed phenotype, whereas gastric phenotype is diminished according to GC progression. A previous report revealed that the incidence of GC showing gastric phenotype decreases as the tumor diameter increases.\(^9\) In submucosal GC, the frequency of gastric phenotype decreases.\(^15\) GC cases at early stages, independent of the histological type, mainly consist of gastric phenotype, and phenotypic shift from gastric to intestinal phenotype is clearly observed with progression of tumor stage.\(^7\) There is no clear correlation between phenotype and clinicopathologic characteristics, including sex, age, location of GC, or macroscopic features.\(^15\)

Prognosis of gastric/intestinal phenotypes of GC has been investigated. However, a definitive conclusion has not been established. One possible explanation is that each study uses different definitions of gastric/intestinal phenotypes. To establish a clear association between prognosis and gastric/intestinal phenotypes, the gastric/intestinal phenotype definitions must be clarified.

Molecular characterization of gastric cancer gastric/intestinal phenotypes

To understand the gastric/intestinal phenotypes of GC at the molecular level, several genetic and epigenetic alterations have been investigated (Table 1). \(TP53\) mutation and allelic deletion of the \(APC\) gene are detected more frequently in the intestinal phenotype of GC.\(^8,16\) In contrast, \(CDH1\) gene mutation is detected in differentiated type GC showing gastric phenotype.\(^17\) Microsatellite instability (MSI) is detected more frequently in the gastric phenotype of GC.\(^18,18\) Alterations of \(TP73\), including loss of heterozygosity and abnormal expression, play an important role in the genesis of the gastric phenotype of GC.\(^19\) Several epigenetic alterations have also been identified. DNA methylation of \(MLH1\) gene frequently occurs in the gastric phenotype of GC,\(^20\) whereas \(MGMT\) gene is frequently methylated in the intestinal phenotype of GC.\(^21\)

Expression of cancer-associated genes has also been investigated by immunohistochemistry (Table 1). Aberrant expression of activation-induced cytidine deaminase is common event in intestinal phenotype of GC.\(^22\) Studies have shown that the cytokeratin (CK) profile is different between GC and colorectal cancer. Colorectal cancer shows a CK7\(^{-}\)/CK20\(^{+}\) expression pattern, whereas adenocarcinomas of foregut origin, including GC, demonstrate a CK7\(^{-}\)/CK20\(^{-}\) expression pattern.\(^23\) In our study, GC cases showing CK7\(^{-}\)/CK20\(^{+}\) were frequently found in intestinal phenotype of GC, whereas GC cases showing CK7\(^{+}\)/CK20\(^{-}\) were commonly found in gastric phenotype of GC.\(^13\) Nuclear \(\beta\)-catenin staining was frequently found in the intestinal phenotype of GC. However, expression of MMP7, laminin \(\gamma2\) or HER2 was not correlated with GC gastric or intestinal phenotypes.\(^24\) Together these observations indicate that in addition to histologic characteristics, genetic, epigenetic and gene expression alterations in the intestinal phenotype of GC are similar to those of colorectal cancer, while those of the gastric phenotype of GC are clearly different from those of colorectal cancer.

Identification of gastric cancer-associated genes by comprehensive gene expression analysis

To identify potential molecular markers for GC and to better understand the development of GC at the molecular level, comprehensive gene expression analysis is useful. Serial Analysis of Gene Expression (SAGE) is used to analyze 14-
AID, activation-induced cytidine deaminase; CK, cytokeratin; EGFR, epidermal growth factor receptor.

Our immunohistochemical study detected LI-cadherin expression in 67% of GC tissue samples, and LI-cadherin expression was significantly more frequent in advanced stage GC than in early stage GC. A previous report showed that LI-cadherin is a marker of intestinal metaplasia of the stomach, suggesting LI-cadherin as a marker for the intestinal phenotype. Indeed, LI-cadherin expression is frequently found in the intestinal phenotype of GC. These results indicate that LI-cadherin is one of the key regulators for the intestinal phenotype of GC.

REG4. Through SAGE analysis, REG4 was found to be one of the upregulated genes in GC. REG4 is a member of the REG gene family and encodes Reg IV protein. REG4 was originally identified by high-throughput sequence analysis of an inflammatory bowel disease cDNA library. REG IV is an activator of the epidermal growth factor receptor (EGFR) signaling pathway and increases expression of Bcl-2, Bcl-xl and survivin, which inhibit apoptosis. We previously reported that Reg IV inhibits 5-fluorouracil (5-FU)-induced apoptosis through EGFR activation in GC cells. It has been reported that Reg IV is upregulated in undifferentiated-type GC, and that the increased tumorigenic ability of ALDH1-positive cells depends on Reg IV. These findings support the notion that Reg IV protein participates in carcinogenesis.

In non-neoplastic human tissue, Reg IV expression is narrowly restricted. In non-neoplastic stomach, foveolar epithelial cells do not express Reg IV, whereas goblet cells of intestinal metaplasia and neuroendocrine cells at the base of intestinal metaplasia express Reg IV, suggesting Reg IV as a marker for the intestinal phenotype. Expression and localization of Reg IV in human cancers have been analyzed by immunohistochemistry. The immunohistochemistry reports show that Reg IV is overexpressed in adenocarcinoma cells that are positive for MUC2. Overexpression of Reg IV is also observed in neuroendocrine neoplasms. Intestinal carcinoid tumors, parathyroidal cell tumors, small-cell carcinomas of the lung, and Merkel cell carcinomas also overexpress Reg IV. In our study, Reg IV expression was detected in 29% of GC cases, and was frequently found in the intestinal phenotype of GC. Together these data indicate that Reg IV activates EGFR and plays an important role in the inhibition of apoptosis in the intestinal phenotype of GC.

**Table 1. Summary of genetic/epigenetic/gene expression alterations in gastric and intestinal phenotypes of gastric cancer**

| Function | Frequency (%) | References |
|----------|---------------|------------|
| **Gastric phenotype** | | |
| SOX2 | 5 | 31 | 8 |
| CDX1, CDX2 | 0 | 38 | 16 |
| TPS3 mutation | 21 | 0 | 17 |
| APC deletion | 80 | 0 | 19 |
| CDH1 mutation | 45 | 0 | 8 |
| TP73 deletion | 74 | 33 | 20 |
| Microsatellite instability | 46 | 82 | 21 |
| MLH1 DNA methylation | 6 | 46 | 13 |
| MGMT DNA methylation | 14 | 38 | 22 |
| AID expression | 12 | 31 | 32 |
| EGFR expression | – | CK7+/CK20− | CK7−/CK20+ |
| Cytokeratin profile | 5 | 63 | 32 |
| Li-cadherin expression | 1 | 77 | 11 |
| Reg IV expression | 25 | 44 | 54 |
| HoxA10 expression | 28 | 45 | 61 |
| Desmocollin 2 expression | 48 | 74 | 83 |
| MDR1 expression | – | Unknown function | 73 |
| Olfactomedin 4 expression | 44 | 74 | 70 |
| Claudin-18 down-regulation | 77 | 11 |
| **Intestinal phenotype** | | |
| Olfactomedin 4 | 1 | 77 | 11 |
| Claudin-18 | 44 | 74 | 70 |
| REG4 | 5 | 38 | 22 |
| CDX1, CDX2 | 0 | 38 | 16 |
| TPS3 mutation | 21 | 0 | 17 |
| APC deletion | 80 | 0 | 19 |
| CDH1 mutation | 45 | 0 | 8 |
| TP73 deletion | 74 | 33 | 20 |
| Microsatellite instability | 46 | 82 | 21 |
| MLH1 DNA methylation | 6 | 46 | 13 |
| MGMT DNA methylation | 14 | 38 | 22 |
| AID expression | 12 | 31 | 32 |
| EGFR expression | – | CK7+/CK20− | CK7−/CK20+ |
| Cytokeratin profile | 5 | 63 | 32 |
| Li-cadherin expression | 1 | 77 | 11 |
| Reg IV expression | 25 | 44 | 54 |
| HoxA10 expression | 28 | 45 | 61 |
| Desmocollin 2 expression | 48 | 74 | 83 |
| MDR1 expression | – | Unknown function | 73 |
| Olfactomedin 4 expression | 44 | 74 | 70 |
| Claudin-18 down-regulation | 77 | 11 |

bp tags derived from defined positions of cDNA without a priori knowledge of the sequence of the genes expressed, and offers an unbiased, comprehensive gene expression profiling approach. Escherichia coli ampicillin secretion trap (CAST) is a signal sequence trap method to identify genes that encode transmembrane or secretory proteins. Schematic outline of the SAGE and CAST methods are shown in Figures S1 and S2. We performed SAGE and CAST on GC samples and identified several genes whose expression was altered in GC. Among them, CDH17, REG4, OLFM4, HOXA10, DSC2, TSPAN8 and TM9SF3 were upregulated in GC, and CLDN18 was downregulated in GC. Importantly, many of these genes are tightly associated with gastric/intestinal phenotypes of GC.
cell function. In fact, olfactomedin 4 expression is detected in crypt base columnar cells, which are intestinal stem cells. Thus, antibody against olfactomedin 4 is useful to identify intestinal stem cells.\(^{(46)}\) Using anti-olfactomedin 4 antibody in APC\(^{(Min/+)}\) mice, we found that dietary suiludac induces apoptosis to remove intestinal stem cells with nuclear or phosphor-
ylated β-catenin.\(^{(47)}\) We also showed that canonical Wnt signals support homeostatic intestinal stem/progenitor cell prolif-eration.\(^{(48)}\) Two studies have investigated OLFM4 knockout (KO) mice. Liu \et\(d\)\(^{(49)}\) demonstrated that Helicobacter pylori colonization in the gastric mucosa of OLFM4 KO mice was significantly lower compared with wild-type mice, and reduced bacterial colonization was associated with enhanced infiltration of inflammatory cells in gastric mucosa. Schuijers \et\(d\)\(^{(50)}\) report that OLFM4 KO mice showed no phenotype.

In non-neoplastic stomach, foveolar epithelial cells do not express olfactomedin 4, whereas olfactomedin 4 is expressed in the basal crypt epithelium in the intestinal metaplasia of the stomach, suggesting that olfactomedin 4 may be a marker for the intestinal phenotype.\(^{(46)}\) In our immunohistochemical analysis,\(^{(46)}\) expression of olfactomedin 4 was detected in 56% of GC cases, and expression of olfactomedin 4 was frequently detected in well-differentiated adenocarcinomas. In well-differ-
entiated adenocarcinomas, patients with olfactomedin 4-posi-
tive GC had a better survival rate than those with olfactomedin 4-negative GC. Expression of olfactomedin 4 was frequently observed in the gastric phenotype. Together this indicates that olfactomedin 4 plays an important role in the gastric phenotype of GC. Similar results were shown in colorectal and endometrioid adenocarcinoma.\(^{(51–53)}\)

Both Reg IV and olfactomedin 4 are secreted proteins, and serum Reg IV and olfactomedin 4 serve as tumor markers for GC. The sensitivity and specificity of serum olfactomedin 4 combined with Reg IV for GC detection were 52% and 95%, respectively.\(^{(46)}\) These data suggest that serum olfactomedin 4 combined with Reg IV is likely to be suitable for screening of GC.

\textbf{HOXA10.} \textit{HOXA10}, which encodes HoxA10 protein, was identified as one of the upregulated genes in GC in SAGE analy-
sis.\(^{(54)}\) \textit{HOX} genes are important regulators of embryonic mor-
phogenesis and differentiation, and control normal development patterning along the anteroposterior axis.\(^{(55)}\) The homeodomain binds to sequence-specific DNA motifs and regulates the trans-
scription of genes relevant to the formation of specific segmental architecture. Overexpression of \textit{HOXA10} has been detected in prostate, lung and ovarian cancer.\(^{(56–58)}\) Forced expression of HoxA10 has been reported to promote cell proliferation, sug-
gesting that overexpression of HoxA10 may participate in the pathogenesis of cancer.\(^{(56)}\) In contrast, another study showed that HoxA10 induces expression of \textit{CDKN1}, which encodes p21 protein,\(^{(59)}\) and downregulation of HoxA10 has been reported in endometrial cancer.\(^{(60)}\) Therefore, the significance of HoxA10 expression in human cancers is still unclear, and further investiga-
tion is required.

Our study by immunohistochemistry revealed that in non-
neoplastic stomach, foveolar epithelial cells do not express HoxA10, whereas HoxA10 is expressed in the intestinal meta-
plasia of the stomach, suggesting that HoxA10 could be a mark-
er for the intestinal phenotype.\(^{(54)}\) HoxA10 expression was detected in 30% of GC cases, and the prognosis of patients with positive HoxA10 expression was significantly better than those with negative HoxA10 expression. In addition, HoxA10 expression is frequently found in the intestinal phenotype of GC. Together this suggests that HoxA10 is a key factor in the intestinal phenotype of GC.

\textbf{DSC2.} Through CAST analysis, DSC2 was identified as one of the upregulated genes in GC.\(^{(61)}\) DSC2 encodes desmocollin 2 protein, one of the three known desmocollin proteins. In the mature organism, desmosomes are most abundant in areas sub-
ject to mechanical stress, including skin, heart and esopha-
gus.\(^{(62)}\) Desmocollins are membrane-spanning glycoproteins that form desmosomes along with desmogleins and function as Ca\(^{2+}\)-dependent cell adhesion molecules.\(^{(63)}\)

In our study, immunohistochemical analysis showed weak or no staining of desmocollin 2 in the foveolar epithelium of the stomach, whereas desmocollin 2 expression was observed in the intestinal metaplasia. Expression of desmocollin 2 was detected in 28% of GC tissue samples, and was frequently found in the intestinal phenotype of GC.\(^{(61)}\)

\textbf{TSPAN8.} Through CAST analysis, TSPAN8 was demonstrated to be one of the upregulated genes in GC.\(^{(64)}\) TSPAN8 encodes tetraspanin 8 protein and is a member of the tetra-
span family. Tetraspanins proteins cross the membrane four times and are involved in numerous biological processes.\(^{(65)}\) Tetraspanins proteins are components of exosomes, and exo-
somes containing rat Tspan8 have been shown to affect tumor cell migration, proliferation and tumor angiogenesis.\(^{(66)}\)

Our immunohistochemical analysis revealed that 34% of GC cases were positive for tetraspanin 8, and microvessels density was higher in tetraspanin 8-positive GC cases compared with tetraspanin 8-negative GC cases.\(^{(64)}\) Furthermore, tetraspanin 8 expression was an independent prognostic classifier of patients with GC. Expression of tetraspanin 8 was not associated with the gastric or intestinal phenotype of GC, indicating that tetra-
spanin 8 plays a crucial role in both the gastric and intestinal phenotypes of GC.

\textbf{TMSF3.} TM9SF3 was identified as one of the upregulated genes in GC by CAST analysis.\(^{(67)}\) TM9SF3, which encodes transmembrane 9 superfamily member 3 protein, is a member of the TM9SF family. TM9SF proteins are characterized by a large noncytoplasmic domain and nine putative transmembrane domains.\(^{(68)}\) TM9SF proteins are required for adhesion and phagocytosis in innate immune responses; however, the biological functions of TM9SF proteins are largely unknown.\(^{(69)}\)

We found that 50% of GC cases were positive for transmembrane 9 superfamily member 3 protein.\(^{(67)}\) Expression of transmembrane 9 superfamily member 3 protein is frequently detected in scirrhous-type GC and associated with poor prog-
nosis. There was no association between expression of trans-
membrane 9 superfamily member 3 protein and the gastric or intestinal phenotype. Together these data suggest that trans-
membrane 9 superfamily member 3 protein is an ideal molecu-
lar target for treatment of scirrhous-type GC.

\textbf{CLDN18.} CLDN18 was one of the downregulated genes in GC identified by SAGE analysis.\(^{(70)}\) CLDN18, which encodes claudin-18 protein, is a member of the claudin family, and a component of tight junctions. The claudin family comprises 27 members, and all claudins are 20–27 kDa proteins with four transmembrane domains.\(^{(71)}\) CLDN18 has two variants in mice: variant 1 is expressed in the lung, whereas variant 2 is expressed in the stomach.\(^{(72)}\)

In our immunohistochemistry analysis in non-neoplastic stomach, foveolar epithelial cells expressed claudin-18 on the cell membrane, whereas claudin-18 was not expressed in the intestinal metaplasia of the stomach. This suggests that clau-
din-18 may be a marker for the gastric phenotype. Downregu-
lation of claudin-18 was observed in 58% of GC cases and was correlated with poor survival. Downregulation of claudin-
18 was frequently found in the intestinal phenotype of GC.
Although the precise function of claudin-18 has not been described, the function of the tight junction is to maintain the luminal barrier, paracellular transport, and signal transduction. Therefore, downregulation of claudin-18 and disruption of tight junctions can cause loss of cell polarity, resulting in an abnormal influx of growth factors, which can provide autocrine and paracrine stimulation to tumorigenic epithelial cells. Indeed, CLDN18 KO mice show paracellular H⁺ leakage, upregulation of interleukin-1β and atrophic gastritis. These results indicate that downregulation of claudin-18 participates in the pathogenesis of the intestinal phenotype of GC.

A recent study demonstrated an interchromosomal translocation between CLDN18 and ARHGAP26. ARHGAP26 is a GTPase-activating protein that facilitates conversion of RHO GTases to the GDP state and has been implicated in enhancing cellular motility. However, the significance of interchromosomal translocation between CLDN18 and ARHGAP26 has not been analyzed.

IMAB362, a highly potent and tumor-cell selective therapeutic antibody, is a medicinal product directed against the tight junction molecule claudin-18 variant 2. A Phase II trial (NCT01630083), in which IMAB362 is combined with standard chemotherapy for first-line treatment of gastroesophageal cancer, is ongoing (NCT01630083).

It is important to note that these genes encode transmembrane/secretory proteins, suggesting that the microenvironment as well as cancer cells are different between gastric and intestinal phenotypes of GC. Although the precise functions of Reg IV and olfactomedin 4 are unclear, these two proteins are upregulated in inflammatory bowel disease. Therefore, inflammatory response may be different between gastric and intestinal phenotypes of GC.

Transcription factors of gastric/intestinal phenotypes of gastric cancer and their target genes

Several transcription factors that induce the gastric/intestinal phenotypes have been identified. In the intestinal phenotype of GC, ectopic CDX2 expression has a key function. In mammals, the CDX1 and CDX2 homeobox transcription factors play critical roles in intestinal development, differentiation, and maintenance of the intestinal phenotype. CDX1 and CDX2 proteins show significant homology to the protein product of the Drosophila caudal gene, a key regulator of anterior–posterior...
ior regional identity. Ectopic expression of Cdx2 in the gastric mucosa of transgenic mice induced intestinal metaplasia.\(^{77}\) In contrast, SOX2 may be an important transcription factor of the gastric phenotype of GC. SOX2 induces expression of MUC5AC and pepsinogen A, both of which are markers for the gastric phenotype.\(^{78,79}\) Furthermore, SOX2 negatively regulates the CDX2 promoter by hampering the action of other transcription factors.\(^{80}\)

To characterize the intestinal phenotype of GC, identification of CDX2 target genes is important. Among the genes aberrantly expressed in GC described above, we showed that CDH17, REG4 and DSC2 are direct targets of CDX2, and these genes are expressed in CDX2-positive GC cells.\(^{81,82}\)

For further characterization of the intestinal phenotype of GC, we investigated CDX2-target genes, and found that ABCB1, which encodes the multidrug resistance protein 1 (MDR1), is a direct target of CDX2.\(^{83}\) Immunohistochemical analysis detected MDR1 expression in CDX2-positive GC cells, and showed that MDR1-positive GC cases are frequently found in the intestinal phenotype of GC. As described above, CDX2 induces Reg IV expression. Reg IV inhibits F-5-induced apoptosis and MDR1 inhibits taxane-induced apoptosis. These observations led us to speculate that chemotherapy, including 5-FU-based or taxane-based chemotherapy, is more beneficial in patients with the intestinal phenotype of GC. For these patients, molecular-targeted therapies could be suitable.

**Conclusions**

Molecular alterations of GC are summarized in Figure 2. Expressions of transmembrane/secretory proteins in cancers are ideal diagnostic biomarkers. Moreover, if these molecules are involved in the neoplastic process, the molecules are not just biomarkers but may also be therapeutic targets. Here we described clinical and molecular characteristics of the gastric/intestinal phenotypes of GC. Expression of transmembrane/secretory proteins, including L1-cadherin, Reg IV, olfactomedin 4, desmocollin 2 and claudin-18, is different between gastric and intestinal phenotypes of GC. These transmembrane/secretory proteins are extracellular proteins, indicating that the microenvironment as well as cancer cells are different between the gastric and intestinal phenotypes. About 10 years ago, oncogenic driver mutations have emerged as major treatment targets for molecular therapies in a variety of cancers. Whole genome or exome sequencing in GC has been performed, and mutation of RHOA gene in undifferentiated-type GC has been reported.\(^{84}\) According to the COSMIC website (http://cancer.sanger.ac.uk),\(^{85}\) the most frequently mutated gene is TP53 (32%), and the second most frequently mutated gene is ARID1A (14%).\(^{86}\) Frequencies of other gene mutations are approximately 10% or below 10%. Although the associations between mutation of these genes and gastric/intestinal phenotypes are unclear, driver gene mutation is a rare event, and it is difficult to plan an effective treatment according to driver gene mutations. In contrast, the Cancer Genome Atlas Network has reported that GC can be classified into four distinct molecular subtypes: GC positive for Epstein–Barr virus; microsatellite unstable GC; genomically stable GC; and GC with chromosomal instability.\(^{87}\) As described above, MSI is detected more frequently in the gastric phenotype of GC.\(^{88}\) GC positive for Epstein–Barr virus are also frequently found in the gastric phenotype of GC.\(^{87}\) However, the mucin phenotypes of genomically stable GC and GC with chromosomal instability remains unclear. Classification of these subtypes may be used to provide personalized medicine.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Schematic outline of the Serial Analysis of Gene Expression (SAGE) method.

Fig. S2. Schematic outline of the Escherichia coli ampicillin secretion trap (CAST) method.