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Gastric Cancer Cell Lines as Models to Study Human Digestive Functions

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Abstract The present investigation aims at defining the functional status of several gastric cancer cell lines in order to assess their usefulness as adequate cellular models to study the regulation of gastric digestive functions. Compared to AGS, Hs746t and KATO-III cells, NCI-N87 exhibited an unique differentiation status. They formed coherent monolayers expressing E-cadherin and ZO-1 junctional proteins; their integrity and epithelial morphology were maintained at post-confluency for up to 10 days. All cell lines synthesized PAS-reactive (mucous-type) glycoconjugates. However, only NCI-N87 cells expressed MUC6 glycoprotein suggesting a mucopeptic phenotype. Immunostaining, enzymatic assays, Western blotting and Reverse Transcriptase polymerase chain reaction (RT-PCR) revealed that all cell lines contained varying levels of pepsinogen (Pg5) and human gastric lipase (HGL). Only NCI-N87 cells were able to express zymogens at higher levels, in granule-like structures, and to efficiently secrete both HGL and Pg5. The addition of epidermal growth factor (EGF) to post-confluent NCI-N87 cells, which exhibit an abundant membrane staining for EGF-receptors, modulated HGL activity without affecting Pg5. In conclusion, this investigation enlightens the potential usefulness of the gastric cell line NCI-N87 as a model for elucidating the cellular and molecular mechanisms involved in the regulation of human gastric epithelial functions. J. Cell. Biochem. 81:241–251, 2001. © 2001 Wiley-Liss, Inc.

Key words: gastric cell lines; gastric secretion; mucin; pepsinogen; gastric lipase; epidermal growth factor

The human gastric glands assume the synthesis and secretion of several bioactive substances into the lumen i.e., mucus, hydrochloric acid and zymogens. Apart from its role regarding the digestion of dietary proteins via the release of pepsinogen from chief cells [Defize, 1980], another important aspect of gastric physiology pertains to the digestion of fat. In humans, this function is assumed by the existence of a gastric lipase (HGL) co-localized with pepsinogen-5 (Pg5) in secretory granules [Moreau et al., 1988]. This co-localization is a unique feature of human gastric chief cells since both zymogens are either expressed in different gastric cell types [Carrière et al., 1992; Descroix-Vagne et al., 1993] or distinct zymogenic cell populations [Bernadac et al., 1991] in animal models. Gastric triglyceride digestion is a prerequisite for optimal intestinal lipolysis [Carrière et al., 1993]. Furthermore, the importance of HGL increases in the context of perinatal physiology [Hamosh et al., 1981; Lee et al., 1993] and pathological conditions associated with pancreatic insufficiency [Abrams et al., 1987]. Therefore, it is important to clearly understand the molecular mechanisms controlling the synthesis and secretion of human chief cell digestive enzymes.

Adequate in vitro models must be made available in order to identify specific regulators of gastric epithelial functions and their underlying mechanisms of action. In vivo, the stem cell population, responsible for renewing the entire gastric epithelium, is located in the isthmus compartment. Following division, the cells migrating upward differentiate into pit and surface mucous cells which secrete MUC5 mucin while those migrating downward differentiate into mucous neck cells expressing MUC6 [De Bolos et al., 1995; Ho et al., 1995;

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Bartman et al., 1998], as well as into parietal, endocrine, caveolated and chief cells. It is important to note that mucous neck cells further differentiate into pre-zymogenic cells which in turn give rise to mature zymogenic chief cells [Karam and Leblond, 1992]. Available epithelial cell lines, isolated from human gastric carcinomas, exhibit different morphological characteristics and have preferentially been used to study the implication of specific hormonal systems in the context of oncogenesis and cellular transformation. For instance, metastatic Hs746t cells display irregular squamous-fibroblastoid phenotypes in culture [Smith, 1979]. KATO-III cells, which grow in suspension and have a rounded phenotype, are known to produce transforming growth factor-β [Mahara et al., 1994], EGF-like substances [Naef et al., 1996], and to express the K-*sam* protein amplified in stomach cancer and highly homologous to the keratinocyte growth factor (KGF) receptor [Hattori et al., 1990]. AGS cells rather form dense monolayers of polygonal cells responsive to insulin-like growth factor-1 (IGF-1) and gastrin [Guo et al., 1993; Ishizuka et al., 1992]. Finally, the poorly characterized NCI-N87 cell line has been shown to produce several ligands of the EGF family [Naef et al., 1996]. The potential usefulness of these gastric cancer cell lines for elucidating the process of human gastric epithelial functional differentiation, especially the chief cell lineage, has never been addressed. Such an application appears important since no normal human gastric epithelial cell line exists. In comparison, our organ culture technique [Ménard et al., 1993] and the new primary culture system of gastric epithelial cells [Basque et al., 1999] have served to study the expression of gastric digestive enzymes [Tremblay et al., 1999; Basque and Ménard, 2000]. However, these culture systems present important limitations regarding the availability of human specimens. Consequently, using representative AGS, Hs746t, KATO-III and NCI-N87 gastric cancer cell lines, we performed a detailed analysis of their functional differentiation status (junctional proteins, tyrosine kinase receptors, mucous, chief cell digestive enzymes) using cytochemistry, immunofluorescence, western blotting and RT-PCR. The current investigation reveals for the first time that gastric carcinoma cells display a multipotentiality for expressing mucigenic and zymogenic differentiation markers. Furthermore, NCI-N87 cell line represents the most promising model in its unique capacity to form tight and coherent monolayers (cell–cell junctions), to be maintained at post-confluency, to express MUC6 protein, and to efficiently synthesize and secrete significant levels of HGL and Pg5.

MATERIALS AND METHODS

Cell Culture and Tissues

AGS (CRL 1739), Hs746t (HBT 135), KATO-III (HBT 103) and NCI-N87 (CRL 5822) cell lines isolated from human gastric carcinomas were obtained from the American Type Culture Collection (ATCC, Manassas, VA). They were initially propagated in RPMI 1640 or Minimal Essential Medium (MEM) (Gibco BRL/Life Technologies, Burlington, Ontario, Canada) supplemented with antibiotics (penicillin/streptomycin from Gibco) and 10% (v:v) heat-inactivated fetal bovine serum (CELLect Gold FBS from ICN, Montreal, Québec, Canada), as recommended. These nutrients supported rapid growth but we observed that a DMEM:F12 (1:1, v:v) mixture optimized cell attachment and well density compared to previous medium formulations (unpublished observations). Therefore, DMEM:F12 was used as a routine culture medium in our experiments. Adult gastric biopsies were available for this study. For each patient (two males and one female; ages 40–64), nondiseased samples from fundic and corpus regions of the stomach were confirmed by a pathologist. None of the patients received treatment before surgery and all tissues were processed within 1 h of procedure. Tissue collection was approved by the Institutional Human Research Review Committee of the Université de Sherbrooke for the use of human material.

Immunofluorescence (IF) and Cytochemistry

For histochemical studies performed on gastric cell lines, cells were seeded on sterile glass coverslips and allowed to reach high density or confluency before processing. For tissue histochemistry, biopsies were dissected, embedded in Optimum Cutting Temperature (OCT) compound and processed for cryomicrotomy as described previously [Tremblay et al., 1997; Tremblay et al., 1999]. Specimens were fixed in 3.7% formaldehyde for 10–15 min and permeabilized with 0.1% Triton X-100 diluted in phosphate buffered saline (PBS) for 3 min.

Non-specific binding was blocked with 0.2% bovine serum albumin (BSA, fraction V) in PBS (pH 7.4) or fish gelatin (both obtained from Sigma, St-Louis, MO). Cells and tissues were successively incubated inside humidified chambers with primary antibodies and fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (see below) diluted in BSA-PBS for 60 min and 45 min respectively. Extensive washing with PBS was performed between each step. Coverslips were mounted on glass slides with glycerol:PBS (9:1) and sealed with nail polish. Fluorescent staining was observed on a Reichert Polyvar microscope (Leica Canada, St-Laurent, Québec, Canada) equipped for epifluorescence and photographed on Kodak Tri-X Pan film (400 ASA). Conventional Bowie and Periodic acid Schiff (PAS) dye stainings were performed on cell cultures to detect pepsinogen granules and glycoconjugates respectively. For Bowie, cells were fixed for 12 min in Regaud's fluid (3% (w/v) potassium bichromate in formaldehyde solution), incubated for 10 s in Bowie solution (Biebrich scarlet/Ethyl violet precipitate dissolved in ethanol), then differentiated in acetone, acetone:xylene and xylene solutions. The presence of pepsinogen was revealed by a dark violet cytpoplasmic staining.

Gel Electrophoresis and Western Immunobloting (WB)

Before collecting cell lysates and culture media, confluent cells grown in 25 cm^2 flasks were maintained without serum for 24 h. Total proteins from cells and media were collected in 20 mmol/ml Tris-HCl (pH 6.8) and processed in conventional 2 × solubilization buffer [Basque et al., 1999]. Aliquots (60–80 μg of prot/well assayed with Folin reagent [Lowry et al., 1951]) were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) using 12% polyacrylamide and transferred to a Trans-Blot membrane (BIORAD, Mississauga, Ontario, Canada). The membranes were blocked overnight in 0.2% casein-PBS, then incubated with the same primary antibodies (see below) and amplification was achieved using the 'Western-Light Plus' chemiluminescence protein kit (Tropix, Bedford, MA).

Antibodies

Primary antibodies: Anti-human EGF receptor (1:100 for IF, 1:1000 for WB, mouse monoclonal, Upstate Biotechnology Inc., Lake Placid, NY); anti-human KGF receptor (anti-*bek* 1:50 for IF, rabbit polyclonal, Santa Cruz Lab., Santa Cruz, CA); anti-human HGF receptor (1:75 for IF, rabbit polyclonal, Santa Cruz); anti-IGF-1 receptor (1:50 for IF, mouse monoclonal, Oncogene Science/Canadian Bioclinical, Scarborough, Ontario, Canada); anti-human E-cadherin (1:800 for IF, Transduction Lab., Lexington, KT); anti-human ZO-1 (1:500 for IF, Zymed Lab., San Francisco, CA); anti-human MUC6 mucin (1:500 for IF; 1:1000 for WB, Novocastra Lab., Newcastle, UK); anti-human fundic pepsinogen or Pg5 (1:150 for IF, guinea pig polyclonal, kindly provided by F. Carrière and R. Verger); anti-human gastric lipase or HGL (1:2000 or IF, 1:1000 for WB, rabbit polyclonal, given by A. de Caro and R. Verger). FITC-conjugated secondary antibodies for IF: Anti-mouse and anti-rabbit Ig (1:30, sheep monoclonal, Boehringer Mannheim Canada, Laval, Québec, Canada) and anti-guinea pig Ig (1:64, goat monoclonal, Sigma). Biotin-conjugated secondary antibodies for WB: Anti-mouse Ig (1:20000, Tropix), anti-rabbit Ig (1:10000, Tropix).

RT-PCR

Cells grown in 25-cm^2 flasks were lyzed in TRIZOL solution (Gibco). Total RNA was quantitated by spectrophotometry and its integrity verified by ethidium bromide staining. Reverse transcriptase Superscript (Gibco) and 0.5 μg of oligo $(dT)_{12-18}$ primer (Amersham Canada, Oakville, Ontario, Canada) were added to 5 μg of total RNA, as described previously [Basora et al., 1999]. HGL was amplified using the following primers: LIP-1 (5′-CTGAGGAAACT-GCAGGTCCA-3′) and LIP-2 (5′-AGAAGCACT-GCA TGTCAAAC-3′) amplifying a band of 1300 pb. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also amplified using the following primers: GAPDH-1 (5′-CCACCCAT-GGCAAATTCCATGGCA-3′) and GAPDH-2 (5′-TCTAGACGGCAGGTCAGGTCCACC-3′). Following reversed transcription, single-stranded cDNA was amplified in PCR buffer (Amersham) containing 0.25 μM of both sense and antisense primers for 25 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C), and extension (1 min at 72°C) in a thermal cycler (Perkin Elmer DNA Thermal cycler model 480) in the presence of 250 μM dNTPs and 2.5 units of Taq (Roche Molecular Biochemicals, Laval, Québec, Canada).

Enzymic Assays

Pepsin and lipase activities were measured in confluent cells and the media collected in 25 cm^2 culture flasks. Briefly, lipolytic activity attributed to HGL in the presence of triglycerides was determined using glycerol [^{14}C]-trioleate (Amersham) as substrate and fatty acid free-BSA (A-6003 from Sigma) as a carrier of released fatty acids. After 60 min of incubation at 37°C, free [^{14}C]-oleic acid was separated by liquid–liquid partition in chloroform:methanol:heptane and quantitated on a beta scintillation system. The specific activity of HGL was expressed as nmol of free fatty acids released per minute per milligram of protein. Pepsin activity resulting from activation of pepsinogen at acid pH was measured using 1 ml of acid-denatured and dialysed hemoglobin (Sigma) as substrate. Reaction was carried out at 37°C for 10 min and stopped with 6.2% (w:v) trichloroacetic acid. The resulting free amino acid products were separated by centrifugation and quantitated by spectrometry using a L-tyrosine standard. Specific pepsin activity was expressed in International Units (μmol/min) per milligram of protein. Protein content of the homogenates was measured using the Folin reagent [Lowry et al., 1951]. Recombinant human EGF (purchased from Collaborative Biomedicals, Becton Dickinson Labware, Bedford, MA) is known to modulate HGL-mRNA and activity in fetal gastric tissue [Tremblay et al., 1997, 1999]. In order to evaluate its effect on carcinoma cell lines, EGF was added for 48 h in 100 ng/ml concentration to 4-day postconfluent NCI-N87.

RESULTS

Cell Growth and Morphology

Gastric carcinoma cells were routinely propagated in DMEM:F12 supplemented with 10% FBS. These conditions optimized cell attachment as well as culture density. In accordance with previous data, AGS, Hs746t, KATO-III, and NCI-N87 cells respectively exhibited polygonal, squamous-fibroblastoid, rounded and tightly knit-polyhedral phenotypes (Fig. 1). A number of phenotypic variants were consistently observed in all cultures, however. At confluency, AGS cells formed dense monolayers containing a proportion (~10%) of flattened multinucleated cells and they remained as sensitive to rounding as sub-confluent cells upon fluid renewal or rinsing (absence of cell–cell junctions) (Fig. 1A). Also, Hs746t cells grew more slowly than other cell lines, exhibited contact-inhibition and contained a sub-population of squamous multinucleated cells and giant cells with ruffled borders at their cell

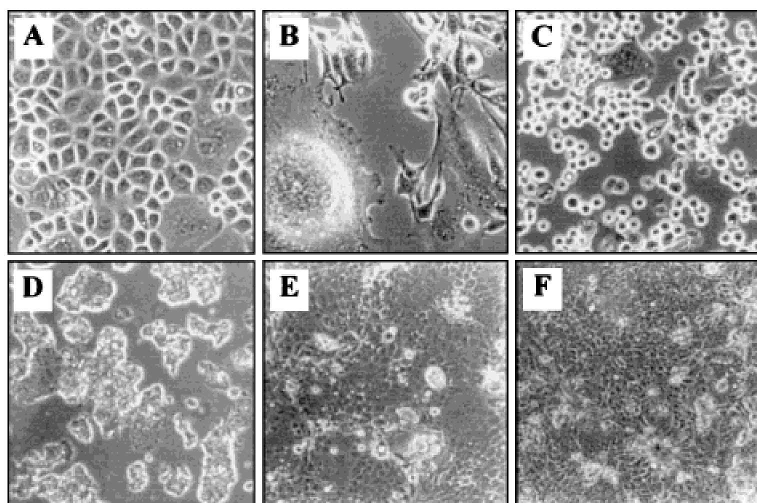

Fig. 1. Morphology of gastric carcinoma cell lines. Cell phenotypes were examined using bright-field and phase-contrast microscopy. Cultured on plastic, AGS formed a dense monolayer of polygonal cells of irregular size (**A**). Hs746t cells display several phenotypic variants of squamous and fibroblastoid cells (**B**). KATO-III cells were rounded and preferentially grew in suspension (**C**). NCI-N87 cells grew as compact colonies (**D**) and formed coherent monolayers. Their integrity was maintained during postconfluency, as shown after 4 days (**E**) and 10 days of culture (**F**). Magnification A–C = 120, D = 16, E–F = 60.

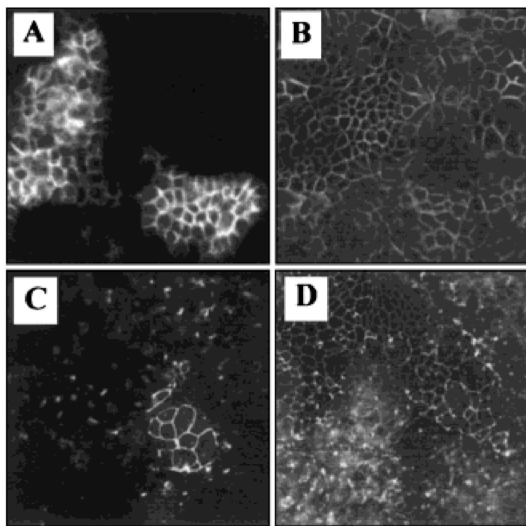

Fig. 2. Detection of cell junction molecules. Indirect immunofluorescence of junctional proteins was evaluated in cultured NCI-N87 cells. After initial attachment, all cells expressed the *zonula adherens*-associated E-cadherin at sites of cell–cell contacts (**A**). E-cadherin became uniformly distributed at cell–cell interfaces after reaching confluency (**B**, 10 days post-confluence). ZO-1 protein associated with *zonula occludens* was detected throughout the growing monolayer and properly localized at intercellular contacts in small clusters (**C**) whose size increased thereafter (**D**, 10 days postconfluence). Magnification = 120.

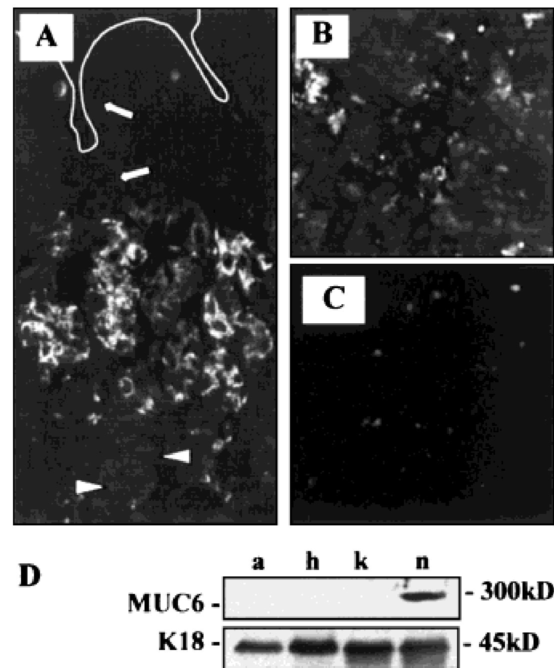

Fig. 3. Immunodetection of MUC6 mucin. Indirect immunofluorescence (**A**) revealed a specific staining in mucous cells in the neck in fundic glands of adult human stomach whereas the superficial epithelium (arrows) and the base of the glands (arrowheads) appear negative (Magnification = 30). MUC6 was also evidenced in isolated cell clusters of NCI-N87 cells (**B**) compared to negative AGS cells (**C**) (Magnification = 120). Western blot analysis (**D**) demonstrated the presence of MUC6 protein only in NCI-N87 (n) compared to AGS (a), Hs746t (h) and KATO-III (k) cells. Keratin-18 (K18) was used as an internal control.

margins (Fig. 1B). KATO-III preferentially grew in suspension with a few isolated cells (no aggregates) attached to the substratum (Fig 1C). NCI-N87 cells formed dense clusters after plating and exhibited a typical colony growing pattern (Fig. 1D). As opposed to previous models, confluent NCI-N87 monolayers displayed a tight and coherent epithelial morphology characteristic of a more polarized cell line and could also be maintained at post-confluency for up to 10 days (Fig. 1E, F).

Functional Epithelial Markers

The functional status of gastric cancer cells was analyzed using several approaches. Firstly, immunofluorescent staining of various epithelial markers confirmed the more polarized and differentiated phenotype of the NCI-N87 cell line. Following initial attachment, all cell colonies were positive for the *zonula adherens*-associated E-cadherin localized at sites of cell–cell contacts (Fig. 2A). At post-confluency, E-cadherin was uniformly distributed at the periphery of cells (Fig. 2B). In addition, the ZO-1 protein associated with *zonula occludens* or tight junctions was similarly localized in small cell clusters (Fig. 2C) that increase in size during post-confluency (Fig. 2D). Secondly, PAS staining was performed which revealed a significant mucigenous reactivity (diffuse and granular) in all cell lines (results not shown). Furthermore, immunostaining against the mucopeptic-type cell marker MUC6 glycoprotein revealed a positive granular reactivity in a sub-population of NCI-N87 cells (Fig. 3B), while AGS (Fig. 3C) and other cell lines were totally negative. As verified herein, the tissue distribution of MUC6 mucin was indeed limited to mucous neck cells in adult gastric (fundic-type) mucosa (Fig. 3A). Western blotting experiments further established the presence of MUC6 immunoreactivity in NCI cell line only (Fig. 3D).

Chief Cell Zymogens

Conventional Bowie staining was performed in all cell lines that revealed a positive cytoplasmic reaction, indicative of the presence of

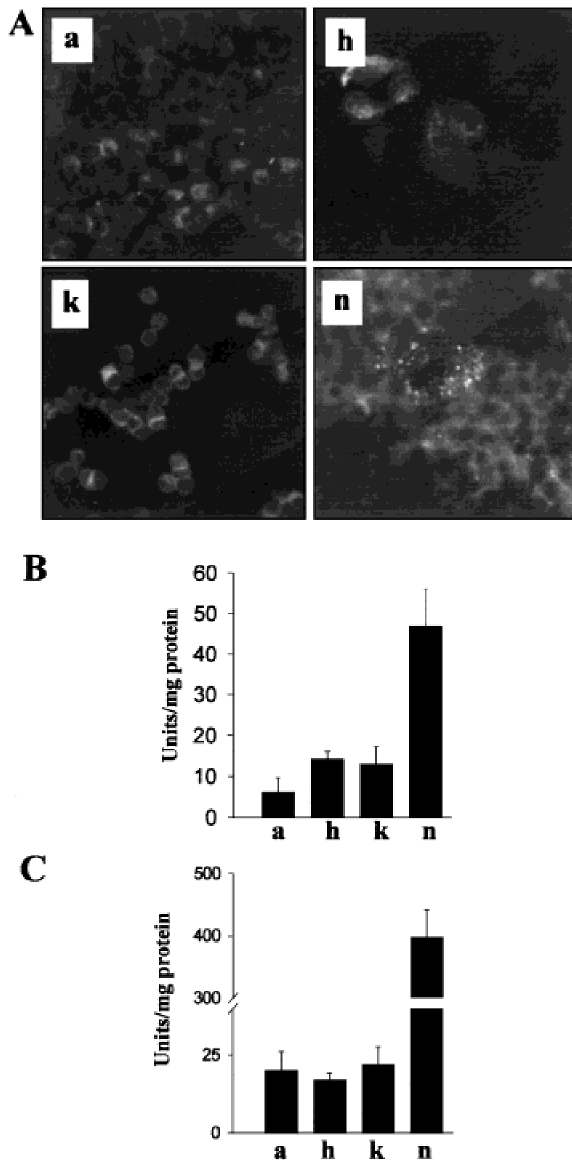

Fig. 4. Immunodetection of fundic pepsinogen (Pg5) in gastric cancer cell lines. Indirect immunofluorescence (**A**) in AGS (a), Hs746t (h), KATO-III (k) and NCI-N87 cells (n). (Magnification = 120). A sub-population of anti-Pg5 positive NCI-N87 cells showed an intense granular staining. Specific pepsin activity measured in cells (**B**) and media (**C**). Results are expressed in pepsin units per milligram of protein and represent the mean ± SEM of three separate experiments.

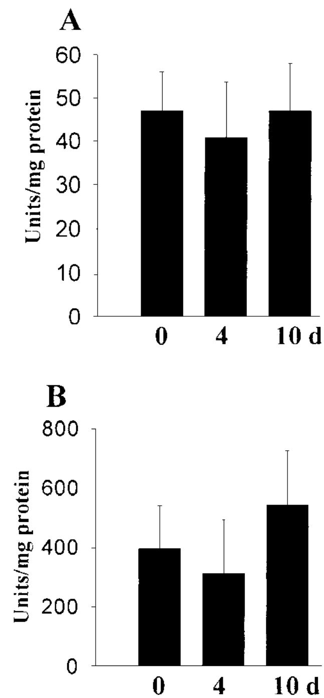

Fig. 5. Variation of pepsin activity in cultured NCI-N87 cells. Specific activity was determined at different post-confluent intervals (0, 4, 10 days) in both cell homogenates (**A**) and culture media (**B**). Results are expressed in pepsin units per milligram of protein and represent the mean ± SEM of three separate experiments.

pepsinogen granules (not shown). Confirming the nature of the latter enzyme, an immunofluorescent, cytoplasmic-diffused anti-pepsinogen (Pg5) staining was observed in subpopulations of AGS, Hs746t and KATO-III cultures (Fig. 4A). Only NCI-N87 cells were ubiquitously stained and showed a granule-like distribution in small clusters. Furthermore, enzymatic assays confirmed the presence of a functional Pg5 protein exhibiting pepsin activity not only in cell homogenates (Fig. 4B) but also in culture media (Fig. 4C) of all studied cell lines, the activity being most abundant in NCI-N87 cells. Of interest, we demonstrate that NCI-N87 cells are able to continuously synthesize and secrete pepsinogen at post-confluency (Fig. 5).

In order to further define the functional status of these gastric cancer cell lines, the presence of HGL, another differentiation marker of human chief cells, was assessed using immunofluorescence (Fig. 6A). A weak cytoplasmic staining was observed in a low proportion of AGS and Hs746t cells (groups of positive cells are shown). Even a smaller fraction ($<1\%$) of KATO-III cells were labeled. By contrast, NCI-N87 cells exhibited an important cytoplasmic reaction for HGL, and a granular localization in specific cell clusters. Western blot analysis and enzymatic assays (Fig. 6B–D) revealed the presence of the 49 kDa HGL-protein in all cell cultures, being less abundant in Hs746t cells and predominant in NCI-N87 cells. Secretion kinetics differed from those of Pg5: only the

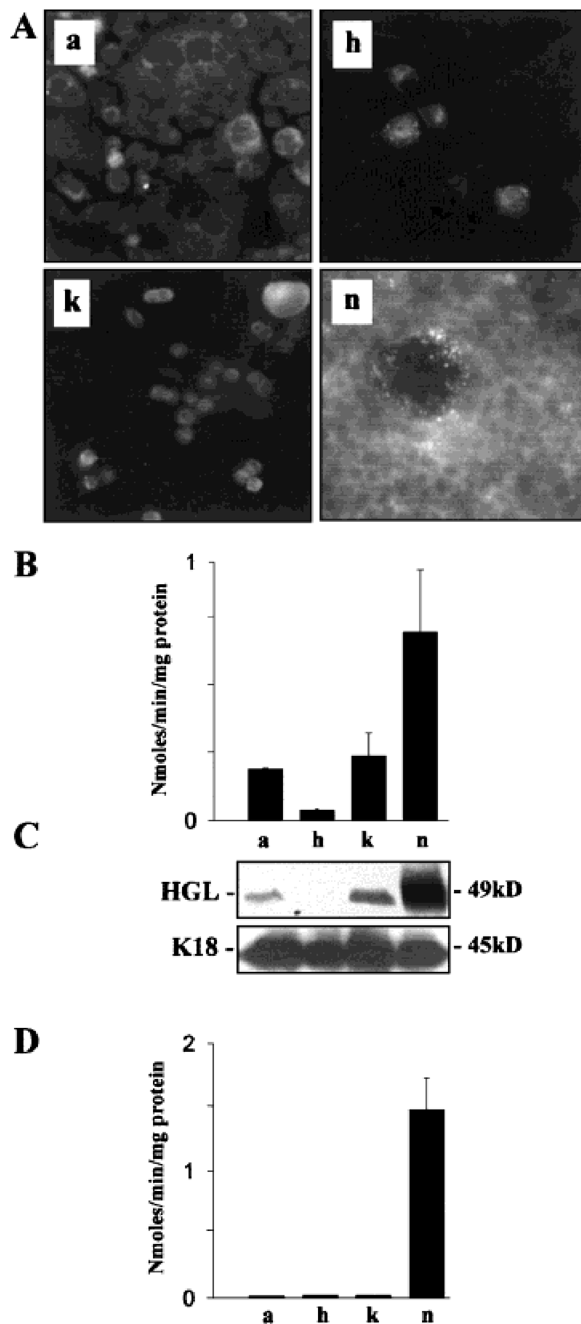

Fig. 6. Immunodetection of human gastric lipase (HGL) in gastric cancer cell lines. Indirect immunofluorescence (**A**) in AGS (a), Hs746t (h), KATO-III (k) and NCI-N87 cells (n) (Magnification = 120). A sub-population of anti-HGL positive NCI-N87 cells showed a granule-like staining. Specific lipase activity measured in cells (**B**) and culture media (**D**). Data are expressed as nmol of free fatty acids released per min per milligram of protein and they represent the mean ± SEM of three separate experiments. Western blot analysis (**C**) revealed the predominance of HGL in NCI-N87 cells.

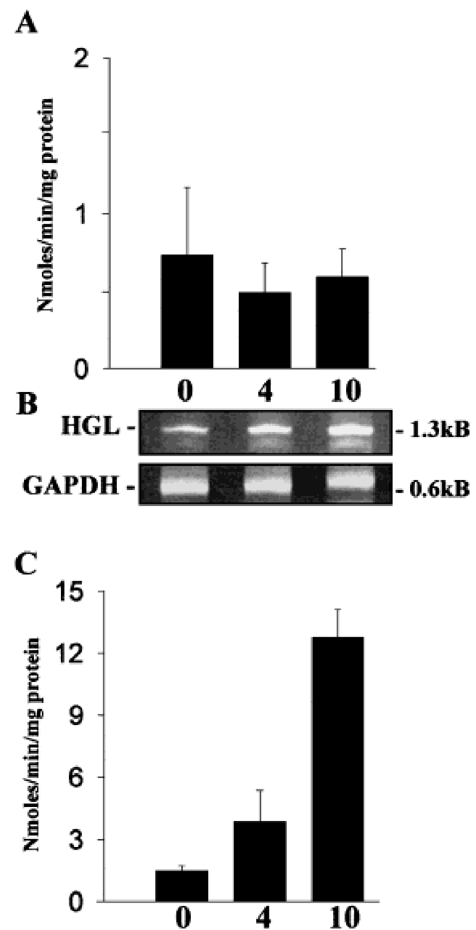

Fig. 7. Variation of lipase activity in cultured NCI-N87 cells. Specific activity was determined at different post-confluent intervals (0, 4, 10 days) in both cell homogenates (**A**) and culture media (**C**). Data are expressed as nmol of free fatty acids released per min per milligram of protein and represent the mean ± SEM of three separate experiments. Representative RT-PCR (**B**) was performed for HGL-mRNA analysis in cell homogenates at the same culture intervals. GAPDH-mRNA probe was used as an internal control.

NCI-N87 cell line could release significant amounts of HGL. Moreover, lipase activity was measured during post-confluency. While cellular HGL levels remained constant for up to 10 days in NCI-N87 (Fig. 7A), the activity continuously increased in culture media (Fig. 7C). Standard RT-PCR analysis performed on total RNA extracted from NCI-N87 cells confirmed a concomitant increase of the transcript according to post-confluency (Fig. 7B).

Growth Factor Systems and Hormonal Regulation

The presence of specific growth factor receptors associated with normal human gastric

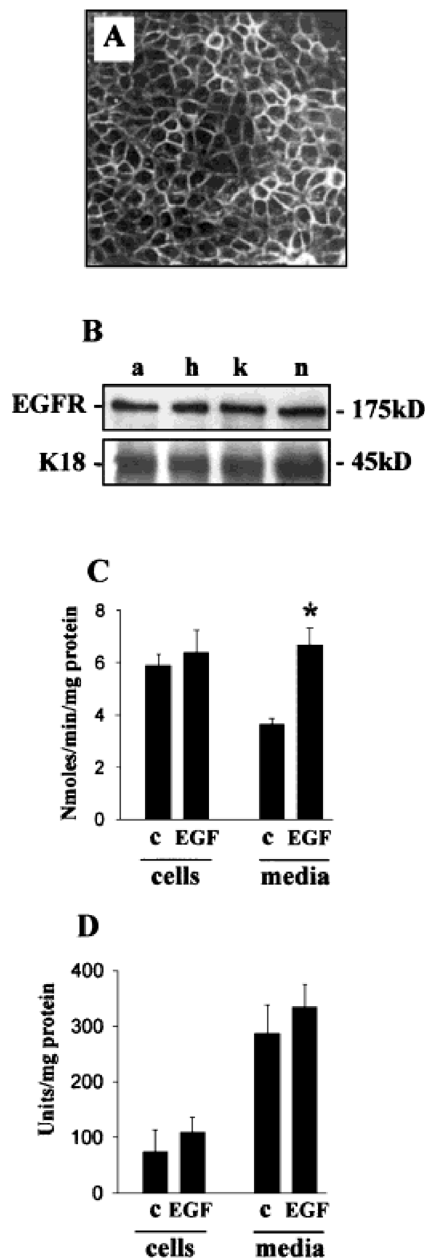

Fig. 8. Immunodetection of EGF-R and influence of EGF-supplementation in gastric cancer cell lines. Indirect immunofluorescence (**A**) in NCI-N87 cells (Magnification = 120). Western blotting of EGF-R (**B**) representative of four experiments in AGS (a), Hs746t (h), KATO-III (k) and NCI-N87 cells (n). Keratin-18 (K18) was used as an internal control. Effect of exogenous EGF (100 ng/ml) on lipase (**C**) and pepsin (**D**) activities in post-confluent NCI-N87 cells. Specific lipase activity is expressed as nmol of free fatty acids released per min per milligram of protein and pepsin activity in pepsin units per milligram of protein. Data represent the mean ± SEM of three separate experiments. Asterisk indicates a significant difference between control and EGF ($P < 0.007$).

epithelial cells [Basque et al., 1999; Basque and Ménard, 2000] was assessed on cultured cells. AGS, Hs746t, KATO-III and NCI-N87 cells were all positive for immunostaining of several tyrosine kinase receptors including KGF-R, HGF-R and IGF1-R (data not shown). Figure 8 illustrates that EGF-R was ubiquitously expressed in all cell lines including NCI-N87 cells in which an intense membrane labeling was observed (Fig. 8A). Western blots revealed the 175 kD EGF-R protein in the studied gastric cancer cell lines (Fig. 8B). Its relative abundance in NCI-N87 cells prompted us to evaluate the effects of EGF ligand on the synthesis of chief cell digestive enzymes. Addition of human recombinant EGF (100 ng/ml) to postconfluent NCI-N87 cells for 48 h resulted in a significant increase (~50%) of HGL activity in culture medium (Fig. 8C). Noted that Pg5 activity remained unaffected by growth factor treatment (Fig. 8D).

DISCUSSION

Cellular models exhibiting normal characteristics of the human gastric epithelium must be developed in order to study, under defined in vitro conditions, the molecular events involved in the regulation of gastric digestive functions. At first hand, the distinct morphology of gastric carcinoma cell lines studied suggests that each possesses unique functional and differentiated characterisics. For instance, KATO-III and Hs746t cells are derived from poorly differentiated gastric carcinomas [Sekiguchi et al., 1978; Smith, 1979] and they either grow in suspension or exhibit an irregular adherent morphology. In contrast, AGS and NCI-N87, which form typical epithelial monolayers, arise from moderate and well differentiated gastric cancers [Barranco et al., 1983; Park et al., 1990]. The current study demonstrates for the first time that, independently of their phenotype, gastric carcinoma cell lines do share a capacity for expressing mucigenic and zymogenic products. The NCI-N87 strain, however, displays a more polarized status and represents a novel promising model, especially regarding the synthesis and secretion of chief cell zymogens.

Since *zonulae adherens* and *occludens* molecules are functional components of apical junctions in all epithelial cells [Shimoyama and Hirohashi, 1991; Hanby et al., 1996], they play

key roles in the maintenance of cell and membrane polarity. Thereby, the presence and cellular distribution of E-cadherin and ZO-1 proteins were verified in subconfluent and postconfluent NCI-N87 cells. Our results indicate that all cells express membrane-associated E-cadherin, supporting the existence of intimate cell–cell contacts in NCI-N87 cultures. Previous studies have shown that KATO-III cells carry E-cadherin point mutations, resulting in the production of aberrant mRNA [Oda et al., 1994]. Such a disfunction probably illustrates their tendency to grow as free-floating isolated cells [Motoyama et al., 1986]. Recently, the absence of E-cadherin expression was observed in AGS cells [Hsieh et al., 1996; Jawhari et al., 1999]. The latter feature would explain, in part, why cultured AGS monolayers eventually loose their integrity after confluency is reached. In contrast, postconfluent NCI-N87 cultures maintain a typical tight coherent epithelial morphology, characterized by the appropriate peripheral distribution of the tight junction-associated ZO-1 in a subpopulation of cells organized in clusters. Thus, it seems plausible that NCI-N87 cells exhibit a more differentiated epithelial phenotype.

To assess the use of cancer cells for studying specific gastric functions, we determined the presence of functional markers normally associated with the human gastric epithelium. Firstly, all cell lines were found to express PAS-positive glycoconjugates in their cytoplasm and granules, suggesting they could be functionally related to gastric mucous cells, as proposed earlier for KATO-III cells [Yamamoto et al., 1990]. In normal gastric epithelium, MUC5 and MUC6 mucins are localized in distinct cell types. MUC5 is expressed in the superficial/foveolar epithelium whereas MUC6 expression is restricted to mucous cells of the neck region [De Bolos et al., 1995; Ho et al., 1995; Bartman et al., 1998]. Recently, the absence of MUC6 mRNA and protein in several gastric cancer cell lines have underlined their limited capacity for mucopeptic differentiation [Carvalho et al., 1999]. Our observations with AGS, Hs746t and KATO-III cells support this conclusion which in fact reconciles with histopathological studies revealing an increased tendency to foveolar cell differentiation in gastric tumors [Fiocca et al., 1987]. However, for the first time, we revealed the presence of MUC6 glycoprotein in NCI-N87 cultures suggesting a similarity with the mucopeptic cell lineage. One has to keep in mind that in the adult stomach, mucous neck cells transdifferentiate into pre-zymogenic and mature zymogenic chief cells as they migrate towards the base of glands [Karam and Leblond, 1992; DeBolos et al., 1995]. Since human chief cells express both pepsinogen (Pg5) and gastric lipase (HGL) [Moreau et al., 1988; Basque and Ménard, 2000], immunostaining and enzymatic assays were performed to detect the latter proteins in cell cultures. We reveal that a subpopulation of AGS, Hs746t and KATO-III cells synthesized both zymogens at various intensities (a diffused cytoplasmic distribution) while no significant HGL activity was detected in their medium. Only NCI-N87 monolayers were intensely positive for Pg5 and HGL and even accumulated these products in granule-like structures, as they were able to efficiently secrete both zymogens into the culture fluid. These characteristics are quite unique for gastric cancer cell lines and are similar to those observed in normal human chief cells either in human gastric explants [Ménard et al., 1995] or in primary cultures [Basque et al., 1999].

The majority of existing gastric cancer cell lines express EGF and its receptor [Kamata et al., 1986; Lewis et al., 1993]. Our results not only confirm the presence of EGF-R in all four studied cell lines but also reveal its abundance and membrane localization in NCI-N87. The addition of human recombinant EGF to postconfluent NCI-N87 cells resulted in a significant increase of HGL activity in culture medium without affecting that of Pg5. This differential response is in total accordance with the concept that gastric digestive enzymes co-expressed in human chief cells are under different regulatory mechanisms [Chew and Hershey, 1982; Kasbekar et al., 1983; Finks et al., 1985; Ménard et al., 1995].

In summary, the gastric cancer cell line NCI-N87 displays a unique differentiation status by its capacity to form tight-coherent monolayers and to be maintained at postconfluency. This study also establishes that NCI-N87 cells exhibit the mixed functional characteristics of pre-zymogenic cells expressing the neck cell (or mucopeptic) MUC6 mucin together with the production and secretion of human chief cell digestive enzymes. In conclusion, the present investigation enlightens the potential usefulness of this specific gastric cancer cell line as a

model for elucidating the cellular and molecular mechanisms involved in the process of human gastric epithelial functional differentiation.

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REFERENCES

Abrams CK, Hamosh M, Dutta SK, Hubbard VS, Hamosh P. 1987. Role of non-pancreatic lipolytic activity in exocrine pancreatic insufficiency. Gastroenterology 92: 125–129.

Barranco SC, Townsend CM, Casartelli C, Macik BG, Burger NL, Boerwinkle WR, Gourley WK. 1983. Establishment and characterization of an in vitro model system for human adenocarcinoma of the stomach. Cancer Res 43:1703–1709.

Bartman AE, Buisine MP, Aubert JP, Niehans GA, Toribara NW, Kim YS, Kelly EJ, Crabtree JE, Ho SB. 1998. The MUC6 secretory mucin gene is expressed in a wide variety of epithelial tissues. J Pathol 186:398–405.

Basora N, Herring-Gillam E, Boudreau F, Perreault N, Pageot LP, Simoneau M, Bouatrouss Y, Beaulieu JF. 1999. Expression of functional distinct variants of β_4A integrin subunit in relation to the differentiation state in human intestinal cells. J Biol Chem 274:29819–29825.

Basque JR, Ménard D. 2000. Establishment of culture systems of human gastric epithelium for the study of pepsinogen and gastric lipase synthesis and secretion. Micros Res Tech 48:293–302.

Basque JR, Chailler P, Perreault N, Beaulieu JF, Ménard D. 1999. A new primary culture system representative of the human gastric epithelium. Exp Cell Res 253:493–502.

Bernadac A, Moreau H, Verger R. 1991. Gastric lipase and pepsinogen during the ontogenesis of rabbit gastric glands. Eur J Cell Biol 55:149–157.

Carrière F, Raphel V, Moreau H, Bernadac A, Devaux MA, Grimaud R, Barrowman JA, Bénicourt C, Junien JL, Laugier R, Verger R. 1992. Dog gastric lipase: stimulation of its secretion in vivo and cytolocalization in mucous pit cells. Gastroenterology 102:1535–1545.

Carrière F, Barrowman JA, Verger R, Laugier R. 1993. Secretion and contribution to lypolysis of gastric and pancreatic lipases during a test meal in humans. Gastroenterology 105:876–888.

Carvalho F, David L, Aubert JP, Lopez-Ferrer A, De Bolos C, Reis CA, Gartner F, Peixoto A, Alves P, Sobrinho-Simoes M. 1999. Mucins and mucin-associated carbohydrate antigens expression in gastric carcinoma cell lines. Virchows Arch 435:479–485.

Chew CS, Hershey SJ. 1982. Gastrin stimulation of isolated gastric glands. Am J Physiol 240:G504–G512.

De Bolos C, Garrido M, Real FX. 1995. MUC6 Apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology 109:723–734.

Defize J. 1980. Development of pepsinogens. In: Lebenthal E, editor. Human gastrointestinal development. New York: Raven Press. p. 299–304.

Descroix-Vagne M, Perret JP, Daoud-el Baba M, Bosshard A, Dechelette MA, Gros I, Desvigne A, Rakotomala H. 1993. Variation of gastric lipase secretion in the Heidenhain pouch of the cat. Arch Int Phys Biochem Biophys 101:79–85.

Finks CS, Hamosh M, Hamosh P, Denigris SJ, Kasbekar DK. 1985. Lipase secretion from dispersed rabbit gastric glands. Am J Physiol 248:G68–G72.

Fiocca R, Villani L, Tenti P, Solcia E, Cornaggia M, Frigerio B, Capella C. 1987. Characterization of four main cell types in gastric cancer: foveolar, mucopeptic, intestinal columnar and goblet cells. A histopathological, histochemical and ultrastructural study of early and advanced tumours. Pathol Res Pract 182:308–325.

Guo YS, Beauchamp DR, Jin GF, Townsend CM, Thompson JC. 1993. Insulin-like growth factor-binding protein modulates the growth response to insulin-like growth factor 1 by human gastric cancer cells. Gastroenterology 104:1595–1604.

Hamosh M, Scanlon JW, Ganot D, Likel M, Scanlon KB, Hamosh P. 1981. Fat digestion in the newborn: characterization of lipase in gastric aspirates of premature and term infants. J Clin Invest 67:838–846.

Hanby A, Chinery R, Poulsom R, Playford RJ, Pignatelli M. 1996. Downregulation of E-cadherin in the reparative epithelium of the human gastrointestinal tract. Am J Pathol 148:723–729.

Hattori Y, Odigiri H, Nakatani H, Miyagawa K, Naito K, Sakamoto H, Katoh O, Yoshida T, Sugimura T, Terada M. 1990. K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor genes. Proc Natl Acad Sci USA 87:5983–5987.

Ho SB, Roberton AM, Shekels LL, Lyftogt CT, Niehans GA, Toribara NW. 1995. Expression cloning of gastric mucin complementary DNA and localization of mucin gene expression. Gastroenterology 109:735–747.

Hsieh MC, Wu CW, Wu LH, Lui WY, Peng FK, Yu CL. 1996. Heat shock and cytokines modulate the expression of adhesion molecules on different human gastric cancer cell lines. Int J Cancer 67:690–694.

Ishizuka J, Martinez J, Townsend CM, Thompson JC. 1992. The effect of gastrin on growth of human stomach cancer cells. Annals of Sur 215:528–534.

Jawhari AU, Noda M, Farthing MJ, Pignatelli M. 1999. Abnormal expression and function of the E-cadherin-catenin complex in gastric carcinoma cell lines. Br J Cancer 80:322–330.

Kamata N, Chida K, Rikimaru K, Horikoshi M, Enomoto S, Kuroki T. 1986. Growth-inhibitory effects of epidermal growth factor and overexpression of its receptors on human squamous cell carcinomas in culture. Cancer Res 46:1648–1653.

Karam SM, Leblond CP. 1992. Identifying and counting epithelial cell types in the corpus of the mouse stomach. Anat Rec 232:231–246.

Kasbekar DK, Jensen RT, Gardner JD. 1983. Pepsinogen secretion from dispersed glands from rabbit stomach. Am J Physiol 244:G392–G396.

Lee PC, Borysewicz R, Struze M, Raab K, Werlin ET. 1993. Development of lipolytic activity in gastric aspirates from premature infants. J Pediatr Gastroenterol Nutr 17:291–297.

Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shepard HM. 1993. Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol Immunother 37:255–263.

Lowry OH, Rosebrough NF, Farr AL, Randall RJ. 1951. Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275.

Mahara K, Kato J, Terui T, Takimoto R, Horimoto M, Murakami T, Mogi Y, Watanabe N, Kohgo Y, Niitsu Y. 1994. Transforming growth factor beta-1 secreted from scirrhous gastric cancer cells is associated with excess collagen deposition in the tissue. Br J Cancer 69:777–783.

Ménard D, Arsenault P, Monfils S. 1993. Maturation of human fetal stomach in organ culture. Gastroenterology 104:492–501.

Ménard D, Monfils S, Tremblay E. 1995. Ontogeny of human gastric lipase and pepsin activities. Gastroenterology 108:1650–1656.

Moreau H, Laugier R, Gargouri Y, Ferrato F, Verger R. 1988. Human preduodenal lipase is entirely of gastric fundic origin. Gastroenterology 95:1221–1226.

Motoyama T, Hojo H, Watanabe H. 1986. Comparison of seven cell lines derived from human gastric carcinomas. Acta Pathol Jpn 36:65–83.

Naef M, Yokoyama M, Friess H, Buchler MW, Korc M. 1996. Co-expression of heparin-binding EGF-like growth factor and related peptides in human gastric carcinoma. Int J Cancer 66:315–321.

Oda T, Kanai Y, Oyama T, Yoshiura K, Shimoyama Y, Birchmeier W, Sugimura T, Hirohashi S. 1994. E-cadherin gene mutations in human gastric carcinoma cell lines. Proc Natl Acad Sci USA 91:1858–1862.

Park JG, Frucht H, LaRocca RV, Bliss DP, Kurita Y, Chen TR, Henslee JG, Trepel JB, Jensen RT, Johnson BE. 1990. Characteristics of cell lines established from human gastric carcinoma. Cancer Res 50:2773–2780.

Sekiguchi M, Sakakibara K, Fujii G. 1978. Establishment of cultured cell lines derived from a human gastric carcinoma. Jpn J Exp Med 48:61–68.

Shimoyama Y, Hirohashi S. 1991. Expression of E- and P-cadherin in gastric carcinomas. Cancer Res 51:2185–2192.

Smith H. 1979. In vitro properties of epithelial cell lines established from human carcinomas and nonmalignant tissue. J Natl Cancer Inst 62:225–230.

Tremblay E, Monfils S, Ménard D. 1997. Epidermal growth factor influences cell proliferation, glycoproteins, and lipase activity in human fetal stomach. Gastroenterology 112:1188–1196.

Tremblay E, Basque JR, Rivard N, Ménard D. 1999. Epidermal growth factor and transforming growth factor α down-regulate human gastric lipase gene expression. Gastroenterology 116:831–841.

Yamamoto R, Iishi H, Tatsuta M, Nakamura H, Terada N, Komatsu K, Matsusaka T. 1990. Enhancement of mucous accumulation in a human gastric scirrhous carcinoma cell line (KATO-III) by fibroblast-tumor cell interaction. Virchows Arch 59:26–31.