Aberrant Expression of TFF1, TFF2, and PDX1 and Their Diagnostic Value in Lobular Endocervical Glandular Hyperplasia

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Abstract

Lobular endocervical glandular hyperplasia (LEGH) is a distinct benign glandular lesion expressing gastric gland mucous cell–type mucin (N-acetylglucosamin$\alpha 1 \rightarrow 4$galactose $\rightarrow R$ [GlcNAc$\alpha 1 \rightarrow 4$Gal $\rightarrow R$]). To investigate histogenesis and diagnostic markers of LEGH, we examined the immunohistochemical expression profile of gastric surface mucous cell (MUC5AC and TFF1), gastric gland mucous cell (MUC6, TFF2, and GlcNAc$\alpha 1 \rightarrow 4$Gal $\rightarrow R$), gastric pyloric epithelial cell (PDX1), and endocervical cell (keratan sulfate) markers in normal endocervix samples and benign glandular lesions (nabothian cysts, tunnel clusters, and LEGHs). MUC5AC and MUC6 were expressed in normal endocervical mucosa and benign glandular lesions. TFF1, TFF2, GlcNAc$\alpha 1 \rightarrow 4$Gal $\rightarrow R$, and PDX1 were expressed only in LEGH. Keratan sulfate was expressed in normal endocervical mucosa and benign glandular lesions. In LEGH, gastric surface mucous cell and gastric gland mucous cell differentiation were demonstrated, and transdifferentiation from endocervical mucosa into gastric pyloric mucosa was suggested. In addition to GlcNAc$\alpha 1 \rightarrow 4$Gal $\rightarrow R$, TFF1, TFF2, and PDX1 are additional useful markers for LEGH.

Mucins are high-molecular-weight glycoproteins consisting of oligosaccharide chains attached to a protein backbone (core protein), and their core proteins (mucin core protein; MUC) have been named according to their corresponding genes (mucin gene, *MUC*).[1] Mucin core proteins are expressed in a cell type–specific manner. In gastrointestinal mucosa, MUC5AC is expressed in gastric surface mucous cells, MUC6 is expressed in gastric gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells) and duodenal gland mucous cells, and MUC2 is expressed in intestinal goblet cells.[2-6] Gastric gland mucous cell–type mucin possesses N-acetylglucosamin$\alpha 1 \rightarrow 4$galactose $\rightarrow R$ (GlcNAc$\alpha 1 \rightarrow 4$Gal $\rightarrow R$), and it is specifically recognized by a monoclonal antibody designated HIK1083.[7,8] In the human endocervix, the prevalent mucin is MUC5B, and in smaller amounts, MUC5AC, MUC6, and MUC2.[9-11]

In addition to mucins, mucous cells secrete trefoil factor family (TFF) peptides, which are mucin-associated peptides that participate in mucosal barrier function and the repair of damaged mucosa.[12] In the gastrointestinal mucosa, TFF1 (pS2) is expressed by gastric surface mucous cells,[13] TFF2 (human spasmolytic peptide [HSP]) is expressed in gastric gland mucous cells and duodenal gland mucous cells,[14] and TFF3 (intestinal trefoil factor) is expressed in intestinal goblet cells.[15] TFF3, but neither TFF1 nor TFF2, is expressed in normal endocervical epithelium.[16,17]

Cell growth and differentiation are controlled by homeobox gene–encoded transcription factors.[18] Among them, pancreatic-duodenal homeobox 1 gene (*PDX1*) regulates the development of the pancreas, duodenum, and gastric antrum. In adult tissues, PDX1 is expressed in the nuclei of pancreatic islet cells and the mucosal epithelial cells of the duodenum

and gastric antrum.[18-22] Expression of PDX1 was reported in gastric cancer[21] and pseudopyloric gland metaplasia of the human gastric fundic mucosa in chronic gastritis[21] and Ménétrier disease.[22] Ectopic PDX1 expression has also been reported in colonic hyperplastic polyps,[23] colonic serrated adenomas,[23] and ulcer-associated cell lineage of ileal mucosa in Crohn disease,[24] which show a gastric pyloric phenotype.

The mucin core proteins, TFF peptides, and homeobox gene–encoded transcription factors are expressed in a cell type–specific manner and, therefore, have been used as phenotypic markers to clarify cell-lineage differentiation.[6]

In 1999, Nucci et al[25] first described lobular endocervical glandular hyperplasia (LEGH), which is a distinct hyperplastic glandular lesion of the uterine cervix that corresponds to a lesion previously described as gastric pyloric metaplasia[26] or pyloric gland metaplasia.[27] LEGHs have been shown to secrete gastric pyloric–type mucin recognized by the monoclonal antibody HIK1083.[26,27]

To investigate histogenesis and diagnostic markers of LEGH, we examined the immunohistochemical expression profile of gastric surface mucous cell (MUC5AC and TFF1), gastric gland mucous cell (MUC6, TFF2, and GlcNAcα1 → 4Gal → R), gastric pyloric epithelial cell (PDX1), and endocervical cell (keratan sulfate) markers in normal endocervix samples, nabothian cysts, tunnel clusters, and LEGHs.

Materials and Methods

Tissue Samples

We retrieved 8 cases of LEGH from the pathology files of the Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan. The patients ranged from 40 to 71 years old (median, 45.5 years). LEGH was defined as follows: (1) proliferation of small to moderate-sized glands with a lobular appearance often with central dilated glands showing wavy contours and papillary infoldings in typical lesions; (2) absence of distinct evidence of cellular atypia and stromal invasion for malignancy on examination of sections with H&E staining; and (3) a positive immunoreaction with the monoclonal antibody HIK1083.

For control samples, we also retrieved uterine endocervix without benign or malignant glandular proliferative lesions from 15 women undergoing hysterectomy because of uterine myoma. These patients ranged from 36 to 49 years old (median, 43.0 years). Cases in which hormonal therapy had been administered in advance were excluded. For analysis of the relationship between menstrual phase and immunoreactivity of cervical mucin, cases in various phases of the menstrual cycle were selected as follows: early proliferative phase, 2; mid to late proliferative phase, 4; early secretory phase, 4; and mid to late secretory phase, 5.

In addition, 8 nabothian cysts and 6 tunnel clusters were examined. In each case, representative tissue blocks of the uterine cervix were used. To examine the immunoreactivity of anti–keratan sulfate in nongynecologic tissues, we retrieved 5 samples each of normal esophagus, gastric fundus, gastric pylorus, small intestine, colon, salivary gland, and lung.

Immunohistochemical Analysis

All materials were fixed in 10% neutral buffered formalin before routine processing and embedding in paraffin. Serial paraffin sections of 3 μm thickness were stained with H&E for histologic examination or were subjected to immunohistochemical staining to investigate the phenotypic expression of normal endocervix samples, nabothian cysts, tunnel clusters, and LEGH. To investigate the characteristics of gastric epithelial cells, the following cell lineage–specific markers were used: anti-MUC5AC (Novocastra, Newcastle upon Tyne, England) and anti-TFF1 (anti-PS2; DAKO, Glostrup, Denmark) for gastric surface mucous cells; anti-MUC6, anti-TFF2 (anti-HSP; YLEM, Rome, Italy), and anti–GlcNAcα1 → 4Gal → R (clone HIK1083, Kanto Chemical, Tokyo, Japan) for gastric gland mucous cells; and anti-PDX1 (guinea pig anti-mouse Pdx1, generously provided by Christopher V.E. Wright, DPhil, Vanderbilt University Medical School, Nashville, TN) for gastric pyloric epithelial cells. Anti–keratan sulfate (monoclonal antibody 5D4, Seikagaku Kogyo, Tokyo, Japan) was used as a marker for endocervical cells. Primary incubation was manually performed for 2 hours at room temperature. Immunohistochemical staining was performed using the immunoenzyme polymer method (Histofine Simple Stain MAX PO Multi, Nichirei Biosciences, Tokyo, Japan) with 3,3'-diaminobenzidine as the chromogen. For immunostaining PDX1, rabbit anti–guinea pig immunoglobulin (DAKO) was used as the secondary antibody before using the Histofine detection system.[23]

Before immunostaining, antigen retrieval was performed using a microwave (0.01 mol/L citrate buffer, pH 6.0) for MUC5AC, MUC6, TFF1, TFF2, GlcNAcα1 → 4Gal → R, and keratan sulfate or a Pascal pressurized heating chamber (DAKO) (0.01 mol/L citrate buffer, pH 6.0) for PDX1. Details of the antibodies and antigen retrieval methods used in this study are summarized in ❙Table 1❙. Pyloric mucosa and cartilage were used as positive control samples for the differentiation markers for gastric mucous cells and for keratan sulfate, respectively. Negative control samples were obtained by omitting the primary antibodies.

Evaluation of Immunostaining

The grade of immunoreactivity of the markers was estimated semiquantitatively as follows: 0, negative; 1, less than one third of cells positive; 2, one third to two thirds of cells positive; or 3, more than two thirds of cells positive. Staining

■Table 1■
Antibodies and Cell Types Recognized

Antigen	Clone	Species	Source	Target Cells	Dilution	Antigen Retrieval*
MUC5AC	CLH2	Mouse	Novocastra Laboratories, Newcastle upon Tyne, England	Gastric surface mucous cells	1:100	MW
MUC6	CLH5	Mouse	Novocastra Laboratories	Gastric gland mucous cells	1:100	MW
GlcNAcα1 → 4Gal → R	HIK1083	Mouse	Kanto Chemical, Tokyo, Japan	Gastric gland mucous cells	1:10	MW
TFF1 (pS2)	BC04	Mouse	DAKO, Glostrup, Denmark	Gastric surface mucous cells	1:100	MW
TFF2 (HSP)	GE16C	Mouse	YLEM, Rome, Italy	Gastric gland mucous cells	1:50	MW
PDX1	Polyclonal	Guinea pig	C.V. Wright, DPhil, Vanderbilt University Medical School, Nashville, TN	Gastric pyloric epithelial cells	1:10,000	Pascal
Keratan sulfate	5D4	Mouse	Seikagaku Kogyo, Tokyo, Japan	Endocervical cells	1:500	MW

HSP, human spasmolytic peptide; GlcNAcα1 → 4Gal → R, N-acetylglucosaminα1 → 4galactose → R; TFF, trefoil factor family.
* Microwaving (MW; 650 W) in 0.01 mol/L citrate buffer (pH 6.0) for 25 minutes; or Pascal pressurized heating chamber (DAKO) (0.01 mol/L citrate buffer, pH 6.0).

scores are nonparametric and are expressed as median scores with interquartile ranges rather than as mean values.

This study was approved by the ethics committee of Shinshu University, Matsumoto, Japan.

Results

The immunohistochemical results are summarized in ■Table 2■.

In normal endocervix, cytoplasmic expression of MUC5AC was found in columnar epithelium in various degrees in 13 (87%) of 15 cases and showed a distinctive spatial expression pattern. MUC5AC tended to be distributed with an increasing concentration gradient toward the ectocervix in the horizontal direction ■Image 1A■ and ■Image 1B■ and toward the luminal surface of the cervical canal in the vertical direction ■Image 2A■ and ■Image 2B■, and TFF1 was not expressed in normal endocervix ■Image 2C■. MUC6 was expressed in the cytoplasm of columnar cells in all cases examined. MUC6 tended to be distributed with an increasing concentration gradient toward
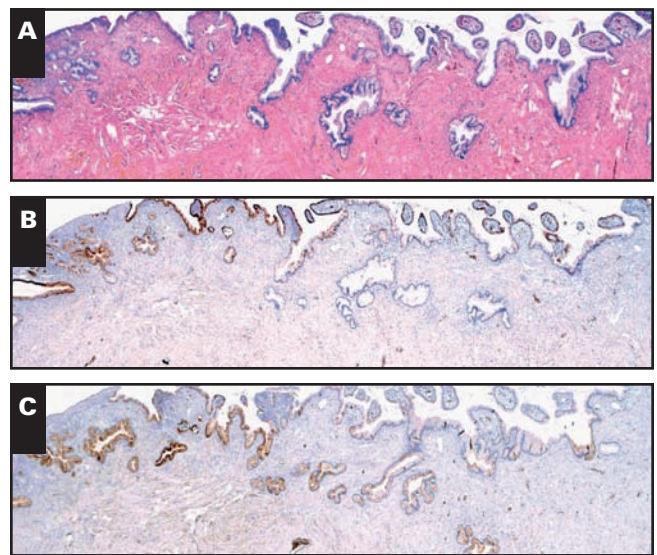

■Image 1■ Normal endocervix. **A**, H&E stain of normal endocervix (×12). **B** and **C**, MUC5AC+ cells (**B**, ×12) and MUC6+ cells (**C**, ×12) are distributed with an increasing concentration gradient toward the ectocervix in the horizontal direction.

■Table 2■
Summary of Immunostaining of Normal Endocervical Mucosa and Benign Endocervical Glandular Lesions*

| | Cell Lineage Markers | | | | | | |
| | Gastric Surface Mucous Cell | | Gastric Gland Mucous Cell | | | Gastric Pyloric Epithelial Cell | Endocervical Epithelial Cell |
Tissue	MUC5AC	TFF1	MUC6	GlcNAcα1 → 4Gal → R	TFF2	PDX1	Keratan Sulfate
Normal endocervix (n = 15)	13 (87)/1 (1-1)	0 (0)/0 (0-0)	15 (100)/2 (1-2)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	15 (100)/2 (2-3)
Nabothian cyst (n = 8)	2 (25)/0 (0-0.75)	0 (0)/0 (0-0)	2 (25)/0 (0-0.75)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	8 (100)/3 (3-3)
Tunnel cluster (n = 6)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	5 (83)/2 (0.75-3)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	0 (0)/0 (0-0)	6 (100)/3 (2.75-3)
LEGH (n = 8)	8 (100)/1.5 (1-3)	8 (100)/3 (1.25-3)	8 (100)/2 (2-2.75)	8 (100)/2 (2-2.75)	8 (100)/2 (1-2.75)	8 (100)/2 (1.25-3)	4 (50)/0 (0-1)

LEGH, lobular endocervical glandular hyperplasia; GlcNAcα1 → 4Gal → R, N-acetylglucosaminα1 → 4galactose → R; TFF, trefoil factor family.
* Data are given as frequency (percentage) of positive specimens/median score (interquartile range).
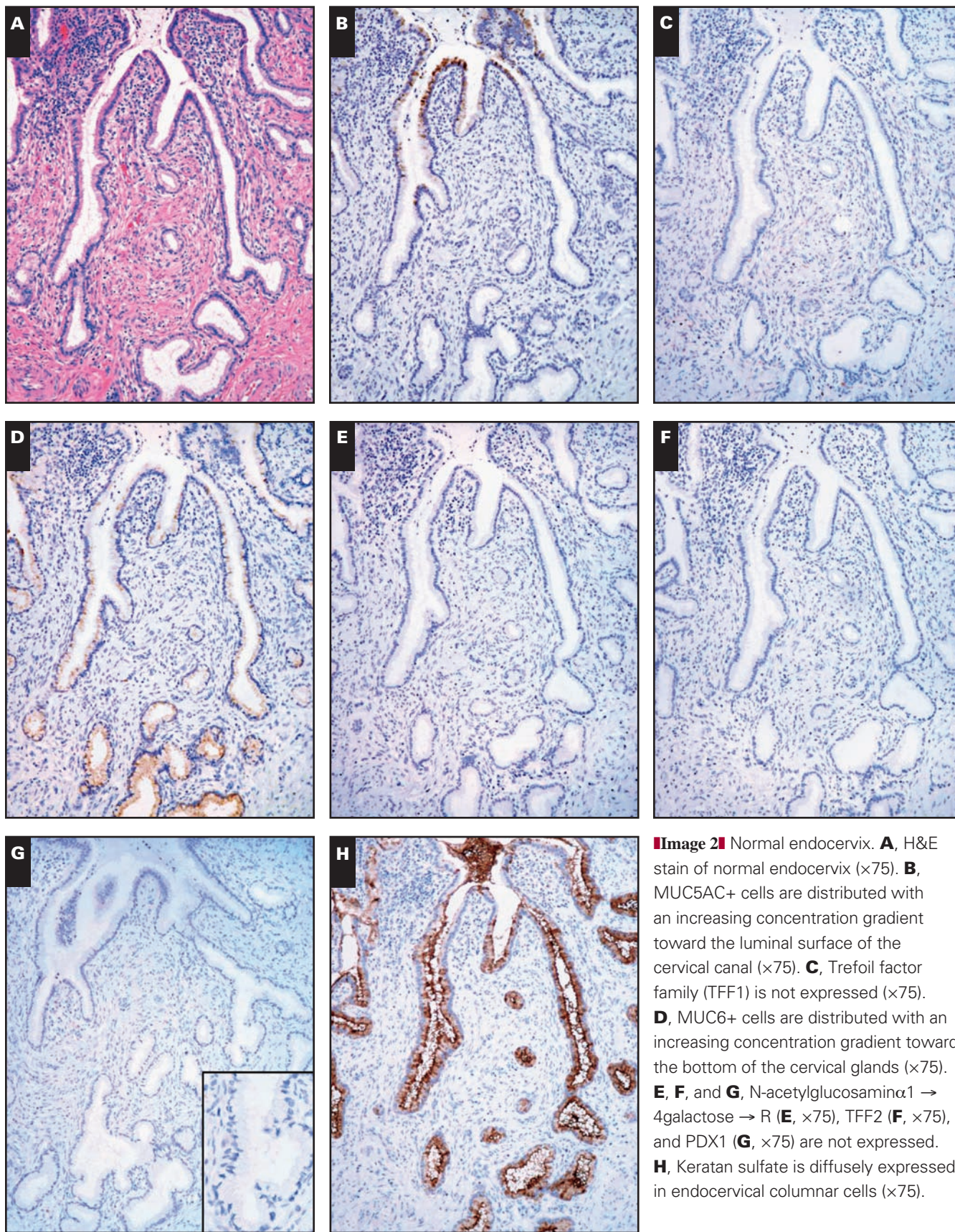
▮**Image 2**▮ Normal endocervix. **A**, H&E stain of normal endocervix (×75). **B**, MUC5AC+ cells are distributed with an increasing concentration gradient toward the luminal surface of the cervical canal (×75). **C**, Trefoil factor family (TFF1) is not expressed (×75). **D**, MUC6+ cells are distributed with an increasing concentration gradient toward the bottom of the cervical glands (×75). **E**, **F**, and **G**, N-acetylglucosaminα1 → 4galactose → R (**E**, ×75), TFF2 (**F**, ×75), and PDX1 (**G**, ×75) are not expressed. **H**, Keratan sulfate is diffusely expressed in endocervical columnar cells (×75).

the ectocervix in the horizontal direction (Image 1A) ▌**Image 1C**▌ and toward the bottom of the cervical glands (Image 2A) ▌**Image 2D**▌. GlcNAcα1 → 4Gal → R ▌**Image 2E**▌, TFF2 ▌**Image 2F**▌, and PDX1 ▌**Image 2G**▌ were not detected in normal endocervix. Keratan sulfate was diffusely expressed by endocervical columnar cells in all cases ▌**Image 2H**▌. These expression patterns of mucins and keratan sulfate did not show any significant change during the menstrual cycle.

In nongynecologic tissues, keratan sulfate was detected on the cilia of ciliated epithelium and in bronchial cartilaginous cells in pulmonary tissues, but not in normal esophago-gastrointestinal epithelial cells and salivary gland cells.

In cases of nabothian cysts, MUC5AC and MUC6 were weakly positive in 2 (25%) of 8 cases; diffuse expression of keratan sulfate was observed in all cases, and other markers

were not detected. Tunnel clusters ▌**Image 3A**▌ showed various degrees of reactivity for MUC6 in 5 (83%) of 6 cases ▌**Image 3B**▌ and showed diffuse reactivity for keratan sulfate in all cases ▌**Image 3C**▌. The other markers were not detected in the tunnel clusters ▌**Image 3D**▌, ▌**Image 3E**▌, and ▌**Image 3F**▌.

Microscopically, LEGH showed a lobular pattern in typical cases ▌**Image 4A**▌ and ▌**Image 4B**▌ or a cluster of variously sized glands in the other cases ▌**Images 5A**▌ and ▌**Image 5B**▌. In the typical cases, LEGH showed a pattern that consisted of a central dilated gland with surrounding smaller glands (Images 4A and 4B). These central dilated glands often showed wavy contours or papillary infoldings (Images 4A and 4B).

In LEGH, MUC5AC, TFF1, MUC6, GlcNAcα1 → 4Gal → R, TFF2, and PDX1 were expressed in all cases examined. Immunoreactivity for MUC5AC was present in the
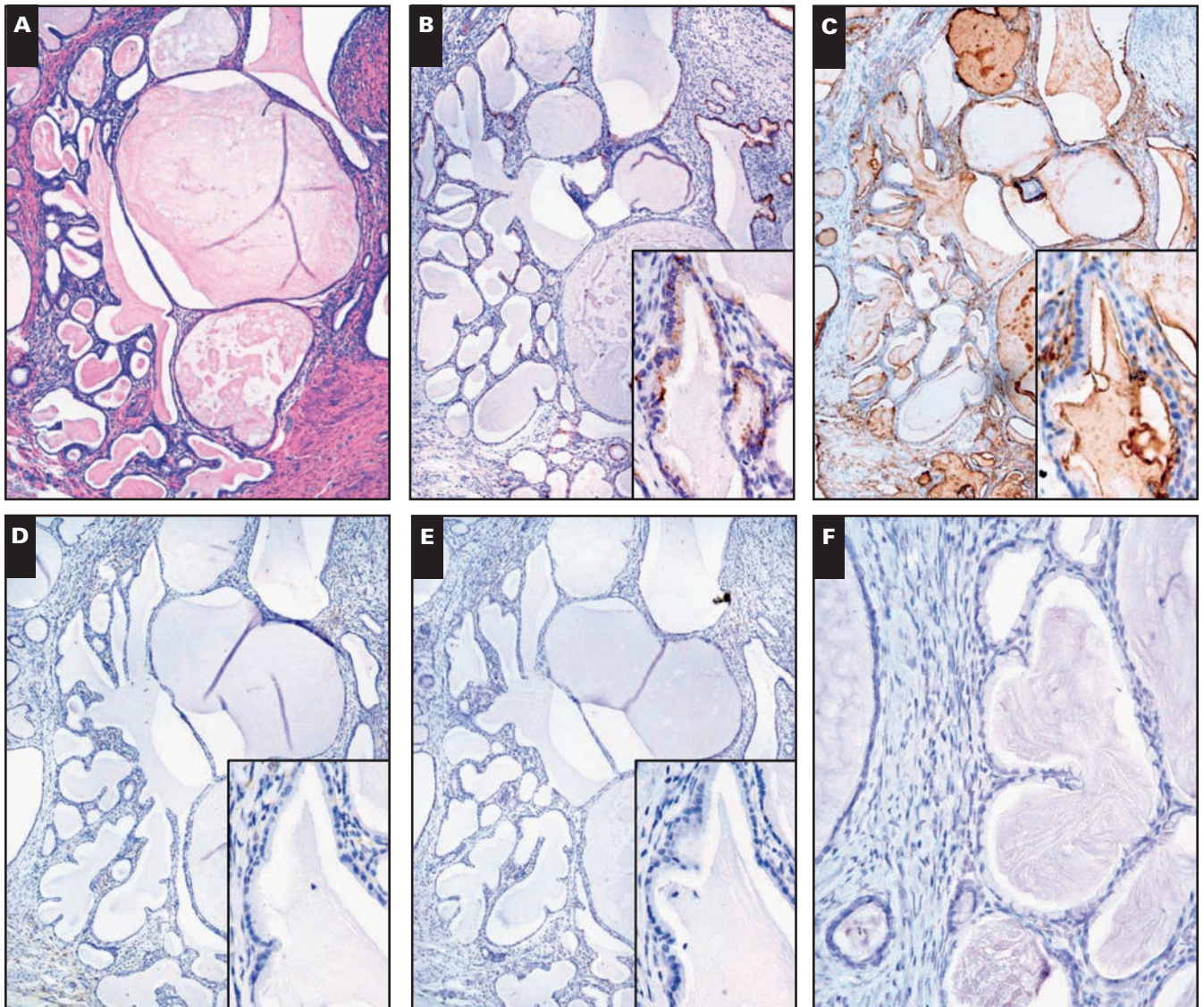

▌**Image 3**▌ Tunnel cluster. **A**, H&E stain of tunnel cluster (×45). **B** and **C**, MUC6 (**B**, ×45) and keratan sulfate (**C**, ×45) are expressed. **D**, **E**, and **F**, Trefoil factor family (TFF1; **D**, ×45), TFF2 (**E**, ×45), and PDX1 (**F**, ×45) are not expressed.
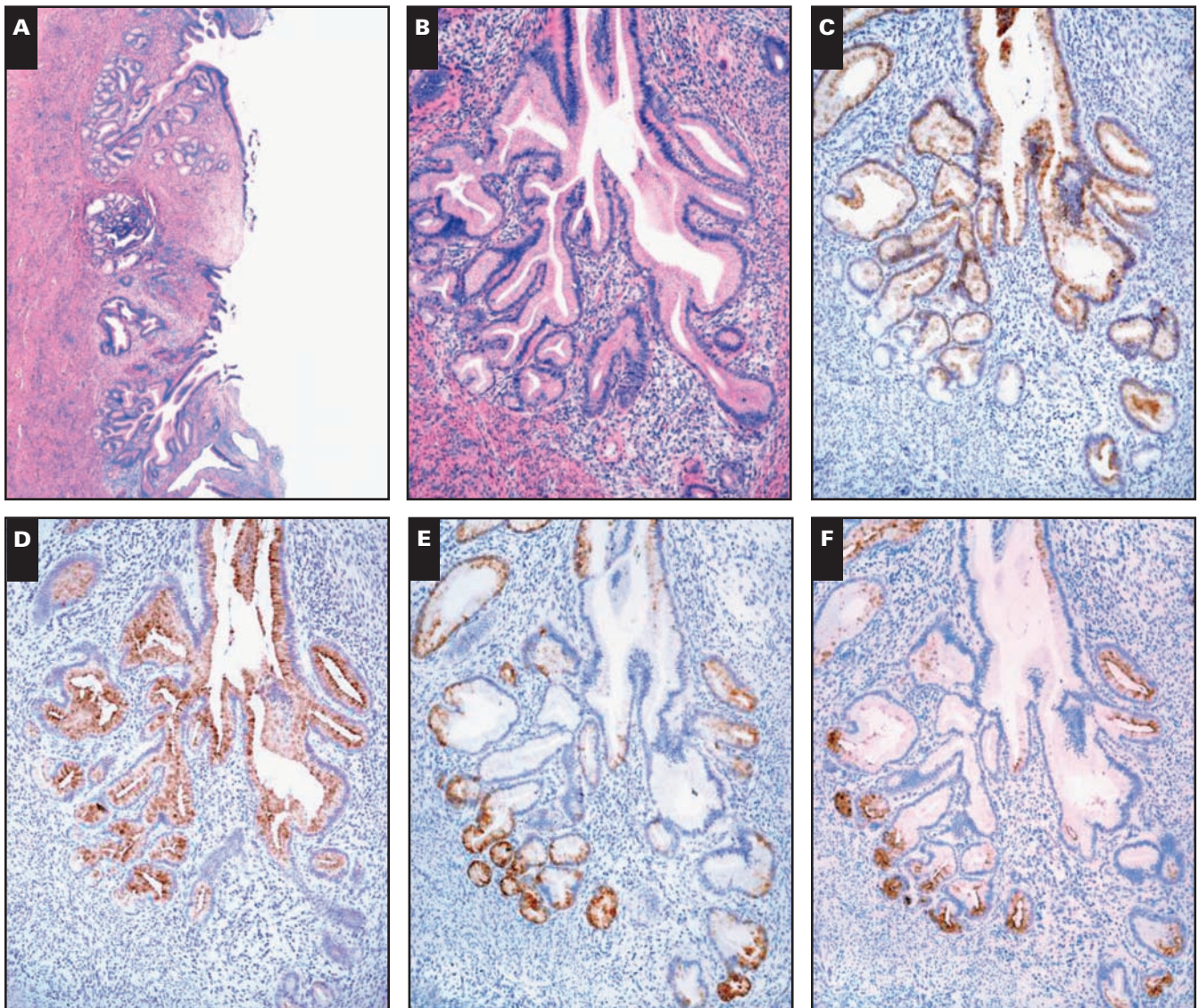
Image 4 A typical lesion of lobular endocervical glandular hyperplasia (LEGH). **A** and **B**, LEGH shows proliferation of small to moderate-sized glands with a lobular appearance with a central dilated lumen showing papillary infoldings (**A**, ×15; **B**, ×65). **C** and **D**, MUC5AC (**C**, ×65) and trefoil factor family (TFF1; **D**, ×65) are expressed in papillary infoldings and some of their adjacent glands. **E**, **F**, and **G**, MUC6 (**E**, ×65), N-acetylglucosaminα1 → 4galactose → R (**F**, ×65), and TFF2 (**G**, ×65) are expressed in glands surrounding the central dilated lumen.

cytoplasm, and that for TFF1 was in the cytoplasmic mucin granules. MUC5AC and TFF1 were found in cells lining central dilated glands with wavy contours or papillary infoldings and in some cells lining clustered small tubules adjacent to the central dilated glands with an increasing concentration gradient toward central dilated glands **Image 4C** and **Image 4D**. In contrast, MUC6, GlcNAcα1 → 4Gal → R and TFF2 were expressed mainly in cells lining the clustered small tubules adjacent to the central dilated glands **Image 4E**, **Image 4F**, and **Image 4G**. Immunoreactivity for MUC6 was present in the cytoplasm and that for GlcNAcα1 → 4Gal → R and TFF2 in the cytoplasmic mucin granules. Immunoreactivity for PDX1 was found in epithelial nuclei in LEGH, and most

of the cells in LEGH were positive for PDX1 **Image 4H**. Keratan sulfate was not expressed in typical lesions of LEGH **Image 4I**. However, in 4 cases of LEGH, some cells showed immunoreactivity for gastric epithelial markers **Image 5C** and **Image 5D** and keratan sulfate **Image 5E**.

Discussion

We showed that MUC5AC and MUC6 were expressed in normal endocervical mucosa, nabothian cysts, and tunnel clusters, as well as in LEGH, but TFF1, TFF2, GlcNAcα1 → 4Gal → R, and PDX1 were expressed only in LEGH. In contrast, keratan sulfate was expressed in normal endocervical
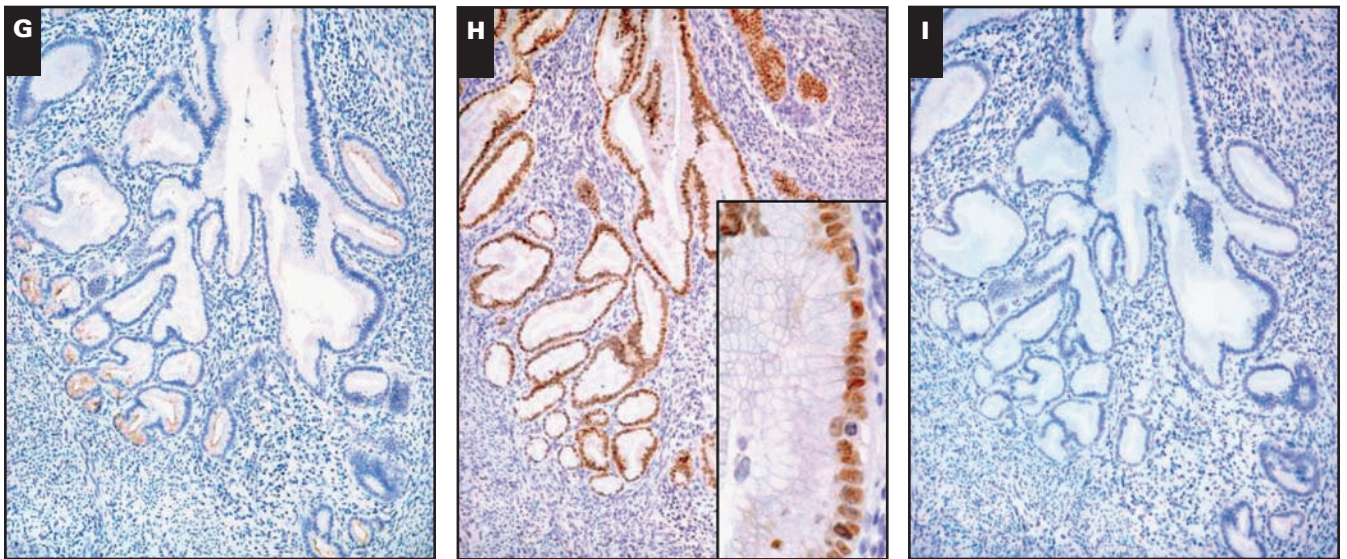
■Image 4■ (cont) **H**, PDX1 is expressed in epithelial nuclei in LEGH (×65). **I**, Keratan sulfate is not expressed (×65).
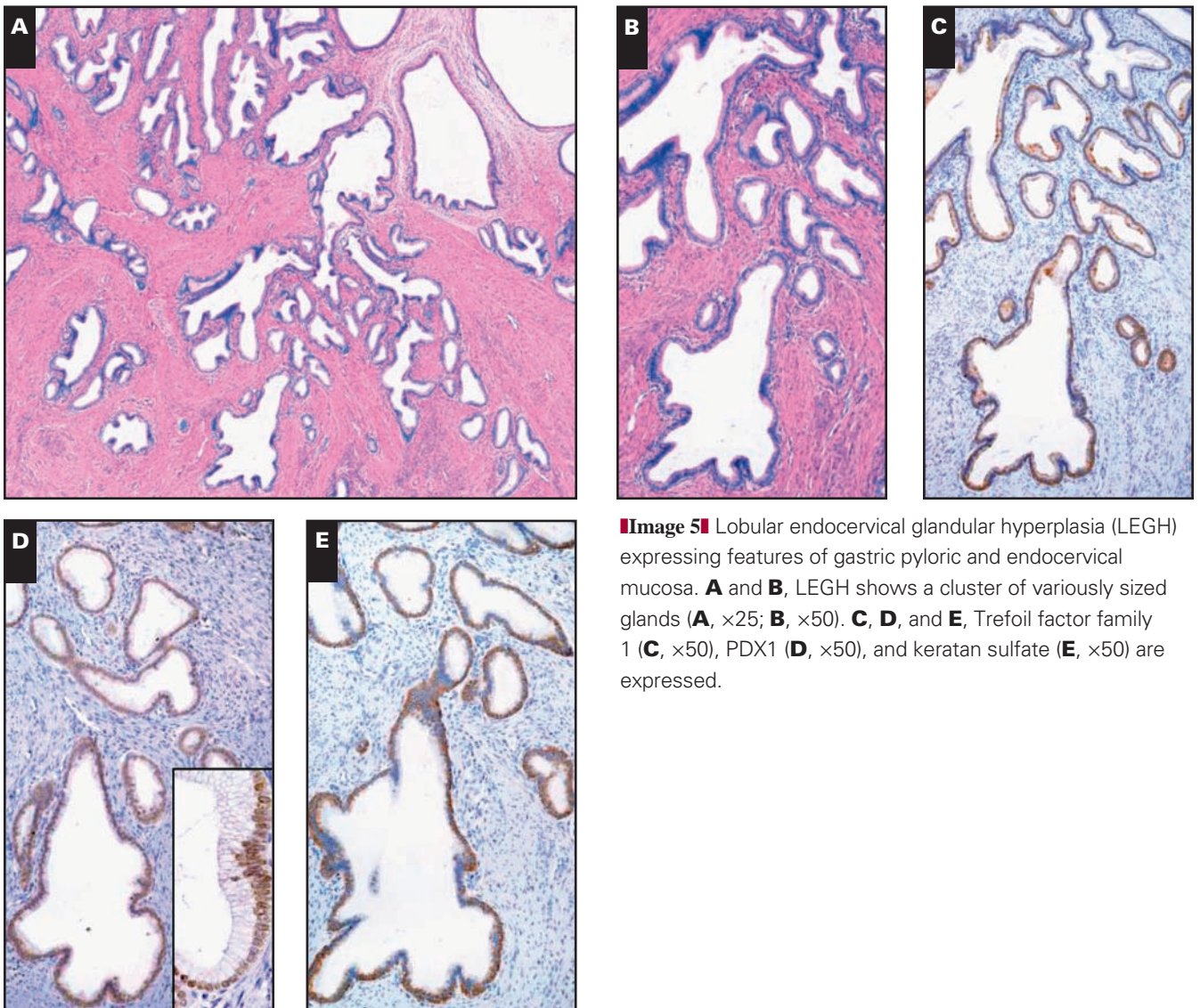

■Image 5■ Lobular endocervical glandular hyperplasia (LEGH) expressing features of gastric pyloric and endocervical mucosa. **A** and **B**, LEGH shows a cluster of variously sized glands (**A**, ×25; **B**, ×50). **C**, **D**, and **E**, Trefoil factor family 1 (**C**, ×50), PDX1 (**D**, ×50), and keratan sulfate (**E**, ×50) are expressed.

mucosa, nabothian cysts, tunnel clusters, and in some cells of LEGH but was absent in typical LEGH. LEGH cases positive for keratan sulfate showed coexpression of keratan sulfate and gastric markers.

A vertical gradient of mucin expression was confirmed in normal endocervix, and this result is consistent with previous reports.[10,28] Thus, MUC5AC tended to be distributed with an increasing concentration gradient toward the luminal surface of the cervical canal, whereas MUC6 tended to be distributed with an increasing concentration gradient toward the bottom of the cervical glands, suggesting that true cervical glands are present within the stroma. This observation is contrary to previous reports in which the deep-sited endocervical epithelium was merely elongated clefts or crypts into the underlying stroma.[29,30] This result can be supported by the following findings. Basically, epithelia are divided into 2 main groups: covering and glandular. The former covers the external surface or lines the cavities of the body, and the latter forms the glands of the body. In nongynecologic tissues, MUC5AC is expressed in the surface epithelium of the stomach (surface mucous cells)[2,3] and upper respiratory tract (goblet cells),[1] whereas MUC6 is expressed in mucous gland cells of the stomach,[2,3,5] duodenum,[2,4,5] and peripancreaticobiliary glands,[4,5] implying that MUC5AC is a marker of covering epithelial cells and MUC6 is a marker of glandular epithelial cells.

In the current study, MUC5AC and MUC6 immunoreactivity were frequently observed in the epithelium of the normal endocervix and in LEGH, which is in agreement with previous reports.[9,10] Immunohistochemical analysis and in situ hybridization revealed frequent expression of MUC5AC and MUC6 in the normal endocervical epithelium throughout the menstrual cycle.[9,10] In LEGH, frequent expression of MUC6 has been reported.[31] These previous data combined with data in this study indicate that neither MUC5AC nor MUC6 can be used to differentiate between normal endocervical epithelium and mucous epithelium in LEGH.

A novel finding in this study was that TFF1 and TFF2 were immunohistochemically detected in mucous cells in LEGH but were not found in nabothian cysts, tunnel clusters, or, as previously reported, in the normal endocervical epithelium.[16,17] TFF1 and MUC5AC showed similar localization in LEGH. In addition, TFF2, MUC6, and GlcNAcα1 → 4Gal → R also showed similar localization in LEGH. TFF1 and MUC5AC expression profiles in LEGH are similar to those of normal gastric surface mucous cells, and TFF2, MUC6, and GlcNAcα1 → 4Gal → R expression profiles are similar to those of gastric gland mucous cells.[2,5,13,14,23,24] This finding suggests differentiation toward gastric surface mucous cells and differentiation toward gastric gland mucous cells in LEGH.

Another noteworthy finding of this study was that PDX1, which is a transcription factor that has an essential role in the genesis and development of the pancreas, duodenum, and gastric antrum,[18-22] was detected in the nuclei of LEGH but not in normal endocervical epithelium, nabothian cysts, or tunnel clusters. Similar ectopic PDX1 expression has been reported in pseudopyloric gland metaplasia (antralization) in humans[21,22] and mice.[22] Ectopic PDX1 expression has also been reported in colonic hyperplastic polyps[23] and colonic serrated adenomas[23] and in ulcer-associated cell lineage of the ileal mucosa in Crohn disease,[24] which shows a gastric pyloric phenotype. Our present finding of PDX1 expression in LEGH, combined with the close association of ectopic PDX1 expression with gastric pyloric differentiation in previous studies,[23,24] demonstrates gastric pyloric differentiation of LEGH.

Gastric pyloric–type mucin recognized by the monoclonal antibody HIK1083 has been detected in minimal deviation adenocarcinoma (MDA) and LEGH,[26,27] suggesting a possible link in the histogenesis between MDA and LEGH. Further studies on the expression of TFF1, TFF2, and PDX1 in MDA would provide useful information to investigate the histogenesis of MDA and the relationship between MDA and LEGH.

In this study, keratan sulfate recognized by the monoclonal antibody 5D4 was detected in normal cervical epithelium, nabothian cysts, and tunnel clusters but not in normal gastrointestinal epithelium. This finding in normal cervical epithelium is in contrast with the results of a previous study in which antibody 5D4 labeled endometrial epithelial cells but not normal endocervical epithelial cells.[32] The discrepancy between studies might be due to different immunostaining methods. In the previous study, no antigen retrieval was used for immunostaining with antibody 5D4.[32] Concerning the expression of keratan sulfate in esophagogastrointestinal mucosa, another study also reported no expression of keratan sulfate defined by monoclonal antibody 5D4 in esophagogastrointestinal epithelial cells.[33] The present study demonstrated that some mucous cells of LEGH expressing gastric phenotype markers also expressed keratan sulfate, suggesting transdifferentiation of endocervical epithelial cells toward gastric pyloric mucous cells in LEGH.

We have demonstrated gastric surface mucous cell differentiation along with gastric gland mucous cell differentiation in LEGH. Our results also suggested transdifferentiation from endocervical mucosa into gastric pyloric mucosa in LEGH. PDX1 expression could be important for inducing the gastric pyloric pattern of cell differentiation in LEGH. In addition to gastric gland mucous cell–type mucin (GlcNAcα1 → 4Gal → R) defined by HIK1083, TFF1, TFF2, and PDX1 are useful immunohistochemical markers for LEGH.

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