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BRIEF ARTICLE

Intestinal stem cell marker LGR5 expression during gastric carcinogenesis

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Abstract

AIM: To investigate the differential expression of leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) in gastric cancer tissues and its significance related to tumor growth and spread.

METHODS: Formalin-fixed biopsy specimens of intestinal metaplasia (n = 90), dysplasia (n = 53), gastric adenocarcinoma (n = 180), metastases in lymph nodes and the liver (n = 15), and lesion-adjacent normal gastric mucosa (controls; n = 145) were obtained for analysis from the Peking University Cancer Hospital's Department of Pathology and Gastrointestinal Surgery tissue archives (January 2003 to December 2011). The biopsied patients' demographic and clinicopathologic data were retrieved from the hospital's medical records database. Each specimen was subjected to histopathological typing to classify the tumor node metastasis (TNM) stage and to immunohistochemistry staining to detect the expression of the cancer stem cell marker LGR5. The intergroup differences in LGR5 expression were assessed by Spearman's rank correlation analysis, and the relationship between LGR5 expression level and the patients' clinicopathological characteristics was evaluated by the χ^2 test or Fisher's exact test.

RESULTS: Significantly more gastric cancer tissues showed LGR5$^+$ staining than normal control tissues (all P < 0.01), with immunoreactivity detected in 72.2% (65/90) and 50.9% (27/53) of intestinal metaplasia and dysplasia specimens, respectively, 52.8% (95/180) of gastric adenocarcinoma specimens, and 73.3%% (11/15) of metastasis specimens, but 26.9% (39/145) of lesion-adjacent normal gastric mucosa specimens. Comparison of the intensity of LGR5$^+$ staining showed an increasing trend that generally followed increasing dedifferentiation and tumor spread (normal tissue < dysplasia, < gastric adenocarcinoma < metastasis; all P < 0.001), with the exception of expression level detected in intestinal metaplasia which was higher than that in normal gastric tissues (P < 0.001). Moreover, gastric cancer-associated enhanced expression of LGR5 was

found to be significantly associated with age, tumor differentiation, Lauren type and TNM stage (Ⅰ + Ⅱ *vs* Ⅲ + Ⅳ) (all $P < 0.05$), but not with sex, tumor site, location, size, histology, lymphovascular invasion, depth of invasion, lymph node metastasis or distant metastasis. Patients with LGR5$^+$ gastric cancer specimens and without signs of metastasis from the original biopsy experienced more frequent rates of recurrence or metastasis during follow-up than patients with LGR5$^-$ specimens ($P < 0.05$).

CONCLUSION: Enhanced LGR5 is related to progressive dedifferentiation and metastasis of gastric cancer, indicating the potential of this receptor as an early diagnostic and prognostic biomarker.

Key words: Leucine-rich repeat-containing G protein-coupled receptor 5; Cancer stem cell; Gastric cancer; Intestinal metaplasia; Tumorigenesis

Core tip: This is the first study to evaluate the expression of leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), a putative cancer stem cell marker, in progressive stages of gastric carcinogenesis. The observation of increasing LGR5 expression in human gastric cancer lesions, following the loss of differentiation (from dysplastic to gastric cancer cases) and risk of spread (metastatic cases), suggests that this receptor may represent an important biomarker for early detection of patients at higher risk for gastric tumorigenesis. The observed distinctive expression pattern of LGR5 in intestinal metaplasia suggests that these cells may represent a precancerous condition but not carcinoma precursors.

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INTRODUCTION

Gastric cancer (GC) is one of the most common cancers worldwide, yet the majority of GC-related deaths occur in less developed countries, including China and other Asian nations[1,2]. Studies to elucidate the tumorigenic processes underlying GC development have revealed a multistep sequential process involving normal gastric tissue progression to chronic gastritis, atrophy, intestinal metaplasia, dysplasia, and carcinoma, with or without metastatic potential[3]. This model supports the possibility of a stepwise accumulation of genetic alterations affecting expression of key molecules, possibly having direct or indirect (*i.e.*, signaling pathways) functional effects on cell growth and movement.

The stem cell origin hypothesis of carcinomas has gained much research attention in the recent decade. Cancer stem cells (CSCs), which express a distinctive profile of cell type-specific surface markers[4], have been detected in a broad range of clinical cancer specimens, including hematological malignancies and solid tumors of the breast, lung, ovary, liver, prostate, pancreas, skin, brain and colon[5-13]. However, few studies to date have investigated the presence of CSCs in GC lesions, and their role in GC tumorigenesis remains largely unknown.

The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5, also known as GPR49) has been proposed as a marker of GC-related stem cells. Under normal conditions, LGR5 is expressed primarily on intestinal stem cells, where it functions as a transducer of Wnt signaling[14,15]. Murine-based investigations to uncover the role of LGR5 in cancer development and progression have also demonstrated its expression on rare, scattered cells in the eye, brain, stomach, mammary gland and reproductive organs[16] and showed that LGR5$^+$ stem cells were much more effective in producing tumorigenesis than more differentiated (LGR5$^-$) cells[17]. In humans, LGR5$^+$ cells have been detected in both the population of crypt stem cells (precursor cells) and gastric mucosal lesions that progressed to cancer[18].

A functional study of LGR5-expressing cells and their age-related distribution using a mouse model revealed that its expression was localized to the base of prospective corpus and pyloric glands in neonatal stomach but predominantly restricted to the base of mature pyloric glands in adult stomach, and demonstrated that a single LGR5$^+$ cell could efficiently generate long-lived organoids resembling mature pyloric epithelium *in vitro*[19]. While the collective findings have increased interest in developing LGR5 as a universal epithelial CSC marker for clinical use[18], the loss of restriction to the stem cell niche is considered an early event in the premalignant transformation of stem cells and suggests that this protein may also be a key contributor to carcinogenesis.

Although many previous studies have investigated the association of perturbed LGR5 expression with tumorigenesis, very few have reported on the differential expression of LGR5 and its role in the multistep sequential process of GC development. Therefore, the present study analyzed LGR5 expression in human clinical specimens of gastric tissues from the non-cancerous condition through gastric adenocarcinoma and in GC-related lymph nodes and liver metastases, and evaluated the relationship between differential LGR5 expression and clinicopathological features. The findings from this study will provide novel insights into the carcinogenic process of GC from the perspective of the stem cell origin hypothesis.

MATERIALS AND METHODS

Patients and tissue samples

Formalin-fixed/paraffin-embedded specimens of in-

testinal metaplasia (n = 90), dysplasia (n = 53), gastric adenocarcinoma (n = 180), metastases in lymph nodes and the liver (n = 15), and lesion-adjacent normal gastric mucosa (controls; n = 145) were obtained for analysis from the Peking University Cancer Hospital's Department of Pathology and Gastrointestinal Surgery tissue archives (January 2003 to December 2011). All specimens had been obtained during endoscopic biopsy or surgical resection. Each specimen was analyzed by routine histopathological analysis and was classified according to the pathological criteria published by the World Health Organization (4th edition) and the tumor node metastasis (TNM) staging system of the American Joint Committee on Cancer Staging Manual (7th edition) and the Japanese Gastric Cancer Association Guidelines (3rd edition).

The biopsied patients' demographic and clinicopathologic characteristics (during the clinical management and follow-up periods) were retrieved from the hospital's electronic records database. If a patient had no record of death but lacked follow-up data, the patient's general practitioner was contacted to obtain the information. None of the GC patients had synchronous cancers or previous gastrointestinal diseases, nor had undergone abdominal surgery, chemotherapy or radiotherapy prior to specimen collection.

This study was performed with pre-approval from the Ethics Committee of Peking University Cancer Hospital. Informed consent allowing for investigative use of tissue samples had been provided by each patient.

Immunohistochemical analysis

Specimen sections (4 μm thickness) were mounted on poly-*L*-lysine coated slides, deparaffinized in xylene, and rehydrated in a descending ethanol-to-water gradient series. Endogenous peroxidase was blocked by exposure to 3% H_2O_2 for 10 min, followed by antigen retrieval via pressurized heating in EDTA buffer (Zhongshan Biotechnology Inc., Beijing, China) for 5 min. After cooling to room temperature, non-specific sites were blocked by exposure to 10% goat blood serum. LGR5 immunodetection was carried out by incubating with purified rabbit polyclonal antibody (AP2745d; Abgent, San Diego, CA, United States), followed by two-step diaminobenzidine visualization (GK500705; Dako, Glostrup, Denmark).

The immunostained sections were counterstained with hematoxylin for 40 s, rinsed in water, dehydrated in an ascending water-to-ethanol gradient series followed by clearance with xylene, and permanently cover-slipped. Negative controls were created using the same procedure but without addition of primary antibody.

Evaluation of immunostaining

The processed immunostained sections were examined by light microscopy. Two experienced pathologists (Sun Y and Dong B), working independently and blinded to the corresponding clinical data, evaluated each sample to calculate and score the percentage of $LGR5^+$ cells [none (negative, -): 0%, 1%-25%: 1, 25%-50%: 2, and > 50%: 3] and to score the intensity of cytoplasmic staining (no staining: 0, mild: 1, moderate: 2, and strong: 3; with the highest intensity score being assigned when > 10% of cells stained with that intensity). Adding the percentage and intensity scores provided a composite expression score (0-6), which was defined as: weakly positive (+): 1-2, moderately positive (++): 3-4, and strongly positive (+++): 5-6. For statistical analysis, a composite score of 0 was classified as negative and 1-6 as positive, with $\leqslant 2$ ranked as low expression and $\geqslant 3$ ranked as high expression.

Statistical analysis

All statistical analyses were carried out using the SPSS software statistical package (version 20.0; SPSS Inc., Chicago, IL, United States). The differences in LGR5 expression between the gastric tissue types were analyzed by Spearman's rank correlation analysis. The relationships between LGR5 differential expression and clinicopathological characteristics were evaluated by the χ^2 test or Fisher's exact test. A two-sided P-value < 0.05 was considered statistically significant.

RESULTS

LGR5 expression and distribution in normal gastric mucosa

Immunostaining of LGR5 showed a predominant localization to the cytoplasm or on the cell membrane in normal gastric mucosa specimens. Morphologically, the $LGR5^+$ cells were localized to the mucous neck region at the base of the gastric crypts between the foveolae and glands (Figure 1A and B). The positive-staining percentages are presented in Table 1.

Differential LGR5 expression in GC-related tissues during tumorigenesis

Immunodetection of LGR5 in GC-related tissues, progressing from non-neoplastic epithelia to gastric cancer and finally metastasis, showed an increasing trend in the number and intensity of $LGR5^+$ cells (all *vs* normal gastric mucosa specimens and *vs* the different GC-related tissues, P < 0.05; Table 1). In addition, the significantly enhanced LGR5 expression in dysplasia specimens (P = 0.019) was largely accounted for by the specimens with low grade dysplasia (roughly twice that of the high grade dysplasia specimens). The GC-related enhanced LGR5 expression was also greater in specimens from patients with lower clinical stage (TNM stages Ⅰ + Ⅱ > Ⅲ + Ⅳ) and the majority of GC cases showed weak staining (with strong cytoplasmic or membranous immunodetection < moderate staining < weak staining < no staining; Figure 1C-J). Morphologically, the distribution of $LGR5^+$ cells was uneven and inhomogeneous in the GC-related specimens and occurred in cohesive patches of a variable number of tumor cells.

Association of immunodetected LGR5 expression with clinicopathological features of GC patients

The patients' demographic and clinicopathologic features

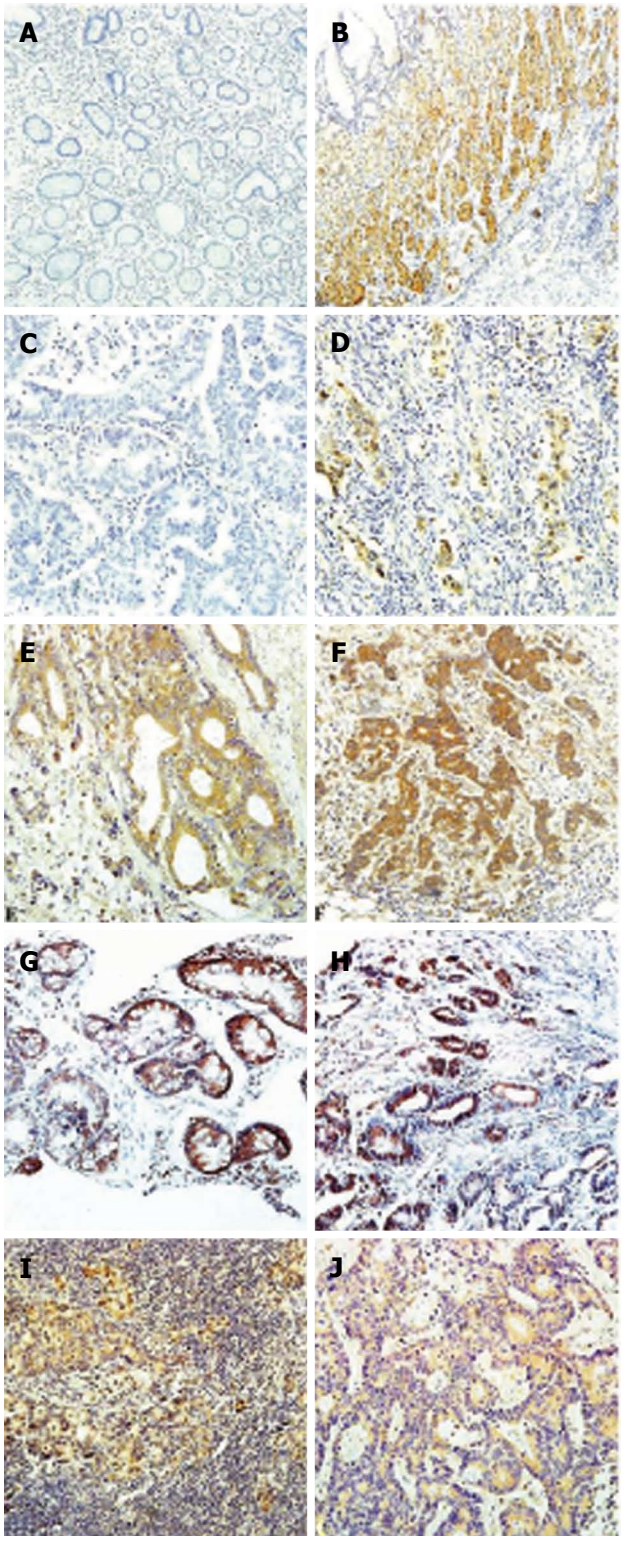

Figure 1 Immunodetected differential LGR5 expression in gastric tissues, following progression of tumorigenesis, and in distant metastases. Representative samples are shown from leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)$^-$ (A) and LGR5$^+$ (B) normal gastric normal tissues, LGR5$^-$ (C) and LGR5$^+$ (D-F) gastric cancer (GC) tissues with weak, moderate and strong expression, LGR5$^+$ gastric intestinal metaplasia and dysplasia tissues (G, H), and LGR5$^+$ lymph node and liver metastases (I, J). A, C-J: Magnification: × 200; B: Magnification: × 100.

Table 1 LGR5 immunostaining in gastric cancer-related gastric tissues and metastases *n* (%)

Pathological type	Total, *n*	LGR5 expression		*P* value
		Negative	Positive	
Normal tissue	145	106 (73.1)	39 (26.9)	0.000
Dysplasia grade	53	26 (49.1)	27 (50.9)	
Low	25	8 (32.0)	17 (68.0)	0.019
High	28	18 (64.3)	10 (35.7)	
TNM stage	180	85 (47.2)	95 (52.8)	
Ⅰ-Ⅱ	71	27 (38.0)	44 (62.0)	
Ⅲ-Ⅳ	109	58 (53.2)	51 (46.8)	
Metastases	15	4 (26.7)	11 (73.3)	
Lymph node	5	1 (20.0)	4 (80.0)	
Liver	10	3 (30.0)	7 (70.0)	

LGR5: Leucine-rich repeat-containing G protein-coupled receptor 5; TNM: Tumor, nodes, metastasis.

Table 2 Association of immunodetected LGR5 expression with clinicopathological features of gastric cancer patients *n* (%)

Clinicopathological feature	LGR5 expression		*P* value
	Negative	Positive	
Sex			0.072
Male	56 (43.1)	74 (56.9)	
Female	29 (58.0)	21 (42.0)	
Age, yr			0.005
≤ 60	48 (58.5)	34 (41.5)	
> 60	37 (37.8)	61 (62.2)	
Location in stomach			0.657
Upper	15 (40.5)	22 (59.5)	
Mid	17 (47.2)	19 (52.8)	
Lower	45 (49.5)	46 (50.5)	
Lesion size in cm			0.612
≤ 4	33 (42.9)	44 (57.1)	
> 4	32 (47.1)	36 (52.9)	
Differentiation			0.006
Differentiated	31 (36.5)	54 (63.5)	
Undifferentiated	54 (56.8)	41 (43.2)	
Histological type			0.579
Adenocarcinoma	67 (46.2)	78 (53.8)	
Others	18 (51.4)	17 (48.6)	
Lauren type			0.035
Intestinal	48 (41.4)	68 (58.6)	
Diffuse/other	37 (57.8)	28 (42.2)	
Lymphovascular invasion			0.288
No	43 (43.9)	55 (56.1)	
Yes	42 (51.9)	39 (48.1)	
Depth			0.833
T1-T2	19 (48.7)	20 (51.3)	
T3-T4	66 (46.8)	75 (53.2)	
Lymph node metastasis			0.934
No	19 (47.5)	21 (52.5)	
Yes	65 (46.8)	74 (53.2)	
Metastasis			0.160
No	71 (45.2)	86 (54.8)	
Yes	14 (60.9)	9 (39.1)	
TNM			0.046
Ⅰ-Ⅱ	27 (38.0)	44 (62.0)	
Ⅲ-Ⅳ	58 (53.2)	51 (46.8)	

LGR5: Leucine-rich repeat-containing G protein-coupled receptor 5; TNM: Tumor, nodes, metastasis.

are summarized in Table 2. There were more males than females (130 *vs* 50), but the percentage of LGR5$^+$ immunodetection was similar between the two and sex was not found to be significantly correlated with LGR5 expression in the GC-related specimens. The overall patients

Table 3 LGR5 expression in gastric cancer tissues of various differentiation *n* (%)

Tissue	LGR5 expression		*P* value
	Negative	Positive	
Intestinal metaplasia	25 (27.8)	65 (72.2)	0.000
Normal tissue	106 (73.1)	39 (26.9)	
Dysplasia with IM			0.004
Yes	3 (18.8)	13 (81.2)	
No	23 (62.2)	14 (37.8)	
Lauren type			0.035
Intestinal	48 (41.4)	68 (58.6)	
Diffuse/other	37 (57.8)	28 (42.2)	
Intestinal type GC			0.019
Metastasis or recurrence	6 (12.5)	21 (31.3)	
No metastasis or recurrence	42 (87.5)	46 (68.7)	

LGR5: Leucine-rich repeat-containing G protein-coupled receptor 5; GC: Gastric cancer; IM: Intestinal metaplasia.

ranged in age from 22-87 years old (median: 62 years old), and age was found to be significantly associated with LGR5$^+$ immunodetection in GC-related specimens (P = 0.005). In addition, differentiation (P = 0.006), Lauren type [P = 0.035, with intestinal type having significantly more LGR5$^+$ cells than the diffuse/other types (58.6% *vs* 42.2%)] and TNM stage (Ⅰ + Ⅱ *vs* Ⅲ + Ⅳ, P = 0.046) were also correlated significantly with LGR5$^+$ immunodetection, but tumor site, location, size, histology, lymphovascular invasion and depth of invasion were not.

Analysis of the follow-up data showed that GC patients without metastases at surgery but with LGR5$^+$ staining specimens experienced a higher rate of recurrence or metastasis than their counterparts with LGR5$^-$ staining specimens (87.35% *vs* 12.7%, P = 0.020). However, the presence of metastases at surgery was not correlated with LGR5$^+$ immunodetection (both P > 0.05; Table 2). The specimens from patients with intestinal type GC also showed a significantly higher rate of LGR5$^+$ immunodetection than those from patients with diffuse or mixed types GC (P = 0.035), and LGR5$^+$ immunodetection in intestinal type GC was associated with more frequent rates of recurrence or metastasis after surgery (P = 0.019; Table 3).

Association of LGR5 expression with transformation of intestinal metaplasia tissues

As shown in Table 3, intestinal metaplasia specimens showed a significantly higher rate of LGR5$^+$ immunodetection than normal gastric tissues (P = 0.000). Moreover, dysplasia specimens with intestinal metaplasia had a significantly higher rate of LGR5$^+$ immunodetection than those without (P = 0.004).

DISCUSSION

Using a standard immunohistochemistry-based method, the differential expression pattern of the putative CSC marker LGR5 in progressively tumorigenic clinical specimens of GC was demonstrated. In particular, an increasing trend was observed in LGR5$^+$ staining intensity that generally followed increasing dedifferentiation and tumor spread (normal tissue < dysplasia < gastric adenocarcinoma < metastasis).

The adenoma-carcinoma progression sequence is well known in colorectal cancer and esophageal adenocarcinoma, and is becoming more generally accepted as the likely mode of tumorigenesis in the gastrointestinal tract as well[20-23]. Recent findings from studies in mammalian (mouse) model systems and with human GC specimens have demonstrated that GC progenitor cells are derived from multipotent stem cells in the highly regenerative and proliferative regions of the stomach, including the isthmus and fundic gland-rich neck[24,25]. Indeed, the subpopulation of stem cells with high LGR5 expression were shown to have the capability to reconstitute crypt structures *in vitro*[26], and LGR5 has been detected on progenitor cells in human gastric mucosa crypts[27,28].

As stated in the Introduction, the multitude of signaling factors that comprise this multistep progression model of GC tumorigenesis also represent a plethora of targets for improved detection and treatment methods. The occurrence of gastric epithelial dysplasia is a well-characterized precursor event to GC, and is currently considered the most dependable marker for such cancer risk. A prospective longitudinal study of gastric epithelial dysplasia and development of GC indicated that high grade dysplasia is associated with rapid development of intestinal type GC[29]. This finding is in line with the current study's observation of similar LGR5$^+$ immunodetection rates in dysplasia and gastric carcinoma specimens (with a slightly higher rate in the latter), and higher rates in well to moderately differentiated intestinal type and lower-staged gastric cancers.

The dynamic undulation of immunodetected LGR5 expression observed in the low clinical stage (enhanced in Ⅰ-Ⅱ) to the high clinical stage (reduced in Ⅲ-Ⅳ) to metastasis (again enhanced) agrees with a previously reported profile of LGR5 expression in tumorigenesis of endometrial, colorectal and ovarian carcinomas (with the high expression demonstrated during the initial stages, being down-regulated in fully developed tumors)[30,31]. Collectively, these findings support the hypothesized clonal selection model of putative stem cells leading to carcinogenesis[32]. In particular, the results from the current study suggest that overexpression of LGR5 may be an early event in tumorigenesis and that immunodetection of such protein is achieved with good reproducibility and tracks with differentiation of tumor specimens.

From a mechanistic perspective, the tumorigenic-related expression profile observed in the current study suggests the existence of a potential tumor promoter regulating LGR5. However, it is important to consider the unexpected observation of higher immunodetected LGR5 expression in low grade dysplasia than in high grade dysplasia; similar results were also reported from another study of esophageal dysplasia lesions[20]. A possible explanation of this result is the fact that the cur-

rent morphologic criteria for different grade dysplasias include a mix of architectural and cytologic features and do not consider functional characteristics[33]. Indeed, low grade dysplasia preserves some of the functions of intestinal metaplasia, which underlies the risk of misdiagnosis for these two conditions[34]. Previous studies have addressed this confusing issue, proposing that the increased amounts of high-intensity LGR5$^+$ cells that are observed in dysplasia may represent a stem cell population that is prone to becoming CSCs[35,36].

Other intriguing findings from the current study are the higher amounts of LGR5$^+$ cells detected towards the crypt base or in the invasive tumor front during the development and progression of GC (although the change in differential expression did not reach statistical significance) and in metastases (both local and distant). Brablez *et al*[37] hypothesized that tumor progression is mediated by two types of CSCs with distinct functions. The first was proposed as a stationary cancer stem (SCS) cell population, which would be present in the area for cell differentiation but which would not promote metastasis. The second was proposed as a migrating (or mobile) cancer stem (MCS) cell population, which may be derived from the SCS cells and located primarily at the invasive tumor front, and which would drive metastasis. Therefore, the observed shift in distribution of LGR5$^+$ cells towards the invasive tumor front that accompanied the development and metastasis of GC in the current study may be related to such MCS cells. This notion may also be in line with the current study's observation of GC patients with LGR5$^+$ intestinal type specimens being at higher risk of recurrence or metastasis after surgery.

Previous studies have demonstrated that Wnt signaling regulates stemness and organ development, as well as the process of epithelial to mesenchymal transition (EMT) that increases the metastatic potential of disseminated cancer cells[38,39]. In addition, EMT may also restart the growth and differentiation programs of stem cells at metastatic sites[37,40]. Studies of human colorectal cancer have demonstrated that aberrant Wnt signaling not only triggers early steps of intestinal carcinogenesis but also malignant tumor progression towards invasive carcinomas and metastasis[41-43]. Therefore, LGR5 (as a Wnt target and a stem cell marker) plays an important role in initiating tumor growth and driving distant metastasis. These functions of LGR5 may also explain the findings in the current study of LGR5$^+$ GC patients without evidence of metastases during the initial surgical treatment being at a greater risk of recurrence or metastasis.

Interestingly, the LGR5-immunodetected expression had higher intensity in gastric intestinal metaplasia, dysplasia with intestinal metaplasia, and intestinal type GC than in the normal tissues examined in the current study; all of these GC-related lesions have the potential to manifest intestinal type differentiation. Intestinal metaplasia has been shown to originate from stem cells of the isthmus, and the crypts possess multiple stem cells[44,45]. Although intestinal metaplasia is regularly detected in the antrum of patients with gastritis and duodenal ulcers related to *Helicobacter pylori* infection, these patients very rarely develop gastric carcinoma[46]. Similarly, Tatematsu *et al*[47] suggested that gastric/intestinal mixed type intestinal metaplasia might be the consequence of abnormal differentiation of stem cells that are capable of producing both gastric and intestinal types of cells.

Only the relatively rare type Ⅲ intestinal metaplasia has been identified as a risk marker for the development of gastric carcinoma, being classified as "low grade dysplasia"[34]. The related findings in our study suggest that intestinal metaplasia may be a precancerous condition, but not a precursor for gastric carcinoma (possibly with the exception of some rare types). Thus, LGR5 may represent a unique and sensitive marker of intestinal stem cells and may be closely related to the intestinal type of GC.

In conclusion, the immunodetectable expression pattern of LGR5, a CSC-related gene, increasing from normal tissues to lesions of dysplasia, gastric carcinoma and finally metastases, suggests potential for this protein to serve as an important biomarker for early detection of patients at higher risk for gastric tumorigenesis. Furthermore, as an intestinal stem cell marker, differential LGR5 expression in conjunction with development of intestinal metaplasia may represent a precancerous condition, but not a carcinoma precursor.

COMMENTS

Background

Cancer stem cells (CSCs) may be the source of various carcinomas, including gastric cancer (GC), and are identifiable by clinically detectable profiles of cell type-specific surface markers. The leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), a target of Wnt signaling, is primarily expressed on normal intestinal stem cells and has been suggested as a putative CSC marker (and contributor to GC tumorigenesis) according to its differential expression on crypt stem cells (precursor cells) and gastric lesions that progress to cancer. Accumulated evidence has suggested roles for LGR5 in both cancer development and progression. Recent studies have also indicated that LGR5 may be a potential marker of gastrointestinal stem cells in humans and that loss of restriction to the stem cell niche is likely an early event in the premalignant transformation of stem cells.

Research frontiers

The differential protein expression of LGR5 in normal gastric tissue, intestinal metaplasia and dysplasia specimens, gastric carcinomas, and distant metastases was determined by immunohistochemistry to provide insights into its potential as a clinical marker for early GC detection. Furthermore, the differential LGR5 expression observed in conjunction with development of intestinal metaplasia suggests that this phenomenon represents a precancerous condition, but not a carcinoma precursor.

Innovations and breakthroughs

An increasing trend in intensity of LGR5 expression was detected in GC-related tissues, following the well-recognized sequential development from normal tissue to dysplasia to gastric carcinoma and finally metastasis, with the exception of the intestinal metaplasia state. The differential expression of LGR5 detected in GC by immunohistochemistry appeared to be significantly associated with age, differentiation, Lauren type, and tumor node metastasis stage. The LGR5$^+$ cells detected in intestinal metaplasia specimens were more prevalent than those detected in normal gastric tissues, and the data indicated that intestinal metaplasia may manifest from differentiation of a population of abnormal stem cells with high expression of LGR5, but may not represent a carcinoma precursor. Collectively, these data indicate that LGR5 expression may serve as an important biomarker for early detection of patients at higher risk for gastric tumorigenesis, and may be a candidate target for future individualized therapeutic

strategies.

Applications

The current poor prognosis of GC is largely associated with the low rate of early diagnosis. The findings from this study of human clinical samples of GC lend to a recommendation that LGR5 should be the focus of further studies to develop its potential as a biomarker for early detection of patients at higher risk for GC and as a manipulable intestinal stem cell marker target for improved management of GC cases.

Terminology

The leucine-rich repeat-containing G protein-coupled receptor 5 is expressed primarily on intestinal stem cells, where it functions as a transducer of Wnt signaling. Cancer stem cells, which express a distinctive profile of cell type-specific surface markers, have been detected in a broad range of clinical cancer specimens and are the basis of the stem cell origin hypothesis of cancer. Gastric cancer development is a multistep sequential process involving normal gastric tissue progression to chronic gastritis, atrophy, intestinal metaplasia, dysplasia, and carcinoma, with or without metastatic potential.

Peer review

This study determined the GC-related expression profile of the putative CSC marker LGR5, using standard immunohistochemistry to detect expression in human clinical samples of normal gastric tissue, intestinal metaplasia, dysplasia, gastric carcinoma, and distant metastases. The observed increasing trend in differential LGR5 expression following progressive tumorigenesis to metastasis suggests that this protein may serve as an important biomarker for early detection of patients at higher risk for gastric tumorigenesis. The data also implicate a role for LGR5 as an intestinal stem cell marker and suggest that intestinal metaplasia may be a precancerous condition but not a carcinoma precursor. The study is well controlled and provides novel insights into this life-threatening disease.

REFERENCES

1 **Filomena A**, Saieva C, Lucchetti V, Santacroce F, Falorni P, Francini V, Carrieri P, Zini E, Ridolfi B, Belli P, Orsini B, Mandi P, Palli D, Scheggi S. Gastric cancer surveillance in a high-risk population in tuscany (Central Italy): preliminary results. *Digestion* 2011; **84**: 70-77 [PMID: 21494036 DOI: 10.1159/000322689]

2 **McCune K**, Bhat-Nakshatri P, Thorat MA, Nephew KP, Badve S, Nakshatri H. Prognosis of hormone-dependent breast cancers: implications of the presence of dysfunctional transcriptional networks activated by insulin via the immune transcription factor T-bet. *Cancer Res* 2010; **70**: 685-696 [PMID: 20068169 DOI: 10.1158/0008-5472]

3 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740 [PMID: 1458460]

4 **Kemper K**, Grandela C, Medema JP. Molecular identification and targeting of colorectal cancer stem cells. *Oncotarget* 2010; **1**: 387-395 [PMID: 21311095]

5 **Ponti D**, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res* 2005; **65**: 5506-5511 [PMID: 15994920]

6 **Eramo A**, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* 2008; **15**: 504-514 [PMID: 18049477 DOI: 10.1038/sj.cdd]

7 **Curley MD**, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR, Foster R. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. *Stem Cells* 2009; **27**: 2875-2883 [PMID: 19816957 DOI: 10.1002/stem.236]

8 **Yang ZF**, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 2008; **13**: 153-166 [PMID: 18242515 DOI: 10.1016/j.ccr.2008.01.013]

9 **Collins AT**, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946-10951 [PMID: 16322242 DOI: 10.1158/0008-5472.CAN-05-2018]

10 **Li C**, Lee CJ, Simeone DM. Identification of human pancreatic cancer stem cells. *Methods Mol Biol* 2009; **568**: 161-173 [PMID: 19582426 DOI: 10.1007/978-1-59745-280-9_10]

11 **Monzani E**, Facchetti F, Galmozzi E, Corsini E, Benetti A, Cavazzin C, Gritti A, Piccinini A, Porro D, Santinami M, Invernici G, Parati E, Alessandri G, La Porta CA. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur J Cancer* 2007; **43**: 935-946 [PMID: 17320377 DOI: 10.16/j.ejca.2007.01.017]

12 **Singh SK**, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003; **63**: 5821-5828 [PMID: 14522905]

13 **Vermeulen L**, Todaro M, de Sousa Mello F, Sprick MR, Kemper K, Perez Alea M, Richel DJ, Stassi G, Medema JP. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. *Proc Natl Acad Sci USA* 2008; **105**: 13427-13432 [PMID: 18765800 DOI: 10.1073/pnas.0805706105]

14 **Pinson KI**, Brennan J, Monkley S, Avery BJ, Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* 2000; **407**: 535-538 [PMID: 11029008 DOI: 10.1038/35035124]

15 **Van der Flier LG**, Sabates-Bellver J, Oving I, Haegebarth A, De Palo M, Anti M, Van Gijn ME, Suijkerbuijk S, Van de Wetering M, Marra G, Clevers H. The Intestinal Wnt/TCF Signature. *Gastroenterology* 2007; **132**: 628-632 [PMID: 17320548 DOI: 10.1053/j.gastro.2006.08.039]

16 **Shackleton M**, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE. Generation of a functional mammary gland from a single stem cell. *Nature* 2006; **439**: 84-88 [PMID: 16397499 DOI: 10.1038/nature04372]

17 **Barker N**, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 2009; **457**: 608-611 [PMID: 19092804 DOI: 10.1038/nature07935]

18 **Brabletz S**, Schmalhofer O, Brabletz T. Gastrointestinal stem cells in development and cancer. *J Pathol* 2009; **217**: 307-317 [PMID: 19031475 DOI: 10.1002/path.2475]

19 **Barker N**, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007; **449**: 1003-1007 [PMID: 17934449 DOI: 10.1038/nature06196]

20 **Becker L**, Huang Q, Mashimo H. Lgr5, an intestinal stem cell marker, is abnormally expressed in Barrett's esophagus and esophageal adenocarcinoma. *Dis Esophagus* 2010; **23**: 168-174 [PMID: 19549212 DOI: 10.1111/j.1442-2050.2009.00979.x]

21 **Takeda K**, Kinoshita I, Shimizu Y, Matsuno Y, Shichinohe T, Dosaka-Akita H. Expression of LGR5, an intestinal stem cell marker, during each stage of colorectal tumorigenesis. *Anticancer Res* 2011; **31**: 263-270 [PMID: 21273608]

22 **Uchida H**, Yamazaki K, Fukuma M, Yamada T, Hayashida T, Hasegawa H, Kitajima M, Kitagawa Y, Sakamoto M. Overexpression of leucine-rich repeat-containing G protein-coupled receptor 5 in colorectal cancer. *Cancer Sci* 2010; **101**: 1731-1737 [PMID: 20384634]

23 **Kleist B**, Xu L, Li G, Kersten C. Expression of the adult intestinal stem cell marker Lgr5 in the metastatic cascade of colorectal cancer. *Int J Clin Exp Pathol* 2011; **4**: 327-335 [PMID: 21577318]

24 **Karam SM**, Li Q, Gordon JI. Gastric epithelial morphogenesis in normal and transgenic mice. *Am J Physiol* 1997; **272**:

G1209-G1220 [PMID: 9176232]
25 **Karam SM**, Straiton T, Hassan WM, Leblond CP. Defining epithelial cell progenitors in the human oxyntic mucosa. *Stem Cells* 2003; **21**: 322-336 [PMID: 12743327 DOI: 10.1634/stem]
26 **Sato T**, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* 2011; **469**: 415-418 [PMID: 21113151 DOI: 10.1038/nature]
27 **Hsu SY**, Liang SG, Hsueh AJ. Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region. *Mol Endocrinol* 1998; **12**: 1830-1845 [PMID: 9849958]
28 **McDonald T**, Wang R, Bailey W, Xie G, Chen F, Caskey CT, Liu Q. Identification and cloning of an orphan G protein-coupled receptor of the glycoprotein hormone receptor subfamily. *Biochem Biophys Res Commun* 1998; **247**: 266-270 [PMID: 9642114]
29 **Rugge M**, Farinati F, Baffa R, Sonego F, Di Mario F, Leandro G, Valiante F. Gastric epithelial dysplasia in the natural history of gastric cancer: a multicenter prospective follow-up study. Interdisciplinary Group on Gastric Epithelial Dysplasia. *Gastroenterology* 1994; **107**: 1288-1296 [PMID: 7926493]
30 **Sun X**, Jackson L, Dey SK, Daikoku T. In pursuit of leucine-rich repeat-containing G protein-coupled receptor-5 regulation and function in the uterus. *Endocrinology* 2009; **150**: 5065-5073 [PMID: 19797400 DOI: 10.1210/en.2009-0690]
31 **McClanahan T**, Koseoglu S, Smith K, Grein J, Gustafson E, Black S, Kirschmeier P, Samatar AA. Identification of overexpression of orphan G protein-coupled receptor GPR49 in human colon and ovarian primary tumors. *Cancer Biol Ther* 2006; **5**: 419-426 [PMID: 16575208]
32 **Visvader JE**, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008; **8**: 755-768 [PMID: 18784658]
33 **Appelman HD**. What is dysplasia in the gastrointestinal tract? *Arch Pathol Lab Med* 2005; **129**: 170-173 [PMID: 15679413]
34 **Tosi P**, Filipe MI, Luzi P, Miracco C, Santopietro R, Lio R, Sforza V, Barbini P. Gastric intestinal metaplasia type III cases are classified as low-grade dysplasia on the basis of morphometry. *J Pathol* 1993; **169**: 73-78 [PMID: 8433217 DOI: 10.1002/path.1711690112]
35 **May R**, Riehl TE, Hunt C, Sureban SM, Anant S, Houchen CW. Identification of a novel putative gastrointestinal stem cell and adenoma stem cell marker, doublecortin and CaM kinase-like-1, following radiation injury and in adenomatous polyposis coli/multiple intestinal neoplasia mice. *Stem Cells* 2008; **26**: 630-637 [PMID: 18055444 DOI: 10.1634/stemcells.2007-0621]
36 **Sureban SM**, May R, Ramalingam S, Subramaniam D, Natarajan G, Anant S, Houchen CW. Selective blockade of DCAMKL-1 results in tumor growth arrest by a Let-7a MicroRNA-dependent mechanism. *Gastroenterology* 2009; **137**: 649-59, 659.e1-2 [PMID: 19445940 DOI: 10.1053/j]
37 **Brabletz T**, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005; **5**: 744-749 [PMID: 16148886 DOI: 10.1038/nrc1694]
38 **Chisholm AD**. Gastrulation: Wnts signal constriction. *Curr Biol* 2006; **16**: R874-R876 [PMID: 17055968 DOI: 10.1016/j.cub.2006.09.028]
39 **Labelle M**, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell* 2011; **20**: 576-590 [PMID: 22094253 DOI: 10.1016/j.ccr.2011.09.009]
40 **Fessler E**, Dijkgraaf FE, De Sousa E Melo F, Medema JP. Cancer stem cell dynamics in tumor progression and metastasis: Is the microenvironment to blame? *Cancer Lett* 2013; **341**: 97-104 [PMID: 23089245 DOI: 10.1016/j.canlet.2012.10.015]
41 **de Lau W**, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ, Clevers H. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* 2011; **476**: 293-297 [PMID: 21727895]
42 **Vermeulen L**, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medema JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; **12**: 468-476 [PMID: 20418870 DOI: 10.1038/ncb2048]
43 **Fodde R**, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol* 2007; **19**: 150-158 [PMID: 17306971 DOI: 10.1016/j.ceb.2007.02]
44 **Hattori T**, Fujita S. Tritiated thymidine autoradiographic study on histogenesis and spreading of intestinal metaplasia in human stomach. *Pathol Res Pract* 1979; **164**: 224-237 [PMID: 461230 DOI: 10.1016/S0344-0338(79)80045-X]
45 **McDonald SA**, Greaves LC, Gutierrez-Gonzalez L, Rodriguez-Justo M, Deheragoda M, Leedham SJ, Taylor RW, Lee CY, Preston SL, Lovell M, Hunt T, Elia G, Oukrif D, Harrison R, Novelli MR, Mitchell I, Stoker DL, Turnbull DM, Jankowski JA, Wright NA. Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. *Gastroenterology* 2008; **134**: 500-510 [PMID: 18242216 DOI: 10.1053/j.gastro.2007.11.035]
46 **Hansson LE**, Nyrén O, Hsing AW, Bergström R, Josefsson S, Chow WH, Fraumeni JF, Adami HO. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. *N Engl J Med* 1996; **335**: 242-249 [PMID: 8657240 DOI: 10.1056/NEJM199607253350404]
47 **Tatematsu M**, Tsukamoto T, Inada K. Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. *Cancer Sci* 2003; **94**: 135-141 [PMID: 12708487]

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