

Original Paper

Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk

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Abstract

Hereditary diffuse gastric cancer (HDGC) is caused by germline E-cadherin (*CDH1*) mutations in 25–40% of tested families. Management options for asymptomatic mutation carriers are fraught, since endoscopic surveillance can miss cancer foci and prophylactic gastrectomy has profound clinical sequelae. The aims of this study were to evaluate the impact of current surveillance practices on pre-operative diagnosis and to characterize the microscopic lesions in gastrectomy specimens to better inform clinical practice. Histological assessment and mapping of endoscopic surveillance and gastrectomy specimens were performed for eight asymptomatic *CDH1* mutation carriers. E-cadherin expression and proliferation were analysed and evidence of epithelial–mesenchymal transition (EMT) was sought by immunohistochemistry for vimentin and cytokeratin 8/18. Four of eight patients had lesions detected at endoscopic surveillance. A median of 20.5 (range 0–66) signet ring foci were identified per gastrectomy (including *in situ* lesions and pagetoid spread). Foci were predominantly identified in the fundus and body (90% endoscopic biopsies and 85% in gastrectomy). The likelihood of detecting foci pre-operatively was positively correlated with the number of biopsies taken and the number of lesions in the gastrectomy specimen. E-cadherin expression in gastrectomy specimens was reduced or absent in all of the foci compared with the intervening gastric tissue, suggesting that these lesions are polyclonal. The foci had a low proliferative index (<2%) and there was no evidence for EMT. Multiple endoscopic biopsy sampling of the gastric mucosa increases the yield of microscopic cancer foci. The low proliferative index and lack of EMT suggests that these foci may represent an indolent stage of HDGC.

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Introduction

Hereditary diffuse gastric cancer (HDGC) is the only genetic cancer syndrome in which the stomach is the primary site affected. The criteria that are currently used by the International Gastric Cancer Linkage Consortium (IGCLC) to define HDGC are: any family with two documented cases of diffuse gastric cancer in first- or second-degree relatives with one case under the age of 50 years; or three or more documented cases of diffuse gastric cancer in first- or second-degree

relatives at any age [1,2]. In 1998, Guildford *et al.* identified germline mutations in the gene encoding the epithelial cell adhesion protein E-cadherin (*CDH1*) in Maori kindred, and mutations have since been identified in 25–40% of families fulfilling the criteria for HDGC [1,3–6]. The average age at cancer diagnosis is 38 years and, according to the most recent penetrance analysis, carriers of a *CDH1* germline mutation have a 67–83% risk (67% men, 83% women) of developing gastric cancer by the age of 80 [7]. However, a more recent study reported lower penetrance values

Table 1. Summary of the published gastrectomy series for patients with germline E-cadherin mutations

Family/mutation	Individual age and sex	Number of foci identified	Cancer identified on previous endoscopy? (Y/N)	Reference
Exon1 bp45 ins T	57 M	0	N	[22]
	34 M	5	Y	[22]
Exon 7 bp1003 C>T	Unknown	0	N	[23]
	Unknown	1+	N	[23]
	Unknown	1+	N	[23]
	Unknown	1+	N	[23]
	Unknown	1+	N	[23]
	Unknown	1+	N	[23]
Exon 7 bp1003 C>T	52 F	1+	N	[10]
	55 F	1+	N	[10]
	49 F	1+	N	[10]
	51 M	1+	N	[10]
	56 M	1+	N	[10]
	53 F	1+	N	[10]
	Exon7 bp1008 G>T	28 M	214	Y
15 F		318	Y	[16,21]
34 F		111	Y	[16,21]
39 M		45	N	[21]
16 M		15	Unknown	[21,25]
45 F		487	Unknown	[25]
27 F		51	Unknown	[25]
20 F		238	N	[21,25]
19 F		115	Unknown	[25]
18 F		15	Unknown	[25]
49 F		18	Unknown	[25]
19 F		19	Unknown	[25]
Exon 8 bp1134 del8, ins5		28F	1+	Y
Exon11 bp1588 insC	41 F	13	N	[9,17]
	39 M	2	N	[9,17]
	37 F	5	N	[9,17]
	47 F	15	N	[9,17]
	40 M	45	N	[9,17]
Exon11 bp1711 insG	40 M	3	Unknown	[17,27,28]
	22 M	2	Unknown	[17,27,28]
	28 F	1	Unknown	[17,27,28]
Exon12 bp1792 C>T	35 F	55	Unknown	[16,26,28]
Exon12 bp1792 C>T	43 F	4	N	[16]
Exon14 bp2287 G>T	33 F	32	Y	[16]
Unknown	53 F	11	Y	[26]
Median (from complete data)	38 11 M:22 F (n = 33)	17 (range 0–487) (n = 27)	7/28 (25%) (n = 28)	

of 40% for males and 63% for female at the age of 75 [8]. It would therefore seem sensible to carry out endoscopic surveillance for individuals with HDGC harbouring germline E-cadherin mutations. However, since diffuse gastric cancer manifests as single cells or small clusters of cells covered by normal appearing epithelium, these lesions may not be visible endoscopically. From the published series in which endoscopic data was reported, 7/28 cases had positive endoscopic biopsies (summarized in Table 1).

The difficulty in endoscopic detection is highlighted by two studies which identified foci of diffuse signet-ring carcinoma in 100% of prophylactic gastrectomy specimens ($n = 5$ and $n = 6$), even though the results of pre-operative biopsies were negative for cancer

in all cases [9,10]. Thorough biopsy protocols may increase the identification of early lesions using endoscopic surveillance; however, considering that early HDGC foci often contain <100 cells, it is still possible that these lesions may go undetected using standard endoscopy protocols. It is in part due to uncertainty over the value of surveillance endoscopy that *CDHI* germline mutation carriers are opting for prophylactic gastrectomy.

The decision to have a prophylactic gastrectomy is not one to be taken lightly, in view of the peri-operative risks and long-term morbidity, including an average weight loss of 4–7 kg, lactose intolerance (50% of cases), fat malabsorption and steatorrhoea (66–100%), micronutrient deficiencies, post-prandial

fullness and bacterial overgrowth [11–15]. In addition, since the penetrance of the *CDH1* germline mutation is not complete (67–83%), cancer will never occur in 20–30% of cases [7]. It is therefore important to identify better methods of surveillance for this disease. Targeted biopsying of the body–antral transitional zone of the stomach during endoscopic surveillance has been suggested to increase the diagnostic yield, on the basis that in six prophylactic gastrectomy specimens the transitional zone contained approximately 40% of foci, despite occupying <10% of the mucosal area (16). The size of the foci was also greatest in this region [16]. However, such a predilection has not been confirmed in other published studies [17,18]. It is therefore important to further analyse the location of foci within prophylactic gastrectomy specimens in order to inform endoscopy protocols.

The natural history of the microscopic foci also remains to be fully understood. In previous studies, E-cadherin expression was shown to be greatly reduced or absent within the signet ring cell foci of prophylactic gastrectomy specimens [9,10,17]. Hence, down-regulation of E-cadherin expression appears to be an early, initiating event in these foci. Humar *et al* have previously identified smaller E-cadherin-negative cells within the larger (>3 mm diameter), predominantly signet ring cell lesions [19]. These smaller cells expressed increased c-Src kinase and its downstream targets fibronectin, Fak and Stat3, compared to the larger signet ring cells within the same lesions [19]. The expression of c-Src coupled with the down-regulation of E-cadherin expression would suggest an EMT-style process and therefore these smaller cells may represent the most invasive component of the lesions [20]. Further analysis of these lesions is required to understand the progression from microscopic foci to invasive carcinoma.

This study comprised eight asymptomatic individuals with germline *CDH1* mutations who had endoscopy performed prior to gastrectomy and in whom the entire gastrectomy specimens were available for analysis. The first aim of this study was to analyse the number and position of endoscopic and gastrectomy specimen signet ring cell foci in order to identify the likelihood of endoscopic detection and zones of cancer predilection. The second aim of the study was to determine the E-cadherin status, proliferative index and evidence for EMT (vimentin and cytokeratins 8/18) within microscopic signet ring foci, particularly with regard to the smaller cells, in order to improve our understanding of the nature of these microscopic foci.

Materials and methods

Patient specimens

All specimens were collected as part of the Familial Gastric Cancer Study (MREC ref. 97/5/32), Cambridge. Families were coded M for mutation, followed

by a digit, and each individual within that family was assigned a letter. For the purposes of this analysis, inclusion criteria were: a clinically confirmed *CDH1* germline mutation; gastrectomy undertaken in asymptomatic individuals; an endoscopy examination prior to surgery (one patient excluded); and availability of the entire stomach for mapping (two patients excluded). One patient (M10B) had three small sections of stomach missing (Figure 1) but was still included in the analysis. Although the Familial Gastric Cancer Study does provide guidelines for endoscopy, the endoscopy and histopathological analyses were performed according to usual clinical practice at the referring hospitals. The entire stomachs were processed to paraffin blocks and one block may span a long strip of the stomach. In addition, for research purposes all of the blocks from the gastrectomy specimens were stained with haematoxylin and eosin (H&E) and analysed by one of two consultant histopathologists (VS, FC). For the purpose of identifying zones of predilection of location of foci, the stomachs were divided into five anatomical regions (Figure 1), using a combination of anatomical landmarks and specialized cell types (ie chief cells and parietal cells) within the normal gastric tissue (VS). Lesions identified were categorized into *in situ* lesion and areas of pagetoid spread. *In situ* lesions were defined as foveolae and glands that have retained an intact basement membrane, which were totally or partially lined by signet ring cells. Pagetoid spread was defined as spreading of the signet ring cells beneath the luminal epithelium [17].

Immunohistochemistry

Paraffin-embedded tissue sections (4 µm) were deparaffinized and rehydrated in xylene and ethanol, respectively. Antigen retrieval was performed by pressure cooking in Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA, USA). Endogenous peroxidases were blocked by incubation in 1.6% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO, USA). Non-specific reactivity was blocked by incubation in 10% normal horse serum (Vector Laboratories), 5% BSA diluted in 0.05% Tween TBS. Samples were incubated with primary antibody [extracellular domain-specific E-cadherin (1:40; clone 36B5, Lab-Vision Corporation, Fremont, CA, USA) or Ki-67 (1:100; clone MIB-1, DakoCytomation, Glostrup, Denmark)], diluted in 1% BSA in 0.05% Tween TBS overnight at 4 °C. Positive (normal gastric tissue with defined staining pattern) and negative controls (omission of primary antibody) were included for each set of slides. Incubation with biotinylated anti-mouse secondary antibody (Vector Laboratories) was for 45 min at room temperature and the avidin–biotin complex (ABC) system (Vector Laboratories) was used to detect antibody binding. The DAB peroxidase substrate kit (Vector Laboratories) was used for colour detection, followed by a haematoxylin counterstain. E-cadherin staining was carried out on every microscopic focus

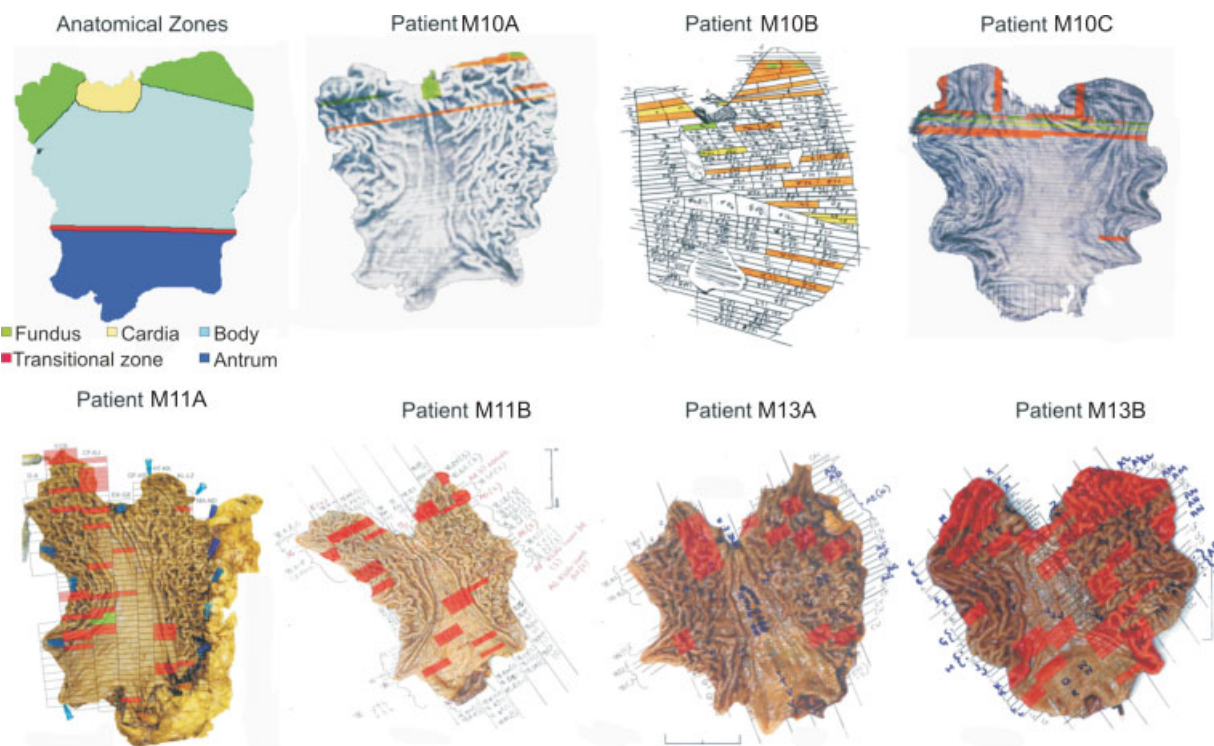


Figure 1. The diagram labelled 'anatomical zones' shows how the stomach was subdivided for analysis. The gastrectomy specimen maps are colour-coded according to the highest degree of abnormality at that location: signet ring cells foci (red/orange rectangles), *in situ* lesions (green) and areas of pagetoid spread (yellow). The number of coloured regions may not correlate with the number of foci identified (Table 2), since more than one focus may be found in each block. Furthermore, when areas of pagetoid spread co-localize with foci of signet ring cells, this is labelled red

of signet ring cells detected from the H&E stain. Staining patterns were described as negative, faintly positive or positive, compared with the surrounding normal gastric tissue. Ki-67 expression was analysed in four sections from each of five patients (M10B, M10C, M11A, M11B and M12A) and the number of positive cells/lesion/high-power field was visually estimated (VS, MB).

Dual labelling immunofluorescence

The immunohistochemistry procedure above was modified as follows. Antigen retrieval was carried out using the Micromed T/T Mega Microwave Processing LabStation (Milestone S.r.l, Sorisole, Italy) with sodium citrate solution (VWR International, West Chester, PA, USA). Mouse anti Cytokeratin 8/18 and rabbit anti-Vimentin (both from Vector Laboratories) diluted in 1% BSA in 0.05% Tween TBS were simultaneously incubated at room temperature for 1 h. The slides were then washed in 0.05% Tween TBS before simultaneous incubation of both secondary antibodies [fluorescein anti-mouse IgG (Vector Laboratories) and Alexa Fluor 546 anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA)] in the dark for 1 h. The slides were then washed in 0.05% Tween TBS before coverslips were added, using Vectashield Mounting Medium for Fluorescence with DAPI (Vector Laboratories). Sections were visualized using an Axion LSM 10 Laser Confocal Microscope (Carl Zeiss, Oberkochen, Germany).

Vimentin and CK8/18 expression and localization was analysed in five patients (M10B, M10C, M11A, M11B and M12A).

Statistics

The Mann–Whitney *U*-test was used to compare the total number of biopsies and the number of signet ring cancer foci between patients who did and did not have lesions detected endoscopically.

Results

Clinico-pathological correlates (Table 2)

The median age of the patients at the time of surgery was 33 (range 20–64) years, with a male:female ratio of 3:5. The germline mutations were all confirmed in a clinical genetics laboratory in accordance with the familial gastric cancer study guidelines. Six of eight individuals from three families (M10, M11 and M12) had truncating germline mutations, and of these patients, M10A, M10B, M11A and M12A opted for gastrectomy, independent of the endoscopy findings. Patients M10C and M11B were undergoing surveillance and had surgery following positive endoscopy findings. Two individuals from family M13 had a missense mutation (Exon5 641T > C) and, due to the uncertainty about the clinical relevance of this mutation, they were not offered

Analysis of gastrectomy specimens from hereditary diffuse gastric cancer

Table 2. Patients' characteristics and foci identified

Patient ID	Germline mutation	Mutation type	Age at surgery (years)	Sex	Length of time in surveillance programme	Number positive endoscopic biopsies/total taken (months prior to surgery)	Number of signet ring cancer foci identified in gastrectomy
M10A	45insT	Frameshift	64	M	8 Years	0/12 (9)	4 3 <i>in situ</i> lesions
M10B			30	F	3 Years	0/24 (<1)	17 2 <i>in situ</i> lesions 5 areas pagetoid spread
M10C			36	M	First endoscopy	1/24 (2)	15 1 <i>in situ</i> lesion 1 area pagetoid spread
M11A	1466insC	Frameshift	48	M	3 Years	0/0 (9)	44 2 <i>in situ</i> lesions
M11B			23	F	First endoscopy	1/38 (3)	21 1 <i>in situ</i> lesion
M12A	191C>T	Non-sense	44	F	6 Months	0/12 (6)	0
M13A	641T>C	Missense	23	F	6 Months	2/24 (2)	16
M13B			20	F	6 Months	6/24 (2)	66
Median	—		33	3M:5F	—	0.5/24	20.5 (0–66)

prophylactic gastrectomy prior to positive endoscopic findings.

Histopathological analysis of the entire mucosa from eight gastrectomy specimens demonstrated a median of 19.5 (range 0–66) foci/specimen (including *in situ* lesions and areas of pagetoid spread). Signet ring cell foci were not found in one of eight gastrectomies. The highest number of foci was found in the fundus (44.7% of all foci) followed by the body (40.2%), (Figures 1, 2A). Very few foci were detected in the cardia (4.0%), transitional zone (7.5%) and antrum (3.5%). All seven patients with microscopic foci of disease had lesions in the fundus and body, whereas only two patients had lesions in the transitional zone (M11B, M13B). There was no correlation between the number or location of foci identified and the age and sex of the patients or their specific germline mutation.

Endoscopy was performed according to the local practice and therefore the number of biopsies taken varied enormously, from 0 to 38 (median 24). Four of eight (50%) patients had lesions detected in endoscopic biopsy specimens (patients M10C, M11B, M13A and M13B), which equates to four of seven (57%) patients with signet ring cell lesions in their gastrectomy specimens (Figure 2B). These biopsy-positive patients had biopsies taken (median 24, range 24–38) compared to those without a pre-surgical diagnosis (median 12, range 0–24; $p = 0.02$; Table 2). Nine of ten positive endoscopic biopsies were from the fundus or body, in keeping with the preponderance of lesions found at these locations in our series. The distribution of biopsies taken was representative and similar for all patients, suggesting that sampling bias did not influence these findings (Figure 2B). There were also a greater number of signet ring foci within the gastrectomy specimens (median 20, range 16–66) of biopsy positive patients compared to those three patients

with negative surveillance biopsies (median 16, range 0–46, including *in situ* lesions and areas of pagetoid spread), although this was not statistically significant ($U = 5$, $p = 0.25$; Figure 2A, B). Overall, as expected, the likelihood of detecting a lesion pre-operatively was related to the number of biopsies taken.

Immunohistochemical characterization

Of the analysable foci in this study, 77% were negative for E-cadherin expression and the remaining foci had membranous expression which was greatly reduced and patchy (Figure 3). Some of the larger foci identified within the gastrectomy specimens contained smaller cells with little mucin, which tended to be located at the base of the lesions, as previously described [19]. Therefore, we determined the immunohistochemical characteristics of these cells in our series. E-cadherin expression appeared to be similar or stronger in these small cells compared to the larger signet ring cells (Figure 4). Ki-67 analysis demonstrated 1–2% of positive cells spread throughout the lesions (Figure 4), compared to an expected proliferative index of 24% in the antrum of normal stomachs (20). Dual-label immunofluorescence demonstrated cytokeratin 8/18 expression but no vimentin expression in all signet ring cells, including these smaller cells. In contrast, there were a significant number of vimentin-expressing stromal cells in the vicinity of the lesions (Figure 4). There was no apparent difference in staining between the small cells and the large signet ring cells. These data suggest that both the signet ring cells and the smaller cells at the base of the lesions are of epithelial origin, with a low proliferative index, and do not exhibit EMT despite the loss of E-cadherin expression.

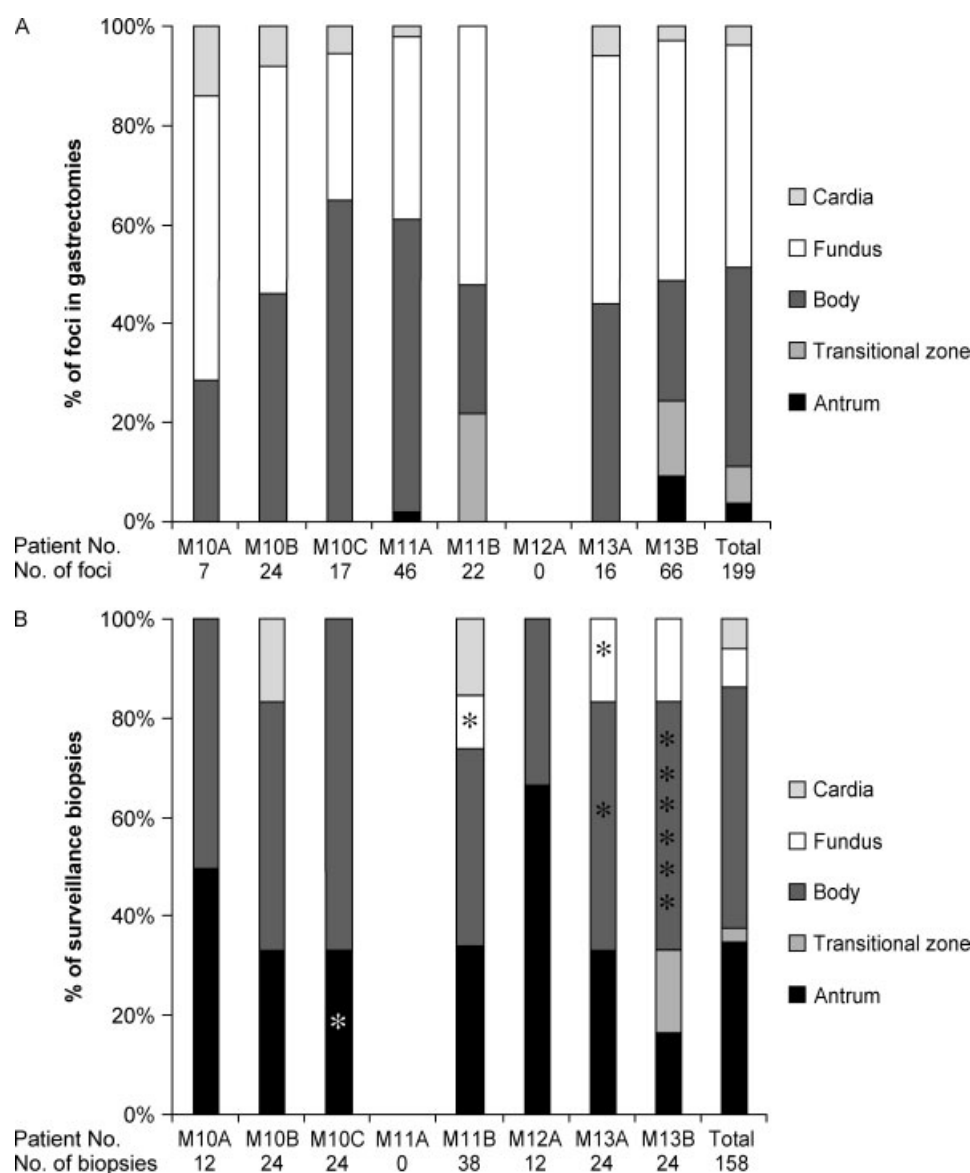


Figure 2. Bar charts show: (A) the anatomical distribution of signet ring foci found within gastrectomy specimens; and (B) the distribution of biopsies taken during surveillance, whether or not they were positive. Each star in (B) indicates a biopsy specimen which contained a focus of signet ring cells

Discussion

In summary, we have shown that in asymptomatic *CDH1* mutation carriers with a normal-appearing gastric mucosa, endoscopic detection of microscopic foci of signet ring cells is possible when multiple biopsies (>20) are taken. The low proliferative index of these lesions and the lack of evidence for EMT suggests that they may be indolent. These findings have potential implications for surveillance protocols, histopathological specimen processing and the timing of gastrectomy, which will be discussed in turn.

Endoscopic surveillance has the benefit of preserving the stomach until lesions are identified. However, there are a number of issues which have made the use of endoscopy in this context questionable, including patient compliance and the sensitivity and specificity of the technique [9,10]. The quality of white light endoscopic images has improved considerably

in recent years, and adjunctive techniques, such as chromo-endoscopy using contrast agents, have been suggested. Congo red–methylene blue test was shown to increase the likelihood of detecting foci >4 mm compared with standard white light endoscopy [21]. However, methylene blue and Congo red have both recently been withdrawn from use, due to concerns over embryotoxicity and carcinogenic potential. The pale lesions identified by chromoendoscopy were predominantly confined to the body–antral transitional zone (six cases fully mapped) [16], but it may be possible to target this area based on endoscopic landmarks without requiring chromoendoscopy. In contrast to these findings, in our series there was a predominance of foci within the fundus, confirming data from a recent study [18]. Antral sparing has previously been noted in prophylactic gastrectomy specimens by Shaw *et al* [21], and this is confirmed in this study.

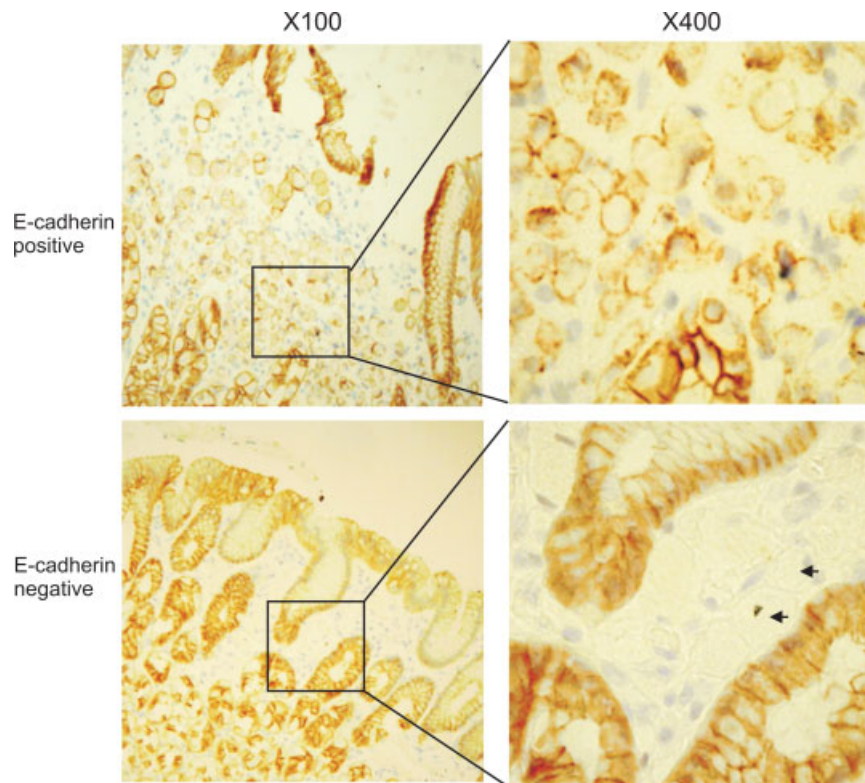


Figure 3. Representative E-cadherin immunostaining of signet ring cell foci in gastrectomy specimens. The top panel demonstrates patchy membranous E-cadherin expression whereas the bottom right panel highlights loss of E-cadherin in the signet ring cells (arrows). In both sections normal membranous E-cadherin staining of adjacent glands is shown for comparison

It is unclear why there should be a difference in the distribution of the foci between these series. Possible explanations include the different ethnic origins of the patients, which may affect other non-*CDH1* genetic susceptibility loci, as well as different environmental exposures, which may influence the nature of the second allele inactivation and hence the distribution of the foci. In light of the regional variation in the location of lesions, caution is required before surveillance protocols advocate targeted biopsies. However, it is clear that, to maximize the yield from endoscopic surveillance, multiple biopsies are required.

Histopathological diagnosis of microscopic cancer foci is a painstaking task and these lesions can cut out on serial sections. This difficulty is highlighted by the different findings from two of the prophylactic gastrectomy specimens (from patients M10A and M10C) analysed in this study, which were previously reported by Gaya *et al* [22]. In patient M10A, who had previously been reported as negative, our research analysis identified four signet ring cell lesions and three *in situ* lesions. In patient M10C, our analysis revealed 15 signet ring cell lesions, one *in situ* lesion and one area of pagetoid spread, compared to the five lesions reported previously. The prophylactic gastrectomy specimen from patient M12A did not contain any foci of signet ring cells. Completely negative prophylactic gastrectomy specimens have only been identified twice previously, and one of these previously reported negative specimens has been shown to contain lesions in this study (patient M10A) [22,23].

The findings of microscopic signet ring foci in two individuals from family M13 with a missense mutation (Exon5 641T>C) is also pertinent for the management of other individuals with non-truncating mutations. PCR and *in silico* analyses did not suggest that the mutation confers cryptic splicing. However, using sorting intolerant from tolerant (SIFT) analysis, this mutation was predicted to have an effect on protein structure, and *in vitro* functional studies demonstrated that this mutation is detrimental to the ability of E-cadherin to mediate cell–cell adhesion and suppress invasion [24]. This family has multiple members affected by diffuse type gastric cancer and the two individuals in our study had the same mutation as their father, who died of the disease. It has been recommended that individuals with missense mutations should have *in silico* and functional studies performed to help guide clinical management [24].

In this study, membranous E-cadherin expression was either low or absent in all of the signet ring cell foci, in keeping with previous studies [9,10,17]. It is possible that the lack of immunohistochemically detectable E-cadherin expression may be a useful diagnostic adjunct. The absence of E-cadherin expression within these cancerous foci suggests that the second wild-type *CDH1* allele must be inactivated within the signet ring cells that make up these lesions. This is likely to be lesion-specific, since E-cadherin expression was normal in the tissue between lesions, suggesting that each lesion is likely to be an independent

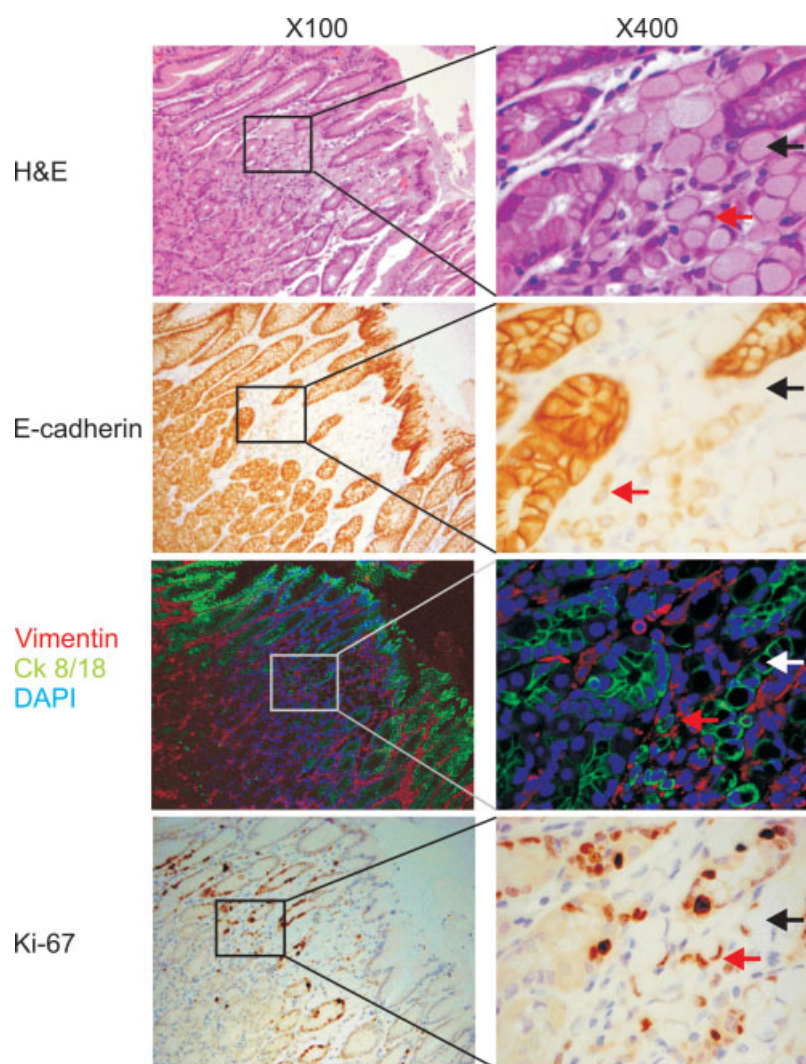


Figure 4. Characterization of the same focus of signet ring carcinoma cells for a range of immunohistochemical markers. The red and black/white arrows point to small signet ring cells and large signet ring cells, respectively. Expression of E-cadherin is higher in small cells than in signet ring cells. Both the small and large cells express cytokeratin 8/18 (green) but do not express vimentin (red), which can be seen in adjacent mesenchymal cells. Both small cells and large signet ring cells express Ki-67 to a lesser extent than the adjacent normal epithelium

occurrence, and hence HDGC cancer foci may be independent clones. This needs to be verified by molecular genetic analyses of individual foci.

The penetrance of the *CDHI* germline mutation has been estimated at 67–83% and the frequent occurrence of these tiny lesions (88% of cases in this study, and 95% of cases identified in the literature; Table 1) suggests that these early lesions may represent an indolent stage that precedes invasion of the mucosa [7–10,16,17,19,21–23,25–29]. The immunohistochemical analysis of proliferation status and markers of EMT performed in this study supports this idea. In addition, the smaller cells identified at the base of the large foci by Humar *et al*, which were thought to represent the invasive front, also appear to be indolent from our analysis, with maintenance of E-cadherin expression and a low proliferative index [19]. In previous studies, cytokeratin expression has been variably reported in the smaller basal cells [19,27]. However, here cytokeratins 8 and 18 were

detected in all signet ring cells, including the smaller cells. Vimentin was only found in non-epithelial cells, which further suggests that EMT is not occurring. Overall these data would suggest a direct conversion from gastric epithelium to mucous containing signet ring cells, rather than progression via an intermediate mesenchymal-like cell. In view of the indolent nature of these microscopic foci, further molecular events, in addition to the loss of E-cadherin, are likely to be required for the indolent foci to turn invasive. However, the nature of these events and the time scale involved for transformation are currently unknown.

Interestingly, there was no correlation in this study between the age of the patient and the number of foci detected. In the absence of data on the natural history of these lesions, therapeutic intervention is inevitable once microscopic foci of signet ring cells have been detected. However, in the future, efforts should be focused on finding algorithms (molecular and/or clinical) to predict cancer progression, so

that gastrectomy can be delayed for as long as possible. This is a particularly important consideration for young individuals in whom the long-term clinical sequelae, including effects on fertility and quality of life, are unknown. Ultimately an endoscopic surveillance protocol with a minimum of number of biopsies and expert histopathological review needs to be devised. A large study investigating the correlation between endoscopic surveillance and therapeutic and/or prophylactic gastrectomies is necessary before such a system could be implemented.

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